

Pentavalent rhenium-188 dimercaptosuccinic acid: a new kit formulation and its initial evaluation in mice

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Summary. Pentavalent rhenium-188 dimercaptosuccinic acid [¹⁸⁸Re(V)DMSA] is a β -emitting radiopharmaceutical that has been proposed for the treatment of painful bone metastases, medullary thyroid carcinoma and other soft tissue tumours. In this study the development of a kit formulation for convenient, routine preparation of ¹⁸⁸Re(V)DMSA is presented. The kit contains all the ingredients in a single vial and its reconstitution with ¹⁸⁸Re perrhenate, up to 4 mL, produces ¹⁸⁸Re(V)DMSA in high radiochemical purity, suitable for clinical application. ¹⁸⁸Re(V)DMSA prepared by this kit method was evaluated in mice and resulted in a biodistribution pattern similar to that of ^{99m}Tc(V)DMSA.

Introduction

Pentavalent oxotechnetium dimercaptosuccinic acid ^{99m}Tc(V)DMSA is a high specificity tumour-seeking agent which is used for the detection of medullary thyroid carcinoma, soft tissue tumours as well as metastatic bone lesions [1–7]. It was also suggested [8] that the rhenium analogues, ¹⁸⁶Re(V)DMSA (¹⁸⁶Re: $t_{1/2} = 90$ h, $E_{\beta} = 1.1$ MeV, $E_{\gamma} = 137$ KeV (ca 10%)) and ¹⁸⁸Re(V)DMSA (¹⁸⁸Re: $t_{1/2} = 16.7$ h, $E_{\beta} = 2.1$ MeV, $E_{\gamma} = 155$ KeV (ca 15%)), which are beta emitters could be useful agents for systemic radiotherapy. Initial therapeutic studies have demonstrated the similar pharmacokinetics between ^{99m}Tc(V)DMSA and ¹⁸⁶Re/¹⁸⁸Re (V)DMSA [9, 10].

¹⁸⁶Re is produced by irradiation of ¹⁸⁵Re in a nuclear reactor and it may contain significant amounts of carrier. Carrier-free ¹⁸⁸Re can be repeatedly obtained from the ¹⁸⁸W/¹⁸⁸Re generator and thus it is more attractive in the preparation of radiopharmaceuticals. To our knowledge there is no single step kit formulation for the instant preparation of ¹⁸⁸Re(V)DMSA. So far, ¹⁸⁸Re(V)DMSA can be prepared using the common DMSA-kit (kidney agent) but this method requires the addition of ascorbic acid to the sodium perrhenate solution in order to prevent autoradiolysis. Moreover, in this method the reaction volume has to be

kept below 1 mL per vial, a limitation which usually requires concentration of the ¹⁸⁸W/¹⁸⁸Re generator eluate to a small volume [8, 10–13]. Other methods refer to the *in situ* preparation of ¹⁸⁸Re(V)DMSA using either ¹⁸⁸ReOCl₃(PPh₃)₂ as precursor [11] or SnCl₂, oxalate ions and γ -cyclodextrin [14]. To meet the requirement for widespread clinical application of ¹⁸⁸Re(V)DMSA it is desirable to develop simplified preparation methods.

The present work describes a convenient procedure for the preparation of ¹⁸⁸Re(V)DMSA on the basis of a single-vial freeze-dried kit. ¹⁸⁸Re(V)DMSA prepared by this formulation was further evaluated in mice and its biodistribution was compared to that of ^{99m}Tc(V)DMSA.

Experimental

Materials and methods

Rhenium-188 was obtained as sodium perrhenate by elution of a ¹⁸⁸W/¹⁸⁸Re generator (Oak Ridge National Laboratory (ORNL), Oak Ridge, Tennessee) with 10–20 mL physiological saline. The solution was sterilised by filtration. Chemicals for the preparation of the kit, *meso*-dimercaptosuccinic acid (DMSA), inositol, stannous chloride and ascorbic acid, were obtained from Sigma. ^{99m}Tc(V)DMSA was prepared using the commercially available kit DMS(V)/Demomed (Institute of Radioisotopes-Radiodiagnostic Products, NCSR "Demokritos"). n-Bu₄NRe^VO(DMSA)₂ was prepared according to the literature [8]. The radioactivity of TLC and biological samples was measured in an automatic γ -counter (Canberra Packard Auto-Gamma 5000 series).

Freeze-dried kit formulation

The kit was prepared under aseptic conditions by the following technique: 500 mg of *meso*-dimercaptosuccinic acid and 2.5 g inositol were dissolved, with slight heating, in 80 ml water for injection. The solution was deoxygenized by bubbling with nitrogen for 15 min. Then, 1.5 g ascorbic acid and 100 mg stannous chloride (dissolved in 0.2 mL 10 N HCl + 1.8 mL water) were added. The pH was adjusted to 2 with 1 N NaOH and the volume was diluted to 100 mL. The solution was filtered using a 0.22 μ m Millipore filter

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for terminal sterilisation. Under aseptic conditions, one mL of the solution was dispensed into 10 mL serum vials and lyophilised. At the end of the lyophilization cycle the vials were stoppered under nitrogen atmosphere and stored in a refrigerator (2–8 °C).

Composition: 5 mg DMSA, 1 mg SnCl₂, 15 mg ascorbic acid, 25 mg inositol.

¹⁸⁸Re(V)DMSA preparation

The lyophilized kit was brought to room temperature and reconstituted with 10 mCi ¹⁸⁸Re perrhenate in saline. The volume of the reaction was 4 mL, except for the studies of the reaction volume on labelling yield, where the volume varied. The reaction vial was immersed in a boiling water bath and heated for 30 minutes. After cooling, the solution was filtered through a 0.22 µm filter in an evacuated vial in order to remove any particulates and effect terminal sterilization. Alternatively, the reconstituted vial can be autoclaved for 20 min. By this method the pH of the final solution was 3.5–4.0.

In order to determine optimum reaction parameters, the effect of the perrhenate volume, activity as well as heating time and temperature were studied.

Radiochemical purity

Free perrhenate was evaluated by using instant thin layer chromatography – silica gel strips (ITLC-SG, Gelman Co) as the stationary phase and acetone as the mobile phase. Reduced-hydrolyzed rhenium and ¹⁸⁸Re(V)DMSA remain at the origin whereas Re-188-perrhenate moves with the solvent front. Reduced-hydrolyzed rhenium was evaluated using ITLC-SG strips as the stationary phase and 5% aqueous solution of glycine as the mobile phase. Reduced-hydrolyzed rhenium remains at the origin while ¹⁸⁸Re(V)DMSA and Re-188-perrhenate moves with the solvent front. The ITLC radioactivity was analysed by cutting the strips in segments of 1 cm and measuring the radioactivity in an automatic gamma counter. Non-radioactive ^{185/187}Re(V)DMSA prepared in macroscopic amounts was used as reference. The observation of the orange colour of non radioactive ^{185/187}Re(V)DMSA was used for its localisation on the ITLC.

Biodistribution studies in mice

For the biodistribution study with ¹⁸⁸Re(V)DMSA, a kit was reconstituted as described above (10 mCi, 4 mL). Then 0.1 mL of the radiopharmaceutical was diluted to 3 mL with saline and filtered. The radiochemical purity of the final solution was checked before administration and found be higher than 98%.

Three groups of five Swiss Albino mice each (male, 30–35 g, 60 days old) were injected with ¹⁸⁸Re(V)DMSA (0.1 mL, 8 µCi) through the tail vein. The animals of the first group were sacrificed by cardiectomy under slight ether anaesthesia 30 minutes after the injection, while the animals of the second and third group were sacrificed 2 and 24 hours postinjection respectively. The organs of interest were excised, weighed and counted in an automatic gamma counter.

Bladder and excreted urine were not weighed. The stomach and intestines were not emptied of food contents prior to radioactivity measurements. The percentage of injected dose per organ (%ID/organ) was calculated by comparison of sample radioactivity to standard solutions containing 10% of the injected dose. The calculation for blood, muscle and bones was based upon measured activity, sample weight and body composition data (considering that blood, muscle and bones comprise 7, 43 and 10% of body weight). The percentage of injected dose per gram (%ID/g) was calculated by dividing the %ID/organ by the weight of the organ or tissue.

For the biodistribution study with ^{99m}Tc(V)DMSA, a kit was reconstituted with 2.5 mL freshly eluted ^{99m}Tc pertechnetate (250 µCi). After a 20-minute incubation period, the radiopharmaceutical (0.1 mL, 10 µCi) was injected into the animals, in a similar manner to ¹⁸⁸Re(V)DMSA.

Results and discussion

^{99m}Tc(V)DMSA is a radiopharmaceutical with high affinity to a wide variety of benign and malignant tumours and it is successfully used in the detection of medullary thyroid carcinoma (MTC) and metastatic bone lesions. Initial studies have demonstrated the similar pharmacokinetics between ^{99m}Tc(V)DMSA and ¹⁸⁶Re/¹⁸⁸Re(V)DMSA. The methods for the preparation of the rhenium complexes require the addition of excess DMSA and SnCl₂ in a commercial DMSA kit (kidney agent) or the use of a precursor. Moreover, the addition of ascorbic acid in this preparation was considered necessary to avoid radiolysis. Another limitation of these methods is the necessity of using concentrated perrhenate solutions.

We have focused our study on the development of a simple method based on a single vial kit formulation which will enable the preparation of ¹⁸⁸Re(V)DMSA. The composition of the kit contains increased amounts of DMSA and SnCl₂ compared to the kit for the preparation of ^{99m}TcDMSA radiopharmaceutical for kidney imaging. Moreover, the kit contains ascorbic acid as an antioxidant while inositol was used as a stabilizer. After lyophilization, the vials were stoppered under nitrogen atmosphere in order to protect stannous ions and avoid the reoxidation of ¹⁸⁸Re(V)DMSA to perrhenate.

The radiochemical purity of the radiopharmaceutical was determined using two systems, which are applied for the quality control of ^{99m}Tc(V)DMSA. ITLC-SG strips developed with acetone give the percentage of free perrhenate (R_f = 1). The percentage of reduced-hydrolyzed rhenium as well as other unidentified impurities were determined using ITLC-SG strips developed with a 5% aqueous solution of glycine (R_f = 0). The chromatographic behaviour of ¹⁸⁸Re(V)DMSA and ^{99m}Tc(V)DMSA on ITLC-SG strips developed with either 5% glycine or acetone are shown in Fig. 1. As expected, ^{99m}Tc(V)DMSA, ¹⁸⁸Re(V)DMSA and non-radioactive Re(V)DMSA have similar chromatographic behaviour.

The effect of various parameters such as temperature, reaction time, volume and activity on the labelling yield were evaluated. At room temperature or even after boiling for

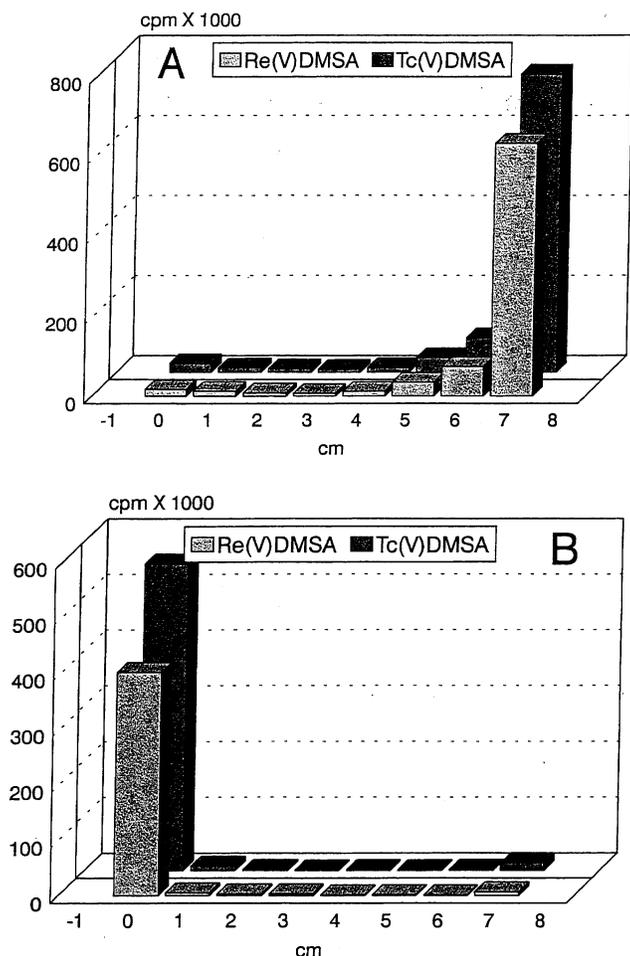


Fig. 1. Chromatographic behaviour of $^{188}\text{Re}(\text{V})\text{DMSA}$ and $^{99\text{m}}\text{Tc}(\text{V})\text{DMSA}$ on ITLC-SG strips developed with: (A) 5% glycine and (B) acetone.

short times, the reaction is not completed in reasonable time. High labelling yields were achieved when the reaction vial was embedded in boiling water for 30 minutes or autoclaved for 20 minutes.

Emphasis was placed on the investigation of the effect of the reaction volume on the radiochemical yield. The lyophilized kits were reconstituted with various volumes (1.0–6.0 mL) of ^{188}Re -perrhenate while the activity was 10 ± 2 mCi (Table 1). Reaction volumes between 1 and 4.5 mL resulted in radiochemical purity over 95%, which is acceptable for radiopharmaceuticals. The labelling yield dropped to 93.8 and 92.7% when the reaction volume was 5 or 6 mL respectively. Thus, the volume of 4 mL was selected as the maximum reaction volume to obtain radiochemical yields higher than 95%. To further investigate the effect of radioactivity on the radiochemical purity of $^{188}\text{Re}(\text{V})\text{DMSA}$, the lyophilized kits were reconstituted with perrhenate at various concentrations of radioactivity (2–40 mCi in 4 mL saline). Interestingly, even at the maximum activity tested, the radiochemical purity was over 95% and no obvious evidence of radiolysis was observed at least 2 hours after labelling.

The *in vivo* kinetics of the radiopharmaceutical in mice are presented in Table 2. After intravenous injection, the radiopharmaceutical is rapidly cleared from the blood stream and excreted into the urine. The highest radioactive con-

Table 1. Product radiochemical purity at specified time following re-constitution of the kit with 1–6 mL perrhenate-188 (10 ± 2 mCi).

Volume mL	Time (hours)	$^{188}\text{ReO}_4^-$ %	$^{188}\text{ReO}_2^*$ %	$^{188}\text{Re}(\text{V})\text{DMSA}$ %
1.0	1	0.6	0.6	98.8
	5	0.2	1.4	98.4
2.0	1	0.5	0.5	99.0
	5	1.7	1.3	97.0
3.5	1	0.2	0.3	99.5
	5	0.6	4.4	95.0
4.0	1	0.9	3.4	95.7
	5	0.6	4.4	95.0
4.5	1	0.2	4.7	95.1
	5	5.0	1.2	93.8
5.0	1	1.4	11.9	86.7
	5	4.7	3.6	92.7

* Including other unidentified species

Table 2. Biodistribution (%dose/gram) of $^{188}\text{Re}(\text{V})\text{DMSA}$ in mice (4–5 animals per group).

Organ	30 min	2 hours	24 hours
Blood	1.43 ± 0.14	0.27 ± 0.05	0.04 ± 0.01
Liver	0.66 ± 0.01	0.32 ± 0.04	0.27 ± 0.02
Heart	0.48 ± 0.04	0.15 ± 0.04	0.03 ± 0.01
Kidneys	4.06 ± 0.12	2.06 ± 0.15	1.42 ± 0.39
Stomach	0.58 ± 0.03	0.35 ± 0.14	0.34 ± 0.01
Intestines	0.55 ± 0.09	0.14 ± 0.03	0.35 ± 0.12
Spleen	0.49 ± 0.08	0.22 ± 0.09	0.08 ± 0.01
Muscle	0.36 ± 0.02	0.09 ± 0.04	0.02 ± 0.00
Lungs	0.94 ± 0.07	0.27 ± 0.03	0.07 ± 0.02
Bones	5.18 ± 0.35	3.75 ± 0.39	2.02 ± 0.32
Urine	51.05 ± 0.61	65.02 ± 2.56	70.44 ± 3.51

centration was observed in bone and kidneys. The uptake in other tissues was not especially high. The *in vivo* data were found to be similar to those of $^{99\text{m}}\text{Tc}(\text{V})\text{DMSA}$ (Fig. 2). These findings are in good agreement to those previously reported for $^{99\text{m}}\text{Tc}(\text{V})\text{DMSA}$ and $^{188}\text{Re}(\text{V})\text{DMSA}$ [3, 10].

The radiopharmaceutical prepared from this formulation is presently in clinical evaluation. The initial results are

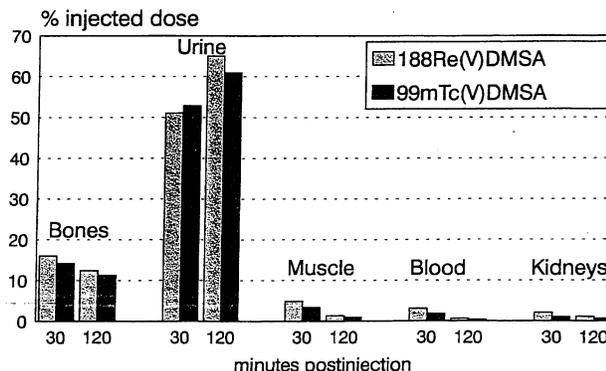


Fig. 2. Comparative biodistribution study between $^{188}\text{Re}(\text{V})\text{DMSA}$ and $^{99\text{m}}\text{Tc}(\text{V})\text{DMSA}$. %Dose per organ at 30 and 120 minutes post injection.

very promising. The distribution of $^{188}\text{Re}(\text{V})\text{DMSA}$ in humans is similar to that of $^{99\text{m}}\text{Tc}(\text{V})\text{DMSA}$. Moreover, the patients showed good response with regard to pain palliation [15, 16].

Conclusions

The formulated kit provides a convenient method for the preparation of $^{188}\text{Re}(\text{V})\text{DMSA}$ for clinical use. Such a method is easily applicable by Nuclear Medicine laboratory departments. It is believed that this method will enhance the wide use of this promising therapeutic agent for the treatment of painful bone metastases, medullary thyroid carcinoma and other soft tissue tumours.

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