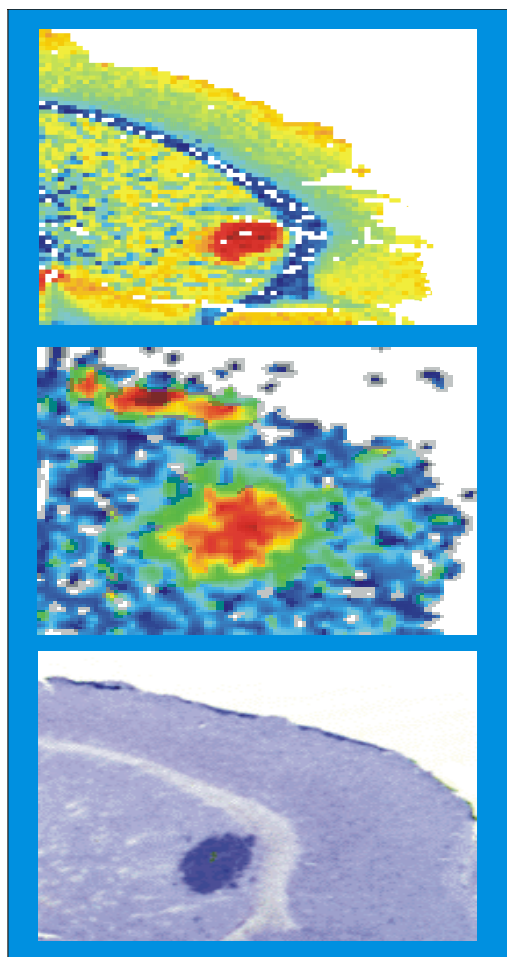
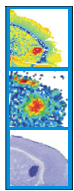


Institute of Bioinorganic and Radiopharmaceutical Chemistry



Annual Report 2001

Cover Picture:



iii Infrared spectroscopic mapping as a new imaging modality:
comparison of i) histological staining, ii) autoradiography and
ii) IR spectroscopic map of a glioblastoma tissue section.

i

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FOREWORD

In 2001 the Forschungszentrum Rossendorf e.V. continued and further developed its basic and application-oriented research. Research at the Institute of Bioinorganic and Radiopharmaceutical Chemistry, one of five institutes in the Research Centre, was focused on radiotracers as molecular probes to make the human body biochemically transparent with regard to individual molecular reactions.

As illustrated by the large number of contributions in this report, the Institute is predominantly engaged in the coordination chemistry and radiopharmacology of technetium and rhenium. The relevant chapter in the report starts with a review article that is meant to help to classify our activities within the flourishing research area of technetium radiopharmaceuticals. Technetium-99m (^{99m}Tc) is of outstanding interest in the development of novel radiopharmaceuticals for routine diagnostics in nuclear medicine. Unlike organic molecules labelled with carbon-11 or radiohalogens, ^{99m}Tc metalloradiodiagnostic agents cannot be straightforwardly derived from known organic lead structures. Technetium is a transition metal and requires a sophisticated coordination chemistry to design complexes that are specifically recognized by target molecules in the human body. It has to be coordinated in such a way that the metal at one of its various oxidation states is tightly bound in a pharmacologically acceptable form. The coordination has a great and not sufficiently predictable impact on the in vivo behaviour of the small molecule into which the technetium-bearing chelate unit is integrated. The design of site-specific technetium molecules is therefore beset with difficulties, including challenges in terms of specific recognition of "artificial", metal-based molecules, penetration through membranes and particularly through the blood-brain barrier, clearance from nontarget sites, and pharmacokinetics in the region of interest which permits imaging and target assessment. Because of similarities in the chemistries of technetium and rhenium, rhenium complexes play an essential part in basic research into technetium complexes, which are radioactive by nature and therefore structurally better to be characterized by analogous non-radioactive rhenium compounds. Most importantly, therapeutic radioisotopes of rhenium are of widespread interest in oncology, nuclear medicine and interventional cardiology. Particularly rhenium-188 (^{188}Re) as an isotope that is easy to obtain in house is of growing interest. Research activities on rhenium-188 have therefore been stepped up at our Institute.

The Institute's chemically and radiopharmacologically oriented activities on technetium- and rhenium-based molecules were complemented by more clinically oriented activities in the Positron Emission Tomography (PET) Centre Rossendorf, which closely links the Institute with the Medical Faculty of the Technische Universität Dresden.

While further pursuing and extending our chemical, biological and medical activities in the PET Centre, new lines of activity have also been opened up. By virtue of its ability to directly assess both pharmacokinetic and pharmacodynamic events in humans and in animals, PET provides a new perspective for drug research. Collaboration with drug companies started in 2001 on the understanding that PET can be used to address bottlenecks in drug discovery and development. The elaborate and time-consuming development of tracer molecules and their careful application are required for successful PET imaging. To make full use of the potentials of PET in drug development, scientists from various disciplines have to be efficiently integrated. For this and other reasons the conceptional orientation and organizational arrangement within the Institute have been modified and adapted.

Closely related to drugs are bioactive substances as they are present in food. Such substances may cause a considerable health risk or may exert effects not yet fully understood. New biotechnological procedures in food processing also give rise to new questions that can be addressed by PET.

Our achievements so far have only been possible thanks to the dedication and commitment of the permanent and temporary staff, the Ph.D. students and collaborators inside and outside the Forschungszentrum Rossendorf. I would like to extend my thanks to all of them.

The Institute wishes to acknowledge in particular the support and assistance received from the Executive Board of the Forschungszentrum Rossendorf, from the competent authorities and funding agencies.



Prof. Dr. Bernd Johannsen

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Tc-99m Chemistry and New Tc-99m Radiopharmaceuticals

Bernd Johannsen

Over the past decade research into the chemistry of technetium continued to progress very rapidly. Aimed at target-specific, smart diagnostic agents, the design of new molecules has largely profited from the introduction of new chelate types as building blocks for new diagnostic molecules.

This review is meant to give an overview of the state of the technetium chemistry and trends in the development of new radiopharmaceuticals based on technetium-99m. It should also be of some help in identifying how our own activities might fit into the modern radiopharmaceutical research and development process.

Introduction

By virtue of its ideal nuclear physical characteristics for routine nuclear medicine diagnostics and its ready availability, technetium-99m (^{99m}Tc) is still of outstanding interest in the development of novel radiopharmaceuticals.

Unlike organic molecules labelled with carbon-11 or radiohalogens, ^{99m}Tc metalloradiodiagnostic agents cannot be straightforwardly derived from known organic lead structures. Technetium is a transition metal and requires a sophisticated coordination chemistry to design complexes that are specifically recognized by target molecules in the human body. It has to be coordinated in such a way that the metal at one of its various oxidation states is tightly bound in a pharmacologically acceptable form. The coordination has a great and not sufficiently predictable impact on the in-vivo behaviour of the small molecule into which the technetium-bearing chelate unit is integrated. The design of site-specific technetium molecules is therefore beset with difficulties, including problems related to the specific recognition of "artificial", metal-based molecules, penetration through membranes and particularly through the blood-brain barrier, clearance from nontarget sites, and pharmacokinetics in the region of interest permitting imaging and target assessment.

From a coordination chemistry point of view, the design of new ^{99m}Tc radiopharmaceuticals starts conceptually with the modification of the coordination environment around the metal with a variety of chelators. Diversity of the chelate unit is needed, and considerable research has consequently been devoted to designing improved and new chelate types, resulting in a flourishing technetium chemistry. The knowledge explosion in the technetium chemistry needs to be translated into targeted radiopharmaceutical research activity. There is no question that novel Tc-99m based radiopharmaceuticals should be target specific and considerably more complex than simple chelates or labelled particles and macromolecules. It is therefore not surprising that the number of new launches is stagnating in a short-term perspective.

Design reflections on specific Tc-99m molecules: choosing the building blocks

Unlike many well established ^{99m}Tc radiopharmaceuticals for routine nuclear medicine diagnostics, such as ^{99m}Tc complexes of DTPA, DMSA, HMPAO, ECD, MAG_3 , MIBI and others, which are quite symmetric small molecules, more specific agents should be asymmetric in a specific manner, integrating building blocks for distinct functions: In general, the technetium-based specific molecule can be considered an entity formed by three constituents: a targeting organic moiety, a linker and the technetium chelate unit (Fig. 1).

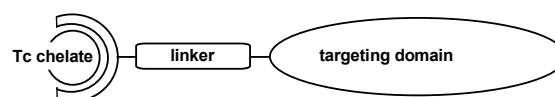


Fig. 1. Schematic representation of a specific ^{99m}Tc molecule

New chelate units

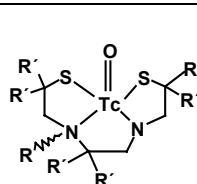
Among the various oxidation states of technetium explored in radiotracer design, Tc(V) proved to be very suitable. One dominant structural element is the oxotechnetium core, $[\text{TcO}]^{3+}$. The presence of the oxo ligand has a significant effect on the structure and properties of the derived complexes. To reduce the synthetic expenditure necessary for tetradentate compounds, particularly for series of complexes for structure-activity relationship studies, *mixed-ligand complexes* were synthesized. A combination of tridentate (S_3 or NS_2) and monodentate (thiol) ligands is employed in the so-called "3+1" complexes. Although stable in vitro, ligand exchange may occur in vivo. The introduction of the Tc(V)HYNIC approach by Abrams *et al.* in the early 90s represented a milestone in the development of Tc-99m radiopharmaceuticals, particularly labelled peptides of very high specific activity. Since the HYNIC only occupies one coordination site, coligands such as tricine, EDDA, etc. are required to complete the coordination sphere of the metal. Ternary ligand systems offer the advantage of high variability for influencing the pharmacokinetic behaviour of the tracers.

A Tc-nitrido heterocomplex has recently been proposed. This is an asymmetric, five coordinate $Tc^V N$ complex containing a bidentate diphosphine ligand and a bidentate chelator associated with the receptor binding domain [1, 2].

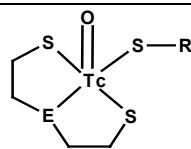
"4+1" complexes at the metal oxidation state +3 no longer possess the oxo ligand as typical

for $Tc(V)$ complexes, thus avoiding the polarity of the complexes caused by this oxo group [3]. Application of this type of chelate is anticipated.

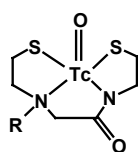
Tc(V)



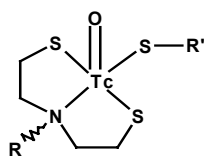
DADT or BAT



(E = S, NCH₃)

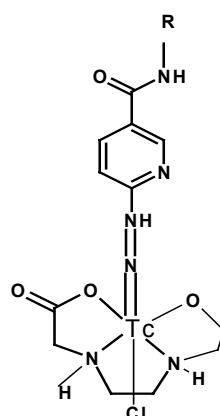


MAMA



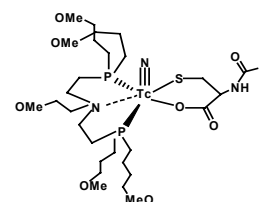
"3+1"

N₂S₂



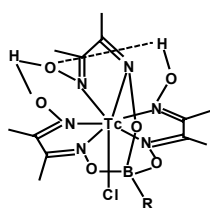
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HYNIC

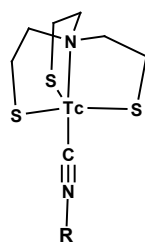


Nitrido-heterocomplex

Tc(III)



BATO



"4+1"

Tc(I)

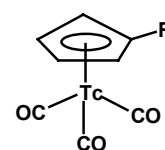
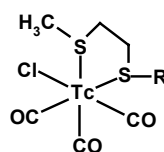
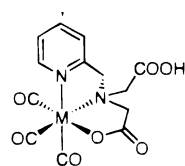
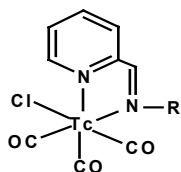


Fig. 2. Main types of chelate units for radiotracer design (R = spacer + targeting domain, R' = H, alkyl, aryl)

A new era of technetium chemistry started when Alberto *et al.* succeeded in making technetium(I) conveniently available as tricarbonyl synthon [4]. Work on the preparation of the core led to a freeze-dried kit formulation [5].

The organometallic aquaion [^{99m}Tc(H₂O)₃(CO)₃]⁺ has been shown to bind to histidine with specific activities of up to 6 TBq/μmol (160 Ci/μmol) [6]. For the functionalization of biomolecules, the influence of the

nature and denticity of ligand systems were systematically studied [7]. Much of recent research was dedicated to developing strategies for exploitation of this promising approach. Based on the results for Tc-tricarbonyl complexes, access to similar but higher-charged compounds was obtained by replacing a neutral [CO] group by an [NO](+)-group. The resulting Re(I)- and Tc(I)-dicarbonyl-nitrosyl complexes show a tendency for co-ordination

at carboxylic and amine groups of biomolecules, e.g. proteins or antibodies [8].

Linker

To attach the chelate unit covalently to the targeting molecule, two strategies are used: the direct bond (N-C, S-C) and the bifunctional approach, using a chelator with an additional coupling group (BFCA). A linker may be helpful or necessary. It may be a simple hydrocarbon chain to maintain a flexible spatial arrangement or to increase lipophilicity. Several complexes have been reported in which the linker had a significant effect on biodistribution and binding. Modified linkers can therefore be used to influence the biodistribution of the complexes. In a special case of CNS-receptor ligands, an ether-oxygen atom, which formally replaced a CH₂ group of the linker, was introduced at an appropriate distance to protonable nitrogen in the receptor-binding domain in order to reduce the pK_a value due to electronic effects [9]. Carbohydration has been introduced as a new tool to improve biodistribution [10]. To reduce the background activity of intravascular targeting molecules, use of a dextran moiety has been proposed [11].

In-vivo stability

In contrast to organic PET tracers, ^{99m}Tc complexes have not yet been subject to extensive metabolic studies. However, as some first examples have shown, both the chelate unit (particularly in certain mixed ligand complexes) and the coordinated organic residues may undergo transformation in vivo. In the case of "3+1" complexes ligand exchange at the metal core leads to instability of the compounds in vivo. The "3+1" mixed ligand complexes containing a monodentate thiol ligand show a remarkable reactivity with other SH-group-containing compounds such as glutathione (GSH) or some proteins [12-14]. The occurrence of ligand exchange reactions with GSH depends on the donor set of the tridentate ligands as well as on the structure of the monodentate ligands.

Complexes with robust tetradentate chelate units could also give rise to metabolism, for example, by splitting off the whole chelate as illustrated in Fig. 4 [15, 16]. Metabolism in both arterial and venous compartments in baboons showed rapid degradation of the parent [^{99m}Tc]TRODAT-1 compound from >80 % intact after injection decreasing to appr. 10 % unmetabolized TRODAT-1 60 min after injection [16].

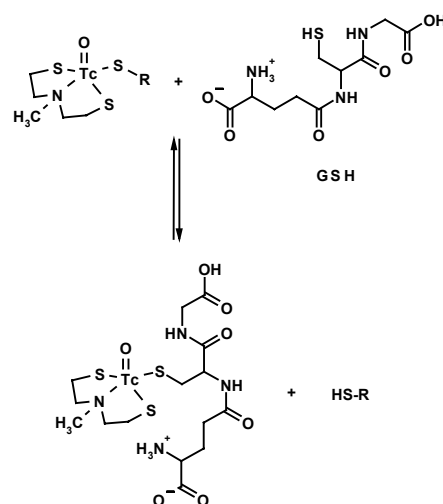


Fig. 3. Ligand exchange of "3+1" mixed-ligand complexes with glutathione (GSH)

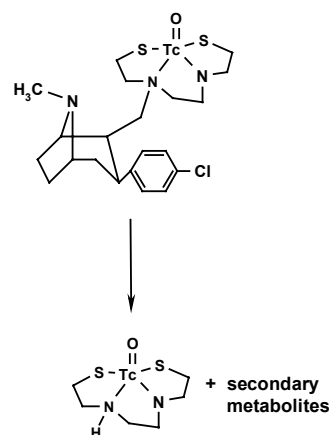


Fig. 4. Release of the chelate unit as the major lipophilic metabolite of [^{99m}Tc]TRODAT-1 in rat plasma [15, 16]

The bond between a tertiary nitrogen of the coordination shell and carbon of the linker is susceptible to splitting also with other chelate units and even during labelling, as was recently reported for ^{99m}Tc tricarbonyl labelled glucose [17].

Targeting domain

The topography of the various receptors appears to accept a relatively wide array of structural types.

Agonist, partial agonist and antagonist structures known from pharmaceutical research and advances in the development of radioligands yield an abundance of potential lead structures. What is rational and predictable in the design of organic drugs or radiopharmaceuticals cannot simply be extended to technetium complexes. New design concepts and strategies have therefore been developed.

For small molecules, merely imagining the inevitable increase in size of the assembled molecule renders the idea of combining a chelate unit with the entire original antagonist molecule unattractive. In this case, it is important to clarify the bulk tolerance and to identify an essential core or fragment(s) of the lead structure, which in combination with the chelate and a spacer (modifier) can be used to assemble molecular structures of retained receptor affinity. It can be concluded from studies of CNS-receptor ligands that various degrees of simplification of the original lead structure are possible or even necessary to obtain a receptor-binding domain as a building block for the design of receptor-binding technetium complexes. Sometimes even quite simple technetium hybrid molecules may exhibit nanomolar affinity for the receptor [18], although additional criteria, such as higher selectivity, metabolic stability, brain uptake and ultimately delineation of the receptor in primates, have so far rendered the plain approach a failure. An illustrative example of successful design strategy are imaging agents that have been derived from tropane structures. There is obviously a large degree of bulk tolerance in the bridgehead nitrogen region of the lead structure. Likewise, the good binding affinity of Tc-99m TRODAT-1 towards the dopamine transporter in the brain indicates that 2 β substitution is well tolerated.

For larger molecules such as peptides and proteins, the choice of the targeting domain is usually less critical and a BFCA - either in the prelabelling or postlabelling approach - can be coupled to the biomolecule often far apart from the receptor or antigen binding motif.

Search for novel radiopharmaceuticals

The progress made in technetium chemistry and impetus from biology and medicine have stimulated exploratory attempts in various directions.

Peptides

Successful developments were made with ^{99m}Tc labelled small peptides. These have been recently reviewed [19]. The successful use of Octreoscan in the diagnosis of somatostatin receptor-positive tumours has intensified the search for improved or new peptide-based agents for imaging thrombi, infection/inflammation and tumours.

Among the chelate units used for peptide labelling the Tc tricarbonyl and Tc HYNIC cores have gained most interest. Other chelating framework has been studied, such as the novel dithia-bisphosphine BFCA [20], or further employed, such as tripeptide N₃S chelators for the

inflammation imaging agent ^{99m}Tc-RP128 [21], a tuftsin receptor-binding peptide [22] and melanocortin receptor-1 specific ligands for targeting melanoma [23]. The N-terminal region of annexin V has been used for attachment of additional peptide sequence of the predicted N₂S₂ or N₃S technetium binding site [24].

Ongoing research into ^{99m}Tc labelled somatostatin analogues has further clarified the effect of labelling methods and peptide sequence [25, 26]. A variety of co-ligands used for labelling HYNIC-derivatised peptides has been explored. The commercial kit NeoTect™ (Diatide) for the preparation of a somatostatin-receptor imaging agent is based on the peptide P829 (depreotide) with the technetium binding N₃S sequence diaminopropionic acid-lysine-cysteine in the molecule [27]. Diatide launched in 1998 the AcuTect™ kit for the preparation of a GPIIb/IIIa receptor ligand for detection of deep vein thrombosis.

Labelled HYNIC-conjugated peptides recently described also involve RGD peptides targeting the integrin $\alpha v \beta 3$ (vitronectin) receptor [28]. A tertiary ligand complex of HYNIC -conjugated peptide, tricine and trisodium triphenylphosphine-3,3',3''-trisulphonate (TPPTS) has been recently described [28]. Interleukin-8, a chemotactic cytokine involved in activation of neutrophils to areas of infection, can be labelled with ^{99m}Tc, using HYNIC, with preservation of its leukocyte receptor-binding capacity [29].

After the introduction of the Tc(I) tricarbonyl approach, its application to peptide labelling has been pursued [30, 6]. Labelling of neurotensin(8-13) derivatives resulted in stable tracers of high specific activities and retained biological properties.

Recently, a first attempt to exploit the chemistry of nitrido(V) complexes for labelling small biomolecules has been described. The tripyrrole peptide distamycin A, an antibiotic agent that binds to DNA, was functionalized with cysteine to obtain a bidentate ligand, which forms a mixed-ligand complex with a [^{99m}Tc(N)(PP)]²⁺ fragment [31].

Proteins, liposomes

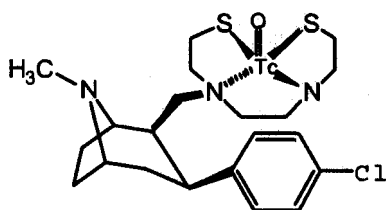
During the past ten years much work has been focused on the development of ^{99m}Tc labelled monoclonal antibodies and their fragments. Three main strategies for labelling can be distinguished: direct labelling, BFCA-based prelabelling and BFCA-based postlabelling. The Tc(I) tricarbonyl approach was developed into a simple one-step labelling of His-tagged recombinant proteins [32].

HYNIC conjugated to form a constituent in the bilayer was used in a novel, alternative labelling method for liposomes [33].

CNS receptor imaging agents

The development of ^{99m}Tc based imaging agents selective for CNS receptors continues to remain an area of considerable research endeavour. Progress has been made both conceptually and in

- development of [^{99m}Tc]TRODAT-1 as dopamine transporter (DAT) imaging agent, in 1997 [34]



[^{99m}Tc]TRODAT-1

- development of another DAT ligand, [^{99m}Tc]O1505T, in a phase I clinical trial [35]
- synthesis of ^{99m}Tc or surrogate rhenium complexes with nanomolar in-vitro affinity to dopamine (D_1 , D_2), serotonin (5-HT $_{1A}$, 5-HT $_{2A}$) and muscarinic acetylcholine receptors

[^{99m}Tc]TRODAT-1 and possibly ^{99m}Tc -labelled O-1505T represent successful developments of SPET ligands for imaging DAT sites. The laboured advances in developing other ^{99m}Tc based CNS receptor-binding radiopharmaceuticals reflect the tremendous challenge posed by the peculiarity of the transition metal technetium. The state of the art of technetium-based CNS receptor ligands has been recently reviewed [36].

A number of potential receptor-binding technetium complexes have been described. Complete data concerning the specific receptor binding in terms of affinity and selectivity and the initial brain uptake have not always been reported. Another problem is the variability of the methods used to obtain in-vitro and in-vivo data. As the high *in-vitro* affinities to various neuroreceptors in the nanomolar and sub-nanomolar range indicate, molecular recognition of technetium complex molecules and their fit into the binding pocket of the receptors are achievable. Therefore, it is not receptor binding that is the extremely difficult or even unattainable goal as could have been expected. The main issue is the very low or absent brain uptake. After two decades of research into brain

^{99m}Tc perfusion agents it is now well feasible to enable certain technetium complexes to cross the blood-brain barrier. Technetium perfusion agents such as HMPAO with a brain uptake of 2.25 ± 0.51 % ID in rats 2 min p.i. [37] may serve to indicate the level of expectations. A suitable combination of a high receptor affinity with a sufficient brain uptake was not achieved, with the only exception of DAT ligands. Systematic studies of model technetium compounds with different log P and pKa values provided rules for selected homologous series of complexes but did not really help to tackle the problem [38].

Other factors such as the nature of the chelate unit may limit the brain transfers. Since a wide variety of chemically diverse compounds, among them lipophilic cations such as ^{99m}Tc MIBI, ^{99m}Tc tetrofosmin or Q-series compounds, may be actively transported out of the cell by P-glycoprotein, it might also affect the transport of potentially receptor-binding ^{99m}Tc agents [39].

Other specific ligands

Because of overexpression of sigma receptors in a variety of tumours, particularly of sigma-2 receptors, a potential Tc-99m radiotracer was synthesized and characterized [40, 41].

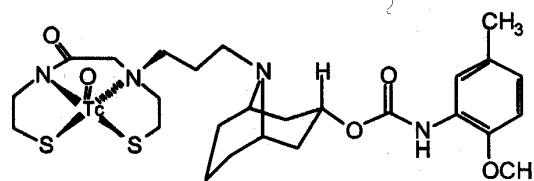


Fig. 5. A sigma-2 receptor ligand as a potential breast tumour imaging agent [40, 41]

The initial results of a series of novel ^{99m}Tc -labelled MIBG derivatives were mixed. At least in principle, it appears feasible to replace the iodine of MIBI with a [$\text{Tc}^{\text{V}}\text{O}$] $^3+$ N_2S_2 complex [42]. Based on this initial attempt, other aromatic substances containing an N_2S_2 chelate have been synthesized and tested [43]. Replacing the aromatic ring of IMBA, a ^{123}I labelled melanom-targeting benzamide, by a MAMA-chelate led to metal complexes that displayed a significant melanoma accumulation in vivo [44].

DTPA-folate conjugate was labelled with $^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3^+$ and tested as folate-receptor ligand for tumour uptake [45].

There is considerable interest in developing a ^{99m}Tc -labelled glucose derivative as an alternative to FDG. All attempt made so far were in vain. Labelling experiments have been per-

formed with either N_3 and N_2O chelators or IDA-functionalized glucose, using the tricarbonyl approach [46, 47].

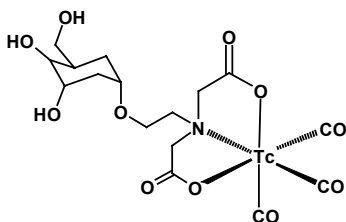


Fig. 6. Tc(I)tricarbonyl labelled iminodiacetic acid /2-deoxyglucose [47]

Technetium research in the field of redox-active complexes (hypoxia and ischemia imaging agents) has developed from two approaches, the hypoxia-dependent reduction of the nitro functionality of nitroimidazoles and the metal electron transfer of copper-containing dithiosemicarbazone (DTS) complexes. A series of 2-nitroimidazoles coupled to peptidic chelators was developed and characterized [48]. A Cu-DTS mimetic agent, Tc-ATSM₂ as an ischemia-damaged myocardium agent has been discussed [49].

A novel class of ^{99m}Tc compounds showing high heart uptake has been described [50]. These complexes were formed by a terminal TcN multiple bond and two bidentate phosphine-thiol ligands. The myocardial accumulation is apparently driven only by their lipophilic character.

Conclusions

- ◆ Flourishing technetium chemistry (new chelate units)
- ◆ Many attempts to open a window into *in-vivo*-biochemistry (specific substrates and ligands)
- ◆ Progress in tuning the biodistribution („pharmacological modifier“ in peptide conjugates)
- ◆ ^{99m}Tc radiopharmaceuticals: scarcely new aunches in a short-term perspective (peptides, TRODAT, ...)

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I. RESEARCH REPORTS

TUMOUR AGENTS AND TUMOUR DIAGNOSIS

Prognostic Value of FDG-PET for the Evaluation of Posttherapeutic Residual Mass in Patients with Hodgkin's Disease (HD) and Non-Hodgkin's Lymphoma (NHL)

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The database for definition of the place of FDG-PET in staging and restaging several tumour entities is constantly increasing. This study addresses the question whether FDG-PET can reliably differentiate between viable tumour tissue and scar formation in lymphoma patients with residual mass after therapy and adds additional prognostic information concerning the disease outcome.

Introduction

Despite a good response to the therapy, residual mass is found by Computer Tomography (CT) in up to 80 % of patients with HD and in up to 40 % of NHL patients. Since CT cannot differentiate between residual viable lymphoma and scar tissue, these findings are a dilemma for the oncologist. Like many tumour entities, lymphoma tissue exhibits an increased glucose metabolism, which can be measured and visualised with FDG-PET. The aim of this study was the evaluation of FDG-PET in terms of differentiation between viable residual lymphoma and non-viable scar tissue.

Patients and Methods

All together 58 patients with histologically proven HD (n = 43) and NHL (n = 15) were evaluated. They all showed residual findings larger than 0.5 cm evaluated by CT. At least 4 weeks after the end of the chemotherapy or 10 weeks after the end of the radiation therapy, the patients were evaluated by FDG-PET (300-370 MBq ¹⁸F-FDG i.v., start of acquisition 60 min p.i. from the proximal femur to the base of the skull. ECAT EXACT HR+ (Siemens/CTI, TN, U.S.A.). Focal increased uptake suspicious of residual disease was analysed, using regions of interest and determining the mean standard uptake value (SUV). PET-positive findings were correlated with CT and clinical information. PET findings were classified as

1. highly suspicious of viable tumour (SUV > 3)
2. no increased FDG uptake
3. questionable pathological findings indicate of tumour (SUV < 3)

All patients were seen at regular intervals for standard follow-up procedures. PET-positive findings were followed clinically and by CT at short intervals or surgically removed. The end points of the study were either clinically or histologically confirmed recurrence or sustained complete remission (CR). The median follow-up of all patients still in CR covers 41 months.

Results and Discussion

Hodgkin's disease:

Highly suspicious of a viable tumour: One out of 4 patients with a positive finding in FDG-PET developed a recurrence 6 months after PET. The 3 other patients are still in CR.

No increased uptake: None of the 36 PET-negative patients developed a recurrence (negative predictive value 100 %).

Questionable pathological findings: 3 patients with questionable positive findings are clinically in CR, in 2 patients FDG-PET turned negative on the follow-up.

Non-Hodgkin's lymphoma:

Highly suspicious of a viable tumour: One out of 4 patients with PET-positive findings developed a recurrent disease.

No increased uptake: One out of 8 patients with negative PET developed a recurrence.

Questionable pathological findings: 2 out of 3 patients with questionable findings developed a recurrent disease.

Including positive and questionable PET findings, FDG-PET showed a sensitivity of 88 %, a specificity of 68 %, an accuracy of 71 %, a positive predictive value of 30 % and a negative predictive value of 97 %. The exclusion of questionable pathological findings (SUV < 3) raised the specificity to 94 % and the accuracy to 91 %. A cutoff value of SUV > 3 for discrimination between malignant and non-malignant PET findings seems to be a useful parameter.

HD and NHL patients with positive PET findings (SUV > 3) had a worse prognosis (5/8 recurrences) than patients without PET abnormalities (2/50 recurrences). The progression-free survival of NHL patients without PET findings was significantly higher than that of NHL patients with positive findings (p = 0,0015).

FDG-PET is a valuable tool with a high prediction concerning early recurrences in patients with residual mass, especially in NHL.

References

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Diagnostic Value of PET and MRI in the Differential Diagnosis of Diseases of the Pancreas – Assessment of their Operability

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To obtain accurate diagnostic results in differential diagnosis of suspected diseases of the pancreas, modern imaging modalities such as magnetic resonance imaging (MRI), computed tomography (CT) or ultrasound (US) are indispensable. In spite of these methods, in many cases the diagnostic findings cannot provide exact information concerning the possibility of surgical treatment. The number of feasible operations is small, but there is, on the other hand, a high rate of explorative laparotomy. To estimate the diagnostic value of [¹⁸F]-FDG PET in these patients (pts.), the results of MRI were registered and compared with the histological findings („gold standard“).

Introduction

Various established imaging modalities, such as computed tomography (CT), ultrasound (US), endoscopic retrograde pancreaticochoangiography (ERCP) and angiography exist for the diagnosis of suspected diseases of the pancreas. Despite these methods, the diagnostic accuracy as to whether surgical treatment is possible or not is often inadequate. Only 20 to 30 % of pts. can be surgically treated, the main reasons for inoperability is the detection of liver metastases during the operation, carcinosis of the peritoneum, lymphomas along the great vessels in the abdomen or tumour infiltration within the wall of the portal vein. The diagnostic impact of MRI on the preoperative diagnosis has been increasing recently. To differentiate between suspected carcinoma of the pancreas and benign disease, PET with 2-[¹⁸F]fluoro-2-deoxy-D-glucose (FDG) was classified as useful by the Third German “onco-PET“ Consensus Conference. In this study we investigated the diagnostic value of PET and MRI and compared the results with histological findings. A PET study was performed on 32 pts., an additional MRI study on 22 of them. In 5 pts. a histological confirmation was not possible due to inoperability of the patient. The mean age was 63 years (f: n = 15; m: n = 12). A resection was performed in 18 pts. Only a palliative operation was possible in 8 pts.. In one of the pts. a benign occlusion of a bile duct was treated.

Results and Discussion

The histological findings are summarized in the table below.

On the basis of these results, the sensitivity of FDG PET for the detection of malignancy was calculated to be 85 % and the specificity 100 %. In the 19 pts. on whom MRI was performed, these values were 71 % and 80 %. The detection of metastases of the liver was not possible with PET (n = 4). Lymph node metastases were detected in only one of 8 cases.

PET with [¹⁸F]FDG is diagnostically relevant for differentiating benign from malignant pancreas findings. The detection of lymph node or liver metastases, which is decisive for the possibility of surgical treatment, seems not to be sufficient. In view of the relatively small number of pts. investigated so far, a final assessment is not yet possible (ongoing study).

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Diagnosis	Ductal CA	Carcinoma of the papilla duodeni	Distal carcinoma of a bile capillary	Neuroendocrine carcinoma	Malignant cystic neoplasia	Benign cystic neoplasia	Chronic pancreatitis	Adenoma of the papilla duodeni
Number	14	2	2	1	1	2	3	2
PET: correct (RP and RN)	12	2	1	1	1	2	3	2

Probing Brain Cancer by Fiber Optic FTIR Spectroscopy

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Introduction

In the past years, Fourier Transform Infrared (FTIR) and Raman spectroscopy has been applied in many cases to the analysis of tissue, blood and other fluids¹. Established methods for histology are currently complemented by optical spectroscopy because many changes occur on the molecular level in tissue before a diseased state can be observed macroscopically. In some diseases, as in cancer, successful treatment is highly dependent on early detection. Since vibrational spectroscopy probes the molecular level, it is a potentially powerful tool for the early *ex-vivo* detection of cancer in tissue samples. However, an *in-vivo* or at least a minimally invasive method is preferred or in some instances, e.g. brain tumors, required.

Results and Discussion

There are mainly two types of IR optical fibers. The first one based on polycrystalline silver halide mixed crystals ($\text{AgCl}_x\text{Br}_{1-x}$). The second fiber type based on chalcogenide glasses such as sulfide and selenide. In this study four types of optical fibers have been tested, two silver halide ($\text{AgCl}_{0.5}\text{Br}_{0.5}$ and $\text{AgCl}_{0.4}\text{Br}_{0.6}$) and two chalcogenide (As_2S_3 and $\text{As}_{40}\text{Se}_{35}\text{S}_{25}$) fibers with a length of approximately 15 cm.

In order to reduce the data volume of a whole map only the fingerprint region from $1000 - 1800 \text{ cm}^{-1}$ was used for data analysis. This spectral region contains the most information regarding the differences between the spectra. The spectra were then surface normalized. The spectra clearly show the strong amide I and amide II bands as well as absorption bands between 1000 and 1400 cm^{-1} arising from lipids and DNA. Because the chalcogenide fiber has a cut-off frequency at approximately 1150 cm^{-1} all spectra were cut at 1170 cm^{-1} in order to avoid strong noise and mistakes in data analysis. Both silver halide fibers have provided a higher transmission than the chalcogenide fibers. Generally, the chalcogenide fiber shows a lower transmission than the silver halide and some additional absorption bands due to impurity of the core material and cladding but can be used. Silver halide fibers have the disadvantage that they lose transmission due to leg modes within the fiber loop and they show an aging process

caused by reaction from contact with metals or by illumination with visible light.

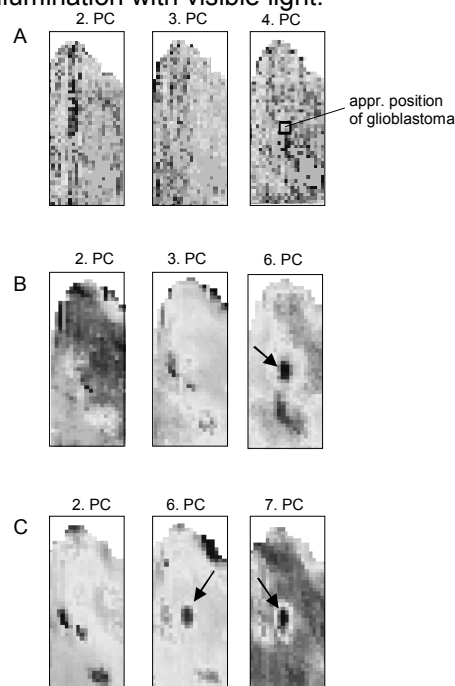


Fig. 1. PCA score plots computed from IR maps of (A) As_2S_3 chalcogenide fiber, (B) $\text{AgCl}_{0.5}\text{Br}_{0.5}$ fiber and (C) $\text{AgCl}_{0.4}\text{Br}_{0.6}$ fiber. The arrows marks the glioblastoma, clearly highlighted by the PC results.

Summary

FTIR spectroscopy with IR optical fibers is suitable to distinguish between tumor and normal tissue. Both of the silver halide fibers produce excellent results. With regard to the fiber materials, the chalcogenide optical fibers were not suitable. The $\text{As}_{40}\text{Se}_{35}\text{S}_{25}$ fiber break too easily, e.g. during bending the fiber, and the As_2S_3 fiber has strong absorption in a spectral region which contains important chemical information for tissue classification. The lateral resolution is limited by the diameter of the fiber, and the distance between fiber end and sample. In our case a lateral resolution of approximately 1 mm is sufficient for detection of tumor. Data analysis performed by using PCA clearly depicts the outline of the tumor.

The authors want to acknowledge the financial support by the Sächsischen Staatsministerium für Wissenschaft und Kunst (SMWK/4-7531.50-03-0370-98/3).

Targeting of Human GGT in a Renal Cell Carcinoma Mouse Model with ^{99m}Tc-HYNIC-mAb 138H11

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The study shows that the specific tumor uptake of ^{99m}Tc-HYNIC-mAb 138H11 in GGT positive tumours was 2 to 5 times as high as in the controls. A high unspecific background activity was observed in the blood and in all organs well supplied with blood.

Introduction

Searching a new tool for targeting the metastatic renal cell carcinoma (RCC), Fischer and Scherberich developed the monoclonal antibody (mAb) 138H11 against human renal gamma-glutamyltransferase (GGT) [1]. This approach is very promising because on RCC, GGT is expressed on the whole cell surface and thereby exposed to the blood. In a normal kidney, GGT is localized at the luminal brush-border membrane of proximal tubules and thus shielded from the blood, resulting in the specific tumour uptake of ex-vivo perfused human kidneys [2]. In vivo, a 138H11-drug conjugate significantly reduced tumour growth [3]. Here, the mAb 138H11 labelled with ^{99m}Tc was to be tested using a new mouse RCC model. ^{99m}Tc-labelling was carried out, using the HYNIC bifunctional coupling agents which constitutes one of the most attractive approaches to preparing ^{99m}Tc-labelled proteins [4].

Results and Discussion

mAb 138H11 was conjugated with HYNIC as described in [5]. For ^{99m}Tc labelling, tricine was used as the coligand. The specific activity was 3.5 GBq/mg; 200 to 250 Mbq were administered to each mouse (i. p.). The test of ^{99m}Tc-HYNIC-mAb 138H11 by TLC, SE-HPLC and PAGE revealed stability in PBS after 24 and 48 h.

Nude mice with human RCC xenografts and Balb/c mice with human GGT-transfected Renca allografts were injected with ^{99m}Tc-HYNIC-mAb 138H11. The controls were SKN cells and Renca wild-type cells. After 0, 24, 48 h p.i. the distribution of activity was determined by gamma-imaging. We measured the relation of intensity between several regions of interest on both days and found that the relative activity over the GGT-positive tumours was increasing, while the relative activity over all other regions was decreasing. That meant that the tumour was still not completely infiltrated with ^{99m}Tc antibody.

After euthanasia the total activity of the organs was measured and the specific uptake per gram calculated as a percentage of the total dose. In both animal models the GGT-positive tumour had a higher activity than the negative ones. The specific tumour uptake was 2 to 5

times higher in GGT positive tumours than in the controls. The specific activity in the tumour was higher than in any other organ with the exception of the kidneys. Surprisingly, the activity in the kidneys was a bit higher in mice bearing transfected Renca tumours than in those with wild-type tumours.

Why we find a lot of activity in the blood and in the kidneys can be attributed to various reasons. In contrast to the immunohistochemistry of ex-vivo perfused human kidneys [2], in mice mAb 138H11 is bound to restricted antigens in the glomeruli. We observed more binding in mice which carried a GGT-positive tumour than in mice with a control tumour. We conclude that either mAb 138H11 formed immun-agglomerates with tumour cells in the blood stream which were retained in the glomeruli or that mAb 138H11 was bound to the Fc receptors of mesangial macrophages. High radioactivity in the blood and kidneys may also be caused by ligand exchange with the amino acid residues of blood proteins or in the lysosomes [6].

These studies show that the mAb 138H11 specifically discriminates between tumours from GGT-transfected cells and wildtype cells as proposed. One problem to solve in the animal model is the unspecific background in the blood and therefore in all organs well supplied with blood. Another aspect is the high renal activity in mice, especially in mice bearing the GGT-positive tumour. In further investigations it will have to be shown, whether radioactively labelled fragments of mAb 138H11 and/or the use of alternative coligands may lead to less activity in the kidneys.

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Molecular Structures of Acyclic Purine and Pyrimidine Derivatives

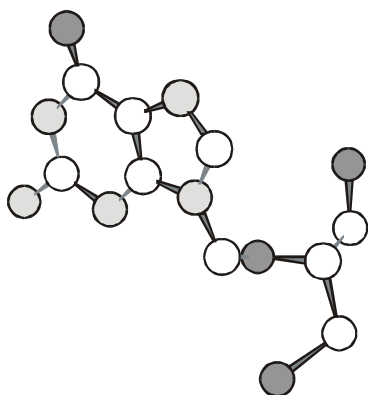
B. Noll, St. Noll, M. Grote, W. Kraus¹
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The molecular structures of ganciclovir, penciclovir, and analogues of interest as therapeutics in gene therapy were determined, and the influence of structural alterations is discussed.

A variety of nucleosides are known to be substrates of herpes simplex virus thymidine kinase (HSV-1) in contrast to the human thymidine kinase which phosphorylates only thymidine. This low specificity makes it possible to modify the molecular structure of the substrate in view of extending the substrate acceptance of the HSV-1 TK.

In our search for fluorine-18-labelled nucleosides that are useful for evaluation of the therapeutic effect of a gene therapy by controlling the selective introduction of genes into tumor cells, we looked for relationships between structural features and substrate properties of respective nucleoside derivatives. After synthesizing ganciclovir and penciclovir which are successfully used today in gene therapy and, in form of F-18-labelled derivatives, also as tracers for monitoring gene expression [1, 2], the X-ray crystal structures of these and two other nucleosides were determined.

Fig. 1.



Ganciclovir (Fig. 1) and penciclovir (Fig. 2) show structural differences in the acyclic side chain and also in their molecular size because of the replacement of the ether oxygen by a methylene group in the side chain. The molecular distances from the oxygen of the purine base to acyclic hydroxylic groups are 10.22 Å / 9.52 Å for penciclovir and 8.89 Å / 7.05 Å for ganciclovir.

In enzymatic studies ganciclovir was proved to be a better substrate for the isolated HSV-1 TK than penciclovir. This may be explained by the structural alteration observed.

The introduction of a methyl group in the N1 nitrogen of ganciclovir (Fig. 3) led to the complete loss of enzymatic affinity. The unsubstituted nitrogen in this position of the purine base thus seems to be an essential condition.

The structure of a new promising tracer, metacyclur, with a purine base is shown in Fig. 4.

Fig. 2.

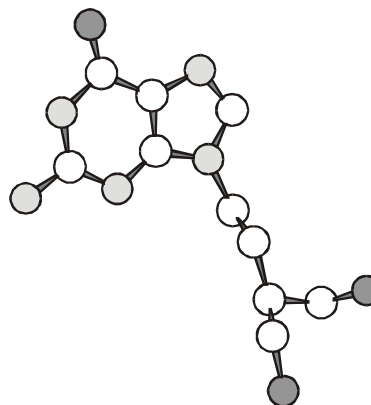


Fig. 3.

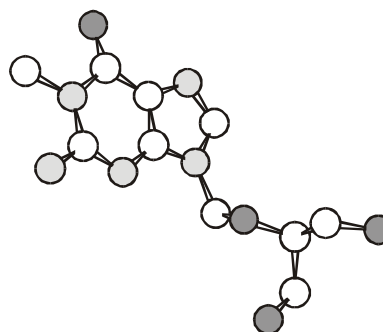
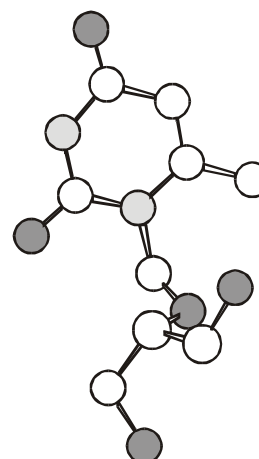


Fig. 4.



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First Results of the Synthesis of [¹⁸F]FMHPU

M. Grote, St. Noll, B. Noll

Substances labelled with positron emitters may become important for monitoring gene transfer of e.g. suicide genes. Our attempts to find new substances for monitoring gene expression led to a promising candidate, the pyrimidine derivative [¹⁸F]FMHPU. We investigated several reaction conditions in order to increase the radiochemical yield of the desired tracer.

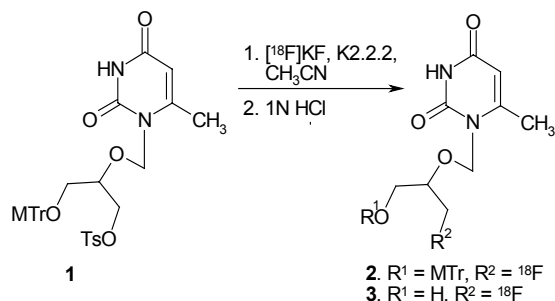
Introduction

The Herpes simplex virus 1 thymidine kinase is an enzyme that phosphorylates nucleosides with a high substrate diversity [1]. That enables us to find, from a variety of nucleosides, substrates that show a high affinity to the HSV 1tk and that are therefore suitable for determining the viral kinase activity *in vivo*.

This requires labelling suitable compounds with fluorine-18 and using Positron Emission Tomography (PET) as a non-invasive imaging method. A possible new compound for this purpose is [¹⁸F]-6-methyl-1-[(1-hydroxy-3-fluoro-2-propoxy)-methyl]uracil [¹⁸F]FMHPU **3**.

Methods

We reported on the synthesis of the precursor **1** and the reference standard **3** with the stable isotope fluorine-19 [2]. Here we describe our experiments to produce the desired tracer in a high radiochemical yield.



The temperature of the reaction was varied to find the ideal conditions for obtaining the intermediate **2**. The dependence of the radiochemical yield on the amount of precursor **1** was also examined. The formation of compound **2** was analysed by TLC (silica gel, CH₂Cl₂/MeOH, 85:15). The protecting groups were subsequently split off by 1N HCl in methanol generating **3** [3] in a range of 5 - 12 % (not decay-corrected).

Results and Discussion

The synthesis of **3** is a two-step procedure which involves fluorination of the precursor, followed by removal of the methoxytrityl group. Reaction temperatures of 120 °C and 140 °C give nearly equivalent yields of **2** (28 % and 26 %, Fig. 1) so that a temperature in between should be suitable for carrying out the addi-

tional experiments. Correlation of the yield and the amount of precursor was determined in the range from 9.6 μmol/ml to 14.5 μmol/ml of starting material in a reaction time of 20 min and a temperature of 130 °C in CH₃CN (Fig. 2).

Fig. 1.

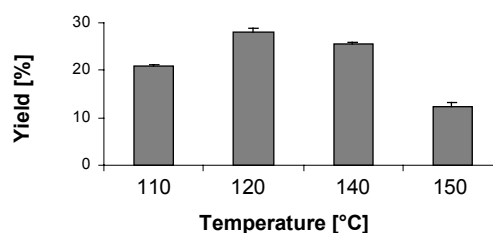
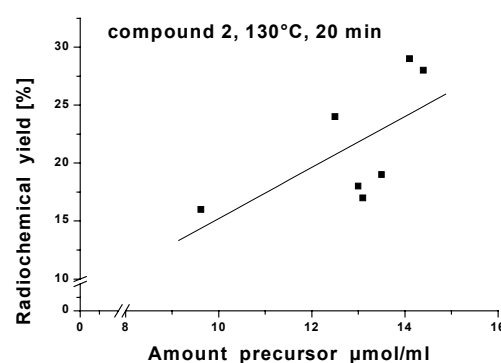


Fig. 2.



Although the breakpoints show great variance, the radiochemical yield was demonstrated to depend on the quantity of **1**. Highest yields were obtained with a precursor concentration of about 14 μmol/ml while a lower concentration (9.6 μmol/ml) yielded a poor result of compound **2** (Fig. 2). Additional experiments have to be performed for more exact predictions. The amount of radiotracer received is sufficient for biodistribution studies which will be performed in addition.

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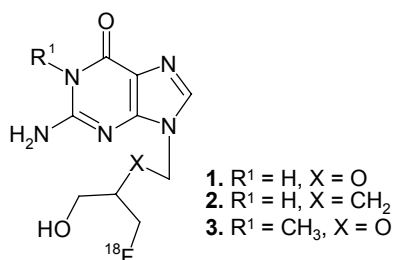
Optimization of the Synthesis of ^{18}F -labelled Compounds for Monitoring Gene Therapy

M. Grote, St. Noll, B. Noll

Several methods have been described for radiolabelling eligible precursors for monitoring gene therapy with the PET isotope fluorine-18. Some problems occurred during the adaptation of the reaction conditions to our compounds. To overcome them, we optimized the synthesis of N^1 -methyl-9-[(1-hydroxy-3- ^{18}F fluoro-2-propoxy)-methyl]guanine (^{18}F FMHPG).

Introduction

Positron Emission Tomography (PET) on the basis of appropriate radiotracers is suitable for noninvasive measurement of processes *in vivo*. For gene therapy applications, several reporter probes such as acycloguanosine analogues labelled with fluorine-18 have been described [1]. Recently, a one-pot preparation of two representatives, ^{18}F FHPG **1** and ^{18}F FHBG **2**, was published [2]. Since a drawback of these procedures are low yields, we optimized the reaction conditions so as to improve the yield, using ^{18}F FMHPG **3** as a model compound, the synthesis of which is described [3].

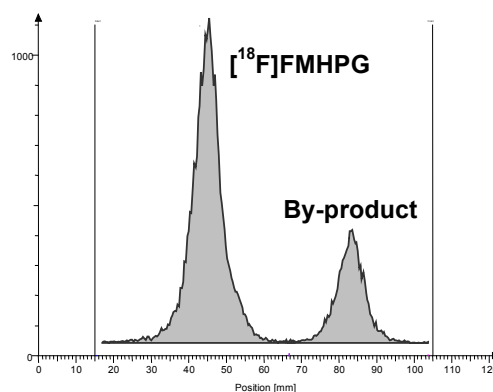


Chemistry

To prepare the labelled compounds **1-3**, nucleophilic substitution at the precursor was performed under standard conditions (K^{18}F -kryptofix 2.2.2 complex in dry acetonitrile at 160°C). Especially splitting off the methoxytrityl protection groups by means of aqueous HCl presented some difficulties. Carried out as a one-pot synthesis with acidification of the reaction mixture and without separating of unreacted fluoride and kryptofix, it produced a low yield of reduced radiochemical and UV purity. We found that purification by a silica gel cartridge (elution with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (85:15)) produces an intermediate in higher purity and yield. Deprotection was performed by methanolic 1N HCl. The mixture was heated to 120°C for 5 min in a closed vial and neutralized. Subsequent purification by semipreparative HPLC (RP-18, water/ethanol 95:5) led to a product with no UV impurities and a radiochemical purity >98 %.

Results and Discussion

The main disadvantage of the original procedure was the application of aqueous HCl as a deprotecting step, because the two-phase system water-dichloromethane did not allow satisfactory control of the reaction temperature. As a consequence the acyclic side chain was split off, resulting a by-product (TLC-plate, RP-18, acetonitrile/water 1:9). Furthermore, removal of dichloromethane before acidification caused high radioactivity absorbance at the glass wall.



These problems were overcome by using methanolic hydrochloric acid as a splitting agent. It is miscible with CH_2Cl_2 and this solution can be heated to 120°C in a vial closed with a screw cap and a teflon coated septum. When the reaction is finished, the solvent can be removed very fast under reduced pressure and a constant flow of nitrogen without any waste of activity. This improved procedure produces **3** in yields between 8-17 % (not decay corrected) within 90 min.

This synthesis was successfully applied to ^{18}F FHPG **1** and ^{18}F FHBG **2**.

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Comparison of the Affinity of Some Potential Substrates Towards HSV 1 TK Wild Type and the Mutant Q125N

M. Grote, St. Noll, B. Noll, L. Scapozza

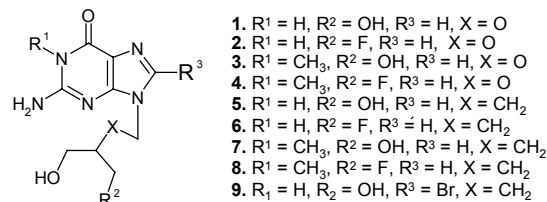
Various nucleosides were determined in terms of their affinity towards viral herpes simplex thymidine kinase (HSV 1 TK). Besides, the HSV 1 TK wild type (wt), a mutant of the enzyme (TK Q125N), was used for this study.

Introduction

The high substrate diversity of herpes simplex virus 1 thymidine kinase is the crucial basis for finding substrates with a high affinity to TK that are useful for monitoring the efficacy of gene expression. In this respect the nucleosides [1] listed in Scheme 1 were tested with HSV 1 TK wt as well as HSV 1 TK Q125N. The Q125N mutant was specifically performed to understand the molecular mode of action of the TK [2].

In this study, the respective nucleoside, HSV 1 TK (wt or Q125N) and ATP as the phosphorylating agent were incubated at 37°C for 1 h. The mixture was analysed by HPLC as already described [3]. The ADP/ATP ratio relates to the substrate activity. Results are shown in Fig. 1 and Fig. 2.

Scheme 1



For further evaluation of the compounds **1**, **3**, **5**, **7** and **9** with regard to their substrate affinity towards HSV 1 TK Q125N, a spectrometric assay was used in addition (Fig. 3) [4].

Results and Discussion

In the reaction with the wild-type enzyme only compounds **1**, **2**, **5**, **6** and **9** showed an increase in the ADP/ATP ratio (Fig. 1) and are substrates of the HSV 1 TK wt. In contrast, the compounds **3**, **4**, **7** and **8** reacting with the mutant HSV 1 Q125N also have a significant ADP/ATP ratio (Fig. 2) and may be potential substrates for this enzyme. It was also noticed that ganciclovir **1** generally showed a higher ratio than penciclovir **5** when reacted with both kinds of enzymes. Surprisingly, it was found when comparing the methylated ganciclovir derivative **3** with the corresponding penciclovir derivative **7**, that **7** is a better substrate to the HSV 1 TK Q125N than **3** due to its higher ADP/ATP ratio. The results mentioned above are also reflected in the spectrometric assay.

Compound **9** showed the best turnover rates, followed by thymidine **10**, ganciclovir **1**, penciclovir **5** and the methylated substances **7** and **3**.

Fig. 1.

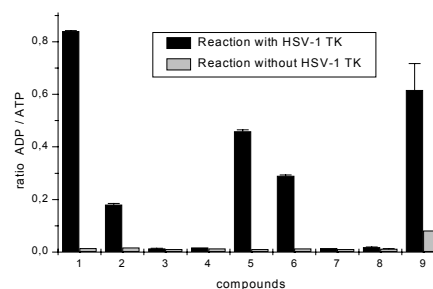


Fig. 2.

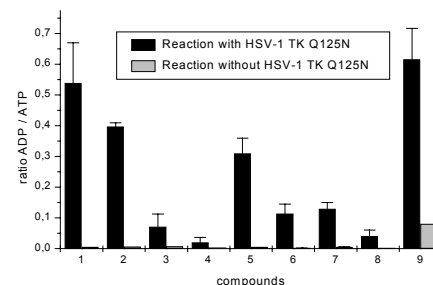
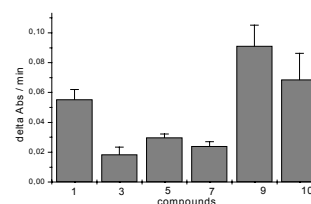


Fig. 3.



References

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A Competitive Reaction Observed during Fluorination of FHPG and FHBG

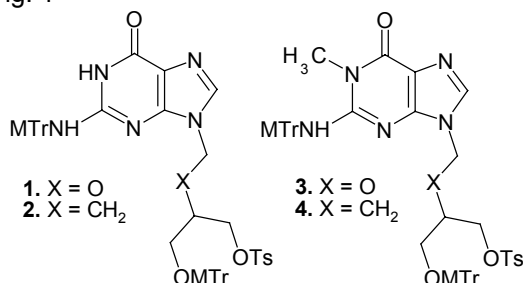
M. Grote, St. Noll, C. Dittmar, B. Noll

The ^{18}F -labelling yield of the tracers $[^{18}\text{F}]\text{FHBG}$ and $[^{18}\text{F}]\text{FHPG}$, which is used to monitor the gene expression of HSV-1 TK, is reduced by competitive reactions. In the synthesis of the reference standards the by-products were isolated and analysed.

Introduction

In our efforts to obtain ^{18}F -labelled compounds for imaging gene therapy, we noticed highly varying yields, depending on the precursor used. Conversion of precursor **1** and **2** into $[^{18}\text{F}]\text{FHPG}$ [1] and $[^{18}\text{F}]\text{FHBG}$ [2], but also into the non-radioactive fluorine compounds resulted in a much lower yield than was obtained by reaction of the methylated derivatives **3** and **4** [3].

Fig. 1

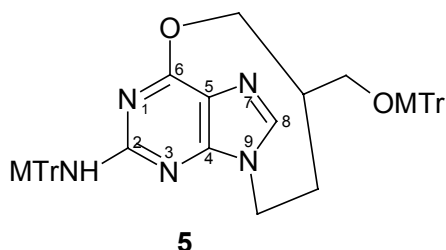


To characterize the by-products, the reaction of the precursor **2** was chosen.

Results and Discussion

Non-radioactive fluorination of compound **2** (KF, K₂CO₃, and K₂2.2 in CH₃CN) produces a by-product **5** in a yield of about 20%, that was characterized by ^1H NMR, IR, MS and elemental analysis.

Fig. 2



^1H NMR showed a shift to lower field of the formerly acyclic side chain and the absence of the proton on N¹, while the other signals had nearly the same position with regard to compound **2**. The IR spectrum confirmed the absence of the N¹-H valence vibration. MS and elemental analysis resulted in the formula C₅₀H₄₅N₅O₄. Examination of these data suggests for **5** the N/O bridged structure shown in Fig. 2.

An analogue substance was obtained from **1** under the same conditions.

In ^{18}F -labelling experiments the reaction mixture was analysed, using HPLC (RP-8, CH₃CN/H₂O 70:30 5 min isocrat., CH₃CN grad. 15 min, CH₃CN 10 min isocrat.). Fig. 3 reveals the formation of **5** in a concurrent reaction which decreases the yield of $[^{18}\text{F}]\text{FHBG}$.

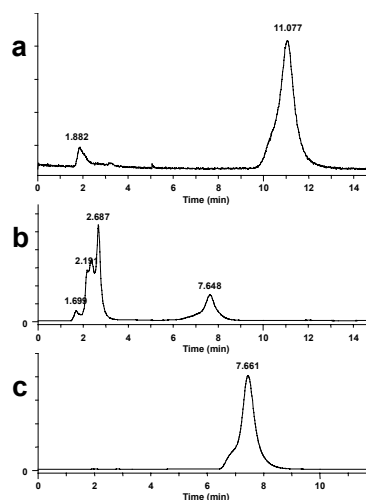


Fig. 3. a: radioactivity, reaction mixture
b: UV, reaction mixture
c: UV, compound **5** (reference)

Chromatogram (a) shows the $[^{18}\text{F}]$ -labelled, methoxytrityl protected FHBG ($R_t = 11.08$ min) as main the product. The corresponding UV chromatogram (b) shows a product with a retention time of 7.65 min corresponding to **5** (c, $R_t = 7.66$ min) was formed among others.

An analogue product was also observed when fluorinating **1** (data not shown). Since the methyl group in the N¹-position inhibits cyclization, **3** and **4** do not form such a by-product. In further experiments we hope to avoid cyclization by introducing a useful protecting group into N¹-position or by varying the reaction conditions.

References

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3-O-Methyl-6-[¹²³I]iodo-L-DOPA (OMID) - an Amino Acid Derivative for Tumour Imaging with SPECT

G. Wunderlich¹, F. Füchtner, R. Bergmann, J. Bredow¹, J. Steinbach, B. Johannsen
¹Department of Nuclear Medicine, TU Dresden

Starting from the convincing first results in clinics and the interesting biological behaviour of 3-O-methyl-6-[¹⁸F]fluoro-L-DOPA ([¹⁸F]OMFD)(1) we synthesize the iodine labelled amino acid analogue [¹²³I]OMID, using the same tin organic precursor. The product is stable *in vitro* and *in vivo*. Initial biological data are described.

Introduction

With the introduction of PET into clinical application, ¹¹C labelled amino acids were put into use for brain tumour imaging. This isotopic labelling was extended to other radionuclides, such as ¹⁸F, in order to improve their usability. To transfer the useful properties of these PET radiotracers to the SPECT application, 3-[¹²³I]iodo- α -methyl-L-tyrosine was successfully developed. In addition to these investigations, the search for ¹²³I labelled amino acids with improved properties is of interest to make them more suitable for clinical investigations especially for tumour studies.

Methods

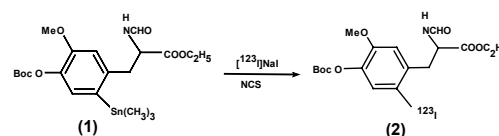
The stereoselective synthesis of ¹²³I labelled OMID is based on electrophilic substitution of the precursor N-formyl-3-O-methyl-4-O-boc-6-trimethylstannyl-L-DOPA-ethyl ester **1** (BOZ Chem, Canada). The reaction was performed in a glass vial, using 1 mg of precursor dissolved in 100 μ l acetonitrile. Commercially available [¹²³I]NaI was added and the pH adjusted to 5 - 6, using 2 % acetic acid dissolved in acetonitrile. Radioiodination of **1** (Fig.1) was carried out, using 5 μ l N-chlorosuccinimide (NCS) (2 mg/ml acetonitrile) as oxidant (**2**). After 10 min the reaction was completed and the intermediate **2** was ready for subsequent hydrolysis of the protecting groups with 100 μ l 12 M HCl at 85 °C **3-6**. For analytical purposes we used HPLC (LiChrospher 100 RP-18, 250x10 mm, gradient: acetonitrile/0.5 % acetic acid in H₂O = 50/50 to 100/0 over 20 min). The separation of the final product was performed by HPLC (PRP-1 column, 125x10 mm, gradient: ethanol/phosphate buffer pH 7 from 0/100 to 70/30 over 20 min). The radiochemical purity was determined by TLC (RP-18 plates) in methanol/H₂O = 50/50.

Results

The radiochemical yield of the iodination reaction is nearly quantitative. The cleavage kinetics of the protecting groups was investigated, revealing a maximum yield of [¹²³I]OMID **5** after about 60 min.

A longer reaction leads to increased amounts of iodine-labelled DOPA **6** and iodide. The total yield of the final product is about 50 %.

Iodination of the precursor



Hydrolysis

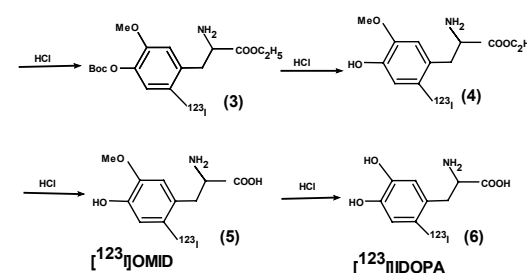


Fig. 1. Reaction scheme for labelling of OMID precursor and hydrolysis

The preparation of [¹²³I]OMID, including iodination, hydrolysis, purification and formulation, is completed after 90 min. The final product is stable for 24 h in EtOH/buffer. No side products are found, no iodine release is observed.

First biological evaluation of the radiotracer

The biodistribution of the labelled [¹²³I]OMID and [¹⁸F]OMFD was compared in Wistar rats one hour after their simultaneous injection. The activity concentration in the stomach and in the thyroid was comparable for both tracers, indicating a low deiodination of [¹²³I]OMID. The distribution patterns of the labelled compounds were similar and show relatively low radioactivity in the main organs, with the exception of the kidneys. The high [¹⁸F]OMFD uptake in the brain and muscle was not confirmed for [¹²³I]OMID at that time. More than 30% of the injected dose was eliminated by the kidneys. The accumulation of [¹²³I]OMID in the kidneys (12.1 \pm 6.2 %ID/g tissue) was higher than that of [¹⁸F]OMFD (4.3 \pm 2.0 %ID/g tissue).

References

- [1] Füchtner, F. *et al.* J. Labelled Comp. Radiopharm. 42 (1999) 267-269.
- [2] Wilbur, D. S. *et al.* J. Nucl. Med. 30 (1989) 216-226.

Development of a PET Tracer for Imaging EGFR Tyrosine Kinase: Evaluation of the Suitability of PKI166

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The suitability of PKI166 for the development of a PET tracer for imaging EGFR tyrosine kinase was investigated. Binding studies using EGFR positive tumour tissue and tritiated PKI166 as the radioligand indicated a low binding affinity of PKI166 to the target tissue. PKI166 is therefore not recommended for PET tracer development.

Introduction

Many tumour types have been shown to express dysfunctional receptor protein tyrosine kinases (RPTK) either as a consequence of excessive production of the growth factor, the receptor or both, or in some cases via mutations in the RPTK's structure. Irrespective of the cause, this leads to hyperactivity of the particular RPTK system and, in turn, to aberrant and inappropriate post-receptor cellular signalling within the cell. Both preclinical and clinical data strongly support the involvement of these receptors in the formation and progression of human cancers and other proliferative diseases. The rationale of inhibiting the epidermal growth factor receptor (EGFR) tyrosine kinase family as an approach to a cancer therapy has continued to grow stronger over the last 10 years.

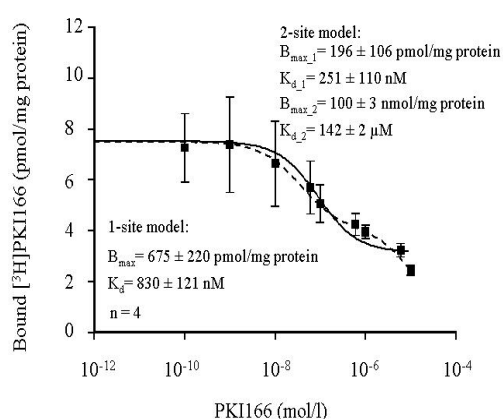
PKI166 is a novel pyrrolo-pyrimidine derivative that inhibits EGFR tyrosine kinase in the low nanomolar range (in vitro IC₅₀ = 0.7 nM). The preclinical profile shows moreover potent anti-proliferative activity in several EGFR-dependent experimental tumour models in nude mice [1].

established methods in the lab [2]. Values of K_d and B_{max} were estimated directly from the binding data by non-linear regression analysis, using the program Fig. P.

Results and Discussion

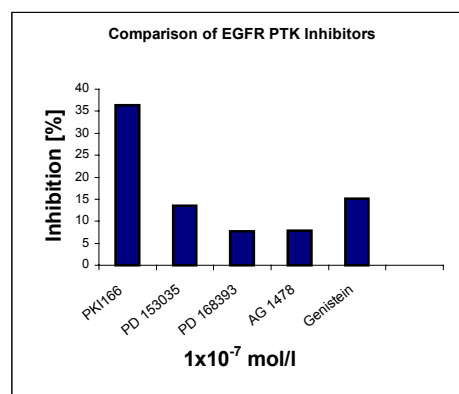
The equilibrium of specific [³H]PKI166 binding was reached after about 60 minutes (data not shown). An incubation time of 75 minutes was therefore chosen for the subsequent studies. Fig. 1 describes the influence of increasing concentrations of PKI166 on the specific binding of the tracer. The best fit to these data was obtained, using a model with two binding sites. Surprisingly, the binding affinity of [³H]PKI166 d(1/K_d) was much lower than expected. Other EGFR-PTK inhibitors were therefore used. In a direct comparison (Fig. 2) it was shown that these compounds are less potent than PKI166. The reasons for the low binding affinity of the EGFR-PTK inhibitors are unclear. It was concluded that PKI166 is not suitable for the development of a PET tracer for imaging EGFR tyrosine kinase. However the tritiated compound may be used in binding assays to select EGFR-PTK inhibitors with higher binding affinities.

Fig. 1.



Methods

In-vitro radioligand binding assays were performed on EGFR-positive FaDu-tumour tissue samples. Binding of [³H]PKI166 to the tissue preparations was performed analogously to



References

- [1] Bruns, C. J. *et al.*, Cancer Res. 60 (2000) 2926-2935.
- [2] Brust, P. *et al.*, Neurosci. Lett. 194 (1995) 21-24.

BRAIN AGENTS AND BRAIN BIOCHEMISTRY

A Novel Tc-99m Radioligand for the 5-HT_{1A} Receptor Derived from Desmethyl-WAY-100635 (DWAY)

I. Heimbold, A. Drews, R. Syhre, M. Kretzschmar, H.-J. Pietzsch, B. Johannsen

Introduction

The large interest in the 5-HT_{1A} receptor today is due to its implicated role in several major neuropsychiatric disorders [1]. In this respect it is of major interest to develop radioligands with a high selectivity and affinity for the receptor to allow brain imaging. In our endeavour to develop 99m-Tc based ligands we used DWAY, an aryl-piperazine ligand, which is successfully used in PET imaging of 5-HT_{1A} receptors [2], as lead structure. Here we describe a new high-affinity Tc-99m ligand for the 5-HT_{1A} receptor with a promising high brain uptake and a satisfying selectivity. The new complex was characterized by several *in vitro* receptor binding assays, biodistribution studies and by *in vitro* autoradiography in rats.

Results and Discussion

Synthesis of the N₂S₂ diamine dithiol (DADT) chelating unit linked to the DWAY-derived piperazine moiety was accomplished as described in Scheme 1 (see also [3]). Technetium complexation was achieved either by SnCl₂ reduction of ^{99m}Tc generator eluate or by ligand exchange from [⁹⁹Tc]technetium gluconate. HPLC analyses indicate the presence of two isomers (*syn* and *anti*), configurationally based on the orientation of the N-substituent in relation to the Tc=O core. For investigations described here we used the mixture of both isomers. With an IC₅₀ value of 1.3 nM the complex [⁹⁹Tc]Tc 1 displays a good affinity for the 5-HT_{1A} receptor, whereas affinity to alpha1-adrenergic receptors is reduced to an IC₅₀ of 8.1 nM.

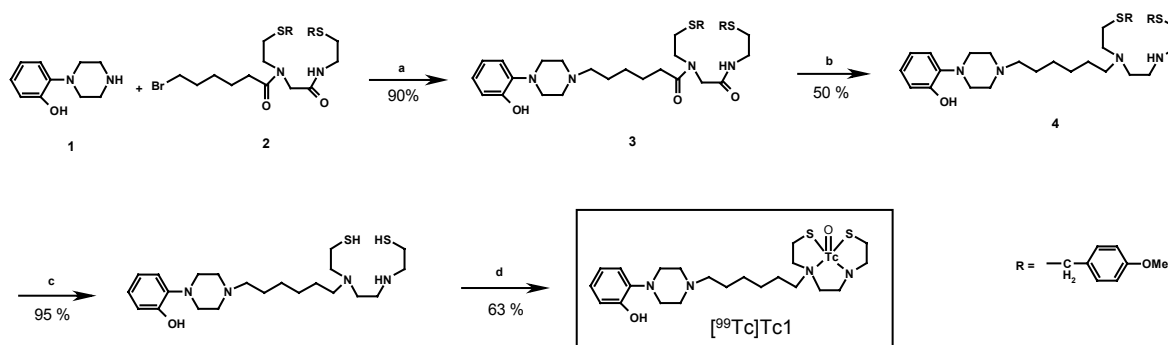
The affinity for the 5-HT_{2A} and the D₂ receptor is considerably lower, the Tc-complex shows more than 700-fold selectivity for 5-HT_{1A} compared to 5-HT_{2A} receptors and about 150-fold selectivity for 5-HT_{1A} compared to D₂ receptors. The results of the *in vitro* receptor binding studies could be confirmed by *in vitro* autoradiographic studies [3]. They clearly indicate the accumulation of [^{99m}Tc]Tc1 in brain areas which are rich in 5-HT_{1A} receptors. This *in vitro* enrichment can be blocked by the 5-HT_{1A} receptor agonist 8-OH-DPAT and is therefore specific.

[^{99m}Tc]Tc1 was taken up in the rat brain up to 0.56 ± 0.07 % ID, 2.5 min p.i.. This value is comparable with that one of [^{99m}Tc]TRODAT-1 (0.43 % ID, 2 min p.i.), which is a useful imaging agent for dopamine transporters in the brain *in vivo* [4]. The relatively slow brain washout accompanied with a fast blood clearance leads to good brain to blood ratios in the time interval of 2.5 to 120 min p.i..

References

- [1] Olivier, B. *et al.*, Prog. Drug. Res. 52 (1999) 103-165.
- [2] Passchier, J. *et al.*, Eur. J. Nucl. Med. 28 (2001) 113-129.
- [3] Heimbold, I. *et al.*, Eur. J. Nucl. Med. 29 (2002) 82-87.
Kretzschmar, M. *et al.*, *this report*, p. 32.
- [4] Meegalla, S.K. *et al.*, J. Med. Chem. 40 (1997) 9-17.

Scheme 1. Synthesis of [⁹⁹Tc]Tc1



Reagents: (a) K₂CO₃, dioxane; (b) BH₃, THF, THF; (c) 1.Hg(OAc)₂, trifluoroacetic acid, anisole, 2.H₂S, ethanol; (d) K⁹⁹TcO₄, SnCl₂, sodium gluconate, H₂O

Biological Evaluation of $^{99m}\text{Tc(V)}$ -labelled Radioligands with High Affinity for the Serotonin 5-HT_{1A} Receptor

A. Drews, I. Heimbold, R. Syhre, H.-J. Pietzsch, S. Seifert, H. Spies, B. Johannsen

Oxotechnetium(V) complexes derived from the selective receptor antagonist WAY 100635 have been characterized by *in vitro* receptor binding assays, biodistribution studies in rats and *in vitro* erythrocytes binding in rat and human blood.

Introduction

For diagnosis of several neuropsychiatric disorders [1] it is of great interest to develop ^{99m}Tc -radioligands with appropriate properties for brain imaging. Here we introduce novel $^{99m}\text{Tc(V)}$ oxocomplexes with "3+1" coordination geometry derived from the 5-HT_{1A} antagonist WAY 100,635 (Fig. 1).

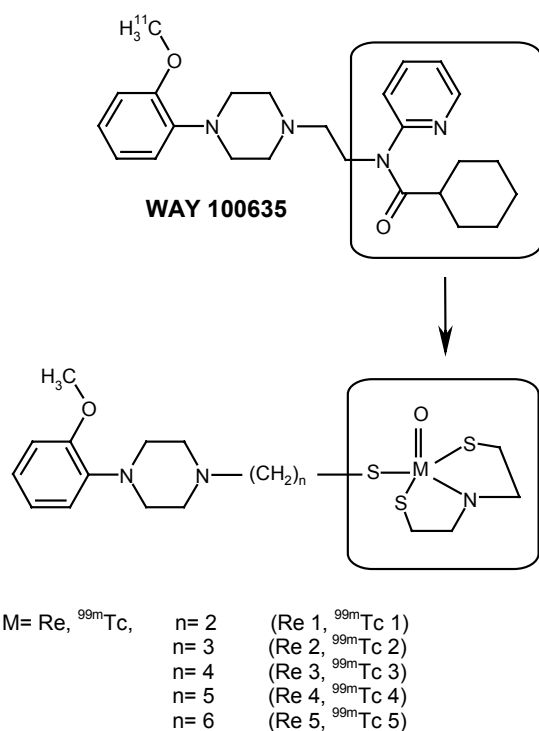


Fig.1. $^{99m}\text{Tc/Re}$ complexes used in this study

Results and Discussion

In vitro receptor binding studies

Table 1. Inhibition constants of Re 1 - Re 5

Complex	IC ₅₀ (nM)	
	5-HT _{1A} [³ H]OH-DPAT hippocampus	5-HT _{2A} [³ H]ketanserin cortex
Re 1	10.3 ± 0.1	19.2 ± 0.2
Re 2	2.6 ± 0.03	24.3 ± 0.3
Re 3	1.25 ± 0.02	34.8 ± 0.5
Re 4	0.47 ± 0.07	35.0 ± 0.4
Re 5	0.13 ± 0.01	103 ± 1

The affinity for the 5-HT_{1A} receptor is improved with increasing length of the alkylspacer. The

selectivity against the 5-HT_{2A} receptor is less pronounced. Two compounds (Re4, Re5) exhibit additional affinity for the α1-adrenoceptor [2,3]. This reactivity has been described for several ligands with an aryl piperazine moiety [4].

Biodistribution in rats

There is no significant dependence of the biodistribution on the alkylspacer length as well as on the receptor affinity. All complexes exhibit a fast washout from the brain (< 0.05 % ID ;120 min p.i.) and a fast blood clearance (0.4-0.2 % ID/g;5 min p.i.).

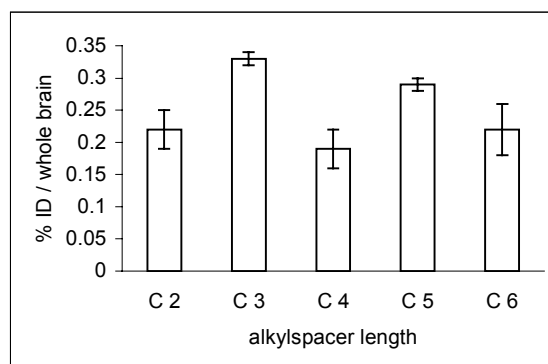


Fig 2. Brain uptake of ^{99m}Tc complexes in Wistar rats (5min p.i.; mean ± SD; n = 4)

The *in vitro* distribution in whole blood is also similar for all complexes but different for rat and human. The RBC fraction of rat blood contains ~20 % of the whole blood activity 5 min after incubation. In human blood only 10 % of the radioactivity was detected on the RBC fraction.

References

- [1] Olivier, B. *et al.*, Prog. Drug Res. (1999) 103-165.
- [2] Heimbold, I. *et al.*, Nucl. Med. Biol. 29 (2002) 82-87.
- [3] Drews, A. *et al.*, J. Labelled Compd. Radiopharm. Suppl. 1 (2001) S544-S546.
- [4] Groß, G. *et al.*, Naunyn-Schmiedeberg's Arch. Pharmacol. (1987) 597-601.

Tc(III) Mixed-Ligand Complexes with Subnanomolar Affinities for the 5-HT_{1A} and the Alpha1-Adrenergic Receptor:

1. Synthesis and Characterization

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Tc(III) mixed-ligand complexes of the general formula $[M(NS_3)(CNR)]$ ($M = Re, {}^{99m}Tc$) have been synthesized and characterized. R bears the receptor-binding domain ($[N-(2-(1-(4-(2-methoxyphenyl)-piperazinyl)-ethyl))-N-(2-pyridinyl)cyclohexanecarboxamide]$) derived from the selective receptor antagonist WAY 100 635.

Introduction

Recently we reported on Tc(V) "3+1" mixed-ligand complexes with subnanomolar affinities for the 5-HT_{1A} receptor. However, the compounds suffer from some in vivo instability due to ligand exchange with endogenous glutathione [1]. Tc(III) "4+1" mixed-ligand complexes are not impaired by such a reactivity [2]. Because of their inertness and stability and an anticipated increase in brain uptake due to higher lipophilicity compared to polar oxygen-bearing chelates, this class of Tc complexes has been chosen by us for an alternative approach towards ^{99m}Tc labelled 5-HT_{1A} receptor ligands.

Results and Discussion

Tc(III) mixed-ligand complexes of the general formula $[M(NS_3)(CNR)]$ ($M = Re, {}^{99m}Tc$) with R bearing a receptor-binding domain have been synthesized and characterized. The receptor-binding domain has been derived from the selective receptor antagonist WAY 100635 and is functionalized with the isocyanide group via an alkyl spacer. Re was used as a Tc surrogate chemical characterization and in vitro receptor binding studies [3]. The synthesis procedure is illustrated in Fig. 1.

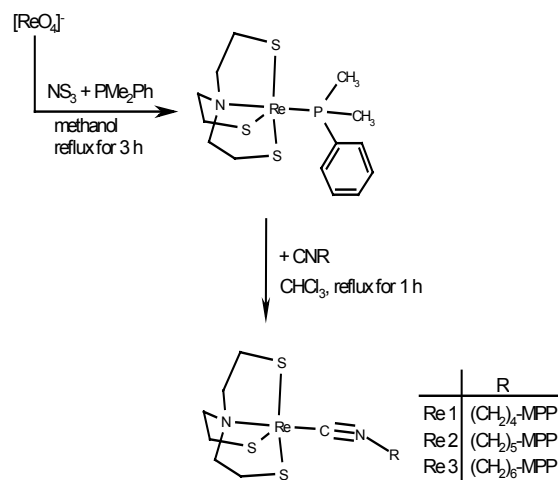


Fig. 1. Synthesis procedures for the Re complexes **Re1** - **Re3**

After preparing the precursor complex, the desired "4+1" complex was formed by addition of the tripodal NS₃ ligand and the functionalized monodentate isocyanide (CN-R).

A perspective drawing of the structure of **Re2** obtained from X-ray structure analysis is presented in Fig. 2. The complex adopt a trigonal-bipyramidal geometry with the trigonal plane formed by the three thiolate sulfurs of the tripodal ligand. The central nitrogen atom of the chelate ligand and the monodentate isocyanide ligand occupy the apical positions.

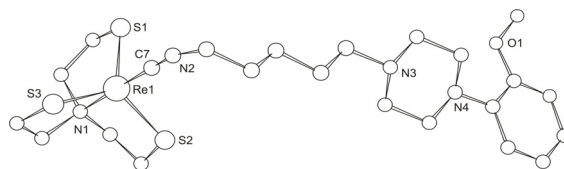


Fig. 2. Molecular structure of complex **Re2**

The appropriate ^{99m}Tc complexes were obtained in a two-step reaction. In the first step the ^{99m}Tc(III)-EDTA complex was formed by reduction of ^{99m}TcO₄⁻ in Na₂EDTA solution. EDTA was substituted in the second reaction step by the tripodal ligand NS₃ and the monodentate isocyanide resulting in the formation of the desired "4+1" complex. The yields obtained by this procedure were between 50 and 70 %. The ^{99m}Tc complexes and the analogous Re complexes exhibited identical retention times in RP-HPLC investigations.

Receptor binding studies and biological evaluation of the complexes in rats will be described in [3].

References

- [1] Heimbold, I. *et al.*, Nucl. Med. Biol (2002), in press.
- [2] Pietzsch, H.-J. *et al.*, Bioconjugate Chem. 12 (2001) 538-544.
- [3] Drews, A. *et al.*, *this report*, p. 30.

Tc(III) Mixed-Ligand Complexes with Subnanomolar Affinities for the 5-HT_{1A} and the Alpha1-Adrenergic Receptor:

2. Receptor Binding and Biological Evaluation

A. Drews, H.-J. Pietzsch, R. Syhre, S. Seifert, H. Spies, B. Johannsen

Tc(III) complexes [M(NS₃)(CNR)] (M = Re, ^{99m}Tc) derived from the selective receptor antagonist WAY 100635 have been characterized by *in vitro* receptor binding assays, biodistribution studies in rats and *in vitro* erythrocytes binding in rat and human blood.

Introduction

Tc(III) complexes derived from the selective receptor antagonist WAY 100635 have been synthesized and characterized [1]. The compounds of the general formula [M(NS₃)(CNR)] (M = Re, ^{99m}Tc) contain alkyl spacers of various chain length linking the "4+1" chelate unit with the receptor-targeting domain (Fig. 1).

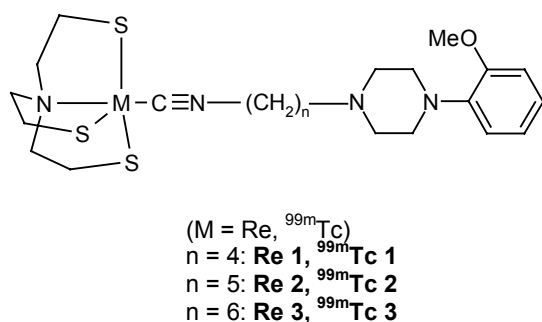


Fig. 1. Re and ^{99m}Tc complexes used in this study

Here we report receptor-binding studies using Re as surrogate for Tc and biological evaluation of the ^{99m}Tc complexes in rats.

Results and Discussion

In vitro receptor binding in rat brain homogenates

Subnanomolar affinity for the 5-HT_{1A} receptor was obtained for **Re 1** (IC₅₀ = 0.29 nM) and **Re 2**, whereas **Re 3** showed a lower affinity (IC₅₀ = 4.5 nM) (Table 1).

Table 1. IC₅₀ values of rhenium "4+1" mixed-ligand complexes **Re 1 – Re 3**

	IC ₅₀ (nM)			
	5-HT _{1A} [³ H]OH-DPAT hippocampus	5-HT _{2A} [³ H]ketanserin cortex	D ₂ [³ H]spiperone striatum	α1 [³ H]prazosin cortex
Re 1	0.29 ± 0.01	156 ± 1	32.7 ± 0.6	< 1
Re 2	0.62 ± 0.17	114 ± 2	18.6 ± 0.2	< 1
Re 3	4.5 ± 0.1	94 ± 2	18.4 ± 0.4	1.13 ± 0.01

All complexes exhibited a higher selectivity for the 5-HT_{2A} receptor as for the D₂ receptor.

Furthermore a high affinity towards α1-adrenoceptors could be observed for all complexes and therefore insufficient selectivity. This "cross reactivity" is already described for arylpiperazine-bearing ligands [2].

Biodistribution studies in rats

^{99m}Tc **1** – ^{99m}Tc **3** were able to cross the blood-brain barrier. The highest brain uptake (0.48 ± 0.09 % ID; 0.32 ± 0.03 % ID/g, 5' p.i) showed ^{99m}Tc **1** bearing the C4-spacer followed by the C5- and C6-homologues. Comparable correlation between spacer length and organ uptake was also observed for the heart. Except for the liver all complexes exhibited similar uptake and elimination behaviour for other organs (Fig. 2).

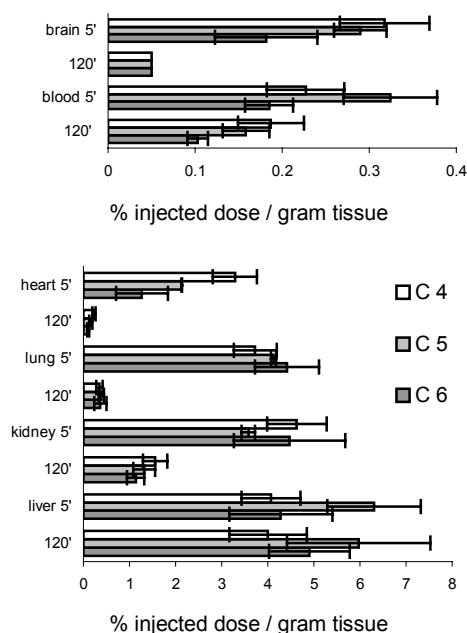


Fig. 2. Biodistribution of ^{99m}Tc **1** – ^{99m}Tc **3** in rats

References

- [1] Pietzsch, H.-J. *et al.*, *this report*, p. 29.
- [2] Groß, G. *et al.*, Naunyn-Schmiedeberg's Arch. Pharmacol. (1987) 597-601

Autoradiographic Evaluation of Novel High-Affinity ^{99m}Tc -Radioligands for the 5-HT_{1A} Receptor (I)

M. Kretzschmar, A. Drews, I. Heimbold, H.-J. Pietzsch, R. Bergmann

5-HT_{1A} receptor binding ^{99m}Tc complexes containing different chelate units with the metal at the oxidation states +5 or +3 have been characterized by *in vitro* autoradiography.

Introduction

Previously described ^{99m}Tc -complexes (Fig. 1) showed subnanomolar affinities to the 5-HT_{1A} receptor on homogenates of hippocampus from rat brain. However, all compounds additionally exhibited affinity to the α_1 adrenoceptors [1, 2, 3].

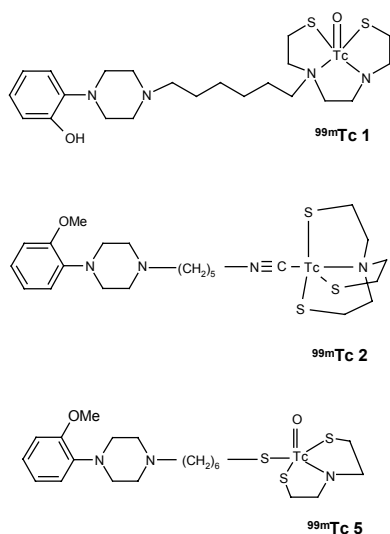


Fig. 1. ^{99m}Tc complexes used in this study

Here the Tc compounds will be evaluated by quantitative digital *in vitro* autoradiography on rat brain sections. The complexes were compared by means of their activity ratios of the lead region for 5-HT_{1A} (hippocampus) and for the α_1 adrenoceptor rich region (thalamus) to the reference region cerebellum according to the following equation respectively for the thalamus.

$$B_r = \frac{(A_{\text{hippocampus}} - A_{\text{cerebellum}})}{A_{\text{cerebellum}}}$$

Results and Discussion

All complexes showed in the *in vitro* autoradiograms of the brain slices (Fig. 2) intense labeling in regions known for 5-HT_{1A} sites such as hippocampus, especially gyrus dentatus, entorhinal cortex and septal nucleus. The binding

was specific to displace with (R)-(+)-8-OH-DPAT. Besides the binding to the 5-HT_{1A} receptor in the autoradiograms occurred a gradually different binding to α_1 adrenoceptor rich regions as thalamus, frontal cortex and cerebellum. The complexes ^{99m}Tc 2 and ^{99m}Tc 5 were additionally accumulated in the caudate putamen, representing a third binding site. The best visualization of the 5-HT_{1A} receptor rich region was achieved by the tetradentate complex with the ratio hippocampus-cerebellum / cerebellum of 1.94 (Table 1).

Table 1. Ratio region-cerebellum/cerebellum

Complex	Hippoc.- Cer. / Cer.	Thal.-Cer./Cer
^{99m}Tc 2	0.41 ± 0.05	0.99 ± 0.32
^{99m}Tc 5	0.97 ± 0.07	1.79 ± 0.20
^{99m}Tc 1	1.94 ± 0.19	0.68 ± 0.09

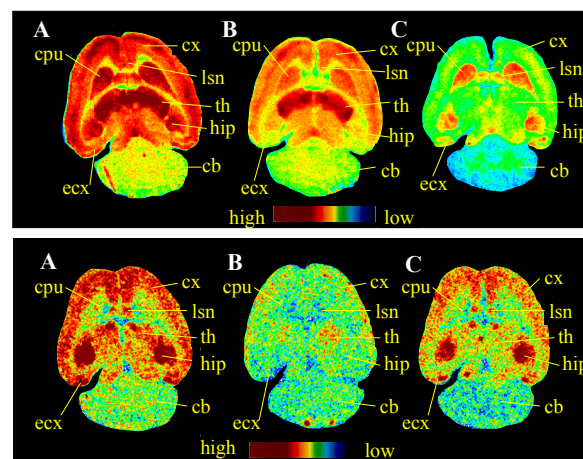


Fig. 2. *In vitro* autoradiograms of rat brains illustrating distribution of ^{99m}Tc 5 (above) and ^{99m}Tc 1 (below) and radioactivity standard. A: control sections, B: sections after displacement by (R)-(+)-8-OH-DPAT, C: sections after displacement by prazosin. Hip = hippocampus, cx = cortex, ls = lateral septal nucleus, th = thalamus, ecx = entorhinal cortex, cpu = caudate putamen, cb = cerebellum.

References

- [1] Drews, A. *et al.*, Nucl. Med. Biol. (2002), in press and *this report*, p. 30.
- [2] Heimbold, I. *et al.*, Nucl. Med. Biol. (2002), in press and *this report*, p. 27.
- [3] Heimbold, I. *et al.*, Eur. J. Nucl. Med. 29 (2002) 82-87 and *this report*, p. 27.

Autoradiographic Evaluation of Novel High-Affinity ^{99m}Tc - Radioligands for the 5-HT_{1A} Receptor (II)

M. Kretzschmar, A. Drews, I. Heimbold, H.-J. Pietzsch, R. Bergmann

The regional *in vitro* distribution in rat brain sections of two $^{99m}\text{TcO(V)}$ "3+1" mixed-ligand complexes derived from WAY 100635 and desmethyl WAY (DWAY) resp. were compared. The DWAY-derived complex ^{99m}Tc 7 showed a higher selectivity for the 5-HT_{1A} receptor than the WAY-derivative ^{99m}Tc 5.

Introduction

The $^{99m}\text{TcO(V)}$ "3+1" mixed-ligand complex ^{99m}Tc 5 derived from the selective receptor antagonist WAY 100635 showed in previous *in vitro* autoradiographic studies significant uptake of radioactivity in regions known for 5-HT_{1A} receptor sites, although the selectivity was not yet satisfying [1].

The aim of this study was to improve the selectivity by desmethylation of the receptor-targeting domain as well as by modification of the chelat unit, resulting in the novel compound ^{99m}Tc 7.

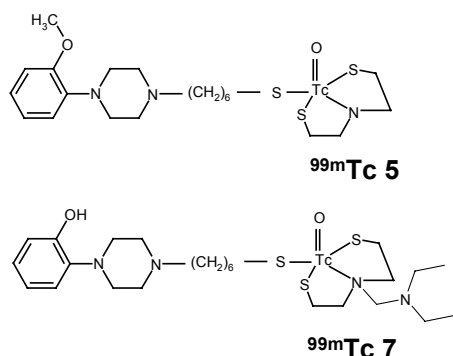


Fig. 1. ^{99m}Tc complexes used in this study

Results and Discussion

Desmethylation of the methoxy group in complex ^{99m}Tc 7 increased the selectivity for the 5-HT_{1A} receptor. Additional binding was observed in brain regions such as thalamus, frontal cortex, external plexiform layer and the internal granular layer of the olfactory bulb. This distribution pattern is characteristic for the alpha1-adrenergic receptor. ^{99m}Tc 7 exhibited only low accumulation in the α_1 -adrenoceptor-rich regions such as thalamus and cerebellum. A third binding site in the caudate putamen as detected for ^{99m}Tc 5 was absent.

Table 1. Ratio region-cerebellum/cerebellum

Complex	Hippoc.-Cer./Cer.	Thal.-Cer./Cer.
^{99m}Tc 5	0.97 ± 0.07	1.79 ± 0.20
^{99m}Tc 7	0.94 ± 0.12	0.51 ± 0.06

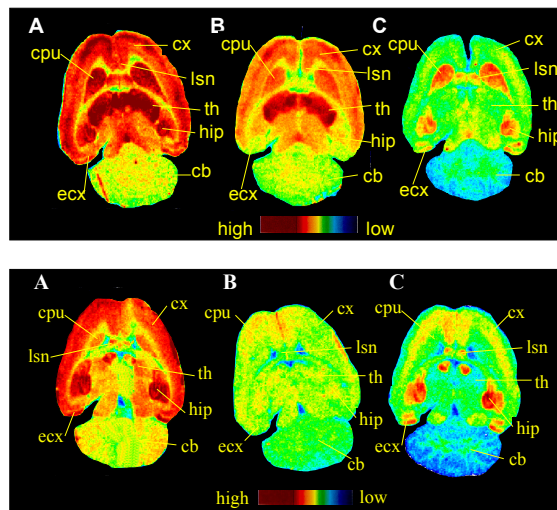


Fig. 2. *In vitro* autoradiograms of horizontal sections of rat brains illustrating distribution of ^{99m}Tc complexes and radioactivity standard. A: control sections, B: sections after displacement by (R)-(+)-8-OH-DPAT, C: sections after displacement by prazosin hydrochloride. Hip = hippocampus, cx = cortex, lsu = lateral septal nucleus, th = thalamus, ecx = entorhinal cortex, cpu = caudate putamen, cb = cerebellum. Above: note the high accumulation of ^{99m}Tc 5 in th, cpu and hip.

Below: ^{99m}Tc 7 shows in A the highest accumulation in hippocampus, little enrichment in thalamus. B shows the small binding to the α_1 adrenoceptor and C exhibits the specific binding to the 5-HT_{1A} receptor.

These results are consistent with the high affinity of ^{99m}Tc 7 to the 5-HT_{1A} ($\text{IC}_{50} = 0.07 \pm 0.01$ nM) measured in the receptor binding assay ($[^3\text{H}]\text{OH-DPAT}$, rat hippocampus). The affinity of ^{99m}Tc 7 for the α_1 -adrenoceptor was one order of magnitude lower than those of ^{99m}Tc 5 ($[^3\text{H}]\text{prazosin}$, rat cortex).

References

- [1] Heimbold, I. *et al.*, Nucl. Med. Biol. (2002), in press.
Kretzschmar, M. *et al.*, *this report*, p. 31.

Improved Synthesis of S-[¹⁸F-Fluoromethyl]-(+)-McN5652

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A modified procedure for the synthesis of S-[¹⁸F-fluoromethyl]-(+)-McN5652 is described. Hydrolysis of the thioester precursor **1** with an aqueous potassium hydroxide solution prevents the formation of a polar side product. Under these conditions the radiochemical yield was increased to 16 ± 4 % (corrected for decay and related to starting [¹⁸F]fluoride).

Introduction

[¹¹C]-(+)-McN5652 is one of the most selective and specific radiotracer for investigation of the serotonin transporter with positron emission tomography (PET). However, the usefulness of this radioligand is limited by its slow pharmacokinetics in the human brain in relation to the short half-life of ¹¹C (20.4 min).

We have introduced the S-[¹⁸F]fluoromethyl analogue of (+)-McN5652 ([¹⁸F]FMe-McN) to avoid this discrepancy [1]. This [¹⁸F]FMe-McN was synthesized by [¹⁸F]fluoromethylation of demethylated (+)-McN5652 with [¹⁸F]bromofluoromethane. The precursor **2** was synthesized by hydrolysis of the thioester **1** with tetrabutyl ammonium hydroxide (TBAH). The radiochemical yield was only about 5 % due to large amounts of a polar side product.

In the present paper we describe an improved synthesis of S-[¹⁸F-fluoromethyl]McN5652 preventing the formation of the polar side product.

version is unknown from reactions with [¹¹C]methyl iodide.

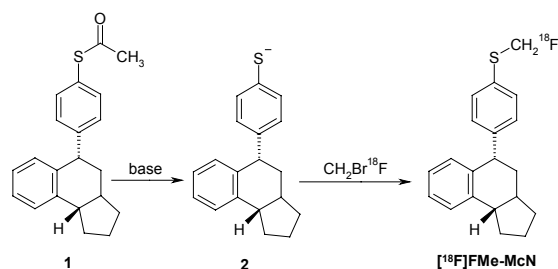
In the case of [¹⁸F]FMe-McN, the undesired competition reaction was prevented by hydrolysis of the thioester **1** with aqueous potassium hydroxide solution. Under these conditions, [¹⁸F]bromofluoromethane was almost completely converted into [¹⁸F]FMe-McN. The radiochemical yield (decay-corrected, related to [¹⁸F]fluoride) was increased to 16 ± 4 % (n = 27). The synthesis, including the synthesis of [¹⁸F]bromofluoromethane, was completed within 45 min. The radiochemical purity was 96 ± 2 %. The specific radioactivity was about 37 GBq/μmol (1 Ci/μmol) at the end of the synthesis.

Analyses of [¹⁸F]FMe-McN by chiral HPLC confirmed that the change of the base did not result in isomerisation reactions.

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Scheme 1. Synthesis of [¹⁸F]FMe-McN



Results and Discussion

Solvents (DMF and methanol) and the base TBAH were individually analyzed for reactions with [¹⁸F]bromofluoromethane, which was prepared according [2]. The formation of the side product was observed during reaction of [¹⁸F]bromofluoromethane with TBAH in methanol. A similar result was obtained by reaction of the [¹⁸F]fluoromethylation reagent with potassium hydroxide in methanol.

These results indicated that the side product was formed by reaction of [¹⁸F]bromofluoromethane with methanol under basic conditions. The product of this conversion could be [¹⁸F]fluoromethyl methyl ether. A similar con-

Autoradiographic Imaging of the Serotonin Transporter, using S-[¹⁸F]Fluoromethyl-(+)-McN5652 ([¹⁸F]Me-McN) in the Brains of several Animal Species

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The [¹⁸F]fluoromethyl analogue of (+)-McN5652 ([¹⁸F]Me-McN) was recently proposed as a new potential PET tracer [1]. To further validate its use in PET, we studied the binding of [¹⁸F]Me-McN in the brains of rats and pigs using autoradiography. The binding was compared with the uptake of the known 5-HT uptake inhibitor [³H]citalopram [2] and the radioligand (+)-[¹¹C]McN5652. The binding of the three compounds was qualitatively identical in the autoradiograms of the individual brains. Intense labelling was observed in regions known to be serotonin uptake sites. The binding was specifically inhibited, using the 5-HT uptake inhibitors citalopram and fluoxetine.

Introduction

The [¹⁸F]fluoromethyl analogue of (+)-McN5652 ([¹⁸F]Me-McN) was synthesized. [¹⁹F]Me-McN shows a similarly high affinity (K_i 2.3 nM) to the serotonin transporter as does (+)-McN5652 (K_i 0.72 nM). The in-vitro distribution of [¹⁸F]Me-McN was therefore determined in the brains of rats and pigs and the ex-vivo distribution in the brains of rats. The regional brain uptake of [³H]citalopram - a highly potent inhibitor of the neuronal serotonin uptake - served as a reference for characterization of the 5-HT uptake sites in the brain. Fluoxetine and citalopram were used in blocking experiments to determine the pharmacological specificity of the binding. The quantitative evaluation of the uptake of [¹⁸F]Me-McN in the various brain regions was studied by radioluminography, using the bioimaging analyser BAS 2000 and AIDA software.

Results and Discussion

The regional brain binding of [¹⁸F]Me-McN and [³H]citalopram in pigs was closely correlated (Figs. 1 and 2). A high uptake was observed in regions known to be serotonin uptake sites, such as the hypothalamic area, the substantia nigra, the superficial layer of the superior colliculus and several nuclei of the thalamus. The tissue-to-cerebellum ratio of [¹⁸F]Me-McN in the 5-HT-rich regions in vitro and ex vivo was between 3 and 5, which corresponded to the value of (+)-[¹¹C]McN5652. Tissue-to-cerebellum values between 8 and 20 were obtained in these regions for [³H]citalopram in vitro. The uptake of all radioligands can be inhibited by fluoxetine or citalopram.

Our data confirm that [¹⁸F]Me-McN is a valid PET tracer for imaging the serotonin transporter.

References

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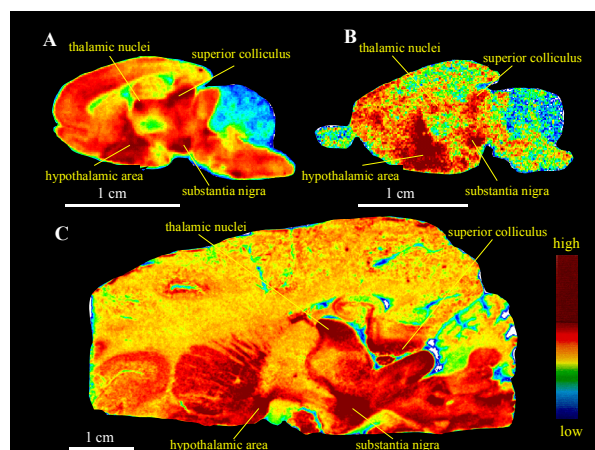


Fig. 1. In-vitro (A, C) and ex-vivo (B) autoradiograms as well as the radioactivity standard illustrating the distribution of [¹⁸F]Me-McN in sagittal sections of rat (A, B) and pig (C) brains. Note that the autoradiographic pattern of [¹⁸F]Me-McN binding sites closely resembles the distribution of [³H]citalopram (Fig. 2).

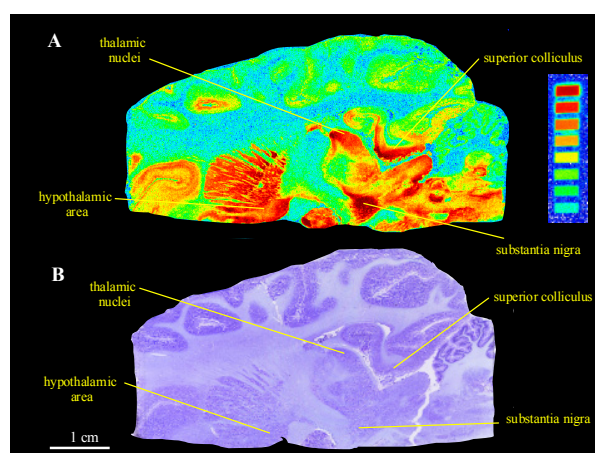


Fig. 2. In-vitro autoradiogram (top) and the corresponding histological image (bottom) as well as the radioactivity standard illustrating the distribution of [³H]citalopram in sagittal pig brain sections. Intense labelling is shown in regions known to be serotonin uptake sites, such as the hypothalamus, several nuclei of the thalamus and the midbrain.

Mapping of Carbonic Anhydrase and Estrone Sulphatase in Rat Brain using 16- α -[18 F]Fluoroestradiol-3,17- β -disulphamate ([18 F]FESDS)

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16 α -[18 F]Fluoroestradiol-3,17 β -disulphamate ([18 F]FESDS) was recently found to display affinities to carbonic anhydrase (CA) and estrone sulphatase (ES), enzymes which are expressed in the CNS and probably play a regulatory role in various brain diseases. In this study the radioligand was used to provide quantitative data on the regional distribution of these enzymes in the rat brain.

Introduction

Carbonic anhydrase (CA; E.C. 4.2.1.1) catalyses the reversible hydration of CO₂ and is widely distributed in the CNS, with CA II as the major isozyme. It has been hypothesized that CO₂ represents a highly diffusible signal in the tonic control of neuronal activity and CA II activity in the CSF has been suggested to be a marker for neurological diseases such as multiple sclerosis. The enzyme estrone sulphatase (ES; E.C. 3.1.6.2) is also highly expressed in various brain regions of primates, such as the cerebral cortex, hypothalamus, midbrain and cerebellum. It is probably involved in the modulatory effect of the neurosteroid dehydroepiandrosterone sulphate (DHEAS) on GABA_A receptors. Preclinical and clinical studies support the potential efficacy of neuroactive steroids as a novel class of drugs for the therapeutic management of epilepsy, anxiety, insomnia, migraine and drug dependence. It is therefore assumed that estrone sulphatase plays a regulatory role in these diseases. We recently discovered that [18 F]FESDS displays affinity to both enzymes. In this study the radioligand was used to provide quantitative data on the regional distribution of these enzymes in the rat brain.

Methods

In-vitro autoradiography was performed as elsewhere described [1].

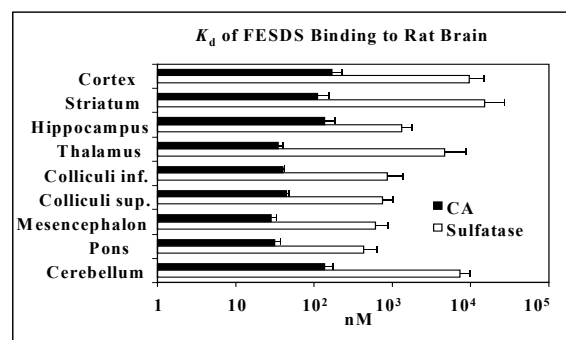
Results

About 80 - 90 % of the total binding of [18 F]FESDS to brain slices was binding to CA (displaceable with acetazolamide). The K_d of CA binding (between 29 and 169 nM in various regions) was similar to the IC₅₀ of FESDS towards human CA II. The K_d of ES binding in the brain is about 50-times higher (between 0.4 and 15 μ M) (see Fig.). The latter values were used as constants to calculate B_{max} of CA and ES binding from displacement studies with FESDS. The B_{max} values were between 2.4

and 6.5 pmol/mg for CA and between 0.6 and 16 pmol/mg for ES. A similar inhibition of FESDS binding to rat brain by the CA inhibitors acetazolamide and ethoxzolamide was observed. Hydrochlorothiazide showed no inhibition. A similar affinity profile was obtained using purified human CA II (data not shown). FESDS inhibited ES in placental microsomes with an IC₅₀ of about 25 nM. Acetazolamide was without effect on ES activity.

Comments

Surprisingly, the binding affinity of FESDS to rat brain ES was found to be much lower than human placental ES. The use of this ligand for



imaging ES *in vivo* is thus ruled out. On the other hand, FESDS has a high affinity to CA II, which is present in the brain in large amounts. In agreement with Gandhour *et al.* [2], the preferable binding of [18 F]FESDS was noticed in highly myelinated brain regions. This encourages further studies to investigate whether this ligand is useful for PET imaging of central myelination processes and their disturbances (e.g. multiple sclerosis, Pelizaeus-Merzbacher disease and adrenoleucodystrophy).

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Mapping of Estrone Sulphatase in Rats

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Estrone sulphatase is a widespread enzyme in mammals. RT-PCR results indicate that this enzyme is expressed in all rat organs. Most enzyme activity was found in the pituitary, the ovaries, the uterus and the fatty tissue but also in the hippocampus of the brain.

Introduction

The enzyme estrone sulphatase (ES; E.C. 3.1.6.2) is expressed in 25 organs of the rhesus monkey [1]. Enzyme activity was also found in some rat organs and in the brain [2, 3].

Methods

RT-PCR and the enzyme assay were carried out as elsewhere described [5].

Results

The mRNA of the enzyme ES was detected in all tissues tested (Fig. 1). This made it necessary to measure the enzyme activity in these tissues. Most ES activity was calculated from the male pituitary (2.670 pmol/mg protein/min) which was 14 times as much as measured in the female pituitary (Table 1). But ES was also split in the corpus callosum (white matter) and in the hippocampus of brain. Organs which are rich in ES are the ovary, the uterus and the fatty tissue (280 – 417 pmol/mg protein/min).

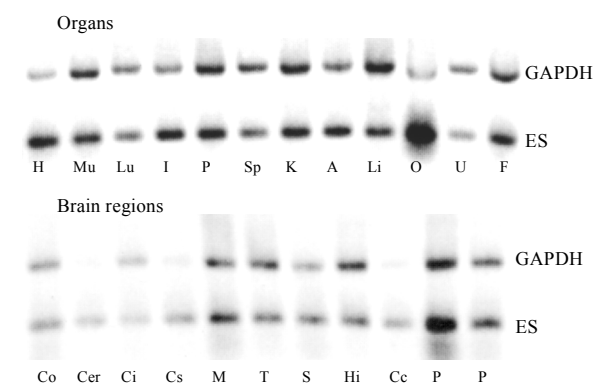


Fig. 1. Agarose gelelectrophoresis of RT-PCR of ES at rat organs (above) and brain regions (below). Organs are from left to right heart, muscle, lunge, intestine, pancreas, spleen, kidney, adrenals, liver, ovary, uterus, fat; brain regions are cortex, cerebellum, colliculi inferiores, colliculi superiores, mesencephalon, thalamus, striatum, hippocampus, corpus callosum, pons, pituitary.

Comments

The mRNA of the enzyme ES was found in all tissues examined. High enzyme activity was found in organs involved in steroidogenesis such as the ovaries, the uterus and the adre-

nals. But fat, too, has a high ES activity, which is important for breast cancer. Another enzyme with a high activity in fat leading to breast cancer is aromatase. Because both enzymes are expressed there, it is important for patients to watch their weight. The ES activities measured in rat brain correlate with the results achieved by *in situ* hybridization of the mouse brain [3].

Table 1. Activity of estrone sulphatase in rat tissues

Tissue	ES-Activity [pmol/mg protein/min]
Heart	51,13 ± 5,46
Muscle	52,32 ± 9,16
Lunge	93,61 ± 14,65
Intestine	63,12 ± 0,85
Pancreas	48,08 ± 4,59
Spleen	35,55 ± 2,50
Kidney	21,85 ± 12,55
Adrenals	211,44 ± 50,92
Liver	92,81 ± 9,17
Ovary	349,12 ± 0,93
Uterus	416,51 ± 85,32
Fat	280,96 ± 0,58
Cortex	50,09 ± 6,28
Cerebellum	36,74 ± 12,74
Colliculi inferior	47,71 ± 9,82
Colliculi superior	239,31 ± 104,20
Mesencephalon	310,52 ± 117,47
Thalamus	162,33 ± 51,49
Striatum	265,75 ± 61,77
Hippocampus	371,98 ± 44,16
Corpus callosum	368,90 ± 90,54
Pons	25,58 ± 7,87
Pituitary, female	195,74 ± 18,98
Pituitary, male	2666,65 ± 95,29

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Kinetics of 16- α -[18 F]Fluoroestradiol-3,17- β -disulphamate ([18 F]FESDS) in Piglet Blood and Brain

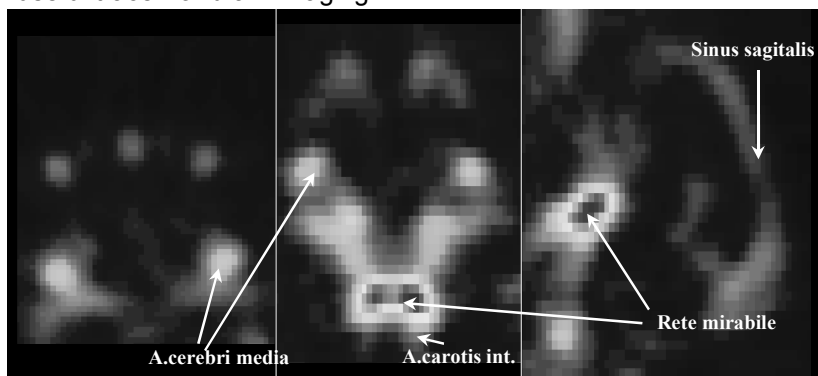
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The suitability of [18 F]FESDS as PET tracer was investigated. After i.v. injection [18 F]FESDS was immediately trapped by the erythrocytes. The PET images did not allow the differentiation of brain regions because of the very high amounts retained in the blood. [18 F]FESDS is therefore not recommended for brain imaging with PET.

Introduction

In view of its high affinity to the enzyme estrone sulphatase (ES), it was suggested to use 16 α -[18 F]fluoroestradiol-3,17 β -disulphamate ([18 F]FESDS) as a potential PET radiotracer for imaging steroid-dependent breast tumours [1]. We recently discovered that this radiotracer also binds to a second target: carbonic anhydrase (CA)[2]. Both enzymes, ES and CA, are widely distributed in the central nervous system. They are involved in the control of neuronal activity. We therefore used [18 F]FESDS in this study to examine whether or not it is a useful tracer for brain imaging.

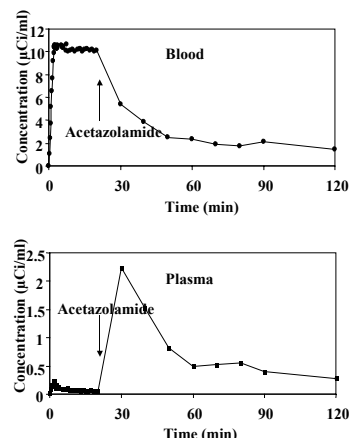


Method

Two six-week-old farm-bred female piglets (13 kg and 15 kg) were studied with PET under general anesthesia. About 490 MBq of [18 F]FESDS was infused into the left jugular vein of both piglets over 2 min. The PET scan was started simultaneously with the infusion. After 24 min the second piglet received an additional i.v. injection of acetazolamide (5 mg kg⁻¹). PET imaging (35 dynamic time frames of between 0.5 min and 10 min length, total length: 120 min) was performed with an ECAT EXACT HR+ (CTI/Siemens) scanner at a spatial resolution (transaxial) of 4-5 mm. During the PET scans arterial blood samples were obtained at defined time points, starting with the beginning of radiotracer infusion and then at intervals between 15 s and 30 min. The samples were immediately stored on ice and centrifuged for plasma sampling and counting in a γ -counter.

Results and Discussion

The [18 F]FESDS concentration in the blood immediately rose to about 0.4 MBq/ml at the end of tracer infusion and remained at this level. The plasma concentration was about 0.012 MBq/ml. It decreased exponentially and reached about 0.002 MBq/ml at the end of the study. After injection of acetazolamide more than 80 % of the radiotracer was released from the cellular blood compartment, resulting in an immediate 35-fold increase of the radiotracer concentration in the plasma (Fig.). However,



no equilibrium between the blood and plasma was established. The blood concentration exceeded the plasma concentration by at least a factor of 2. In the PET images of the piglets only the major blood vessels of the brain were visible without any substantial change after the acetazolamide injection (Fig.). Evidently [18 F]FESDS was trapped in the blood immediately after injection. It was rapidly taken up by the cellular compartment. The PET images (Fig.) did not allow differentiation of brain regions because of the very high amounts of [18 F]FESDS retained in the blood. [18 F]FESDS is therefore not a successful candidate for brain imaging with PET.

References

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Evaluation of S-([¹⁸F]Fluoromethyl)-(+)-McN5652 (FMcN) as a PET Tracer for the Serotonin Transporter

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The suitability of FMcN as a PET tracer was studied in six-week-old piglets. The binding equilibrium in piglet brain was reached about 60 min after i.v. administration of FMcN. The binding was significantly reduced by citalopram but not by maprotiline. It was concluded that FMcN may be suitable for use in humans.

Introduction

The loss of serotonin transporter (SERT) sites has been implicated in various neurodegenerative diseases. (+)-[¹¹C]McN5652 has been demonstrated to be suitable for PET imaging of SERT in clinical studies [1]. The disadvantage of (+)-[¹¹C]McN5652 is the relatively high non-specific binding combined with rather slow kinetics in vivo. There is therefore still a need for the development of new radioligands for SERT imaging. The [¹⁸F]fluoromethyl analogue of (+)-McN5652 was recently proposed as a new potential PET tracer [2]. Its suitability for PET imaging of SERT was studied in piglets.

Method

Twenty six-week-old farm-bred female piglets (between 13 kg and 17 kg) were studied with PET under general anaesthesia. About 300 MBq of FMcN was infused into the left jugular vein over 2 min. The PET scan was started simultaneously with the infusion. Ten piglets received an additional i.v. injection of the SERT inhibitor citalopram or the noradrenaline uptake inhibitor maprotiline (5 mg kg⁻¹) before or after tracer infusion. PET imaging (35 dynamic time frames of between 0.5 min and 10 min length, total length: 120 min) was performed with an ECAT EXACT HR+ (CTI/Siemens) scanner. Arterial blood samples were obtained at defined time points, immediately stored on ice and centrifuged for plasma sampling, γ -counting and HPLC analysis.

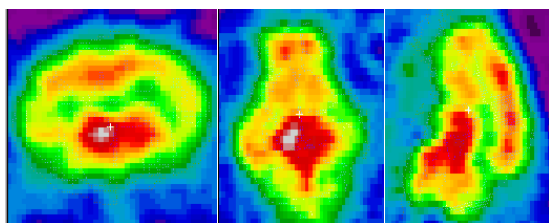


Fig. 1. Coronal, transversal and a sagittal plane (left to right) of a summed PET image (0 to 120 min) after injection of FMcN.

Results and Discussion

The fraction of unmetabolized FMcN in the plasma decreased throughout the study and was approximately 70 % after 10 min and 10 % after 2 h. Most metabolites were more polar

than the parent compound and thus unlikely to pass the blood-brain barrier. One lipophilic metabolite represented less than 2 %. After i.v. infusion of FMcN the highest radioactivity concentration was observed in the thalamus and midbrain. Modest levels were measured in cortical regions and the cerebellum (Fig. 1), which is in agreement with the distribution of SERT in the pig brain [3]. The thalamus/cerebellum ratio reached a steady state after about 60 min (Fig. 2A).

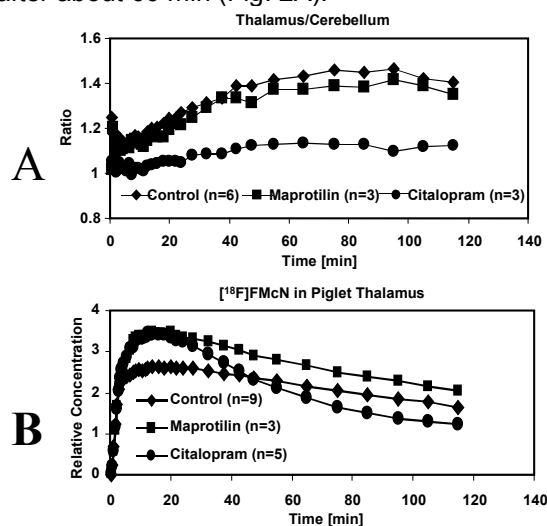


Fig. 2. Time course of the thalamus/cerebellum ratio (A) and the relative tracer concentration (B) after injection of FMcN.

The binding of FMcN in the piglet thalamus was significantly reduced by preinjection of citalopram but not by maprotiline (Fig. 2B). Moreover, injection of citalopram 25 min after the start of the PET scan displaced the binding of FMcN, while maprotiline had no effect. The data suggests that FMcN binds to the SERT in vivo. It is concluded that FMcN is a successful candidate for brain imaging with PET.

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**RADIOTRACERS IN DRUG DEVELOPMENT AND
FOOD SCIENCE**

Synthesis of HYNIC-labelled Immunoglobulins

J.-U. Kuenstler, S. Seifert, B. Johannsen

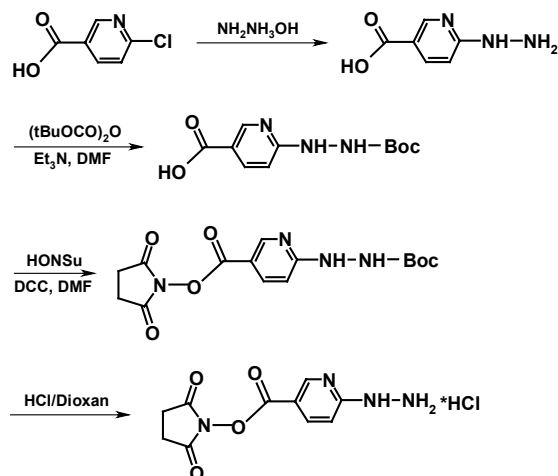
Immunoglobulins (IVIG: Intravenous Immunoglobulin and mAb 138H11) were HYNIC-functionalized. The influence of pH value, protein concentration and molecular ratio protein/HYNIC-ONSu was investigated. The derivatized proteins were characterized by SE-HPLC, SDS-PAGE and determination of the number of bound HYNIC-groups.

Introduction

There is considerable interest in labelling proteins and peptides with ^{99m}Tc for the development of target-specific imaging agents. ^{99m}Tc -labelling via HYNIC derivatives (HYNIC: 6-hydrazino-pyridine-3-carboxylate) was introduced by Abrams *et al.* [1]. The HYNIC group is of particular importance because it can be labelled with high efficiency, and the resulting complexes possess high stability [2]. Here we describe the synthesis and characterization of two HYNIC-functionalized proteins.

Results and Discussion

Several methods for coupling HYNIC to a biomolecule are described in the literature. Labelling proteins requires mild conditions, therefore HYNIC-ONSu (HONSu: N-hydroxysuccinimide) as coupling agent was synthesized according to the following scheme [1].



Immunoglobulin solutions (IVIG-C, 10 %, (BAY 41-1000), provided by Bayer; labelling and analysis supported by Bayer Vital) and mAb 138H11 were derivatized using HYNIC-ONSu. The IgG solutions were dialysed to remove low molecular mass additives (IgG preparations mostly contain additives e.g. glycine to stabilize the solution), concentrated to 10 mg/ml, the pH value was adjusted to 8.0

and HYNIC-ONSu (10fold molar excess) added in small portions. After a reaction time of 4 h the solution was dialysed, sterilized by membrane filtration, aliquots of 1 or 2 mg protein were filled into vials and lyophilized. The vials were capped under nitrogen and stored at $+4\text{ }^\circ\text{C}$.

The coupling reaction depends on the pH value, the protein concentration and the molecular ratio protein/HYNIC-ONSu, among other factors. The pH value of 8.0 is assumed to be an optimum between hydrolysis and aminolysis of HYNIC-ONSu, as revealed by HPLC analysis of the reaction of a model peptide with HYNIC-ONSu. No coupling was observed in solutions with a protein concentration smaller than 1 to 2 mg/ml. A molecular ratio protein/HYNIC-ONSu of 1/20 or 1/40 did not result in a higher number of HYNIC-groups being bound to the protein than a molecular ratio of 1/10. The number of HYNIC groups determined per protein molecule was 3.1 (HYNIC-IgG) and 2.6 (HYNIC-mAb). A threefold molar excess of HYNIC-ONSu compared with the protein resulted in less than one HYNIC group bound to the protein.

HYNIC-IgG and HYNIC-mAb were characterized by SE-HPLC, SDS-PAGE (without a reducing agent) and photometric determination of the number of bound HYNIC groups. Neither of the HYNIC proteins exhibited any significant differences between the pattern in SDS-PAGE and the chromatograms compared with the unmodified proteins. That means that no fragments or oligomers were formed by the modification.

After storage of the two HYNIC-modified proteins for half a year, no differences compared with the freshly derivatisized proteins were observed by SE-HPLC, SDS-PAGE and after ^{99m}Tc labelling.

References

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Stability of ^{99m}Tc and ^{188}Re labelled HYNIC-IVIG

S. Seifert, J.-U. Kuenstler, B. Johannsen

A HYNIC-modified immunoglobulin IVIG was labelled with ^{99m}Tc as well as ^{188}Re . The optimum labelling conditions were determined and stability studies performed. While ^{99m}Tc -HYNIC-IVIG is stable for 24 h, the corresponding ^{188}Re -HYNIC-IVIG preparations largely decompose.

Introduction

Nowadays the HYNIC (hydrazino nicotiny derivatives)-based method introduced by Abrams *et al.* [1] is the most frequently used method of labelling biomolecules with ^{99m}Tc or ^{188}Re . This procedure involves the synthesis of an aromatic hydrazine linker, efficient conjugation of this linker to a biomolecule and labelling. The degree of modification, the stability of the hydrazine-modified molecule, the choice of the proper Tc or Re precursor, rapid and efficient radiolabelling, and *in-vivo* stability of the conjugate are points of consideration.

For labelling a HINYC-conjugated immunoglobulin (IVIG-C provided by Bayer) as well as a monoclonal antibody, we synthesized the derivatized biomolecules and used tricine or EDDA (ethylenediaminediacetic acid) as co-ligands in the form of freeze-dried kit preparations. The stability studies of ^{99m}Tc and ^{188}Re preparations were mainly performed with the HYNIC-IVIG using TLC, HPLC and PAGE (polyacrylamide gel electrophoresis) as described in the article above [2].

Results and Discussion

^{99m}Tc labelling of HYNIC-IVIG

1.3 mg of lyophilized HYNIC-IVIG was dissolved in 2 ml pertechnetate eluate and added to a vial containing either 20 mg tricine and 0.1 mg SnCl_2 or 5 mg EDDA and 0.1 mg SnCl_2 in freeze-dried form, using a syringe. The stability of the preparations was studied between 15 min and 24 h after reconstitution.

^{188}Re labelling of HYNIC-IVIG

The same vials with the lyophilized HYNIC-IVIG and the co-ligands tricine or EDDA were used for the preparation. In addition, 0.9 mg SnCl_2 was added to the reaction mixture. Despite this large amount of the reducing agent, 1.5 – 2 h at room temperature (RT) were required for quantitative reduction of perrhenate. The reaction was not much accelerated by incubation at 37 °C.

Stability of ^{99m}Tc -HYNIC-IVIG

The ^{99m}Tc -labelled HYNIC-IVIG was stable at RT and 37 °C for at least 24 h in 1:3 dilutions of saline, 0.1 M phosphate buffer solution of pH 7.4, and rat plasma.

Challenge experiments with 10 mM solutions of glutathione, cysteine and DTPA confirmed the stability of the preparations.

Stability of ^{188}Re -HYNIC-IVIG

No stability differences were found between ^{188}Re -HYNIC-IVIG complexes with tricine (**1**) or EDDA (**2**) as co-ligands. Both preparations showed similar instabilities after dilution with saline, phosphate buffer, and rat plasma as well as against cysteine in challenge tests (Table 1). In buffer and plasma solutions only perrhenate was observed as decomposition product ($R_f = 0.9 - 1.0$). The challenge experiments with cysteine resulted in the formation of extra hydrophilic components in addition to perrhenate (R_f values between 0.3 and 0.5).

Table 1. Stability of ^{188}Re -HYNIC-IVIG **1** and **2** determined by TLC (Silufol//0.1 M sodium acetate pH 5.8 (A) or acetone (B)).

Time [h]	0.1 M phosphate buffer, pH 7.4		10 mM cysteine	
	A	B	A	B
1	85	91	45	50
2	84	88	28	28
3	80	82	19	19
4	77	80	12	14
24	46	56	5	5

Acknowledgement

IVIG-C was provided by Bayer, labelling and analysis supported by Bayer Vital.

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Biodistribution of the ^{18}F -labelled Advanced Glycation End Products N^ε-carboxymethyllysine (CML) and N^ε-carboxyethyllysine (CEL)

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After synthesis of fluorine-18 labelled analogues by [^{18}F]fluorobenzoylation at the α -amino group, biodistribution and elimination of individual advanced glycation end products, namely N^ε-carboxymethyllysine and N^ε-carboxyethyllysine, was studied in comparison to lysine in rats after intravenous injection using positron emission tomography (PET).

Introduction

Several amino acid derivatives resulting from glycation of peptides or proteins, so called "advanced glycation end products" (AGEs), were found to accumulate in vivo during aging as well as during diabetes and uremia, pointing out to pathophysiological implications for age-related disorders such as atherosclerosis, nephropathy, retinopathy, neurodegenerative diseases as well as the development of long-term complications of diabetes.

Since the amount of AGEs ingested from certain heated foods substantially exceed the total AGE concentration formed "endogenously", recent interest has focused on absorption, renal handling and excretion of dietary AGEs. Data obtained by an unspecific immunoassay, demonstrated an increase in AGE-level of plasma for human volunteers after ingestion of heated mixtures of fructose and egg-white. Elimination of AGEs from plasma was slower for diabetic patients compared to healthy controls. Studies based on more realistic food systems proved that urinary excretion of Amadori products is significantly affected by daily food consumption. About 2 % of applied protein-bound lactuloselysine could be found in urine, which pointed to fast elimination kinetics as well as to currently unknown metabolism of dietary Maillard compounds. The aim of our study, therefore, was to make use of the PET modality to study the in vivo behaviour of well-defined radioactive amino acid derivatives of the Maillard reaction. N^ε-carboxymethyllysine (CML) and N^ε-carboxyethyllysine (CEL) were chosen as first candidates, both from the nutritional and physiological point of view, because these lysine derivatives are formed in proteins during food processing as well as under physiological conditions in vivo.

Results and Discussion

N-succinimidyl-4- ^{18}F fluorobenzoate [^{18}F]SFB was used to modify CML and CEL at their free α -amino group, resulting in the corresponding 4- ^{18}F fluorobenzoylated derivatives, which

should be an useful model for CML-/CEL-containing dipeptides possibly resulting from the digestion of glycated food proteins [1]. The fluorobenzoyl moiety acts as a surrogate for the second amino acid of the dipeptides. 4- ^{18}F fluorobenzoylated lysine served as reference substance. After preparation of fluorine-18 labelled lysine, CML and CEL, preliminary PET- and biodistribution experiments were undertaken for measuring biodistribution and biokinetics in rats.

The compounds were rapidly excreted through the kidneys into the urine. There was no remaining accumulation of the radioactive compounds in the kidney tissue over 2 hours.

For the liver, an obvious temporary accumulation of [^{18}F]FB-CML and [^{18}F]FB-CEL, but not of [^{18}F]FB-Lys was observable. The maximum concentration of radioactivity was reached at about 20 minutes after injection of the labeled compounds. The liver was cleared from the radioactivity to less than 1 % ID/g tissue after 2 hours. At the end of the experiment, only 0.6 % ID (FB-Lys), 1.7 % ID (FB-CML) or 1.2 % ID (FB-CEL) were detected in the intestine, which is a strong evidence for a low transport of the investigated compounds or possible metabolic conjugates through the liver into the bile. As measured by RP-HPLC, only [^{18}F]FB-Lys, not [^{18}F]FB-CML or [^{18}F]FB-CEL, was decomposed to [^{18}F]FB-OH, which was found in the analyzed blood and urine samples in case of the lysine experiments. In contrast to this, no metabolites of [^{18}F]FB-CML or [^{18}F]FB-CEL were detectable. There is also no indication that [^{18}F]FB-CML, [^{18}F]FB-CEL or [^{18}F]FB-Lys are utilized for the protein biosynthesis in the rat.

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RADIOPHARMACEUTICAL CHEMISTRY

Preliminary Biological Evaluation of a Urea-functionalized Dendrimer

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A new third generation ethylurea-functionalized polypropyleneamine dendrimer was prepared. After labelling this dendrimer with ¹¹C the biodistribution in rats was studied. The highest level of radioactivity was found in the liver (30-35 % ID). The ¹¹C-labelled dendrimer was well tolerated by the rats.

Introduction

Their unique structural features and properties make dendrimers ideally suited for many fields of application. The state of reports in the current literature have referred to their use in a broad range of application, including material engineering, industrial, pharmaceutical, and biomedical uses [1]. We recently showed that lipophilic urea-functionalized dendrimers can serve as efficient carriers for oxyanions [2]. Since dendrimers having neutral surface groups are neither haemolytic nor cytotoxic [3], these special urea-containing substances seem to be interesting candidates for in-vivo studies. A more hydrophilic urea-containing dendrimer was prepared for this purpose. We report on the labelling procedure for a third generation ethylurea-functionalized dendrimer (cf. Fig. 1) with carbon-11 and its biodistribution in rats.

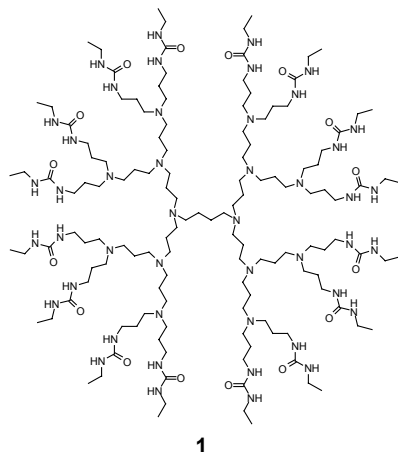


Fig. 1. Chemical structure of third generation ethylurea-functionalized dendrimer 1

Results and Discussion

¹¹C Labelling procedure

The dendrimer 1 was obtained by reaction of polypropyleneamine dendrimer-(NH₂)₁₆ with ethylisocyanate according to [2]. Subsequently, [¹¹C]methyl iodide prepared according to [4] was trapped in a solution of 1 (1,2 mg) and sodium hydroxide in ethanol. After reaction at 110 °C, the pH was adjusted to 7 by hydrochloric acid. This mixture was dissolved in saline yielding the injection solution. HPLC

analysis of this solution confirmed the complete conversion of [¹¹C]methyl iodide.

Biodistribution studies

¹¹C-labelled dendrimer 1 (0.5 ml) was injected into male Wistar rats by various injection paths (i.v.; i.p.). The tracer was well tolerated by the animals and the distribution data were found to be similar in both cases of injection.

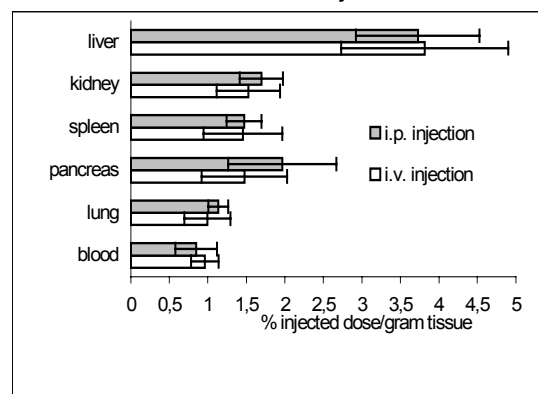


Fig. 2. Comparison of biodistribution data after intravenous and intraperitoneal injection of ¹¹C-labelled dendrimer 1 into Wistar rats (60 min p.i.; mean ± SD; n = 3)

A low level of radioactivity in the circulating blood was observed. The highest accumulation of activity was found in the liver. Its tolerability and biodistribution pattern recommend this dendrimer for in vivo application after the additional introduction of biomolecules as carbohydrates and peptides, by which we want to modify in particular the lipophilicity and the biodistribution of dendritic hosts.

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Molecular Mechanics Calculations of Rhenium “3+1” Complexes

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A novel molecular mechanics force field for rhenium “3+1” mixed-ligand complexes has been developed. The ligand-metal-ligand (L-M-L) bending interaction has been modelled with 1,3-nonbonded interactions (point on a sphere approach) and a harmonic M-L stretching potential is used for the stretching modes.

Introduction

Molecular mechanics is a routine tool in organic chemistry [1]. On the other hand the development of novel approaches to optimizing transition metal ion coordination geometries and of extensive parameterization schemes has led to a situation where many problems involving inorganic compounds may now be solved satisfactorily with the support of molecular mechanics calculations [2, 3].

In previous studies we successfully developed novel force fields for a series of lanthanoid complexes [4], for novel acidic organo-phosphorus extractants having a high selectivity among adjacent lanthanoids.

In this study we present preliminary molecular mechanics force field parameters for rhenium “3+1” complexes. The molecular structure of the complexes calculated is shown in Fig. 1.

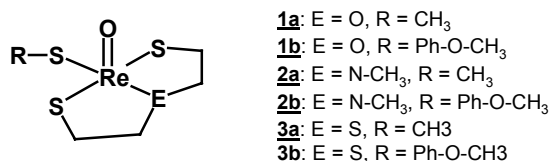


Fig. 1. Chemical structures of rhenium “3+1” complexes investigated in this study

Results and Discussion

The molecular mechanics calculations were performed with the strain minimization program MOME97 [5]. Within the molecular mechanics framework, the structure of a molecule is modified in order to minimize its total strain energy consisting of bond length deformation, valence angle deformation, torsion angle deformation and non-bonded interaction. Starting with a parameter set obtained from the crystal X-ray structures of the rhenium “3+1” complexes **1a** – **3c** [6], we developed a force field for rhenium complexes of the oxidation state +5.

Fig. 2 shows the RMS (root mean square) overlay of the complex structure calculated by molecular mechanics with MOME97 and the

crystal X-ray analysis of **1b**. The good agreement of the crystal and calculated structures indicates that our force field parameters are of sufficient quality. We now want to refine the force field parameters by adding more data from crystal structures of rhenium(V) complexes. Furthermore, we plan to calculate partial charges of rhenium(V) complexes, using the program package *Gaussian98*. A simple and efficient method of predicting stabilities, reactivities and the electronic properties of rhenium(V) complexes will be derived from these data.

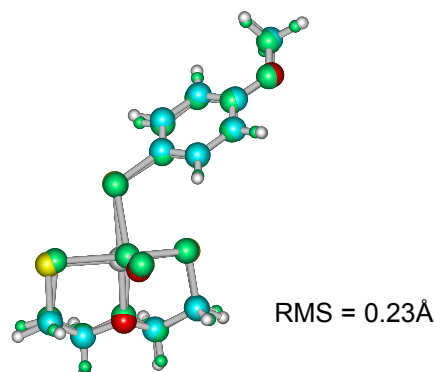


Fig. 2. RMS overlay of computed MOME97 and crystal structure of **1b**

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An Improved Method for the Preparation of n.c.a. '4+1' ^{99m}Tc Complexes

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'4+1' Mixed-ligand ^{99m}Tc complexes were obtained in yields of about 90 % using a two-step procedure. At first the Tc-EDTA complex was prepared and in a second step the '4+1' complex was formed by a ligand-exchange reaction with the tripodal NS_3 ligand and isocyanide.

Introduction

Technetium(III) and rhenium(III) mixed-ligand complexes with 2,2',2''-nitriлотris(ethanethiol) (NS_3) and isocyanides as co-ligands (so-called '4+1' complexes), which are neutral and non-polar since they contain sterically well shielded oxo-free M(III) ions, are suitable alternatives to the well-known more polar M(V) oxocomplexes [1, 2].

The ^{99}Tc complexes were synthesized by a two step procedure starting from TcO_4^- via a phosphine-containing precursor. To a mixture of pertechnetate and NS_3 an excess of dimethylphenylphosphine was added as a reducing agent and co-ligand. The subsequent exchange of phosphine by appropriate isocyanide ligands in chloroform led to the formation of the desired '4+1' complex.

The non-carrier-added preparation of the complexes was first performed by a one-step procedure. 0.5 mg of NS_3 oxalate dissolved in alkaline ethanol and 0.3 mg of isocyanide also dissolved in ethanol were added to the ^{99m}Tc generator eluate and reduced with 0.01 ml stannous chloride solution (1 mg/ml ethanol). The yields were between 50 – 75 %. A by-product occurring in the reaction mixtures was not eluted by HPLC. It was therefore necessary to perform TLC to determine the yields of the preparation. Variations of the ligand amounts did not improve the yields.

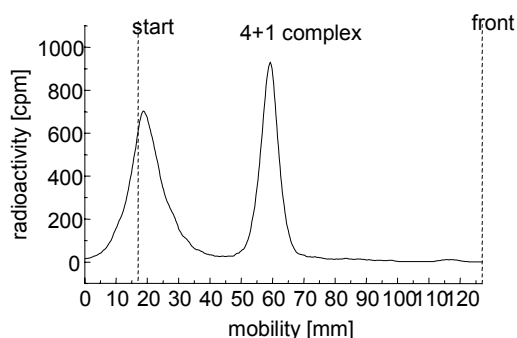


Fig. 1. TLC pattern of a n.c.a. preparation. RP-18 F_{254s} (Merck), methanol/water (80/20)

Results and Discussion

The large amounts of reduced-hydrolysed technetium formed when using the one-step preparation were the motive for us to search for a better reaction route. To avoid the formation of Tc hydroxide we prepared the '4+1' complexes in a two-step procedure. At first the Tc(III)-EDTA complex was formed, which in a second step reacts with both ligands to the desired '4+1' complex.

To prepare the Tc-EDTA complex 0.2 mg of EDTA was dissolved in the generator eluate (1-5 ml) and TcO_4^- was reduced with 0.01 ml SnCl_2 solution (1 mg/ml ethanol) at room temperature. The complex formation was completed after 5 min with yields nearly 100 % determined by TLC (Silufol/acetone; $R_f = 0$ and Silufol/water; $R_f = 0.6$).

For the following ligand exchange 0.5 mg NS_3 and 0.3 mg of isocyanide (benzylisonitrile, cyclohexylisonitrile, isocyano acetic acid ethyl ester) were added and the mixture was held at 50 °C for 15 min. The resulting pH was 4 – 5.

The ^{99m}Tc complexes were analysed by HPLC (Hypersil ODS (250 x 4 mm), flow rate of 1 ml/min, eluants: methanol (A)/0.01 M phosphate buffer pH 7.4 (B); [t(min)/A(%): [5/50], [5/100], [10/100]) and by TLC (Silufol/chloroform/methanol (9:1,v/v), $R_f \sim 0.7$). Under these conditions yields of about 90 % were obtained.

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Efforts to Synthesize a Carboxyl Group Containing Tripodal NS₃ Ligand

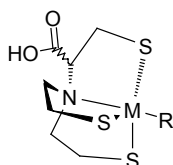
E. Schiller, H.-J. Pietzsch, H. Spies

Tris-(2-mercapto-ethyl)amine (NS₃) is known to form stable technetium(III) and rhenium(III) chelate units suitable for binding lipophilic biomolecules. It is our aim to modify the ordinary NS₃ ligand by introducing a carboxyl group in order to create hydrophilic technetium and rhenium complexes for binding hydrophilic biomolecules, such as peptides.

Introduction

Tris-(2-mercapto-ethyl)amine NS₃ is a chelator whose coordination chemistry to technetium and rhenium has been investigated over the past years [1, 2]. The experience gathered can be used to design a more hydrophilic derivative of NS₃.

The intended introduction of a carboxylic group into the NS₃ framework will result in increased hydrophilicity of the related '4+1' mixed ligand complexes.



The synthesis of hydrophilic ligands aims at the following:

- Hydrophilic chelate units should be especially suited to creating complex systems allowing fast renal excretion of its metabolites.
- Coupling of hydrophilic technetium or rhenium chelates to hydrophilic receptor-binding biomolecules will enhance the possibilities of radiotracer design.

Results and Discussion

A crucial point in the synthesis strategy of a carboxylic group bearing NS₃ ligand is the introduction of sulphur. Sulphur is either introduced with the starting substances or in a later reaction step. There are two possible ways of preparing the desired compound:

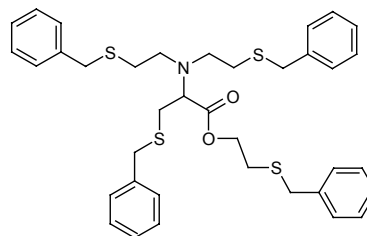
The first way is according to the synthesis of the non-functionalized NS₃ ligand, using the hydroxyl compound triethanolamine as the starting substance. Alkylation of diethanolamine with 2-bromo-3-hydroxy-propionic acid leads to the analogous carboxylic group containing trialcohol. Reaction with thionyl chloride produces the related chloro compound in a suitable yield. However, the subsequent sub-

stitution by a sulphur nucleophile such as thio-urea or 4-methoxybenzyl mercaptane was not

possible. This may be due to inductive effects of electron-accepting groups causing destabilization of the molecule structure.

An alternative route makes use of the possibility of introducing sulphur with the starting substances. The root structure of the carboxyl group containing NS₃ ligand was prepared by reaction of *L*-S-benzyl cysteine methylester with 1-benzylsulphanyl-2-chloroethane. 2-Chloroethanol was converted into 2-benzylsulphanylethanol by reaction with benzyl mercaptane. Subsequent treatment with thionyl chloride in chloroform/pyridine led to 1-benzylsulphanyl-2-chloroethane. Alkylation of *L*-S-benzyl cysteine methylester by 1-benzylsulphanyl-2-chloro-ethane in methanol/NaOH produced *L*-3-benzylsulfanyl-2-[bis-(2-benzylsulfanyl-ethyl)-amino]-propionic acid 2-benzylsulfanyl-ethyl ester in a 1.5 % yield.

The compound was characterized by ¹H NMR, ¹³C NMR and mass spectra.



Efforts are currently under way to increase the yields. Then it will be possible to synthesize the carboxyl group containing NS₃ ligand by separation of the protecting groups.

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Technetium and Rhenium Complexes with Modified Fatty Acid Ligands

4. Evaluation of two New Classes of ^{99m}Tc -Labelled Fatty Acids as Potential Tracers for Myocardial Metabolism Imaging

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^{99m}Tc -labelled fatty acids were synthesized according to the '3+1' mixed-ligand approach and investigated as potential tracers for myocardial SPECT diagnostics on the model of the isolated guinea pig heart. The results indicate a low but specific myocardial uptake of the ^{99m}Tc fatty acid derivatives subject to chain length and structure.

Introduction

Under physiological conditions long chain fatty acids (FA) serve as a major energy source for the normoxic myocardium. During ischemia, however, the extraction of FAs is reduced and glycolysis is enhanced. Regional changes in the FA metabolism can therefore be taken as evidence of myocardial ischemia. The routine use of SPECT for the assessment of cardiac injury by labelled FAs was hampered in the past by the lack of an appropriate low-price radiotracer. To assess regional cardiac metabolism, potential tracers require a high first pass extraction as well as a high myocardial retention.

In an attempt to study the myocardial profile of new ^{99m}Tc -labelled FAs, we synthesized '3+1' mixed-ligand complexes with "SNS" and "SSS" tridentates and ω -functionalized FA monodentates of various chain lengths (C_{11} , C_{15} , C_{16}). In addition, ε -thiaheptadecanoic (C_{16}S) and ε -thiaundecanoic (C_{10}S) acid derivatives of both complex types were synthesized. The thioether unit inhibits β -oxidation and therefore reduces back-diffusion and clearance of the extracted FA.

Myocardial uptake was determined, using the isolated constant-flow-perfused guinea pig heart. Similar to the *in vivo* heart, this experimental model makes it possible to analyse both the effluent perfusate and the radioactivity of the myocardial tissue [1].

Results and Discussion

Isolated guinea pig hearts were retrogradely perfused via the cannulated aorta with Krebs-Henseleit-buffer (KHB, 10 mL/min). The ^{99m}Tc FA analogues were incubated for 30 min with KHB containing 6 % albumin. After 30 min normocapnic perfusion ($p\text{O}_2 = 588$ mmHg, $p\text{CO}_2 = 40$ mmHg, $\text{pH} = 7.38$), the ^{99m}Tc FA analogues were coinfused for 3 min at a rate of 0.01 mL/min. After 3 min the hearts were removed, washed and prepared for counting of γ -activity.

The heart model was validated with established radiotracers. ^{99m}Tc -MIBI and the Iodophenyl FA derivatives IPPA and BMIPP served as positive controls and revealed a high myo-

cardial uptake (15.31 ± 2.35 , 13.34 ± 2.11 , 16.18 ± 1.64). In contrast, DTPA is known not to be extracted by the myocardium and displayed only a very low myocardial activity equivalent to background contamination (Table 1a).

Table 1a

Tracer	Myocardial extraction (%)	Number of experiments
DTPA	0.83	1
^{99m}Tc -MIBI	15.31 ± 2.35	3
IPPA	13.34 ± 2.11	3
BMIPP	16.18 ± 1.64	3

Table 1b

^{99m}Tc labelled "SNS" fatty acids	Myocardial extraction (%)	Number of experiments
C_{11} "SNS"	5.41 ± 2.19	5
C_{15} "SNS"	1.42 ± 0.74	4
C_{16} "SNS"	1.84 ± 0.07	3
C_{16}S "SNS"	4.23 ± 0.45	3
C_{10}S "SNS"	3.49 ± 0.55	3

Table 1c

^{99m}Tc labelled "SSS" fatty acids	Myocardial extraction (%)	Number of experiments
C_{11} "SSS"	2.94 ± 0.44	3
C_{15} "SSS"	2.33 ± 0.23	3
C_{16} "SSS"	2.18 ± 0.05	3
C_{16}S "SSS"	5.07 ± 0.17	3
C_{10}S "SSS"	2.57 ± 0.07	3

The ^{99m}Tc -labelled "SNS" C_{11} derivative revealed the highest ventricular activity (5.41 %). Insertion of a thioether group did not significantly influence the myocardial profile (Table 1b).

The highest myocardial activity among the ^{99m}Tc "SSS" FAs (5.07 %) was determined for the C_{16}S derivative (Tab. 1c).

The comparatively low myocardial extraction rate of all tested ^{99m}Tc complexes shows that the '3+1' mixed ligand design is obviously not suitable for labelling FAs. Nevertheless, the differentiation in the cardiac profile of the particular FA analogues indicates a specific uptake, depending on chain length and structure.

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Technetium and Rhenium Complexes with Modified Fatty Acid Ligands

5. Crystal Structures of three Novel Rhenium Fatty Acid Complexes

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Coordination of Re and Tc to biomolecules often effects a significant change in the structural integrity of the underlying head structure. The influence of various chelate units on the molecular geometry is demonstrated by the crystal structures of three Re fatty acid complexes containing the central metal core in the oxidation states +5, +3 and +1.

Introduction

In our efforts to develop technetium-labelled fatty acid analogues for myocardial metabolism imaging we reported the synthesis of rhenium model compounds according to the 'n+1' mixed-ligand approach and the metal(I)-tricarbonyl design [1]. In this abstract we want to describe the results of the relevant single-crystal X-ray analyses which impressively demonstrate a general problem in Re/Tc tracer design, namely the significant structural alterations of bioactive molecules by coordination to even small-sized metal chelates.

Results and Discussion

Re fatty acid complexes with metal(V) core

The crystal structure of a '3+1' fatty acid mixed-ligand complex with "SSS" tridentate

was elsewhere described [2]. The X-ray analysis of an alternative "SN(Me)S" fatty acid analogue revealed that a change in the central tridentate donor atom from sulphur to nitrogen results in the transition of the coordination sphere from square pyramidal to distorted trigonal bipyramidal (Fig. 1). The axial positions are occupied by the nitrogen donor of the tridentate ligand and the sulphur atom of the monodentate fatty acid derivative; the corner sites of the triangular plane are taken up by the oxygen of the Re=O³⁺ unit and the two mercaptides of the "SN(Me)S" ligand. As frequently observed in this type of complex, the tridentate chelator can change its conformation by a flip-flop mechanism [3], giving rise to a statistical distribution of two isomers within the crystal packing.

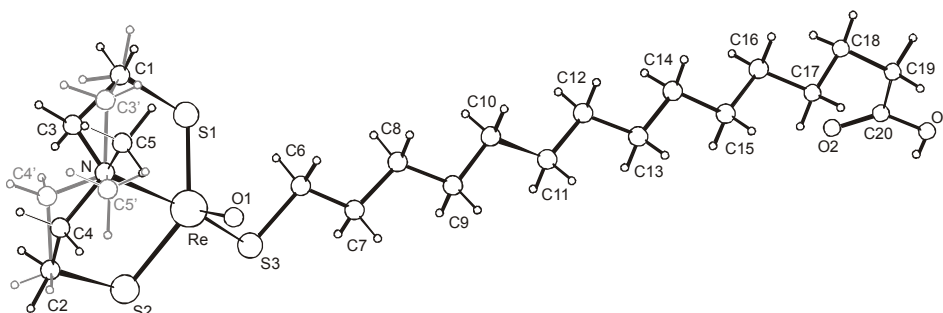


Fig. 1. "SN(Me)S" rhenium fatty acid complex.

Re fatty acid complexes with metal(III) core

The '4+1' mixed-ligand design permits an extensive shielding of the central metal core and is therefore expected to allow a better imitation of the lipophilic fatty acid chain. Fig. 2 shows the crystal structure of a pentadecanoic acid complex (hydrogen atoms are omitted for the sake of clarity). In this type of complex the metal(III) centre is coordinated in a more or less perfect trigonal bipyramidal geometry, indicating the high stability of the coordination

sphere. The apical positions are occupied by the nitrogen atom of the tetradentate NS₃ ligand and the bivalent carbon of the isocyano group. The trigonal surface of the bipyramid is stretched by the three mercaptides of the tripodal chelate. In analogy to Fig. 1 an order-disorder phenomenon was observed in the crystal packing of the pictured '4+1' complex. In this case not the chelate ligand but the monodentate fatty acid chain was arranged in two equivalent orientations.

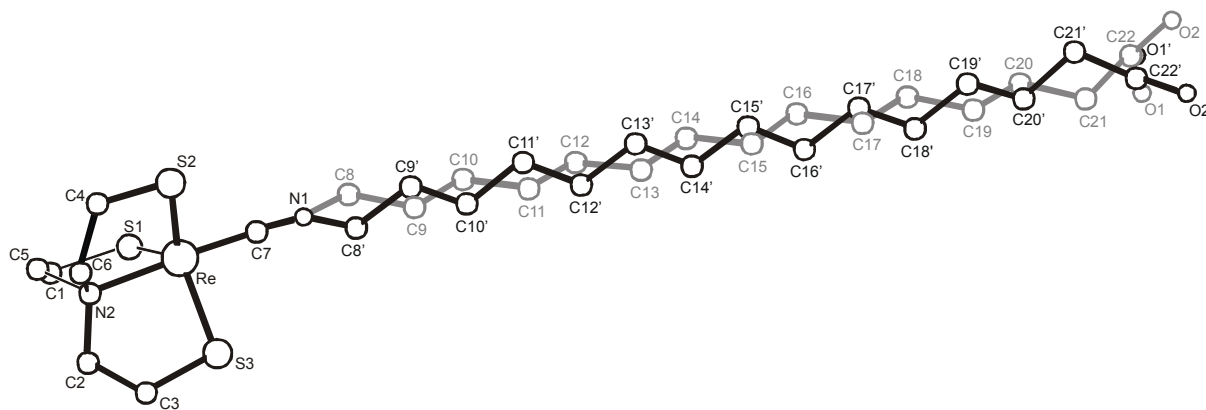


Fig. 2. '4+1' rhenium fatty acid complex.

Re fatty acid complexes with metal(I) core

ALBERTO *et al.* recently introduced the metal(I)-tricarbonyl/Schiff Base design as a promising coordination system for labelling small biomolecules [4]. The X-ray structure of such a complex derived from an ω -functionalized lauric acid derivative is shown in Fig. 3. As

expected for this type of complex, the molecular structure shows an octahedral coordination sphere around the central metal(I) core. The three carbonyl moieties are arranged facial to each other, the remaining coordination sites are occupied by the Schiff Base ligand and a bromine atom.

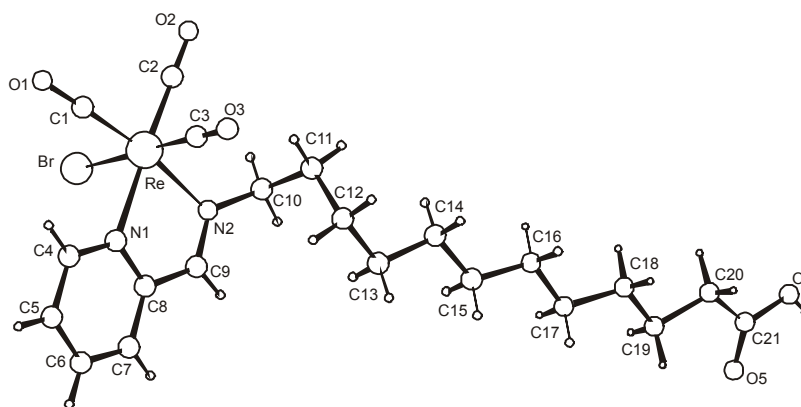


Fig. 3. Rhenium(I) tricarbonyl fatty acid complex.

Conclusion

Particularly conspicuous in the molecular structure of all three complexes (Fig. 1-3) are the proportions between the chelate moieties and the fatty acid ligands. The drastic structural alteration of the biomolecules by coordination to even small-sized metal chelates certainly accounts for the laborious progress in the development of new technetium-based radiopharmaceuticals. Nevertheless, structural integrity is only one of several parameters influencing the *in vivo* behaviour of potential ^{99m}Tc radiotracers. The actual medicinal value of the analogous technetium-99m labelled fatty acid complexes will therefore be investigated in subsequent biological studies.

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Potentially Redox-Active Rhenium and Technetium Complexes Based on the Pyridinium/Dihydropyridine System

5. Synthesis of Quinolinium/Dihydroquinoline-Bearing Rhenium(V) '3+1' Mixed-Ligand Complexes and Kinetics of the Reoxidation of their Dihydro Form

A. Rother, T. Kniess, H. Spies, H. Jungclas¹

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NAD⁺/NADH-analogous pyridinium/dihydropyridine and related redox pairs are frequently used as brain-specific drug delivery system for hydrophilic drugs [1]. In our attempt to use this principle to develop redox-active rhenium and technetium complexes we report on the synthesis of quinolinium/dihydroquinoline-bearing rhenium(V) '3+1' mixed-ligand complexes and on investigations into the stability of their dihydroquinoline form to reoxidation.

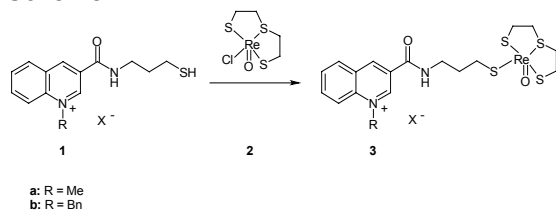
Introduction

In previous studies series of pyridinium/dihydropyridine bearing rhenium(V) '3+1'-mixed-ligand complexes were synthesised, but their 1,4-dihydropyridine form proved to be very unstable to reoxidation with half-lives of only 1 to 6 min at 37 °C [2], which makes them unsuitable for radiopharmaceutical applications. According to the literature quinolinium/dihydroquinoline systems often show higher stability of the dihydro form due to mesomerism. We therefore decided to synthesize quinolinium/dihydroquinoline bearing rhenium(V) complexes and to perform kinetic measurements.

Results and Discussion

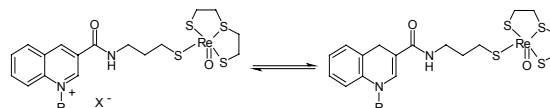
The quinolinium bearing thiols used by us(1) were synthesized in five steps by organic standard reactions starting with quinoline-3-carboxylic acid. The appendant '3+1' rhenium mixed-ligand complexes (3) were prepared by ligand exchange with the precursor chloro (3-thiapentane-1,5-dithiolato) oxorhenium(V) (2) (Scheme 1). The complexes were characterized by ¹H NMR, ¹³C NMR and elemental analysis.

Scheme 1.



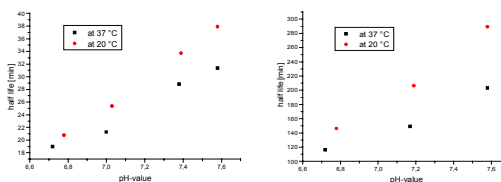
Selective reduction to their 1,4-dihydroquinoline form (Scheme 2) was achieved by an excess of sodium dithionite in an alkaline biphasic system of ethyl acetate/water [3]. After evaporation of the organic layer, the kinetics of reoxidation to the pyridinium form in aqueous phosphate buffered solutions with pH values between 6.7 and 7.6 were followed by UV-vis spectrometry using a diode array spectrometer.

Scheme 2



Scheme 3 shows the half-lives of the reoxidation of the complexes a (left) and b (right) at 20 and 37 °C as a function of the pH-value.

Scheme 3



Previous investigations into the stability of the analogous dihydropyridine complexes had already shown, that the substitution of the N-methyl group by benzyl results in a sixfold increase of the half-life. Furthermore the change from dihydropyridine to dihydroquinoline caused the expected stabilization by a factor of 10 to 20. In addition, the temperature dependence of half-life is less in comparison to the dihydropyridines.

Thus the quinolinium/dihydroquinoline complexes might be suitable as redox-active radiotracers. Further investigations (enzymatic inhibition tests as well as n.c.a. preparations with ^{99m}Tc) are being carried out at present.

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Potentially Redox-Active Rhenium and Technetium Complexes Based on the Pyridinium/Dihydropyridine System

6. Pyridinium/Dihydropyridine-Bearing Rhenium Mixed-Ligand Complexes with Increased Stability to Ligand Exchange

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In view of redox-active rhenium and technetium complexes we report on the synthesis of pyridinium/dihydropyridine bearing rhenium(V) '3+1' and rhenium(III) '4+1' mixed-ligand complexes. The influence of the chelate system and the oxidation state of the metal core on the redox behaviour of the pyridinium/dihydropyridine system was investigated by UV-vis spectrometric measurements.

Introduction

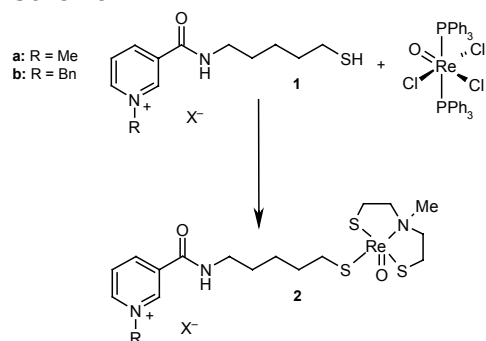
Previous reports on the redox-active ^{99m}Tc-labelled radiotracers based on the pyridinium/dihydropyridine system described rhenium(V) '3+1' mixed-ligand complexes with bis-(2-mercaptoethyl)-sulphide ('SSS') and pyridinium/dihydropyridine bearing thiols as co-ligands [1]. Here we resume the synthesis of pyridinium/dihydropyridine bearing rhenium(V) '3+1' complexes with bis-(2-mercaptoethyl)-methyl amine ('SNS') as a tridentate ligand and rhenium(III) '4+1' complexes with tris-(2-thiolatoethyl) amine and isocyanides as co-ligands. Investigations of their redox behavior were performed. Of particular interest in this regard was the influence of the lower oxidation state of the '4+1' complexes on the stability of the dihydropyridine form of the complexes as well as the stability of the chelate to ligand-exchange reactions with naturally occurring thiols [2].

Results and Discussion

'3+1' rhenium(V) complexes

The pyridinium-bearing thiols (1) were synthesized in four steps by organic standard procedures starting with methyl nicotinate. Reaction of the thiols and the precursor tri-chloro-bis-(triphenyl-phosphino) oxorhenium(V) with di-[bis-(2-mercaptoethyl)-methyl ammonium] oxalate in methanolic solution of sodium acetate forms the corresponding rhenium(V) complexes (2) (Scheme 1). The complexes were characterized by ¹H NMR, ¹³C NMR and elemental analysis.

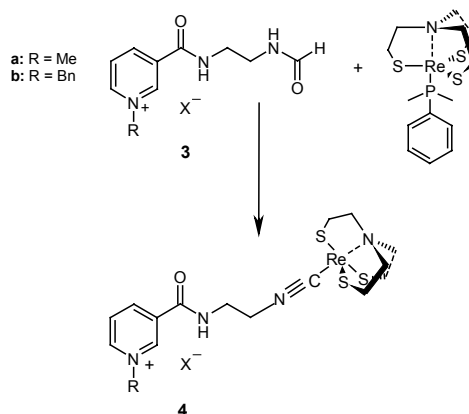
Scheme 1.



'4+1' rhenium(III) complexes

Again organic standard procedures led to the corresponding formamides (3). These were transformed in situ into isocyanides with triphenyl phosphine, carbon tetrachloride and triethyl amine and reacted with the precursor tris-(2-thiolatoethyl) amino triphenylphosphino rhenium(III) into the '4+1' complexes (4) (Scheme 2).

Scheme 2.



The reduction of the pyridinium compounds (2) and (4) to their 1,4-dihydropyridine form and measurement of the reoxidation kinetics was performed according to [1]. The change from the 'SSS' chelate to the 'SNS' type resulted in stabilization of the dihydropyridine form by a factor of 2 or 3.

The kinetics of the '4+1' complexes proved that the oxidation state of the metal core influences the stability of the dihydropyridine form of the complexes only marginally. In comparison with the '3+1' complexes of the 'SSS' type, the half-lives increase only by a factor of 1.2 to 1.6.

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Synthesis of Hydroxyl Silylated (^{99m}Tc)Technetium "3+1" Mixed Ligand Complexes

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Synthesis of silylated "3+1" mixed-ligand [^{99m}Tc]oxotechnetium(V) complexes is described.

Introduction

The lipophilicity of organic compounds is increased by the introduction of silyl groups and it was demonstrated that silylation of biologically active molecules facilitated their transport in the organism [1-4]. We want to use silylation as a tool to direct the transport of metal complexes in vivo. After the description of silylated "3+1" rhenium(V)oxo-complexes [5,6], we intended to use the experience gathered in the synthesis of silylated (^{99m}Tc) mixed ligand complexes in aqueous solution.

Results and Discussion

The silylated (^{99m}Tc)(3-thiapentane-1,5-dithiolato)oxo-technetium(V) complexes **2** were prepared via ^{99m}Tc gluconate in aqueous solution (Fig. 1). The two-step procedure was carried out at a pH of about 7 with phosphate buffer to avoid hydrolysis. First the monodentate ligand **1**, then the 3-thiapentane-1,5-dithiol was added to the ^{99m}Tc gluconate solution. The mixture was heated to 50 °C. The silylated "3+1" ^{99m}Tc complexes **2** were detected by HPLC at retention times corresponding to those of the appropriate rhenium compounds. For the triphenyl- and triethyl-silylated [^{99m}Tc]technetium(V)oxocomplexes **2b**, **2d** and **2e** labelling yields of between 80 and 90 % were found, together with some unreacted ^{99m}Tc gluconate. In any case, no hydrolysed ^{99m}Tc compounds were formed. This was proved by HPLC, by a comparison with the retention times of the hydrolysed rhenium complexes.

In case of the trimethyl-silylated ligands **1a** and **1c** all efforts at labelling were in vain. Attempts

react these ligands with ^{99m}Tc gluconate and 3-thiapentane-1,5-dithiol in the appropriate buffer/acetonitrile mixture resulted in several peaks in HPLC, among them the hydrolysed complex. The trimethylsilyl group was thus found to be too unstable for labelling under aqueous conditions.

Acknowledgements

The authors thank the Deutscher Akademischer Austauschdienst (DAAD) and the Instituto de Cooperação Científica e Tecnológica Internacional (ICCTI) for supporting a bilateral project.

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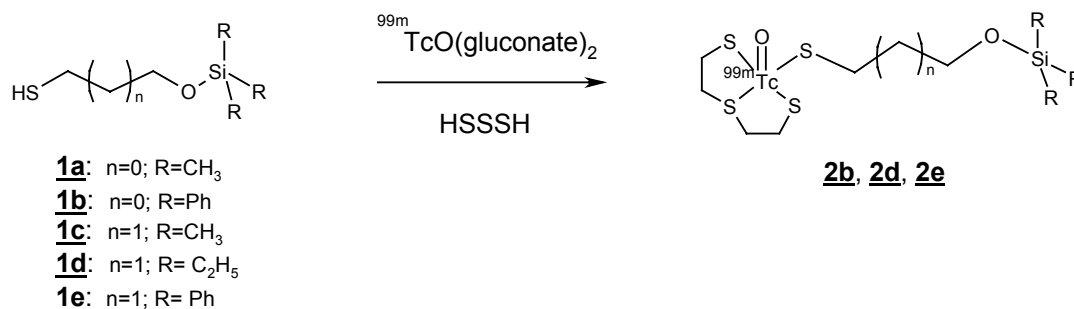


Fig. 1. Synthetic pathway for silylated ^{99m}Tc mixed ligand complexes

New Hydroxyl Silylated "3+1" Rhenium Complexes with the PNS System as a Tridentate Ligand

T. Kniess, H. Spies, J.D.G. Correia¹, I. Santos¹
Departamento de Quimica, ITN, Sacavem Codex, Portugal

Hydroxyl silylated mixed-ligand Re(V) complexes are in the focus of interest in connection with the design of radiotracers. Investigations were carried out to see whether the introduction of organosilicon groups into Re and Tc complexes will improve their molecular properties.

Introduction

Having written about silylated "3+1" mixed-ligand Re and Tc complexes with thiapentane-1,3-dithiole as the tridentate ligand [1-3], we now report on the synthesis of new silylated "3+1" Re complexes based on the tridentate PNS ligand 2-(diphenyl-phosphanyl)-N-(2-thioethyl)benzamide, which was recently described as a new tridentate chelator [4].

Results and Discussion

New silylated "3+1" mixed-ligand Re complexes **2a-e** with the PNS tridentate ligand were synthesized in a one-pot procedure by reacting the monodentate thiolate ligand **1**, the tridentate PNS ligand and the rhenium precursor $[\text{NBU}_4][\text{ReOCl}_4]$ [3-5] in equimolar amounts (Fig. 1). A slight excess of triethylamine is required to bind the hydrochloric acid formed and to avoid hydrolyzation of the silylated ligand. The products are purified by column chromatography. A greyish-green solids results after lyophilization.

One reason for the relative low yields is the formation of a concurrent product, for which the PNS itself reacts as a monodentate ligand. This mixed ligand complex $[\text{ReO}(\kappa^3\text{-PNS})(\kappa^1\text{-PNS})]$ was described in [4].

The trimethylsilylated complexes **2a** and **2c** were partly hydrolysed under the preparation conditions, as was detected byTLC. The triethyl and triphenyl silylated species were stable. The ¹H NMR spectra of **2a-e** involve four multiplet signals of the chelate's methylene protons, showing that the protons became diastereotopic in the complex.

Acknowledgements

The authors thank the Deutscher Akademischer Austauschdienst (DAAD) and the Instituto Cooperacio Cientifica e Technologica Internacional (ICCTI) for supporting a bilateral project.

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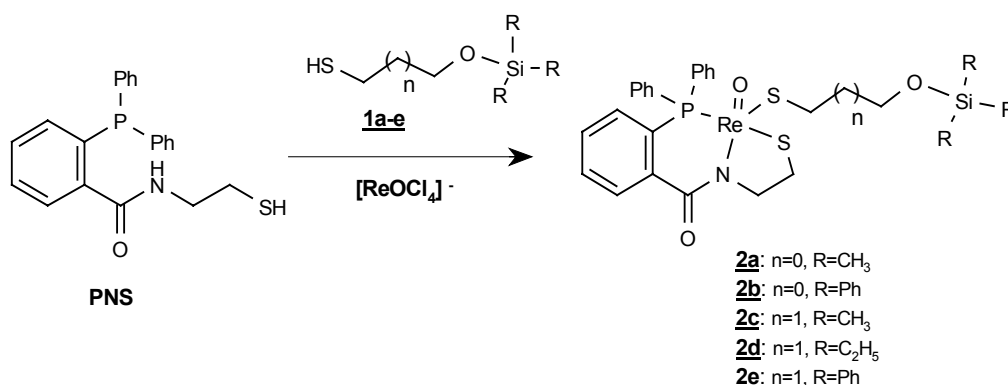


Fig. 1. Synthetic route for silylated Re-PNS complexes

Synthesis of 4-[¹⁸F]Fluorobenzaldehyde – an Important Labelling Moiety in ¹⁸F Chemistry

P. Mäding, J. Steinbach

The synthesis of 4-[¹⁸F]fluorobenzaldehyde (4-[¹⁸F]FBA) was investigated. Starting from 4-trimethylammoniumbenzaldehyde triflate (TMABATf), 4-[¹⁸F]FBA was obtained in radiochemical yields of up to 78 %. 4-nitrobenzaldehyde was also tested as a precursor, but without success.

Introduction

4-[¹⁸F]FBA is a valuable labelled compound in ¹⁸F chemistry. It will be used as a starting material for the synthesis of 4-[¹⁸F]fluorobenzyl halides via 4-[¹⁸F]fluorobenzyl alcohol [1]. Another possibility for the application of 4-[¹⁸F]FBA is its reductive amination to obtain the corresponding 4-[¹⁸F]fluorobenzylated amino compound [2]. Earlier it was also used to synthesize N-succinimidyl 4-[¹⁸F]fluorobenzoate ([¹⁸F]SFB) for labelling peptides [3].

Two main precursors are described in the literature for synthesizing 4-[¹⁸F]FBA: the 4-trimethylammoniumbenzaldehyde triflate (TMABATf) [4] and the 4-nitrobenzaldehyde [4, 5, 6]. These syntheses are based on the nucleophilic substitution of [¹⁸F]fluoride for the 4-trimethylammonium or the nitro group.

Haka [4] describes conversions of TMABATf (6.4 μmol) in DMSO (100 μl), using Kryptofix222/K₂CO₃ activated [¹⁸F]fluoride at 160 °C and 25 min reaction time with radiochemical yields (r.c.y.) of up to 74 % 4-[¹⁸F]FBA (70 % at 120 °C). The same conversion in DMSO (350 μl) at 80 °C for 4 min with r.c.y. of 60 to 70 % is described in [2]. Mach investigated the nucleophilic [¹⁸F]fluorination of TMABATf (120 °C, 10 min; r.c.y.: 28 - 68 %) and of 4-nitrobenzaldehyde (150 °C, 40 min; r.c.y.: 26 - 68 %), using [¹⁸F]fluoride/Cs₂CO₃ in aqueous DMSO [5]. The utilization of the nitro compound is also described in [6], using electrochemically separated [¹⁸F]fluoride and Kryptofix222/K₂CO₃ in 1 ml DMF (130 °C, 5 min; r.c.y.: 97±3 %).

Results and Discussion

Precursors

TMABATf was synthesized according to [2] starting from 4-dimethylaminobenzaldehyde and methyl triflate.

The 4-nitrobenzaldehyde is commercially available.

Radiochemistry

[¹⁸F]Fluorination using TMABATf

Reactions of TMABATf (7 - 8.6 μmol) with dried [¹⁸F]fluoride/K₂CO₃/K222 mixtures were

carried out in several solvents, such as DMSO, DMF, MeCN and N,N'-dimethylethyleneurea (DMEU), at several temperatures and reaction times.

It was found that MeCN and DMEU are not suitable as solvents for this nucleophilic substitution reaction. No conversion was observed in DMEU (250 μl, 160 °C). A fluorination reaction took place in MeCN (1 ml, 160 °C, 5 min) but the [¹⁸F]fluorinated product (about 72 %) was not identified as the desired 4-[¹⁸F]FBA.

Conversions in DMSO (250 μl) at 160 °C yielded 32 % of 4-[¹⁸F]FBA within 5 min reaction time. Under the same conditions but in DMF, the radiochemical yield was only 13 %. Further optimization was carried out in DMF as DMSO may interfere or prevent consecutive reactions. For considerable yields repeated addition of the precursor is necessary at 95 °C: 10.5 μmol precursor, 400 μl DMF, 5 min + 6.0 μmol precursor, 100 μl DMF, 5 min. In this way 70 to 76 % 4-[¹⁸F]FBA was obtained. The best option was only one addition of a relatively large amount of the precursor (27.1-34.5 μmol) in 1 ml DMF and heating at 120 °C for 10 min, which yielded 76 - 78 % 4-[¹⁸F]FBA.

[¹⁸F]Fluorination using 4-nitrobenzaldehyde

We also investigated conversions of 4-nitrobenzaldehyde (19.8-21.2 μmol) with [¹⁸F]fluoride/K₂CO₃/K222 mixtures in DMF (250 - 400 μl) at 130 °C. After 5 to 10 min heating, no formation of 4-[¹⁸F]FBA was observed.

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A Novel Approach for a C-¹¹C Bond Formation: Synthesis of 17 α -([¹¹C]Prop-1-ynyl)-3-methoxy-3,17 β -estradiol

F. Wüst, J. Zessin

A novel method for a ¹¹C-C bond formation was developed, employing a cross-coupling reaction between a terminal acetylene and [¹¹C]methyl iodide. The method was used for the synthesis of 17 α -([¹¹C]prop-1-ynyl)-3-methoxy-3,17 β -estradiol.

Introduction

The majority of ¹¹C-labelling reactions are mainly heteroatom methylations, using [¹¹C]methyl iodide. However, to expand the scope of ¹¹C-labelled compounds, novel ¹¹C-C bond-forming reactions gain more and more attention [1].

The versatile availability of [¹¹C]methyl iodide makes this labelling precursor favourable in several transition-metal mediated cross-coupling reactions. Its technically simple, high-yielding and functional group tolerating reactions are of particular interest. The Sonogashira copper-palladium catalysed coupling of terminal alkynes with aromatic and vinylic halides [2] represents such a reaction. To the best of our knowledge, the Sonogashira-reaction has not yet been employed in ¹¹C-chemistry. In this report we describe a modified Sonogashira-like reaction [3] to label the terminal alkyne group of the potent contraceptive steroid mestranol **3** with [¹¹C]methyl iodide.

Results and Discussion

Synthesis of the standard compound

The standard compound **2** was easily prepared by the reaction of 3-methoxy-estrone **1** with propynyl magnesium bromide (Fig. 1) in a 52 % yield.

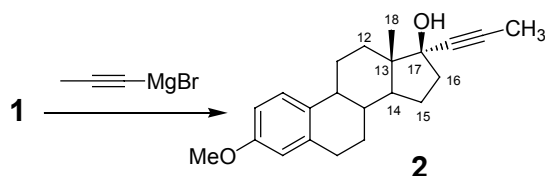


Fig. 1. Synthesis of the standard compound

The stereoselective Grignard reaction with the 17-Keto group in **1** from the α -face of the steroid was assessed by ¹³C NMR. The observed shielding(-) and deshielding(+) effects for the carbon atoms 12-18 (C12: -4.9; C13: +3.0; C14: -1.6; C15: -1.0; C16: +8.4; C17: -1.8; C18: +1.1) prove the 17 α -substitution in comparison with 3,17 β -estradiol.

C-11-labelling

The classical conditions of the Sonogashira reaction (Pd⁰, CuI and TEA or DIPA as the base) can not be employed for ¹¹C-labelling with [¹¹C]methyl iodide due to the rapid quaternization of the amine base. Therefore, we tested alternative catalyst/base combinations. By using tetrakis(triphenylphosphine)-palladium(0) (TTPP), copper(I) iodide, and 1,8-bis-(dimethylamino)naphthalene as a non-nucleophilic base, about 5 % of [¹¹C]methyl iodide was converted into [¹¹C]**2**. Similar results were obtained using the catalyst TTPP and silver oxide as the base.

The reaction of **3** with [¹¹C]methyl iodide catalysed by tris(dibenzylideneacetone)dipalladium(0), triphenylarsine, and tetrabutylammonium fluoride gave [¹¹C]**2** in 42 – 53 % yield (Fig. 2).

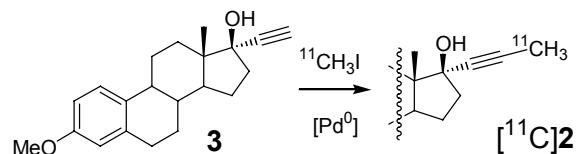


Fig. 2. ¹¹C-labelling of steroid **3**

In summary, we developed a new method for a ¹¹C-C bond formation, employing the cross-coupling of terminal alkynes with [¹¹C]methyl iodide via a Sonogashira-like reaction in sufficient radiochemical yields.

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Synthesis and Reactivity of the Electrophilic Fluorinating Agent N-Fluoro-bis[(perfluoroalkyl)sulfonyl]imide

A. Jordanova, J. Steinbach

N-Fluoroimide ($(CF_3SO_2)_2NF$) was synthesized by solid-phase fluorination of the lithium salt of the trifluoromethanesulfonylimide. Reactions of the *N*-fluoroimide with various model aromatic compounds and carbanions were carried out. C.a. fluorination with $[^{18}F]F_2$ was also performed.

Introduction

Nowadays there is still need for the development of appropriate labelling methods for introducing of n.c.a. ^{18}F into electron rich aromatic systems. Electrophilic fluorination with electrophilic fluorinating reagents can be one alternative. A number of electrophilic aromatic radiofluorination techniques have been developed, but all these methods are performed on the c.a. level and involve the reactions of rather aggressive fluorinating agents. Several groups recently described *N*-fluoro compounds as useful fluorinating reagents [1, 2]. *N*-fluoro reagents surpass *O*-fluoro and *S*-fluoro reagents in stability, reactivity, safety and handling convenience. *N*-fluorosulfonylimide ($(CF_3SO_2)_2NF$) was reported as the most reactive electrophilic fluorinating agent known today [2]. The synthetic procedure includes fluorination of $(CF_3SO_2)_2NH$ with pure F_2 under pressure at $-196^\circ C$, conditions which require special equipment and experience and are not applicable in the case of radioactive synthesis. An alternative route for obtaining of $(CF_3SO_2)_2NF$ was therefore found. This method involves solid-phase fluorination of the lithium salt $(CF_3SO_2)_2NLi$ with diluted fluorine. Reactions of *N*-fluoroimide with various model aromatic compounds and carbanions were also carried out. The reaction conditions for preparation of *N*-fluoroimide were adapted to a radioactive synthesis and c.a. fluorination with $[^{18}F]F_2$ was performed.

Results and Discussion

Synthesis of $(CF_3SO_2)_2NF$ and reaction with the model substances

The *N*-Fluoroimide was prepared in an 80 % yield by slowly passing a fluorine gas 10 % F_2/N_2 through the upper end of a glass column filled with the lithium salt. The second end of the column was connected to a cooling trap at $-55^\circ C$ in which pure *N*-fluoroimide was collected. The compound was confirmed by NMR and the data agreed with the literature data [3].

Electron-rich aromatics were chosen as model substances: anisole, phenol, benzene, phenyllithium and sodium diethylmalonate as a carbanion. The reactions were performed, varying the parameters solvent, molar ratio, reaction temperature and optimised reaction conditions were found.

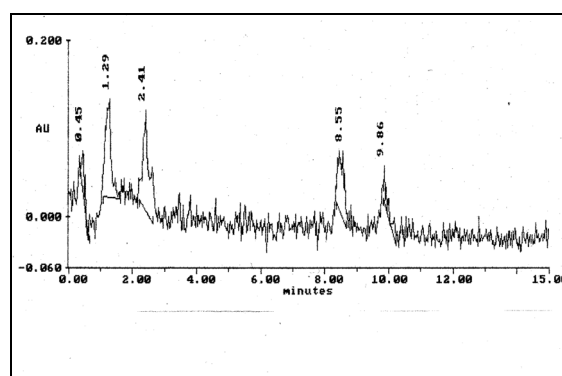


Fig. 1. Radio-HPLC of the reaction of c.a. $(CF_3SO_2)_2NF$ to anisole

Synthesis of c.a. $[^{18}F](CF_3SO_2)_2NF$

The radioactive synthesis of the compound was performed under modified reaction conditions, by passing c.a. $[F_2]F_2/Ne$ through the solution of the lithium salt in CH_3CN at $-40^\circ C$ within 30 min. The compound was not isolated and the anisole solution was directly added to the reaction mixture containing the *N*-fluoroimide. The mixture was reacted within 15 min at $80^\circ C$. 24 % of c.a. $[^{18}F]2$ -fluoroanisole and 7 % of c.a. $[^{18}F]4$ -Fluoroanisole were obtained as shown in Fig. 1.

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Quantification of [¹⁸F]FDOPA and [¹⁸F]-3-OMFD in Pig Serum - a New TLC Method in Comparison with HPLC

B. Pawelke, F. Füchtner, R. Bergmann, P. Brust

A novel TLC method for convenient quantification of [¹⁸F]FDOPA and [¹⁸F]-3-OMFD in routine operation was developed and the results assessed in comparison with an HPLC analysis. The two methods were found to correlate well. [¹⁸F]fluoride which resisted determination on HPLC RP-18 columns was also quantified by TLC.

Introduction

Quantification of [¹⁸F]FDOPA and its labeled metabolites in the arterial plasma is a prerequisite for the kinetic analysis of brain PET studies using [¹⁸F]FDOPA as a tracer. In this context, HPLC is a well-established method for determination of [¹⁸F]FDOPA and its main metabolites [e.g. 1,2]. However, for routine operation it tends to be necessary to minimize the analytical input. Therefore, we developed a TLC method for the quantification of [¹⁸F]FDOPA and its main metabolite, [¹⁸F]-3-OMFD, and compared the results with those obtained by an established HPLC method.

Results and Discussion

Analyses methods

Arterial blood samples were taken from anaesthetized piglets pretreated with carbidopa (0.2 mg/kg). The serum was separated and analyzed after precipitation of plasma proteins.

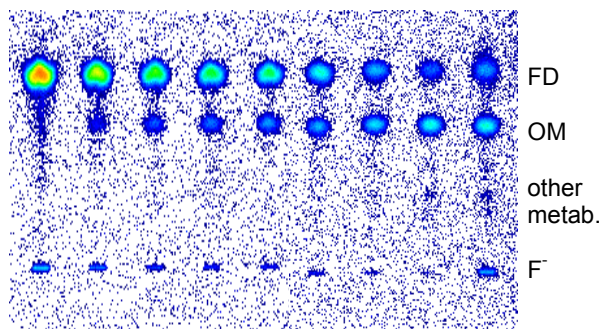
HPLC analysis

HPLC analysis was performed according to [3] in a slightly modified procedure on a Zorbax 300SB-C18 column using an acetonitrile/buffer (70 mM KH₂PO₄ + 0.1 mM Na₂EDTA, pH 3.4) gradient and a radioactivity detector with PET flow cell.

TLC analysis

TLC was performed on HPTLC RP-18 WF_{254s} plates with 0.1 M H₂SO₄/ 15 % (vol) acetonitrile/ 1 mM EDTA as the mobile phase. The chromatograms were analyzed by means of a Bio-Imaging Analyzer BAS 2000 (Fig. 1).

Fig. 1. FD: [¹⁸F]FDOPA, OM: [¹⁸F]-3-OMFD



Series of six independent experiments (between 5 and 9 samples each) were analyzed. The resulting diagrams of correlation (TLC value vs. HPLC value) indicated linear correlations with slopes between 0.94 and 0.98 (Fig. 2). After a correction of the HPLC results by the amount of fluoride determined by TLC, only minor changes in slopes and correlation coefficients were observed (gray symbols).

From the results it was concluded that the TLC method represents a simple analytical option for the quantification of [¹⁸F]FDOPA and [¹⁸F]-3-OMFD in routine applications. It also allows quantification of [¹⁸F]fluoride which is a major advantage over HPLC on an RP-18 column.

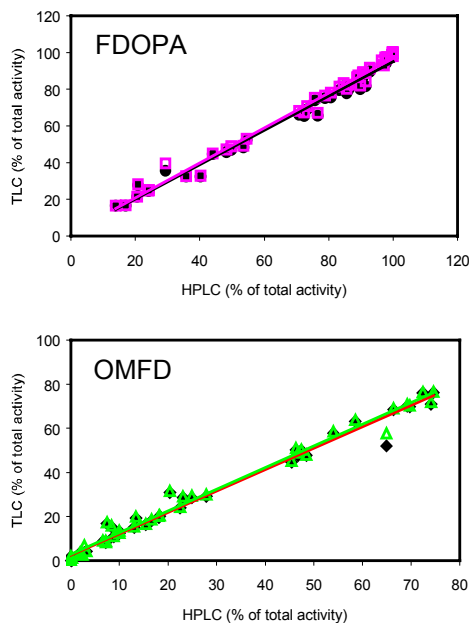


Fig. 2.

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PHYSICS AND INSTRUMENTATION

Operation of the Rossendorf PET Cyclotron "CYCLONE 18/9" in 2001

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Routine operation

The radionuclides produced in routine operation in 2001 were F-18, C-11, O-15 and N-13, available as [¹⁸F]F⁻, [¹⁸F]F₂, [¹¹C]CO₂, [¹⁵O]H₂O and [¹³N]NO₃. Table 1 gives an overview of the 2001 radionuclide production. There were only few demands for production of the radionuclide ¹³N.

The daily operating time of the CYCLONE 18/9 varied between two and four hours.

Table 1. Radionuclide production in 2001

RN	Radionuclide production	
	Number of Irradiations	SumA _{EOB} GBq
[¹⁸ F]F ⁻	328	14770
[¹⁸ F]F ₂	181 ^{*)}	922
¹¹ C	82	2274
¹³ N	10	55
¹⁵ O	88	1748

^{*)} including pre-irradiations

Fig. 1 shows the number of irradiations of our radionuclides and Fig. 2 the total amount of activity produced from 1997 to 2001.

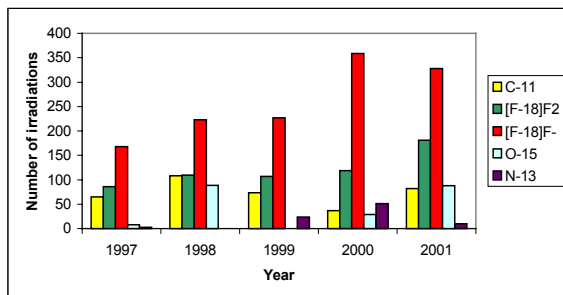


Fig. 1. Number of irradiations of radionuclides produced

Improvements at the cyclotron

- Modification of the ¹⁸F water target filling procedure

Based on our experience with the manually driven syringe system for recycled low-grade enriched [¹⁸O] water [1, 2], we installed a second IBA syringe system for it.

- Procedure for rinsing the ¹⁸F water target with deionized water

A procedure for rinsing the ¹⁸F water target with deionized water was tested and added to the daily target handling routine [3].

- He flow as an interlock signal in the He cooling loop

We installed an electrically driven flow meter in the He cooling loop with series connection to the existing He pressure interlock to improve the protection of the target and vacuum windows in case of a He circuit failure [4].

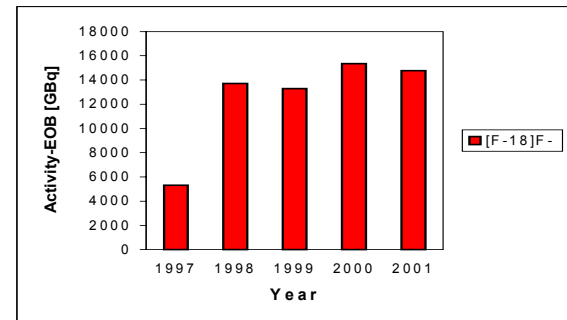
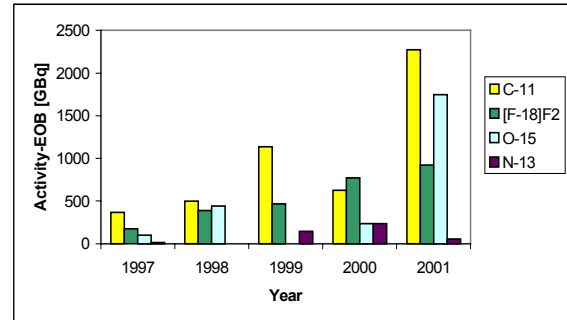


Fig. 2a and b. Total amount of activity produced

Maintenance and service

The main reasons for venting and opening the cyclotron were problems with the highly stressed proton ion source as well as maintenance and service work at both ion sources.

Unplanned turn-off periods:

Three days: problems with both ion sources (short circuits, low beam current).

The annual check of the CYCLONE 18/9 facility by the TÜV Sachsen organization (TÜV = Association for Technical Inspection) under § 66 of the new German Radiation Protection Order was carried out in the second half of September. There were no objections to the further operation of the cyclotron.

Radiation protection

- Emission of radionuclides with the exhaust air
 The emission of radionuclides with the exhaust air is routinely monitored. As shown in Table 3, it is well below the limit.

Table 3. Emission of radionuclides with the exhaust air in 2001 as a result of operation of the CYCLONE 18/9

Radionuclide	Emission [Bq/a]
⁴¹ Ar	6.60E09
¹⁸ F	1.80E10
Sum	2.44E10
Percentage of the Annual limit	12.2

- Exposure to radiation of the cyclotron staff

The cyclotron staff belong to category A of occupational exposed persons. The average exposure to radiation was 1.8 mSv in 1997, 2.9 mSv in 1998, 3.5 mSv in 1999, 6.2 mSv in 2000 and 4.3 mSv in 2001 (Jan. to Nov.).

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Procedure for Rinsing the ^{18}F Water Target with Deionized Water

St. Preusche, F. Füchtner, H. Ross

A procedure for rinsing the ^{18}F water target with deionized water was tested and added to the daily target handling routine.

Introduction

A pneumatically driven Rheodyne valve controls all functions of the IBA ^{18}F water target (rinsing with He, loading, unloading, irradiation mode). Previous investigations showed that drops of $[\text{}^{18}\text{F}]\text{F}/[\text{}^{18}\text{O}]\text{H}_2\text{O}$ remaining in the target and unloading tube after unloading the ^{18}F water target lead to radiolysis effects and moreover to greatly reduced radiochemical yields of $[\text{}^{18}\text{F}]\text{FDG}$ on the next day [1].

Our IBA ^{18}F water target system is not intended to rinse the target or unloading tube with deionized water.

As a first measure a 3-way valve was inserted into the unloading tube close to the Rheodyne valve for rinsing the tube with deionized water. Based on this experience we changed the IBA ^{18}F water target system and added a new procedure to the daily target handling routine.

Solution

A six-port valve (Upchurch Scientific/USA) with LOAD/INJECT positions together with a 1.5 ml loop and a related filling unit were inserted into the He purge line of the ^{18}F water target (Fig. 1). The new valve was placed into the target accessories box and the diameter of the He

purge tube (box to the target, 10 m in length) was reduced from 1/16 to 1/32 inches.

The 1.5 ml loop is filled with deionized water in the LOAD position of the Upchurch valve. To rinse the target the valve is switched to the INJECT position. Then the target can be rinsed by pressing the UNLOAD/RINSE button on the computer screen. The He stream unloads the loop and rinses the target.

As we had no access to the programs of the control system, the procedure was carried out without changing the IBA software.

Results

It takes approximately two minutes to rinse the ^{18}F water target to the pneumatic rabbit. The activated water drops are removed and radiochemical $[\text{}^{18}\text{F}]\text{FDG}$ yields of 45 to 55 % (EOS) are achieved.

Due to the reduced diameter of the He purge tube the time it takes to unload the activated water after the irradiation in the rabbit increased from 1.5 to 2 minutes and was thus within the 5 minutes range predicted in the control software.

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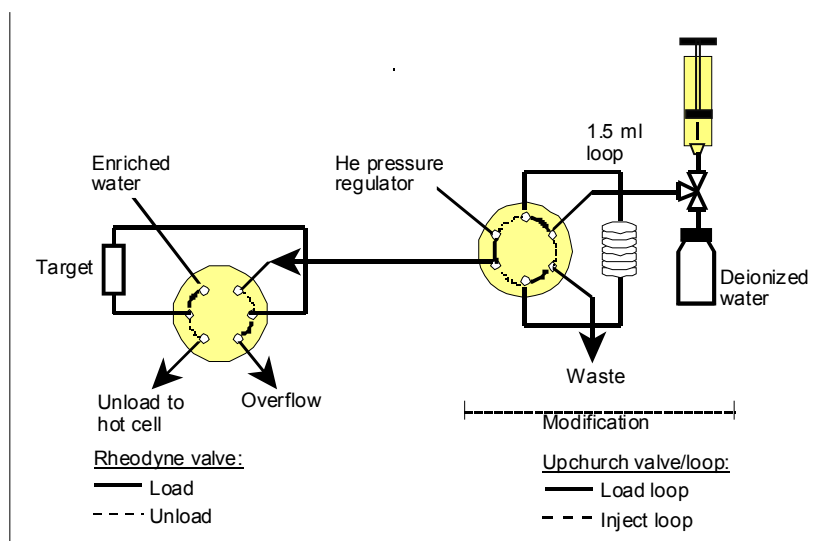


Fig. 1. Modified ^{18}F water target system

He Flow Interlock in the CYCLONE 18/9 He Cooling Loop

St. Preusche, H. Ross

An electrically driven flow meter was installed in the CYCLONE 18/9 He cooling loop with series connection to the existing He pressure interlock to improve protection of the target and vacuum windows in case of a He circuit failure.

Introduction

Both the target and vacuum windows of the CYCLONE 18/9 target systems are cooled with helium. The He cooling loop consists of a metal bellow compressor, the He heat exchanger with the He drain-reload unit and the He manifold.

Originally, only the He pressure (ca. 0.3 bar) was used as an interlock signal via a 24 V relay contact to the PLC. The relay contact is closed during normal operation of the He cooling loop and opened when there is no He pressure so as to switch off the ion beam by switching off the ion source.

In case of problems (e.g. with the He compressor) the He flow goes immediately down to zero but there is still a He pressure in the system. As a result the target and vacuum windows are destroyed because they are no longer cooled due to the He flow having been switched off.

Solution

The He cooling loop already contains a flow meter. It is not equipped with electric contacts

because it is only used to check the He flow under operating conditions.

An electrically driven flow meter was inserted into the return flow tube of the He circuit to the He compressor, in series connection to the existing flow meter (s. Fig. 1). The relay contacts of the existing He pressure interlock and the new He flow interlock were connected in series. When one of the two conditions fails, the system will switch the ion source off.

Result

The new interlock signal was tested under operating conditions but with a low ion beam current on the target. The power of the He compressor was switched off to demonstrate the effect of the He flow interlock. As there was no He flow, the ion source was switched off by the PLC in less than one second.

In spite of the fact that there was no He flow, the He pressure kept values of 0.3 to 0.2 bar for more than five minutes. The He pressure interlock did not act in this case.

Thus, the He flow interlock is better suited to protecting the target and vacuum windows.

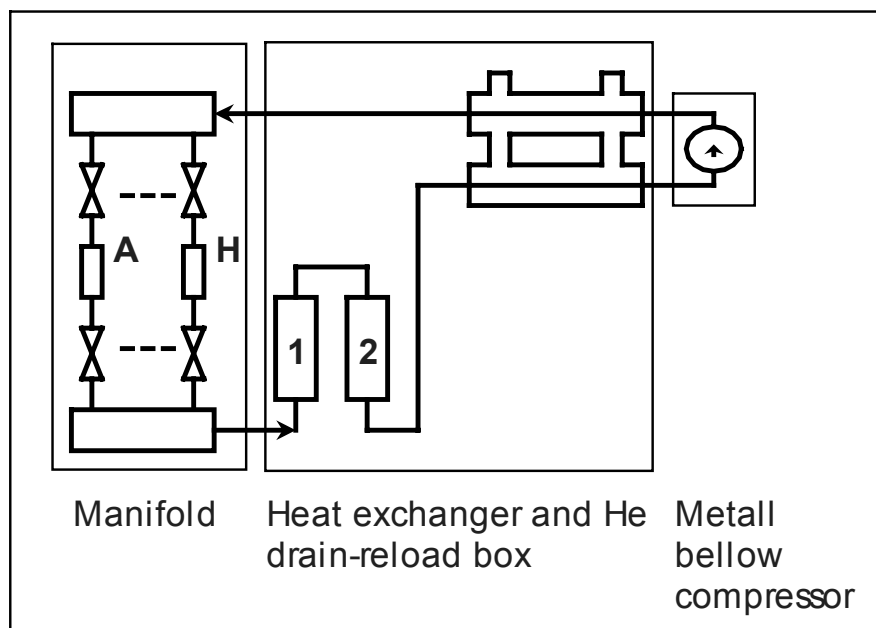


Fig. 1. Modified He cooling loop; 1: existing flowmeter (Brooks Instruments), 2: electrically driven flowmeter (Kobold), A, H: target window holders

CLICKFIT – A Flexible and Powerful PET Data Modelling Tool

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Positron Emission Tomography (PET) is an *in vivo* tracer technique which allows the estimation of physiological and pharmacological parameters based on the tracer's kinetic behaviour. The transformation of the raw data into the physiological parameters of interest requires the application of mathematical models. Mathematical modelling also plays an essential role in the processing of data peripheral to the scan itself, such as blood data used to generate input functions for the tissue data. Here we describe a modelling package specifically designed for the analysis of dynamic PET scan data.

Introduction

Many procedures in PET require mathematical modelling of one-dimensional data. The analysis of tissue time-activity curves, for example, is often based on compartmental models which relate tracer kinetics to the underlying physiological processes. In addition, processing data from whole blood samples taken during a PET study allows the generation of input functions describing delivery of the tracer to the tissue. To generate plasma input functions from the series of whole blood samples, they have to be interpolated according to the most appropriate model of the ratio of plasma activity to the whole blood activity and the parent tracer fraction. For modelling these types of PET data, an easy-to-use software package *CLICKFIT* has been developed.

Material and Methods

To provide portability across various computer platforms, *CLICKFIT* is implemented on the basis of the widespread commercial mathematical toolbox MATLAB (The MathWorks, Inc., Natick, MA, USA). The general data input format is a simple table of formatted data consisting of various columns, typically one column with the values of the independent variable, one column with the values of the dependent variable, one column with the individual weights of each data point and one column to give the data points a code.

Emphasis was put on offering a broad variety of data models. The collection of blood models comprises very simple functions, e.g. a constant ratio or a straight line, as well as expressions of two exponentials or the convolution of the independent variable with an exponential function.

For modelling PET time activity curves, either blood or reference-region data can be used as the input function. A wide range of PET models is implemented: two, three or four rate constant compartmental models with or without blood volume correction [1], the reference region model [2], the simplified reference region model [3], graphical analysis after Patlak and Logan [4] and spectral analysis [5].

The optimum parameter set along with its standard errors for a specified model are computed by weighted nonlinear least square techniques. A numerical approximation of the covariance matrix is calculated as well. Values derived from the estimated parameters, e.g. estimates of the volume of distribution (*VD*) or the binding potential, can be therefore computed with their variances.

Comfortable plotting facilities allow visual inspection of the fitted curves (Fig. 1).

This package is currently being applied to PET data from clinical research programmes carried out at the Medical Research Council Clinical Sciences Centre, Hammersmith Hospital, in London.

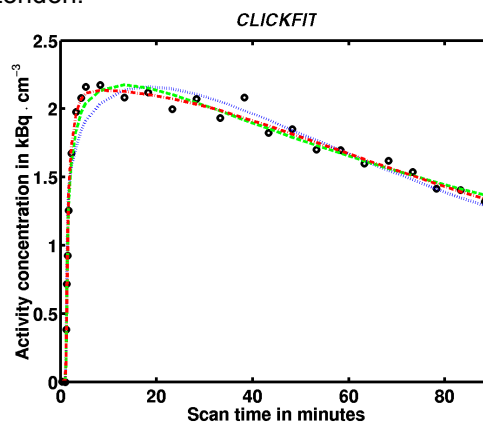


Fig. 1. Results of fits of a measured time activity curve (o) to three PET models: one-tissue compartment model (....., $VD = 12.8 \pm 0.5$), two-tissue compartment model (---, $VD = 18.6 \pm 10.0$) and spectral analysis (-.-.-, $VD = 14.9$).

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Parametric Images Evaluation of Selected Phases of the Heart Cycle with PET

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The standard evaluation of dynamic heart acquisitions with PET uses image data not corrected for heart wall movement. The evaluation of parametric data sets (Patlak Plot) was investigated for gated studies of selected heart phases (diastolic, systolic) and compared to the standard evaluation. Parametric images of selected heart phases have improved resolution. The values for metabolic rate are different for a "normal" and gated evaluation, up to 50% more for the systole compared to the normal one.

Introduction

The normal parametric data set evaluation of dynamic heart acquisitions (Patlak Plot) [1] is based on an integral of the activity distribution over the whole heart cycle. The inherent information about the heart wall movement is eliminated. Listmode [2] acquisition enables the evaluation of selected heart phases (e.g. diastolic, systolic) with gated studies. The resulting quantitative values for the myocardial glucose metabolism of a standard evaluation were compared with the selected phases.

Methods

The listmode study was acquired on an ECAT EXACT HR+ scanner (CTI/Siemens). An ECG device attached to the gate input of the ECAT PET scanner sends trigger signals for the gated measurement at the R-peak of the heart cycle. Sinogram files for a „normal“ study and studies of selected heart phases (systole + diastole) were created from the measured listmode data set using the listmode sorting software. The reconstruction was done with standard ECAT7 software.

The Patlak procedure requires also the progress of the blood activity distribution over the time as input data. The blood activity input is obtained from a ROI located in the centre of the left ventricle. The blood ROIs are outlined at their maximum possible size for a large statistic coverage. In a "normal" study, the contents of the voxels are averaged values over the whole heart cycle. The blood pool size appears therefore larger than in a study of the systole. This leads to a ROI exceeding the real blood pool size in systolic phase of the heart.

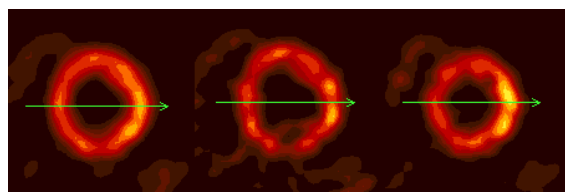


Fig. 1. Slice images showing the metabolic rate per pixel in a "normal" study (left) and in studies of the diastole (middle) and the systole (right). The green line marks the position of the created profiles.

To estimate the error the metabolic rate values determined from such oversized blood ROI was compared with the values obtained from a ROI derived from the systole. For all study types a profile through the left ventricle in a selected slice (Fig. 1) shows the difference in heart wall position and blood pool size.

Pixel Patlak volume images were created to obtain the glucose metabolic rate for all studies. Finally the Patlak volume images were reorientated to obtain a short axis view of the heart.

Results

The activity profiles through the left ventricle (Fig. 2) points out the large difference in position and thickness of the lateral wall between the images of the „normal“ study and the study of the systole. The range of the blood ROI's used for the Patlak procedure is also shown. The blood ROI derived from the „normal“ study overlaps the systolic lateral wall up to 20 % of the ROI width (Fig. 2). The Patlak calculation with the "normal" ROI gives higher metabolic rate values (up to 5 % -10 %) compared to the same study with a blood ROI derived from the systole (Fig. 3).

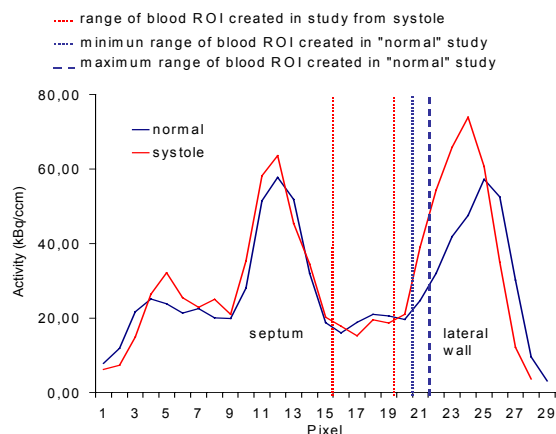


Fig. 2. The profile through the left ventricle in a selected slice image shows the activity distribution in the "normal" study and in the study of the systole.

Heart wall movement causes loss in image resolution due to blurring. Analysis of selected heart phases provide more detailed information on viability of the heart wall and may help to detect smaller lesions as it would be possible

using “normal” evaluation (Fig. 4). The improved image resolution of selected phases of the heart cycle is visible. The evaluation of smaller lesions requires further investigations with recovery corrected images [3].

The different position and thickness of the heart wall between diastole and systole is represented also in the profile of the Patlak images (Fig. 3). The value of metabolic rate depends on the movement of the heart wall. The thickness of the septum does remain almost unchanged for all study types. The width of the lateral wall in the diastolic study is about 20 % smaller than in the “normal” study. The profile of the dynamic study with heart in systolic phase shows higher values for the metabolic rate compared to the „normal“ study, with an about 20 % higher peak value in the septum and up to 50 % higher peak value in the lateral wall. The metabolic rate values of the diastolic study are lower than the values of the normal study.

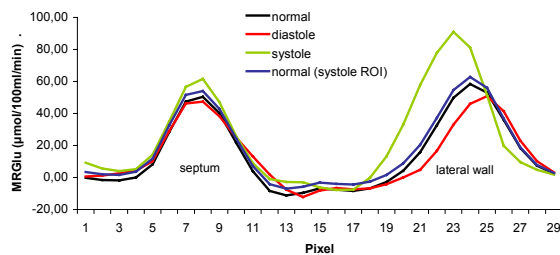


Fig. 3. Profile of the metabolic rate through the left ventricle in a selected Patlak image slice. The results for studies of the systole, the diastole and the “normal” study with different blood ROI’s (“normal” and systolic) are shown.

In a heart cycle the duration of the diastole is longer than the systole. The difference between the study of the diastole and the normal study (which represents an average of the whole heart cycle) is smaller. The peak value compared to the normal study is about 5 % lower at the septum and about 15 % lower at the lateral wall.

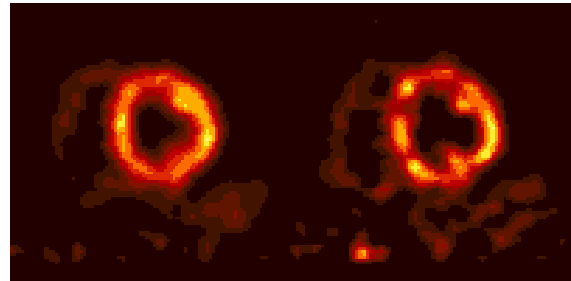


Fig. 4. The image shows corresponding slices of parametric images between apex and base of a human heart. Left not gated, right the diastolic heart phase of a gated study.

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Sorting Software for Listmode Acquisition Data in PET

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Software for sorting listmode acquisition data in positron emission tomography (PET) was developed. It allows repeated evaluation of the measured data and the specification of flexible sorting criteria. The combination of sorting criteria for dynamic and gated studies offers new opportunities for evaluation of the measured data.

Introduction

In conventional acquisition procedures in positron emission tomography (PET) the measured coincidence events are processed in sinogram files immediately on acquisition. Repeated evaluation of the measured data according to various sorting criteria, e.g. dynamic or gated acquisition, is not possible. In such cases new measurements are necessary. Listmode acquisition provides more flexibility since the individual events are sequentially saved in listmode data files which include time markers and gating signals. The measured data can be repeatedly sorted according to different criteria.

Methods

A software package was developed to sort listmode data measured with an ECAT EXACT HR+ scanner (CTI/Siemens) into ECAT7 sinogram files. Further reconstruction and evaluation is possible with the ECAT software. The software is usable for acquisition in the 2D or 3D mode.

The sorting process is controlled by command line parameters to allow batch mode processing. Depending on the selected program options, the sorting parameters are read from files. For evaluation of dynamic studies, frames are freely set by specification of their starting time and width. For easier use the software provides a set of frame numbers and widths. The specified time windows are saved as

frames in the sinogram file. Sorting is also possible according to sections of periodically repeated processes (e.g. heartbeats), which are saved in the sinogram output file as gates. The necessary time information is obtained from external sources and sent as a trigger signal to the PET scanner. For example, an ECG device sends one trigger signal per heartbeat at the time of the R-peak. The point in time of the trigger signal is included in the listmode data stream. The setting options for gates are equivalent to the dynamic frames. The time window specifications can be additionally chosen as absolute values or as percentages of the cycle length. Sorting criteria for dynamic measurements and for sections of periodically repeated processes can be combined. An interactive graphical user interface was developed to simplify operation. Desired sets of sorting parameters can be saved for reuse in the graphical user interface or with the command line program.

Results

Listmode acquisition allows evaluation of dynamic heart studies (e.g. glucose metabolism) at freely specified time windows of the heart cycle. Identification of paradox movement of the myocardium should also be possible. The resolution of images of individual heart phases is substantially improved as a result of the decreased movement of the heart wall.

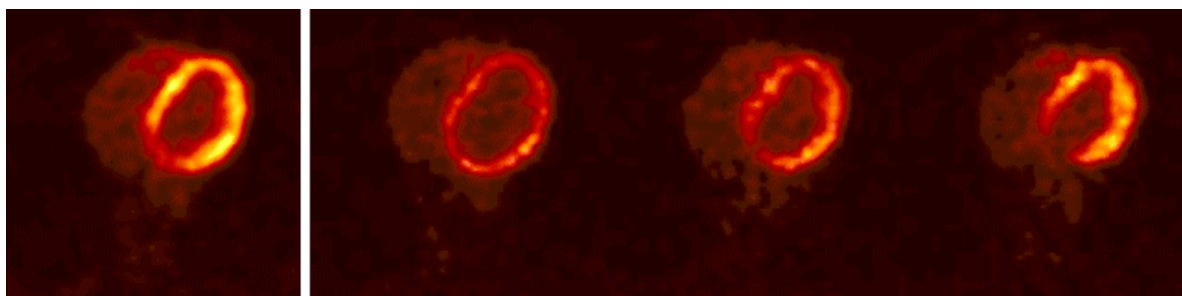


Fig. 1. [¹⁸F]FDG PET images of a heart acquisition: the left slice image resulted from a normal dynamic PET study. The three slices on the right are the result of an identical dynamic study, additionally sorted according to selected phases of the heart cycle. The heart is shown at the same acquisition time but in various phases of the heart cycle; wall thickening is clearly demonstrated.

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AWARDS

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DIPLOMA

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Verhaltens- und neurochemische Veränderungen durch SSRI Behandlung im Modell der sozialen Isolation von Mäusen unter besonderer Berücksichtigung von 5HT Rezeptorveränderungen durch Isolation in Abhängigkeit von der Isolationsdauer.

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Hochschule Zittau-Görlitz (FH), University of applied Studies, 14.12.2001.

III. SCIENTIFIC COOPERATION

COOPERATIVE RELATIONS AND JOINT PROJECTS

In multidisciplinary research such as carried out by this Institute, collaboration, the sharing of advanced equipment and, above all, exchanges of ideas and information play an important role. Effective collaboration has been established with colleagues at universities, in research centres and hospitals.

The *Technische Universität Dresden* has been a major partner in our cooperative relations. Cooperation with various groups in the Department of Chemistry and the Faculty of Medicine was again significantly extended last year. Common objects of radiopharmacological and medical research link the Institute with the Dresden University Hospital, above all with its Department of Nuclear Medicine (Prof. Franke). A joint team of staff members from both the Institute and the Clinic of Nuclear Medicine are currently working at the Rossendorf PET Centre. The Institute of Analytical Chemistry (Prof. Salzer) plays an important part in tumour research. A new area of application of the positron emission tomography modality has been inaugurated in collaboration with the Institute of Food Chemistry (Prof. Henle). Bioinorganic research activities are closely linked with the Department of Coordination Chemistry (Prof. Gloe).

Special thanks go to *Schering AG Berlin* (Dr. Dinckelborg) for the long-standing valuable collaboration in radiotracer development. Recently, cooperation with the pharmaceutical industry and regional enterprises has been intensified and extended. Joint projects exist with *Bayer AG*, *ABX advances biochemical compounds*, *ROTOP Pharmaka GmbH* and *Wälischmiller GmbH*.

Very effective cooperation exists with the *Federal Material Research Institute in Berlin* (Mr. Leibnitz, Dr. Reck, Mr. Kraus), whose staff members carried out X-ray crystal structure analysis of new technetium and rhenium complexes.

Our Institute is linked with the Institute of Pathology (Prof. Zwiener, Dr. Bauer) of *Jena's Friedrich-Schiller University* by long-standing fruitful cooperation on the pathophysiological aspects of brain functions.

Cooperation in Technetium chemistry exists with the *University of Ferrara* (Prof. Duatti, Dr. Bolzati), *CNR Padova* (Dr. Tisato, Dr. Refosco), and, more recently, with the *Instituto Tecnológico e Nuclear (ITN)*, Lisbon (Dr. Santos), as well as with the *University of Kitakyushu*, Japan (Prof. Yoshizuka). The Institute works with the *Humboldt University Berlin*, Charité Hospital (Dr. Fischer), on radioimmuno-scintigraphy and therapy.

In the field of supramolecular chemistry, successful cooperation exists with the Kekulé Institute of Organic Chemistry and Biochemistry (Prof. Vögtle) of the *University of Bonn*.

Cooperation in PET tracer chemistry and radiopharmacology has been established with the *Turku Medical PET Centre* (Dr. Solin).

The identification of common objects in PET radiopharmacy has led to collaborative research with the *Institut für Interdisziplinäre Isotopenforschung Leipzig* (Prof. Steinbach). Both institutes constitute an alliance of research in radiopharmaceutical sciences.

Effective cooperation also exists with the *Riga Institute of Organic Chemistry* (Dr. Zablotskaya), Latvia.

LABORATORY VISITS

A. Drews
Karolinska Institute Stockholm; Sveden
January 28 - February 4, 2001

T. Knieß
Instituto Tecnológico e Nuclear (ITN) Sacavem, Portugal
March 12 - 24, 2001

M. Grote
ETH Zürich
June 10 - July 7, 2001

H. Spies
Russian Academy of Sciences, Novosibirsk, GUS
June 16 - 23, 2001

H. Stephan
Oak Ridge National Laboratory (ORNL), Oak Ridge, USA
August 31 – September 4, 2001

H. Spies
Instituto Tecnológico e Nuclear (ITN) Sacavem, Portugal
October 11 - 16, 2001

J.-U. Künstler
ESFR Grenoble, France
November 6 - 13, 2001

S. Seifert
ESFR Grenoble, France
November 6 - 11, 2001

S. Seifert
Instituto Tecnológico e Nuclear (ITN) Sacavem, Portugal
November 22 - 28, 2001

GUESTS

Dr. A. Zablotskaya
Latvian Institute of Organic Synthesis Riga, Latvia
January 15 - February 6, 2001

Dr. C. Dittmar
Max-Planck-Institut Köln
May 17 - 18, 2001

Dr. T. Pajpanova
Institute of Molecular Biology Sofia, Bulgaria
May 22 - July 21, 2001

C. Fernandes
ITN Sacavem, Portugal
August 6 - September 1, 2001

Prof. E. Golovinsky
Institute of Molecular Biology Sofia, Bulgaria
August 22 - September 3, 2001

Prof. K. Yoshizuka
University Kitakyushu, Japan
October 2 - 13, 2001

Dr. I. Vevere
Latvian Oncology Centre Riga, Latvia
October 18 - 19, 2001

P. Sergejs
Latvian Oncology Centre Riga, Latvia
October 18 - 19, 2001

Y. Hu
China Institute of Atomic Energy Beijing, China
October 27, 2001 - January 26, 2002

Dr. E. Gniazdowska
Institute of Nuclear Chemistry and Technology Warsaw, Poland
November 5 - December 14, 2001

MEETINGS ORGANIZED

Workshop "Development of novel peptide based radiopharmaceuticals for in vivo receptor associated tumor diagnosis and therapy"
Dresden-Pillnitz, February 22-24, 2001.

2nd Rhenium-188 Meeting
Rossendorf, October 5, 2001.

TEACHING ACTIVITIES

B. Johannsen
One-term course on Metals in Biosystems
(Introduction into bioinorganic chemistry).

Contributions to the postgradual course (Nachdiplomkurs) on Radiopharmaceutical Chemistry/Radiopharmacy of the ETH Zurich, Switzerland.

Graduiertenkolleg
"Bioinorganic and Radiopharmaceutical Chemistry"
Rossendorf, October 1 – 2, 2001.

OTHER ACTIVITIES

B. Johannsen
Chairman of the DGN Working Group on Radiochemistry and Radiopharmacy.

B. Johannsen
Co-editor of the Journal "Nuclear Medicine and Biology".

IV. SEMINARS

TALKS OF VISITORS

Dr. H.-J. Wester, TU München
Pharmakologische Konzepte zur Modifikation bioaktiver Peptide
09.02.2001

Prof. K. Neubert, Martin-Luther-Universität Halle
Struktur-Wirkungs-Untersuchungen an biologisch aktiven Peptiden und Enzymeffektoren - eigene Forschungsergebnisse
01.03.2001

Prof. Theuring, Charité Berlin
Funktionelle Analyse des 5-HT_{1A}-Rezeptors in transgenen Tieren
09.03.2001

Zentrumskolloquium
Dr. J. Teller, Micromod Rostock
Synthese und Charakterisierung funktioneller Nano- und Mikropartikel
15.03.2001

Priv.-Doz. Dr. H.-J. Holdt, Universität Potsdam
Koordinationschemie acyclischer und makrocyclischer Derivate des "Bährschen" und des "Steimeckeschen" Dithiolates
24.04.2001

Kolloquium des PET-Zentrums
Dr. S. Krüger, Universitätsklinikum Carl Gustav Carus Dresden
Veränderungen des zerebralen Blutflusses bei bipolaren depressiven Patienten nach Stimmungsin-
duktion gemessen mit PET
31.05.2001

Dr. A. Jacobs, Max-Planck-Institut für Neurologische Forschung Köln
Molekulares Imaging: Von den Grundlagen zur klinischen Anwendung
13.07.2001

Prof. L. F. Lindoy, School of Chemistry, University of Sydney
New supramolecules with copper and other transition metal ions.
13.08.2001

Dr. L. Kronrad, Dr. Viklický, UJV Prag
Possibilities of development and production of radiopharmaceuticals based on monoclonal antibodies
and peptides in Czech Republic.
06.09.2001

Prof. A. Buschauer, Universität Regensburg
Neuropeptid Y-Rezeptorliganden: Design, Synthese und Pharmakologie von Y1-Antagonisten
26.10.2001

Dr. C. Decristoforo, Universität Innsbruck
^{99m}Tc-HYNIC-Octreotid: Von der präklinischen Entwicklung zur Anwendung am Patienten
02.11.2001

Dr. U. Rothe, Martin-Luther-Universität Halle
Selektive als Target für eine antiinflammatorische Therapie
09.11.2001

Zentrumskolloquium
Prof. H. Wiesmeth, BIOTEC TU Dresden
Aufbau der Biotechnologie in der Region Dresden
13.11.2001

Zentrumskolloquium
Prof. F. Vögtle, Universität Bonn, Kekulé-Institut für Organische Chemie und Biochemie
Dendrimere - Fraktale Moleküle, ihre supramolekulare Chemie und Anwendungsmöglichkeiten
29.11.2001

INTERNAL SEMINARS

Preusche S.
Betriebserfahrung mit der neuen Steuerungssoftware des CYCLONE 18/9
07.03.2001

Pawelke B.
Methodenentwicklung zur Synthese von C₁-markierter Benzoesäure und o-Tolylsäure: Fortschritte und Probleme
21.03.2001

Wardwilai C.
¹⁸F-markierte Aminosäuren
28.03.2001

Heichert C.
¹⁸F-Markierung von Neurotensinderivaten: Fortschritte und Probleme
04.04.2001

Jordanova A.
Herstellung und Reaktivität von [N-¹⁸F](DF₃-SO₂)₂NF - [N-¹⁸F]DesMarteau Reagenz
25.04.2001

Smolinka K.
Etablieren einer HPLC-Methode am Beispiel des [¹⁸F]FES: Validierung für die Qualitätskontrolle
02.05.2001

Mäding P.
Stand des CCR-1-Projektes
16.05.2001

Füchtner F.
OMID - Eine SPECT-Alternative zum OMFID?
30.05.2001

Zessin J.
Aktueller Stand und Fortschritte zu Derivaten des McN-5652
06.06.2001

FIRST ALUMNI MEETING

Approaching ten years of existence of the Forschungszentrum Rossendorf and hence of the Institute, it was considered appropriate to initiate regular alumni meetings to keep and intensify the contact and to provide in a pleasant and relaxed atmosphere a platform for exchange of ideas and experience, and in particular encounter of former and present PhD students.

The first alumni meeting was held at Wolfsberg/Sächsische Schweiz over the weekend of 30th November-2nd December 2001.

Alumni reported on their postdoc activities and careers. Topics under discussions involved:

- risk and risk perception
- radiopharmaceutical chemistry for positron emission tomography
- radiopharmaceutical research in the drug industry
- biotechnology enterprises
- testing of software
- hospital radiopharmacy



V. ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS FOR FINANCIAL SUPPORT

The Institute is part of the Research Center Rossendorf Inc., which is financed by the Federal Republic of Germany and the Free State of Saxony on a fifty-fifty basis.

Two projects were supported by Commission of the European Communities:

Peptide radiopharmaceuticals in oncology

BIOMED II

in collaboration with Belgium, Greece and Switzerland
(PL 963198-SC), 04/1998 – 03/2001.

Radiotracers for *in vivo* assessment of biological function

COST B12

in collaboration with Sweden, Italy and Switzerland
02/1999 – 02/2004.

Three research projects concerning technetium tracer design, biochemistry and PET radiochemistry were supported by the Deutsche Forschungsgemeinschaft (DFG):

Tc labelled fatty acids for myocardium diagnosis

Sp 401/6-1 (H. Spies), 06/1999 – 05/2001.

Redox transport system for ^{99m}Tc radiopharmaceuticals

Sp 401/5-1 (H. Spies), 06/1999 – 06/2001.

¹⁸F labelled substrates of bacterial cytosindeaminase for monitoring gene expression of cancer cells

NO 418/1-1 (B. Noll), 06/2001 - 05/2003

The Sächsisches Staatsministerium für Wissenschaft und Kunst provided support for the following project:

Development and characterization of nanoscale metal-based drugs targeting tumors

SMWK-No. 4-7531.50-03-0370-01/4, 06/2001 – 12/2003.

The Bundesministerium für Bildung und Forschung provided support for the following project:

Laboratory for innovation and start-ups

PTJ/03GL0024, 08/2001 - 12/2001

Three projects were supported by cooperations with the pharmaceutical industry:

Cooperation in nuclear diagnostic

Schering AG Berlin

07/1996 – 06/2002.

Cooperation in drug targeting

BASF Ludwigshafen

03/2000 - 02/2001

Cooperation in functional diagnostics

ABX advanced biomedical compounds GmbH Dresden

04/2001 - 03/2003

Bilateral cooperation with Latvia:

Silyl group-functionalized Tc complexes
WTZ, LET-008-97, 07/1997 – 12/2001

Silyl group-functionalized Re complexes
NATO, 05/2000-04/2002

VI. PERSONNEL

Director

Prof. Dr. B. Johannsen

Administrative Staff

L. Kowe

G. Neubert

M. Kersten

Scientific Staff

Dr. Bergmann, R.
Dr. Brust, P.
Dr. Füchtner, F.
Dr. Heichert, C.*
Dr. Heibold, I.*
Kretzschmar, M.
Mäding, P.

Dr. Müller, M.*
Dr. Noll, B.
Dr. Noll, St.
Dr. Pietzsch, H.-J.
Preusche, S.
Dr. Seifert, S.

Dr. Smolinka, K.*
Dr. Spies, H.
Dr. Stephan, H.*
Dr. Syhre, R.
Dr. Will, E.
Dr. Wüst, F.

Technical Staff

Beyer, B.*
Dohn, N.
Gläser, H.
Görner, H.
Große, B.
Hentges, A.*
Herrlich, R.
Herzog, W.*

Kasper, H.
Kolbe, U.
Krauß, E.
Krauß, T.*
Kreisl, B.
Kunadt, E.
Landrock, K.

Lehnert, S.
Lenkeit, U.
Lipps, B.*
Lücke, R.
Roß, H.
Sterzing, R.
Suhr, A.

Post Docs

Hinz, R.*

Dr. Pawelke, B.*

Dr. Zessin, J.*

* term contract

PhD Students

Drews, A.
Grote, M.
Habala, L.

Jordanova, A.
Jung, Ch.
Just, U.

Künstler, J.-U.
Rodig, H.
Wardwilai, C.

Former Personnel

(who left during the period covered by the report)

Scientific Staff:

Dr. Steinbach, J.

Dr. Knieß, T.

Technical staff:

Fischer, K.

PhD Students:

Friedrich, A.

Post Docs:

Dr. Wittrisch, H.