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Radiolabeling of magnetic particles with rhenium-188 for cancer therapy

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Abstract

A one-step radiolabeling procedure of highly magnetic particles with the therapeutic β-emitter rhenium-188 (^{188}Re) has been developed. Magnetic targeted carriers (MTCs) are composites of metallic iron and activated carbon that can be labeled in the presence of the reducing agent $SnCl_2$. As MTCs are effectively targeted to solid tumors, the ^{188}Re-MTC complex has the potential to deliver therapeutically relevant doses of radiation to tumors while minimizing radiation exposure to surrounding tissues or organs.

Keywords: Magnetic microsphere; MTC; Magnetically targeted carriers; Carbon; Iron; Rhenium-188 (^{188}Re); Radiolabeling; Brachytherapy; Technetium-99m (^{99m}Tc)

1. Introduction

Chemotherapy is the single most common method for cancer treatment. Although chemotherapeutic drugs are very effective, their potency leads to many undesirable and even toxic side effects. Replacing the systemic delivery of chemotherapeutic drugs with regional cancer treatment approaches is one way of overcoming side effects [1]. Furthermore, the therapeutic efficiency can be improved since higher drug concentrations at the tumor sites are possible when using regional therapy. In the search for a more generally applicable drug delivery method for cancer therapy, magnetically controlled targeted chemotherapy has been proposed [2]. In this approach, a physical method of capturing magnetically responsive particles present in a patient's circulatory system is combined with the particles' ability to bind, transport and later release a chemotherapeutic drug. Tumor targeting of magnetic particles has been tested, to date, in only a few clinical trials [3–5].

Magnetic targeted carriers (MTCs) have been developed by FeRx Incorporated and are magnetic microparticles composed of metallic iron and activated carbons [6]. Using a small permanent magnet, externally positioned to create a localized magnetic field in the body, MTCs can be targeted to specific sites in the body following intra-arterial administration [7]. Because of their high magnetic susceptibility, MTCs are responsive to the physical force of the magnetic field and extravasate into the targeted area without any detectable physiologic damage to the arterial wall. Once localized, the MTCs do not redistribute and start releasing the adsorbed chemotherapeutic drug, resulting in

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a site-specific therapy. A product based on this technology and doxorubicin, a well-known chemotherapeutic drug, is currently under clinical investigation [5].

A chemotherapeutic drug has to reach and penetrate each tumor cell to be effective. Its therapeutic treatment range is dependent on diffusion, which is influenced by factors such as high tumor pressure, hypoxia and necrosis [8]. On the other hand, radiation therapy is only minimally affected by these tumor-physiological factors. In addition, a local radiation treatment might also be an effective mode for mono- or combination therapy of chemoresistant tumors. As previously described for chemotherapeutics, a therapeutic radiopharmaceutical given systemically would also cause serious side effects. Thus, the challenge is to irreversibly bind the radionuclide(s) to a drug delivery vehicle such as the MTCs so that the tumor could then be magnetically targeted with the radiolabeled MTCs resulting in effective localized radiation therapy with little whole body exposure. The β-emitting radionuclide ^{188}Re was chosen because its β^- radiation with a maximum energy of 2.12 MeV is effective in tumor therapy, it has an average penetration range of 2.6 mm and can destroy tumor cells up to a maximum range of 11 mm in tissue [9]. Beyond this range, β-electrons are completely attenuated and present no danger to adjacent organs and healthy tissue. In addition, ^{188}Re has a γ-line at 155 keV (15%). This energy is close to that of ^{99m}Tc and can thus be imaged easily with a γ-camera [10].

The aim was to find a method to radiolabel the MTCs with ^{188}Re in a stable fashion. Since charged species bind differently to carbon and iron, we tested both negatively and positively charged compounds and compared them to neutral radioactive species for their radiolabeling efficiencies and stabilities.

2. Materials and methods

MTC (FeRx Incorporated, San Diego, CA) are made using a high-energy milling process, range in diameter from 0.5 to 5 μm and contain very high amounts (up to 80%) of metallic iron, with the remaining weight consisting of activated carbon [6,7].

^{99m}Tc, about 2 mCi (74 MBq), eluted from a ^{99}Mo/^{99m}Tc-generator was used to label the MTCs. Technetium was used over rhenium because available radiopharmaceutical kits for the preparation of positively charged and neutral complexes are designed for use with the diagnostic radioisotope ^{99m}Tc. The metallorganic chemistry of technetium is similar to that of rhenium, although the latter often requires larger amounts of reducing agents, longer incubation times and higher temperatures to arrive at the final products [11,17]. It thus made sense to first evaluate a reaction with ^{99m}Tc and then adapt it for ^{188}Re-labeling. The neutral but more lipophilic Tc-exametazime complex (Ceretec from Nycomed/Amersham) and the positively charged Tc-sestamibi complex (Cardiolite from DuPont) (see Fig. 1) were prepared according

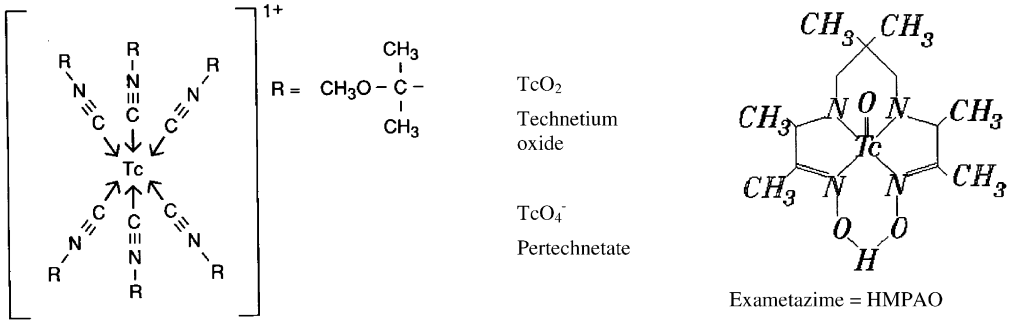

Fig. 1. Chemical structure of the investigated compounds for labeling with ^{99m}Tc and ^{188}Re.

to the kit insert. The labeling efficiency for ^{99m}Tc was $>90\%$, as tested using the thin layer chromatography methods described in the kit. Each of these two radiopharmaceuticals, as well as the negatively charged pertechnetate TcO_4^- were then added in 750 μl of phosphate buffered saline to 10 mg of MTC in a 2 ml polyethylene vial and shaken at 37°C for 30 min. As an additional control, 0.1 mg of $SnCl_2 \cdot 2H_2O$ was added to another portion of pertechnetate, since both radiopharmaceutical kits contain small amounts of this reducing agent. The stability was then evaluated in 3.0 ml of phosphate buffered saline (= PBS) at 37°C.

^{188}Re with a half-life of 17 h was eluted daily from a 500 mCi $^{188}W/^{188}$Re-generator (Oak Ridge National Laboratories, Oak Ridge, TN) [12] in the form of the highly water-soluble perrhenate, ReO_4^-, in 0.9% saline. The eluate from the generator was directly used without a concentration step. Typically, 20 mg of MTC were weighed into 1.9 ml screw cap polyethylene vials and up to 2 mg $SnCl_2$ in 100 μl of 0.01 N HCl was added, followed by 750 μl of radioactive ^{188}Re perrhenate in 0.9% saline (1–2 mCi ^{188}Re). Labeling was also done with the radiolabeled positively charged sestamibi complex, as described above. The vials were placed on an Eppendorf Thermomixer R (Fisher, Pittsburgh, PA, USA) for 5–120 min at temperatures ranging from room temperature to 99°C. Time, temperature, total perrhenate concentration and incubation volume were varied as part of the labeling process optimization. The supernatant was discarded, while the MTC were magnetically held in the vials. The procedure was repeated once more. The labeling efficiency is the ratio of the activity after the final wash to the total activity at the beginning. A 100% labeling efficiency thus means that no activity was washed off the MTC. The stability of the final radioactive MTC was measured in 1.5 ml of PBS at pH 7.4 or human plasma at 37°C. At time points of typically 1, 3, 6, 20, 28, 48 and 72 h, each vial was measured in a RadCal calibrator (total activity), the MTC magnetically retained in the vial and 2×500 μl of supernatant removed for activity counting. The bound ^{188}Re was then expressed as $1.5\times$ the sum of the measured supernatant activity divided by the total activity at each time point.

3. Results and discussion

Labeling efficiencies of ^{99m}Tc-labeled MTCs from highest to lowest were the positively charged Tc-sestamibi (97.4%), the neutral Tc-exametazime (94.1%), the tin-containing pertechnetate (85.7%) and, finally, the pertechnetate (78.2%). Stability measured in PBS over 24 h followed the same pattern (see Fig. 2). From these results, we decided to further investigate the radiolabeling of ^{188}Re with the highly stable sestamibi, but also with $SnCl_2$ added to the perrhenate solution. Although the latter was not yet stable enough for clinical applications, further optimization steps might allow for its clinical use. The labeling of MTCs would thus be straightforward and cheap, without having to first prepare another radioactive compound that requires quality control. Furthermore, different authors recently described the use of ^{188}Re-tin colloids for radiosynovectomy [13,14] and radioembolization therapy [15,16].

The direct adsorption of ^{188}Re-perrhenate, the negatively charged rhenium oxide, onto the MTC resulted in a nonhomogeneous $53.6 \pm 19.8\%$ labeling efficiency. Also, the release of ^{188}Re in the form of the highly water-soluble perrhenate was far too rapid (100% released in 24 h) to be useful in therapy and meet safety criteria (see Fig. 3).

The labeling efficiency of MTCs with the positively charged ^{188}Re-sestamibi was also low with $46.3 \pm 11.5\%$. Since the chelation kinetics of rhenium is slower than with technetium and often requires higher amounts of the reducing agent, this experiment was repeated with an additional 0.2 mg of $SnCl_2 \cdot 2H_2O$. The radiolabeling efficiency stayed the same at 48.1%, and the stability (Fig. 3) did not improve either. Thin layer chromatography confirmed that not more than a few percent of the sestamibi-complex had formed. Almost 80% of the radioactivity was still perrhenate, and about 10–15% was ^{188}Re-tin colloid. Because of the complexity of further developing rhenium-sestamibi, we decided to first investigate the labeling of ^{188}Re-MTC using only tin chloride.

The labeling of MTCs with ^{188}Re at room temperature in the presence of 0.5 mg of tin chloride produced the first labeling efficiencies ($90.7 \pm 0.8\%$) and stabilities ($86.5 \pm 2.0\%$ after

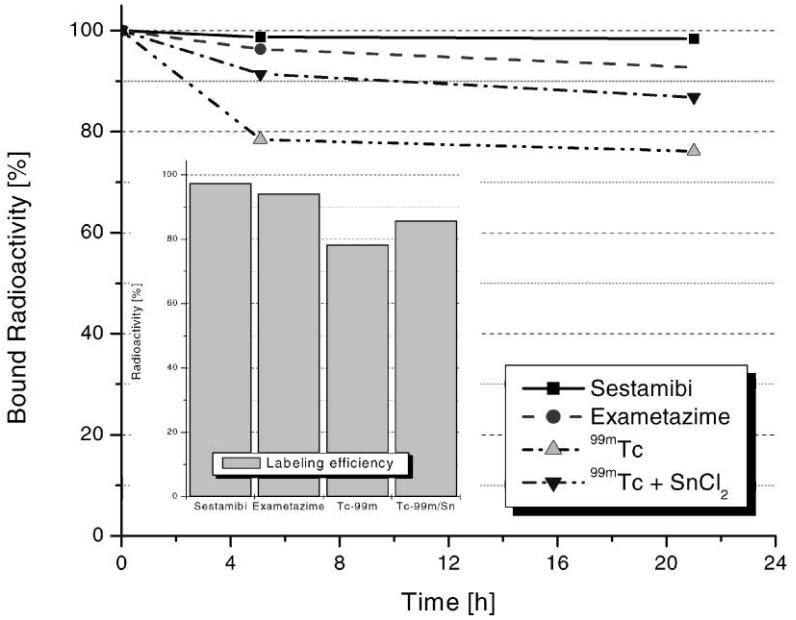

Fig. 2. Radiolabeling efficiencies (inset) and stability of ^{99m}Tc-labeled MTC ($n = 1$). The stability was measured in PBS pH 7.4.

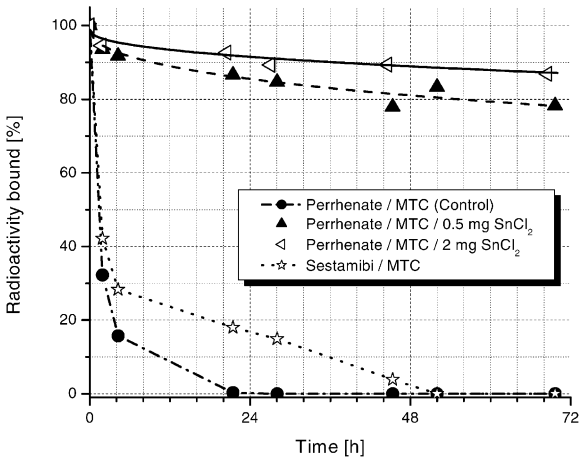

Fig. 3. Radiolabeling efficiencies and stability of ^{188}Re-labeled MTC ($n = 1$). The stability was measured in PBS pH 7.4.

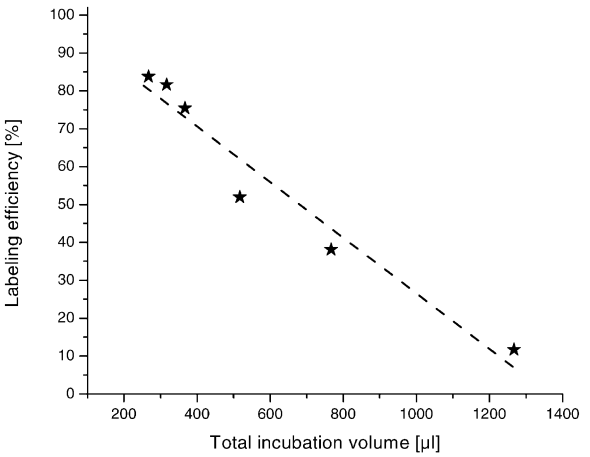

Fig. 4. Radiolabeling efficiencies of ^{188}Re-labeled MTC as a function of the total labeling volume. Labeling was done at room temperature for 30 min.

24 h) adequate for clinical use (Fig. 3). The ^{188}Re-MTC complex formed with 2.0 mg $SnCl_2 \cdot 2H_2O$ was even better, both in labeling efficiency and stability. The exact nature of reductive binding processes is not known, but very likely insoluble Re(IV) and Re(V) oxides have formed, or tin-rhenium-colloids have precipitated into the carbon pores of the MTCs.

Next, different radiolabeling parameters were tested. In general, the labeling efficiency is highest with the smallest possible incubation volume. This rule was confirmed when MTCs were labeled at room temperature for 30 min (Fig. 4). The lowest labeling volume of 300 µl allowed for complete suspension of the MTCs and showed the highest

labeling efficiency. We therefore used this volume to optimize temperature and length of incubation. Fig. 5 shows that longer incubation times result in not only higher labeling efficiencies, but also in better stability over time. The highest temperature of 99°C resulted in the best labeling efficiency, with maximum labeling achieved after 1 h of incubation.

The initial labeling stability characterizations were done over 4 half-lives of ^{188}Re (~68 h) at 37°C in PBS at pH 7.4, followed by testing in human plasma to mimic conditions expected in vivo. The stability of ^{188}Re-MTCs in plasma is comparable to the stability measured in PBS (data not shown). From the labeling efficiencies and stabilities obtained, it seems that the optimized radiolabeled ^{188}Re-MTCs can be directly used for injection. The amount of free ^{188}Re-perrhenate after labeling is less than 5%, which is better than what is required for radiopharmaceuticals to be ready for injection. The labeling stability of ^{188}Re-MTCs is critical because it is linked to the safety of this product, preventing redistribution from the targeted tumor, to normal, non-diseased sites.

We also investigated the influence of additional perrhenate on the labeling efficiency. Fig. 6 shows that doubling the amount of the carrier-free ^{188}Re-perrhenate (=979.4 Ci/mg rhenium) does not influence the labeling efficiency. A 10 times higher

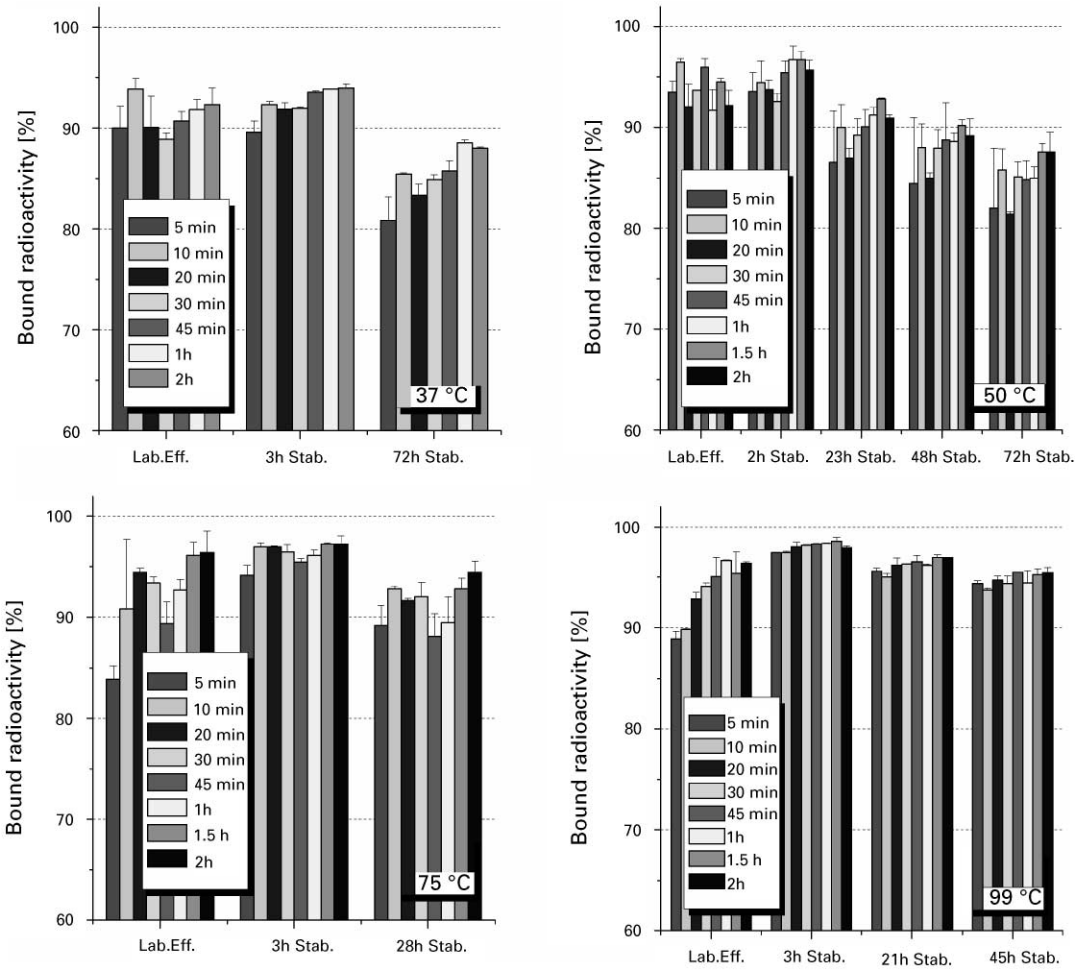

Fig. 5. Radiolabeling efficiencies and stability of ^{188}Re-labeled MTC incubated for 5–120 minutes at 37°C to 99°C ($n = 3$).

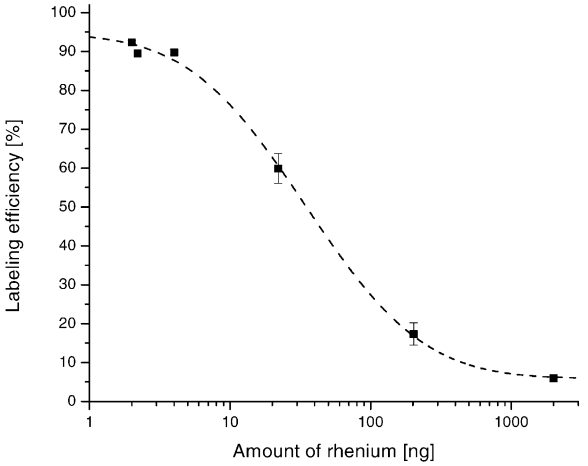

Fig. 6. Radiolabeling efficiencies of ^{188}Re-labeled MTC as a function of added non-radioactive perrhenate (2 mCi of carrier-free ^{188}Re-perrhenate contain 2.0 ng of rhenium).

amount, however, decreases the labeling efficiency to 60%. This experiment was performed before the labeling protocol was optimized and will be further investigated in due time.

4. Conclusions

The radiolabeling of MTCs with ^{188}Re at 99°C with tin chloride results in a higher than 95% labeling efficiency. The labeling process is a simple one-step procedure requiring no additional separation, filtration or sterilization steps, thus it could be done at any hospital licensed to prepare radiopharmaceuticals. Although ^{188}Re-MTC has a relatively short half-life of 17 h, its stability would also allow central preparation and overnight shipping. We are planning next to investigate the in vivo characteristics of these radiolabeled microparticles as well as their potential efficacy in relevant tumor models.

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