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Radiolabeling of MAG₃-morpholino oligomers with ¹⁸⁸Re at high labeling efficiency and specific radioactivity for tumor pretargeting

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Abstract

We are investigating a novel pretargeting approach involving an initial IV injection of antitumor antibody conjugated with a phosphorodiamidate morpholino oligomer (MORF, a DNA analog) and the subsequent IV injection of the radiolabeled complement oligomer (cMORF). In this paper, the cMORF was labeled with ¹⁸⁸Re using MAG₃ as chelator for therapeutic applications. Since (c) MORFs are unstable in acidic condition, an optimal labeling pH was first selected and the other labeling factors were then examined. A labeling efficiency of greater than 90% can be achieved even at a concentration of MAG₃-cMORF as low as 0.8 μM. The labeled cMORF is stable and capable of hybridizing to its complement.

Keywords

Rhenium-188; Radiolabeling; DNA analog; Pretargeting

1. Introduction

The objective of this investigation was to develop a method of labeling phosphorodiamidate morpholino oligomers (MORFs) with ¹⁸⁸Re for tumor pretargeting investigations. Rhenium-188 is often the radiotherapeutic nuclide of choice especially for biomolecules already capable of being labeled with technetium-99 m (^{99m}Tc). We reported earlier on method (Liu et al., 2003) for the labeling of MORFs with ¹⁸⁸Re using MAG₃ as chelator, which was modified only slightly from methods used to radiolabel with ^{99m}Tc (Mang'era et al., 2001; Liu et al., 2002a). However, in vivo instability leading to ¹⁸⁸Re-perrhenate was at level that would be unacceptable for radiotherapy. As an alternative, labeling via ¹⁸⁸Re-tricarbonyl [¹⁸⁸Re(CO)₃⁺] was considered (He et al., 2004; Liu et al., 2004a), to take advantage of the reported chemical inertness against re-oxidation. However, while in vivo stability was improved, it was not possible to achieve the high specific radioactivity levels required for radiotherapeutic applications (Liu et al., 2004a). This paper describes the results obtained in a revisit of the MAG₃ approach for ¹⁸⁸Re labeling of the cMORF useful in pretargeting studies.

The N₃S chelators with a coordinating site consisting of one thiol sulfur and three amide nitrogens have been widely employed for ¹⁸⁸Re and ¹⁸⁶Re labeling. For antibodies and other carriers that are sensitive to heating, a common strategy is to adopt pre-conjugation labeling. In this strategy, the ¹⁸⁸Re- or ¹⁸⁶Re-MAG₃ active ester is first prepared and then conjugated to biomolecule carriers. Radiolabeled in this fashion, a ¹⁸⁶Re-MAG₃-antibody was shown to

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be stable in plasma for up to 30 h (Kievit et al., 1997). Different N₃S chelators have been used in this way, such as MAG₂-GABA (*S*-ethoxyethyl mercaptoacetyl-glycylglycyl aminobutyrate) (Vanderheyden et al., 1989; Goldrosen et al., 1990), *S*-benzoyl-MAG₃ (Visser et al., 1993; Gerretsen et al., 1993), and MAGIPG (*N*-(*S*-acetylmercaptoacetyl)(*p*-NCS) phenylalanylglycine ethyl ester) (Ram et al., 1997). The disadvantages of pre-conjugation labeling include the extensive manipulations with highly radiotoxic therapeutic nuclides and the need for post-labeling purification. In the case of carriers that can withstand harsh environments, post-conjugation labeling should be possible and more practical. However, although a few post-conjugation ¹⁸⁸Re labeling have been reported (Guhlke et al., 1998; Pearson et al., 1996; Zinn et al., 2000; Polyakov et al., 2000; Gestin et al., 2001), to our knowledge, none describes ¹⁸⁸Re labeling of a biomolecule at high labeling efficiency and high specific radioactivity.

This laboratory has been using *S*-acetyl protected and *N*-hydroxysuccinimide-activated MAG₃ ester for ^{99m}Tc labeling. It is in routine use for post-conjugation labeling with ^{99m}Tc of proteins, peptide and oligomers at slightly basic pH and at room temperature (Winnard et al., 1997; Hnatowich et al., 1997, 1998; Rusckowski et al., 2000). However, the labeling efficiency is usually not quantitative and therefore post-labeling purification is often necessary. In recent investigations of the ^{99m}Tc labeling of MAG₃-cMORF, heating was found to increase the labeling efficiency to about 80% but with the appearance of radiolabeled impurities (Mang'era et al., 2001; Liu et al., 2002a). Further investigations showed that the impurities were not due to incomplete purification of the MAG₃-cMORF after conjugation but were generated during ^{99m}Tc labeling (Liu et al., 2002b) and led to the conclusion that these impurities could be removed by introducing the preliminary purification procedure described below. As a result, labeling efficiency of MAG₃-cMORF with ^{99m}Tc now routinely exceeds 95% (unpublished observations). A MAG₃-cMORF preparation has now been stored for more than 1 year at -20 ° C and still provides labeling efficiencies with ^{99m}Tc exceeding 95%.

In the previous method from this laboratory on ¹⁸⁸Re labeling of MAG₃-cMORF (Liu et al., 2003), the MAG₃-cMORF was prepared without this preliminary purification procedure and the labeling method was not optimized. In this present study, the preliminary purification procedure has been included.

2. Material and methods

The MORF (MW 6164) and its complement (cMORF, MW 6331) of this investigation were each purchased with a primary amine on the 3' equivalent end linked via a 6-member linker. As defined for the purposes of this report, the base sequences of MORF and cMORF were respectively 5' -TCTTCTACTTCACAATA and 5' -TAGTTGT-GAAGTAGAAGA (Gene Tools, Philomath, OR). The *S*-acetyl NHS-MAG₃ was synthesized in house (Winnard et al., 1997) and the structure was confirmed by elemental analysis, proton NMR and mass spectroscopy. The anti CEA antibody MN14 (MW 160,000) was a gift from Immunomedics (Morris Plains, NJ). The ¹⁸⁸Re perrhenate eluate was obtained from an ¹⁸⁸W/¹⁸⁸Re generator (Oak Ridge National Laboratory, Oak Ridge, TN) and the ^{99m}Tc pertechnetate was from a ⁹⁹Mo/^{99m}Tc generator (Perkin Elmer Life Science Inc, Boston, MA). The Bio-Gel P4 Gel (medium, Bio-Rad Laboratories, Hercules, CA) and all other chemicals were reagent grade and were used as received.

2.1. HPLC analysis

Both the reverse phase (RP) and size exclusion (SE) high-performance liquid chromatographs (HPLC) were equipped with an in-line radioactivity detector and an inline Waters 2487 dual wavelength absorbance detector (Milford, MA). Recovery of radioactivity was routinely

measured with co-injection of about 10 μg unlabeled cMORF and was always over 85%. Both perrhenate and pertechnetate are retained on SE column but not RP C-18 column.

The RP HPLC was used to evaluate the labeling of cMORF and the stability of ^{188}Re -cMORF in phosphate buffer and was performed on a C-18 column (5 μm , 250×4.6 mm, Vydac, The Nest Group, Inc, Southboro, MA) with 0.05 M TEAP (triethylamine phosphate, pH 4.6) as solvent A and methanol/acetonitrile ($v/v = 80/20$) as solvent B at a flow rate of 1.0 mL/min. Gradient table: 0–3 min, 100% A; 3–6 min, 100–75% A; 6–9 min, 75–66% A; 9–20 min, 66–0% A; 20–37 min, 0% A; 37–40 min, 0–100% A; 40–50 min, 100% A.

The SE HPLC was used along with paper chromatography to investigate the integrity of ^{188}Re -cMORF and its stability in serum and was performed on a Superose-12 HR10/30 column (Amersham Pharmacia Biotech, Piscataway, NJ) with an optimal separation range between 1×10^3 – 3×10^5 Da. The 0.10 M pH 7.2 phosphate buffer was used as eluant at a flow rate of 0.6 mL/min. In addition, paper chromatography was performed on Whatman No. 1 paper with acetone as the mobile phase to detect ^{188}Re perrhenate. In this system, perrhenate migrates to the solvent front ($R_f = 0.9 - 1.0$) while labeled cMORF and other labeled impurities stay at the application point ($R_f = 0.0 - 0.1$).

2.2. Preparations of $^{99\text{m}}\text{Tc}$ MAG₃-cMORF and MORF-MN14

The MAG₃-cMORF conjugate was prepared as follows: 1.5 mg of cMORF was dissolved in 0.2 M, pH 8.0 HEPES to a concentration of 0.5 mg/100 μL and the solution was added to a vial containing *S*-acetyl NHS-MAG₃ at a MAG₃/amine molar ratio of 10–25. After 2 h incubation at room temperature, the reaction mixture was purified over a P4 column (0.7×20 cm) using 0.25 M, pH 5.2 NH_4OAc buffer as eluant. The peak fractions at 265 nm with an OD greater than 1.5 were pooled. A preliminary purification procedure was performed by mixing the pooled cMORF solution with a pH 9.2 tartrate buffer (50 $\mu\text{g}/\mu\text{L}$ $\text{Na}_2\text{tartrate} \times 2\text{H}_2\text{O}$, 0.5 M Na_2HCO_3 , 0.25 M NH_4OAc , 0.175 M NH_3) and a fresh tin solution (10 $\mu\text{g}/\mu\text{L}$ $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, 1 $\mu\text{g}/\mu\text{L}$ Na ascorbate, 10 mM HCl) at a volume ratio of cMORF solution/tartrate buffer/tin solution of 15/5/1, followed by heating for 20 min and then a final purification over a P4 column (1.0×50 cm). The peak fractions with OD values greater than 1.5 were pooled again and were stored at -20°C . This preparation has now been used for $^{99\text{m}}\text{Tc}$ labeling for over 1 year with minimal loss in labeling efficiency and is now in use for ^{188}Re labeling.

Radiolabeling with $^{99\text{m}}\text{Tc}$ of the MAG₃-cMORF was as follows: 50 μL of $^{99\text{m}}\text{Tc}$ -pertechnetate eluant was introduced into a combined solution consisting of 30 μL (preferably no less than 0.5 μg) of MAG₃-cMORF in pH 5.2 NH_4OAc buffer, 10 μL of 50 $\mu\text{g}/\mu\text{L}$ $\text{Na}_2\text{tartrate} \cdot 2\text{H}_2\text{O}$ in the pH 9.2 buffer and 3 μL of 4 $\mu\text{g}/\mu\text{L}$ $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in ascorbate-HCl solution (1 $\mu\text{g}/\mu\text{L}$ Na ascorbate in 10 mM HCl), followed by heating at 100°C for 20 min. The final pH was 7.8 and the labeling efficiency was routinely greater than 95%.

The antiCEA IgG antibody MN14 was conjugated with MORF and used to confirm the hybridization property of cMORF after labeling with ^{188}Re . The conjugation was achieved using a commercial Hydralink method (Solulink, San Diego, California).

2.3. Initial labeling of MAG₃-cMORF with ^{188}Re

Initially, 50 μL of ^{188}Re perrhenate was added to a combined solution of 30 μL of MAG₃-cMORF (0.4 $\mu\text{g}/\mu\text{L}$) in pH 5.2, 0.25 M NH_4OAc buffer, 10 μL Na_2 tartrate $\cdot 2\text{H}_2\text{O}$ (50 $\mu\text{g}/\mu\text{L}$) in 0.5 N HCl, and 20 μL of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (10 $\mu\text{g}/\mu\text{L}$) in 20 mM HCl containing 1 $\mu\text{g}/\mu\text{L}$ of Na ascorbate, followed by heating in a boiling water bath for 1 h. The pH was 3.3 as suggested (Guhlke et al., 1998).

The influence of additional heating for 0, 1, 2 and 3 h on the formation of radiolabeled impurities during the preparation of ^{188}Re -cMORF was examined by RP HPLC. To help establish whether the observed radiolabeled impurities were due to instability of the ^{188}Re label or the cMORF itself, the study was repeated with $^{99\text{m}}\text{Tc}$ as label. The $^{99\text{m}}\text{Tc}$ -cMORF was labeled at pH 7.8 and was adjusted to pH 3.4 before heating for an additional 0, 1, 2, or 3 h.

2.4. Optimization of the labeling procedure

The optimization procedure was as follows: 30 μL of MAG_3 -cMORF at various concentrations in the pH 5.2, 0.25 M NH_4OAc buffer was added to a combined solution of 10 μL of Na_2 tartrate $\cdot 2\text{H}_2\text{O}$ (50 $\mu\text{g}/\mu\text{L}$) in either HCl (0.0, 0.1, 0.2, 0.3 or 0.4 M), NaOH (0.2, 0.3, 0.4, 0.5 M), or the above pH 9.2 buffer and 20 μL of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ at various concentrations in 20 mM HCl containing 1 $\mu\text{g}/\mu\text{L}$ of Na ascorbate. Subsequently, 50 μL of ^{188}Re perrhenate generator eluant was added and the solution with a total volume of 110 μL was heated in a boiling water bath.

The conditions of the optimization studies are summarized in Table 1. In brief, the influence of labeling pH on labeling efficiency in the range from 3.3 to 8.2 was first examined by changing the composition of the tartrate solution. Once the optimum pH was established, the influence of heating time at that pH value (4.6) was evaluated followed by the influence of the concentration of MAG_3 -cMORF and finally the influence of stannous ion concentration.

2.5. In vitro stability and integrity of ^{188}Re - MAG_3 -cMORF

Since excess stannous ion will prevent perrhenate formation and therefore provide an unrealistic assessment of stability against oxidation, the ^{188}Re -labeled cMORF solution was purified from excess stannous ion on a P4 column with 0.1 M, pH 7.0 phosphate buffer as eluant and analyzed by RP HPLC over 24 h. The stability in serum was also examined by incubating 100 μL of the purified labeled preparation with 400 μL fresh human serum at 37 °C over 24 h followed by analysis for protein binding by SE HPLC and for perrhenate by paper chromatography.

The hybridization property of MAG_3 -cMORF after labeling was evaluated by a SE HPLC shift assay in which 15 μCi (about 0.1 μg) of ^{188}Re -labeled cMORF was analyzed with or without the addition of MN14-MORF in excess (20 μg , 0.53 MORF groups per antibody). Hybridization is evident by a shift in the radioactivity profile to earlier retention time.

2.6. Biodistribution in normal mice

Four normal CD-1 mice (Charles River, Wilmington, MA) each received 10 μCi (4.0 μg) of ^{188}Re -labeled cMORF via a tail vein. The mice were sacrificed 3 h later by exsanguination following cardiac puncture under halothane anesthesia. Blood and other organs were removed, weighed, and counted in a Cobra II automatic gamma counter (Packard Instrument Company, CT) along with a standard of the injectate.

3. Results

3.1. Radiolabeling of MAG_3 -cMORF

Radioimpurities were observed in the RP HPLC radio-chromatograms of ^{188}Re - MAG_3 -cMORF labeled at pH 3.3 as shown in Fig. 1A (top panel). The labeling efficiency was less than 80% instead of over 90% as expected. The ^{188}Re - MAG_3 -cMORF elutes in one main peak at 19 min with the impurities at 13–18 min. As shown (remaining panels of Fig. 1A), these impurities increase with increasing post-labeling heating time. Because cMORFs are unstable at pH 1 [Yongfu Li, GeneTools, private communication, 2005], these impurities were suspected to result from morpholino oligomer backbone fracture at acidic pH rather than from loss of the

radiolabel. Since it is known that ^{99m}Tc -MAG₃ is stable to heating at acid pH (unpublished observations), this instability of cMORF was further confirmed by heating ^{99m}Tc -MAG₃-cMORF. When the pH was immediately adjusted to 3.3 from 7.8 (Fig. 1B, top panel), ^{99m}Tc -cMORF shows no impurity peaks. However, with increasing time of heating, radiochemical impurity peaks appear (remaining panels of Fig. 1B) in a similar manner to that observed for ^{188}Re -cMORF. These results indicate that the formation of these radioactive impurities is not a consequence of ^{188}Re label instability but rather a decomposition or hydrolysis of cMORF at low pH especially upon heating.

The radiochemical purity determined by RP HPLC reached a maximum of greater than 90% at pH 4.6. Furthermore, as shown in Fig. 2, labeling at this pH also provides the cleanest RP HPLC radiochromatograph. At lower pH, hydrolysis of the cMORF backbone becomes more pronounced, while at higher pH perrhenate becomes evident by the peak at 8 min. As shown in Fig. 3, 1 h of heating is sufficient, in agreement with the literature (Guhlke et al., 1998).

Fig. 4 shows the influence of MAG₃-cMORF concentration on labeling efficiency. As shown (open circles), labeling efficiency exceeds 90% at concentrations above 5.0 ng/μL of MAG₃-cMORF (about 0.5 μg, 0.8 μM). However this concentration limit is misleading since at low concentration a significant fraction of MAG₃-cMORF is lost from solution by adsorption to the vial wall. Fig. 5 presents the weight of MAG₃-cMORF adsorbed to the vial wall of 0.5 mL micro-centrifuge tube (VWR, Bridgeport, NJ) when added in 60 μL at different MAG₃-cMORF concentrations. As shown (open circles), the adsorption is saturated at about 5 ng/μL. However, after two washes with 1% human serum albumin, the MAG₃-cMORF left on the wall drops to insignificance (open triangles). Unfortunately, the addition of HSA during labeling is impractical because HSA cannot withstand boiling water temperature, but its addition after labeling is useful to minimize adsorption during dose preparation and analysis. If the adsorption of MAG₃-cMORF is avoided by adding native cMORF, as shown in Fig. 4, a labeling efficiency of greater than 90% may be achieved at even lower concentrations of MAG₃-cMORF (closed triangles), as a reflection of the robust chelating ability of MAG₃.

Fig. 6 shows that at least 1.5 μg/μL (160 μg) of SnCl₂ · 2H₂O is necessary to achieve a labeling efficiency of greater than 90%, but concentrations of SnCl₂ · 2H₂O in excess of 2.8 μg/μL (300 μg) resulted in precipitation of stannous hydroxide.

Upon optimization of all labeling conditions, a ^{188}Re labeling efficiency of greater than 90% can be achieved with the following procedure: 50 μL of ^{188}Re perrhenate was added to a combined solution of 30 μL of pH 5.2, 0.25 M NH₄OAc buffer containing more than 0.2 μg but preferably 0.5 μg MAG₃-cMORF, 10 μL Na₂ tartrate · 2H₂O (50 μg/μL) in H₂O and 20 μL of SnCl₂ · 2H₂O (10 μg/μL) in 20 mM HCl containing 1 μg/μL of Na ascorbate, followed by heating in a boiling water bath for 1 h.

3.2. In vitro stability and integrity of ^{188}Re -MAG₃-cMORF

The RP HPLC radiochromatograms of Fig. 7 show a single peak (panel A) that remains essentially unchanged at least over 24 h at room temperature (panel B), serving as evidence of in vitro stability against oxidation. The ^{188}Re -cMORF was prepared according to the above-optimized procedure and the stannous ion was removed as earlier mentioned. As shown by SE HPLC (panel C), the label is also stable in 37 °C human serum for at least 24 h. The recovery in these analyses was over 90%. No evidence for perrhenate in these samples was found by paper chromatography (data not presented). Finally, almost the entire ^{188}Re -labeled cMORF peak was shifted to the position of the antibody by the addition of MN14-MORF (panel D), indicating that the hybridization property of cMORF was unchanged after conjugation and labeling.

3.3. Biodistribution of ^{188}Re -cMORF

The biodistribution data in normal mice obtained at 3 h post intravenous administration of ^{188}Re -cMORF are listed in Table 2. Historical results in an identical study of $^{99\text{m}}\text{Tc}$ -cMORF (Liu et al., 2004b) are also included for comparison. Statistical analysis shows significant differences only for salivary gland and gastrointestinal tract with values for ^{188}Re lower in both cases (Student's t test $P < 0.05$). The low accumulations of ^{188}Re labeled cMORF in salivary gland and stomach indicate the ^{188}Re -cMORF is stable to oxidation in vivo since perrhenate strongly accumulates in these two organs (Zuckier et al., 2004). The stomach, large and small intestines were weighed and counted with contents, therefore values for these organs are reported only as %ID/organ.

Biodistribution of ^{188}Re -cMORF in normal mice after an additional P4 purification to remove the radioimpurities was also performed but showed no obvious differences (data not presented). This indicates that the impurities clear rapidly through the kidneys along with the labeled cMORF. Most probably the impurities are labeled and truncated cMORF and/or labeled tartrate. Therefore, to avoid unnecessary radiation exposure to personnel, the additional purification step was eliminated.

4. Discussion

Radiotherapy by MORF pretargeting will require that cMORF be radiolabeled with a therapeutic radionuclide. The choice of ^{188}Re was dictated in part by the successful radiolabeling of cMORF with $^{99\text{m}}\text{Tc}$ and the chemical similarities between technetium and rhenium. However, while technetium and rhenium share similar chemical properties, there are important differences. For example, much more stannous ion and/or much lower pH values are needed to reduce perrhenate compared to pertechnetate. Furthermore, the radioactivity concentration of ^{188}Re perrhenate in the eluant of the $^{188}\text{W}/^{188}\text{Re}$ generator is usually lower than that of $^{99\text{m}}\text{Tc}$ pertechnetate from the $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator. Because of the higher oxidation potential of rhenium and the lower radioactivity concentration of ^{188}Re , a higher concentration of both stannous ion and chelator concentration are often required for efficient ^{188}Re labeling compared to $^{99\text{m}}\text{Tc}$ labeling.

Because of certain perceived advantages of technetium and rhenium tricarbonyl complexes, such as the expected chemical inertness, these complexes were previously considered by us as a method of labeling (c)MORFs (He et al., 2004; Liu et al., 2004a). Though high labeling efficiency with $^{99\text{m}}\text{Tc}$ was achieved, the specific activity was two orders of magnitude lower than that achievable using MAG_3 as chelator. For example, a labeling efficiency in excess of 90% was achievable at chelator concentration no lower than about 10^{-4} M with the tricarbonyl approach while comparable labeling using MAG_3 as chelator can be obtained at a chelator concentration as low as 0.8×10^{-6} M. In this regard, our experience with the tricarbonyl labeling is consistent with the literature (Egli et al., 1999; Schibli et al., 2000).

As mentioned above in connection with Fig. 4, adsorption has raised the concentration required to achieve a particular labeling efficiency. The decreased labeling efficiency with decreasing concentration is not due to the chelating ability of MAG_3 on cMORF but to the loss of MAG_3 -cMORF from solution such that the cMORF concentration is lower than indicated therein. By adding unconjugated native cMORF to keep the total cMORF concentration constant, the concentration limit to achieve a labeling efficiency of over 90% is reduced to 2 ng/ μL or 0.3 μM , as indicated in Fig. 4 (triangles). Late in this investigation, it was found that the use of non-stick surface micro-centrifuge tube (VWR, Bridgeport, NJ) could minimize the degree of adsorption during labeling.

In this present study, the MAG₃ strategy for ¹⁸⁸Re labeling of cMORF used unsuccessfully in the past (Liu et al., 2003) has been revisited. Reconsideration of this approach was driven by three factors: (1) the recent development of a labeling protocol for MAG₃-cMORF which results in a ^{99m}Tc labeling efficiency of greater than 95% without postlabeling purification; (2) the failure to reach a high specific radioactivity with tricarbonyl approaches using histidine and dipicolylamine (Liu et al., 2004a); and (3) a literature survey suggesting that ¹⁸⁸Re or ¹⁸⁶Re-labeled MAG₃ conjugate is stable against reoxidation.

For radiotherapeutic application of ¹⁸⁸Re-labeled cMORF, besides the requirements that the radiolabel be stable both in vitro and in vivo and that the specific radioactivity be sufficiently high, the hybridization property of cMORF must be preserved. By using SE HPLC in a shift assay, we have demonstrated that the ¹⁸⁸Re-radiolabeled cMORF of this investigation maintained hybridization affinity for its complement.

This investigation was performed with an aged ¹⁸⁸W–¹⁸⁸Re generator providing ¹⁸⁸Re perrhenate at a concentration of only about 0.5 mCi/mL compared to about 10–100 mCi/mL for a new of 250–1000 mCi generator. Because of the no-carrier nature of ¹⁸⁸Re, the labeling efficiency should not be influenced by the practical amount of radioactivity. Since radiolysis has not been found to be a problem with morpholino oligomer, a specific radioactivity of 2–25 mCi/μg cMORF should be achievable.

5. Conclusion

The MAG₃ chelator for post-conjugation labeling of morpholino oligomers with ¹⁸⁸Re has been shown to provide a stable label with specific radioactivity sufficiently high for radiotherapeutic applications without the need for postlabeling purification.

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Fig. 1. RP HPLC radiochromatograms showing the influence of heating time on the profile of cMORF radiolabeled at pH 3.3 with ^{188}Re (column A) and with $^{99\text{m}}\text{Tc}$ (column B). Radiochemical purity is indicated.

Fig. 2. RP HPLC radiochromatograms of ^{188}Re -cMORF prepared at pH 3.3, 4.6, and 5.9. Radiochemical purity is indicated.

Fig. 3.
Influence of heating time on the efficiency of labeling ^{188}Re -cMORF at pH 4.6.

Fig. 4. Labeling efficiency of ^{188}Re -MAG₃-cMORF with increasing weight of MAG₃-cMORF with (○) and without (▲) the loss from solution of MAG₃-cMORF to the vessel wall.

Fig. 5.
Adsorption behavior of 60 µL-radiolabeled cMORF on the vessel wall.

Fig. 6. Influence of stannous ion on the labeling efficiency of ^{188}Re -cMORF after 1 h of heating at pH 4.6.

Fig. 7. RP HPLC radiochromatograms of ^{188}Re -cMORF purified from excess stannous ion analyzed immediately (panel A) and 24 h post-labeling (panel B) and the SE HPLC radiochromatograms of the same ^{188}Re -cMORF preparation after incubation in 37 °C human serum for 24 h before (panel C) and after (panel D) the addition of MN14-MORF. Radiochemical purity is indicated.

Table 1

Parameter values during optimization of labeling conditions

Optimization of:	Labeling pH	Heating time (h)	MAG ₃ -cMORF (μg)	SnCl ₂ · 2H ₂ O (μg)
pH	3.3–8.2	1	12	200
Heating time	4.6	1–1.2	12	200
Concentration of MAG ₃ -cMORF	4.6	1	0.2–12	200
Concentration of Tin (II)	4.6	1	1.6	0–400

Table 2
Biodistribution of ¹⁸⁸Re- and ^{99m}Tc-labeled cMORF in normal mice 3 h post IV administration

Organ	%ID/g		%ID/organ	
	¹⁸⁸ Re-cMORF	^{99m} Tc-cMORF	¹⁸⁸ Re-cMORF	^{99m} Tc-cMORF
Liver	0.1470.04	0.1270.06	0.1970.06	0.2170.09
Heart	0.0370.01	0.0470.00	0.0070.00	0.0170.00
Kidneys	4.1170.75	3.2370.48	1.5970.43	2.0470.24
Lungs	0.0770.01	0.1170.02	0.0170.00	0.0370.00
Spleen	0.0670.03	0.0670.00	0.0170.00	0.0170.00
Stomach	—	—	0.2770.09	0.4270.08
Sm. Int.	—	—	0.1170.05	0.1370.03
Lg. Int.	—	—	0.1370.04	0.6170.05
Muscle	0.0270.01	0.0170.01	0.2170.06	0.2570.09
Salivary gland	0.3470.04	0.9070.30	0.0670.00	0.2770.10
Blood	0.0470.01	0.0670.01	0.0970.01	0.2070.02

Mean ± SD (*n* = 4).
The ^{99m}Tc values taken from (Liu et al., 2004b).