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Contrast Agents III

Radiopharmaceuticals – From Diagnostics to Therapeutics

Springer

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Radiopharmaceuticals
From Diagnostics to Therapeutics

Volume Editor: Werner Krause

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Preface

Radiopharmaceutical chemistry is currently in a transition state. It has come a long way from using radioisotopes as they are, e.g. iodine isotopes, via the synthesis of organometallic complexes to molecular imaging with the administration of highly complicated radiolabelled biomolecules and to very recent applications of targeted therapeutic radiopharmaceuticals, the "magic bullets" of Ehrlich, labelled with radioisotopes.

The process of transition can also be derived from the still-valid definition of radiopharmaceuticals as "radioactive drugs that, when used for the purpose of diagnosis or therapy, typically do not elicit any physiological response from the patient". Apart from being a contradiction in itself, since any therapy should result in a physiological response, otherwise we would be using a placebo, experience with the newly introduced radiopharmaceutical therapeutics, Zevalin and Bexxar, radiolabelled monoclonal antibodies for the treatment of non-Hodgkin lymphoma, has shown that these drugs are indeed capable of eliciting significant beneficial, therapeutic effects but also result in physiological responses experienced by the patient as adverse reactions. Both, the beneficial and the undesired effects are expressions of pharmacological activity, an aspect that is new in the field of radiopharmaceuticals.

This volume on radiopharmaceutical contrast agents, which also covers new developments on radiopharmaceuticals used as therapeutic drugs is the third of a series on diagnostics following a volume that was exclusively dedicated to magnetic resonance imaging and a "mixed" volume on optical, ultrasound, X-ray and radiopharmaceutical agents.

The transition from diagnosis to therapy is also reflected in the contributions to this volume, where the first chapters are dedicated exclusively to diagnosis and the chapters at the end of the book cover both aspects, dealing with compounds for diagnostic imaging and with drugs used for therapy.

The first chapter of this volume is dedicated to bioorganometallic chemistry with special focus on diagnostic imaging using technetium complexes, particularly Tc-CO complexes. The state of the art in this field is described in great detail covering the basics of Tc complexes and their synthesis and characterization, new Tc compounds, and requirements for practical applications. The last part of this chapter deals with labelling of small molecules for the use in CNS imaging and with labelled biomacromolecules for cancer applications. In the second chapter, complexes of technetium and rhenium are reviewed with special emphasis on heterofunctional phosphines and borates (scorpionates). Synthetic procedures, results on reactivity, and structural characterisation are supplemented with data on the labelling of biomolecules.

Nitrido complexes of technetium and rhenium are covered in the third chapter. An extensive discussion of electronic configurations and of the geometry of these complexes and their spatial requirements at the molecular level is provided. Applications in molecular imaging include cardiac investigations and studies in degenerative diseases.

Chapter 4 addresses Tc labelling of small biomolecules using bifunctional coupling agents, in particular 6-hydrazinonicotinamide (HYNIC). Activities relevant for successful labeling are discussed, starting from the conjugation procedure, the protection of the hydrazine moiety, via development of a suitable formulation to examples for the diagnosis of various diseases. Additionally, new techniques for the characterization of technetium-labelled drugs at the tracer level are discussed.

In the fifth chapter, one of the major problems in cancer therapy, the development of multi-drug resistance (MDR) is addressed. In MDR, cancer patients who have responded to their therapy, develop resistance against this treatment making further administration useless. Unfortunately, this phenomenon is not restricted to individual drugs but, instead, resistance is developed across different classes of drugs without the patient ever having been treated with them. As a consequence, not only is treatment with the drug against which MDR has initially developed futile but also with a complete array of other anti-cancer drugs. Analysis whether or not MDR plays a role in the patient's reaction to treatment is an important piece of information for the physician. Technetium complexes useful for PET or SPECT analysis of this phenomenon are extensively discussed in this chapter.

Chapter 6 deals with the radiolabelling of somatostatin analogs for both diagnosis and therapy of cancer. Somatostatin is a small peptide of 14 amino acids regulating the release of certain hormones such as growth hormone, TSH or prolactin. Somatostatin receptors are expressed in several organ systems. In some tumor types, the receptor is overexpressed and may be used for diagnostic and therapeutic purposes. The review describes the production of radionuclides suitable for labelling somatostatin analogs and their radiochemistry and gives details on preclinical and clinical studies with these compounds.

The last chapter is exclusively dedicated to radiotherapy. It describes adhesion molecules out of the integrin family labelled with yttrium-90 via a chelator. Integrins are proteins that are expressed on epithelial and endothelial cells facilitating cellular adhesion on membranes. Some tumor types show high expression of integrin $\alpha_v\beta_3$ making this protein a useful target for the detection and therapy of these tumors. The chapter focusses on radiopharmaceutical design, selection of the radionuclide and the bifunctional chelator and on the pharmacokinetics of yttrium-labelled integrin $\alpha_v\beta_3$ radiopharmaceuticals.

The current transition state clearly provides chances for new directions in this exciting field of research. The future will show whether and when a new volume on this topic is needed.

Montville, October 2004 Werner Krause

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New Organometallic Technetium Complexes for Radiopharmaceutical Imaging

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Abstract It is the objective of this review to introduce the reader to the current status of organometallic chemistry, oriented towards the development of novel ^{99m}Tc-based imaging agents. This type of inorganic chemistry has nowadays become part of so-called bioorganometallic chemistry.

The article describes the current state of research and development of radiopharmaceutical imaging agents based on organometallic moieties and complexes. Organometallics are, in general, not considered to play an important role in inorganic medicinal chemistry. An introductory section describes the requirements for practical application of a radiopharmaceutical to underline in the second part that currently available compounds might well be the basis for the development of completely new imaging agents. The introduction gives also a short outline of basic organometallic technetium compounds as well as organometallic aqua ions from other elements which are currently under investigation in the new field of bioorganometallic chemistry.

In the following section, major focus is put on technetium–CO complexes. The compound $[^{99m}Tc(OH_2)_3(CO)_3]^+$ is the prototype of a precursor, the special behaviour of

which is based on its organometallic character. The synthesis and some fundamental properties are discussed. Within this section substitution reactions of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ are presented according to the denticity of the ligands. The reactions are critically highlighted in respect of their versatility for the development of novel radiopharmaceuticals. Combining monodentate and bidentate ligands leads to the potential of a new [2+1] mixed-ligand concept.

In the last section, the labelling of biomolecules with organometallic moieties is described. Since most of the organometallic complexes are still being researched this section focuses on concepts, and some of the most important developments towards new CNS receptor ligands and labelled peptides and proteins are given. This section aims at motivating researchers from radiopharmaceutical chemistry to experience new approaches which are probably superior to the currently available ones. Throughout the text, examples from other organometallic compounds are described; however, since the carbonyl concept is the most advanced, the review is oriented towards the chemistry of $[^{99m}Tc(OH_2)_3(CO)_3]^+$.

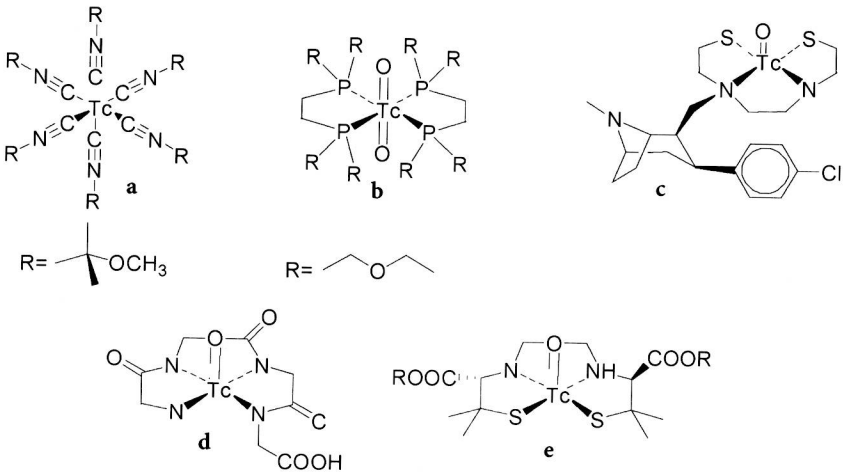
Keywords Technetium · Rhenium · Bioorganometallics · Carbonyl · Radiopharmaceuticals · Labelling

1 Introduction

The artificial element technetium was discovered by Perrier and Segré in 1937 [1]. In those days, nobody suspected that this element would become an essential part of medical health care some 40–50 years later. At that time, X-rays, which had been discovered by Röntgen some 40 years earlier, were the most important tool for diagnosis by imaging. The application of radionuclides had already been introduced by deHevesey but this was more a toy than a serious instrument. In fact patients usually suffered severely from the radiation of administered radionuclides such as radium or thorium and, not surprisingly, a number of them even died as a consequence of the treatment. Nowadays, this situation has changed completely. With growing knowledge about the interaction of ionizing radiation with tissues the properties of a radionuclide to be useful for and not dangerous for a patient became more defined. In parallel, the development of sensitive cameras for the detection of single photons increased. A selection of radionuclides whose decomposition characteristics would allow high-resolution images in single-photon-emission computer tomography without the burden of a high dose to patients became possible. Among these few versatile radionuclides, ^{99m}Tc is probably the most important one and a large portion of nuclear medicine diagnostics is carried out with it. The main reasons for the versatility of ^{99m}Tc are not only its decay characteristics but also its price and availability. Costs of public health care are steadily increasing and play a major role in the selection of a particular diagnostic procedure. The cost for a diagnostic amount of ^{99m}Tc is very low compared with that for other radionuclides, which might even have better decay characteristics. Another important feature of ^{99m}Tc is its availability. In the 1960s researchers at Brook-

haven National Laboratories developed a generator in which ^{99}Mo, the mother of ^{99m}Tc, is loaded on an alumina column [2–4]. The radionuclide ^{99}Mo decays with a 67-h half life time to ^{99m}Tc, which can be continuously eluted with saline from the generator. Mother and daughter are in a so-called secular equilibrium. Brookhaven National Laboratories did not patent the generator, anticipating that the system would not have any application in the future. Considering the number of generators sold so far, it has become obvious that a large amount of money did not reach the inventors owing to this wrong decision.

Price and availability are the favourable features that help to keep the clinical importance of ^{99m}Tc alive. The disadvantages arise from the chemical side owing to the artificial nature of technetium and the difficulty of combining a transition metal with targeting biomolecules. From a chemical point of view, other radionuclides are more versatile, in particular the positron emitters ^{11}C, ^{18}F and ^{123}I, which are most important in positron emission tomography and are strongly competing with single-photon-emission computer tomography despite other disadvantages not discussed here. Whereas a number of so-called technetium-essential radiopharmaceuticals have been successfully introduced onto the market, labelled vectors such as peptides or brain receptor imaging agents are still subjects of research and, with a very few exceptions, in phase I clinical trials only. The radiopharmaceutical chemistry of technetium-based coordination compounds has been reviewed without considering organometallic compounds systematically [5–9]. A selection of perfusion agents introduced onto the market and some ^{99m}Tc-labelled targeting vectors in phase I clinical trials are given in Scheme 1.



Scheme 1 Important representatives of commercially available ^{99m}Tc-based radiopharmaceuticals and advanced research compounds: **a** Cardiolite, **b** Myoview, **c** TRODAT-1, **d** Tc-MAG3 Technescan, **e** Tc-ECD Neurolite

Labelling of targeting vectors is the main object of modern radiopharmaceutical research. Inorganic chemistry is challenged to find convenient ways to introduce physiologically stable ^{99m}Tc cores into biomolecules without affecting their bioactivity. The labelling of biomolecules must inherently be possible with the facilities of hospitals and the skills of their personnel. Beyond these very fundamental requirements, targeting radiolabelled biomolecules accumulate, in general, in nontargeted organs such as kidney or liver; hence, getting clear images from whole body scans is spoiled. Since the biodistribution of an unlabelled biomolecule is not sufficiently well known, quantification of organ uptake becomes obvious only after labelling the biomolecule.

Besides the essential requirements pointed out before, it now becomes the task of the radiopharmaceutical chemist to change the nature of the tagged complex in a way that accumulation in nontargeted organs is reduced and in targeted organs enhanced. Hence, the attached ^{99m}Tc complex has to influence the biological behaviour of the native biomolecule in a positive direction. From a pharmaceutical point of view, such a procedure is called "compound finding" and is comparable to what is performed with nonradioactive organic compounds to enhance, for example, the therapeutic index whilst reducing side effects, and allow the administration of higher amounts of the drug. Consequently, compound finding in radiopharmacy means altering the structure of a metal complex to achieve a better in vivo distribution. This is a new situation and demands a flexible metal core with respect to the chelators forming stable complexes under physiological conditions.

Radiopharmaceutical chemistry is a topic in life science and the related coordination chemistry must be aqueous chemistry. Accordingly, the main focus related to the labelling of biomolecules with ^{99m}Tc is classical "Werner-type" chemistry. Coordination chemistry of technetium has recently been the focus of several reviews [10–16]. As is obvious from Scheme 1 the vast majority of complexes comprises the "$[Tc{=}O]^{3+}$" core in which technetium is in the oxidation state +V. Although a hypothetical aqua ion of $[Tc{=}O]^{3+}$ does not exist, it can be stabilized with weak mulitdentate ligands present in large excess. They can then be replaced via ligand exchange to give stronger multidentate chelators. Another core that merits attention is "$[Tc{\equiv}N]^{2+}$", also with technetium in oxidation state +V and similar chemical behaviour in respect of aquo ions as in $[Tc{=}O]^{3+}$ [17–20]. Besides these two central moieties, the mixed-ligand [3+1] concept based on the $[Tc{=}O]^{3+}$ core is widely applied in research [21, 22]. The approach is problematic for certain biomolecules since in vivo instability of complexes has been observed.

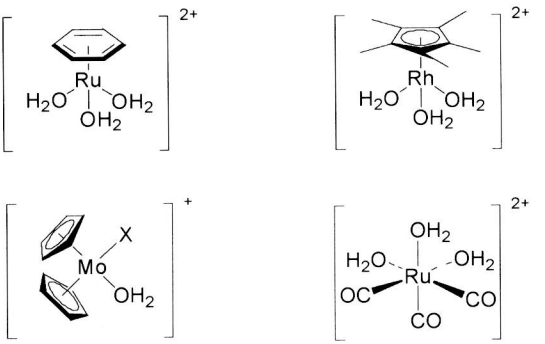
2
Organometallic Complexes in Life Science

At a first glance it seems that the special properties of *M*–C bonds are not involved in biological systems. The well-known coenzyme B12, in which a cobalt-

carbon (to adenosyl or methyl) is present, seems rather to be the exception that confirms the rule. In the recent past however, more and more metalloenzymes that contain *M*–CO fragments have been structurally characterized. These enzymes represent a classical sort of organometallic compound [23–27]. It is probably not just by chance that CO is applied as a coligand in biology and it can be anticipated that more organometallic enzymes will be discovered in the near future.

Owing to the pure aqueous nature of radiopharmaceutical chemistry, organometallic compounds are rare in this field and in life science, in general. It is even a fact that nonradioactive drugs in inorganic medicinal chemistry rarely contain a *M*–C bond. Recent research on cytotoxic agents with $[(C_6H_6)Ru(OH_2)_3]^{2+}$ represents a valuable research extension in the organometallic direction [28–32]. Actually, to our knowledge there is only one compound containing *M*–C bonds which has successfully been introduced onto the (radio)pharmaceutical market. This concerns Cardiolite, a compound which will be discussed later and is shown in Scheme 1. On the other hand, the rapidly growing research field of "bioorganometallic chemistry" is focused on the application of organometallic compounds in life science [33]. Bioorganometallic chemistry will not be discussed here but it merits emphasis that organometallic compounds such as ferrocene, $[Cp^*Rh(OH_2)_3]^{2+}$, $[(C_6H_6)Ru(OH_2)_3]^{2+}$, $[(Cp^*)_2MoX_2]$, where Cp^* is pentamethylcyclopentadienyl, and others proved to be superior to coordination compounds (Scheme 2). Many water- and air-stable organometallic compounds have been described in detail and in respect of their biological versatility. Still, the use of organometallics in life science remains a niche although, for example, ferrocifene and some cytotoxic ruthenium-based agents have progressed to phase I clinical trials.

In radiopharmaceutical chemistry, the only exception is the Tc(I) isonitrile complex $[^{99m}Tc(CN\text{–}R)_6]^+$ (*R* is methoxy isobutyl isonitrile) which has found its way to routine clinical application for myocardial imaging [34, 35]. This organometallic complex is probably the most successful radiopharmaceutical



Scheme 2 Examples of organometallic aqua ions that are currently under investigation for application in life science

ever produced. The complex merits special attention not only owing to its radiopharmaceutical application but, probably more importantly, owing to its surprising preparation and unexpected properties. Originally, the complex was formed in quantitative yield within a short time after heating and in the presence of the isonitrile ligand and dithionite as a reducing agent. It is remarkable that a low-valent organometallic compound is thermodynamically favoured under such conditions and that the complex is completely stable over the pH range and at high temperature. It is a drawback of the stability that the monodentate ligands can hardly be substituted by other ligands and, thus, the complex can be taken as a starting material for further complexes. The synthesis matches with the conditions as required from routine application and in vitro and in vivo stabilities are excellent. The stability must be of kinetic origin and this allows application of a compound that contains exclusively single bonds. This structural feature would not be expected with classical coordination compounds of higher valences.

The preparation of low-valent complexes such as $[^{99m}Tc(CN{-}R)_6]^+$ from water might still initiate research in the field of organometallic compounds for life science. The paradigm that organometallic compounds are not compatible with conditions imposed by biological systems could clearly be repudiated with this compound.

2.1 Particular Properties of Organometallic Compounds

Organometallic complexes applied in life science must be stable in water and air. Additionally, in radiopharmaceutical chemistry they have to be prepared directly from $[^{99m}TcO_4]^-$ in a one-pot synthesis from aqueous saline. Low-valent organometallic complexes are, in general, closed shell and obey the 18-electron rule as, for example, in $[^{99m}Tc(CN{-}R)_6]^+$ as mentioned before. These two important features essentially govern all complexes under investigation in life science, they have 18 electrons and have d^3 or d^6 electronic configuration. Often, the organometallic ligands are not involved in pH equilibria. The electronic configuration is responsible for kinetic stability towards full or partial ligand replacement. The *M*–C bonds are reasonably stable and prevent dissociative substitution and the lowest unoccupied molecular orbital is too high in energy to enable an interchange or an associative reaction pathway. This makes, for example, d^6 organometallic complexes robust if the ligand bond energies are sufficiently high, which is the case for CO or CN–*R*, for example. In contrast to Werner-type ligands, organometallic ligands are not prone to protonation and the corresponding complexes are stable over a broad pH-range. Werner-type ligands, as applied for the Tc(V) cores, are σ and/or π donors which can be protonated and are pH-sensitive. The combination of donors is limited since, for example, weak donors such as thioethers have to be combined with a set of electronically "correct" ligands in order to achieve thermodynamic stability. As a drawback of the robust nature of organometallic complexes, they are more