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Research Article

Kidney uptake of $^{186/188}$Re(V)-DMSA is significantly reduced when the reducing agent is changed from stannous ion to metabisulfite

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Summary

We describe the work carried out on the preparation of $^{186/188}$Re(V)-DMSA, which showed lower kidney retention, formulated by using metabisulfite as the reducing agent. The complex was prepared by reducing $^{186/188}ReO_4^-$ (100 μg, 0.54 μM, ~150 MBq) in the presence of $Na_2S_2O_5$ (30 mg, 0.15 mM) and reacting with DMSA (10 mg, 0.05 mM) in saline at pH 3.5 and at 80°C for 1 h. The complex was characterized by TLC using acetone and saline as two different solvent systems. Reverse phase HPLC carried out using isocratic system with 90:10 water/acetonitrile mobile phase showed the existence of four species. Biodistribution studies were carried out in albino mice with $^{186/188}$Re(V)-DMSA prepared via metabisulfite reductant [$^{186/188}$Re(V)-DMSA (MBS)] as well as stannous chloride reductant [$^{186/188}$Re(V)-DMSA ($SnCl_2$)]. The distribution patterns were similar except for kidney uptake. Kidney retention in case of $^{186/188}$Re(V)-DMSA (MBS) was lower [0.68 (± 0.06)%/g] than that observed in case of $^{186/188}$Re(V)-DMSA ($SnCl_2$) [2.93(± 0.93)%/g] after 24 h p.i. Though bone uptake was initially similar with both the preparations, there was substantial decrease in bone activity after 24 h p.i. with

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$^{186/188}$Re(V)-DMSA (MBS). *In vitro* cell uptake studies were carried out with $^{186/188}$Re(V)-DMSA (MBS), $^{186/188}$Re(V)-DMSA ($SnCl_2$) and ^{99m}Tc(V)-DMSA using Ehrlich Ascites Tumor Cell Lines. Approximately 15% cell uptake was observed with $^{186/188}$Re(V)-DMSA (MBS) as well as with $^{186/188}$Re(V)-DMSA ($SnCl_2$) and was comparable with that of ^{99m}Tc(V)-DMSA (19%). Our studies indicate that $^{186/188}$Re(V)-DMSA prepared by using metabisulfite as a reducing agent has potential for use in targeted radiotherapy of medullary thyroid carcinoma. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: rhenium 188; ^{186}Re(V)-DMSA; ^{188}Re(V)-DMSA; medullary carcinoma; tumor therapy

Introduction

^{99m}Tc(V)-DMSA has been extensively studied as a tumor-imaging agent and is being used in imaging medullary thyroid carcinoma,[1–3] head and neck tumors[4,5] and metastases from breast carcinoma in liver, brain and skeleton.[6–8] Rhenium analogues $^{186/188}$Re(V)-DMSA (^{186}Re $t_{1/2} = 90$ h, $E_\beta = 1.07$ MeV, $E_\gamma = 137$ keV and ^{188}Re $t_{1/2} = 17$ h, $E_\beta = 2.12$ MeV, $E_\gamma = 155$ keV) have been synthesized and characterized for targeted radiotherapy of cancer.[9–12]

Preparation of ^{99m}Tc(V)-DMSA and $^{186/188}$Re(V)-DMSA for clinical studies is performed using stannous chloride as the reducing agent.[11, 13–16] Both the complexes are similar in their tumor-targeting behavior but differ in localization in kidney. An early study in a single patient reported greater kidney accumulation of ^{186}Re(V)-DMSA than ^{99m}Tc(V)-DMSA.[17] However, the higher kidney uptake of ^{186}Re(V)-DMSA observed in a single patient[17] has not been substantiated in more recent studies,[18,19] where both tracers showed similar renal uptake. Applications of this promising therapeutic agent are limited due to high kidney retention.

Attempts to decrease the renal uptake of $^{186/188}$Re(V)-DMSA pharmacologically by inhibition with blocking agents have proved to be difficult.[14,20] One study reported the preparation and biodistribution of tin-free ^{188}Re(V)-DMSA.[21] Although the HPLC radiochromatogram of the complex revealed no significant difference in isomeric composition of tin-free and tin-containing ^{188}Re(V)-DMSA, a significant decrease in kidney uptake was observed in case of tin-free ^{188}Re(V)-DMSA. We have earlier carried out preparation of $^{186/188}$Re(V)-DMSA using tin as reducing agent. *In vivo* studies with the preparation in rats

exhibited marginally higher kidney uptake (5–6% 24 h p.i.) compared to that of ^{99m}Tc(V)-DMSA (2–3% 24 h p.i.)[14] and hence the present studies were directed towards the preparation of $^{186/188}$Re(V)-DMSA with low kidney uptake.

Here we report the results of our studies on the preparation, biodistribution and *in vitro* tumoral cell uptake of $^{186/188}$Re(V)-DMSA prepared using metabisulfite as reductant, denoted as [$^{186/188}$Re(V)-DMSA (MBS)], and its comparison with $^{186/188}$Re(V)-DMSA prepared with tin as reductant, denoted as [$^{186/188}$Re (V)-DMSA ($SnCl_2$)].

Results and discussion

Preparation of $^{186/188}$Re (V)-DMSA (MBS)

Several experiments were carried out to optimize the conditions for obtaining a maximum complexation yield. The effect of pH on complexation yield was studied by adjusting the reaction mixture to different pH values using 1 M NaOH solution. DMSA complexes with Re could be obtained in high yields in acidic medium. Complexation yield decreased in alkaline pH. The major radiochemical impurity indicated by TLC chromatogram was perrhenate. The optimum pH used for complexation was 3.5 (Figure 1) and a complexation yield of 98% could be acheived.

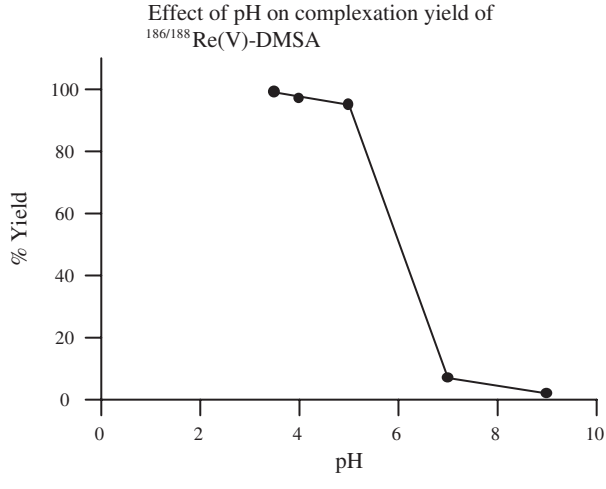

Figure 1. Effect of pH on complexation yield of $^{186/188}$Re(V)-DMSA

The effect of DMSA concentration on complexation yield was studied at pH 3.5 using 30 mg of $Na_2S_2O_5$. The optimum amount of DMSA required for complexation was 10 mg/vial (Figure 2). Thirty millligrams of the reducing agent $Na_2S_2O_5$ was required to achieve quantitative complexation while 10 mg DMSA and 100 μg (0.54 μM) rhenium was used (Figure 3). All studies on optimization of reaction pH and reagent concentration were carried out at 80°C for 2 h. At the optimal pH and reagent concentrations, complexation studies were carried out for different time periods at 80°C. The yields increased with time and optimum time required for complexation was 1 h.

TLC and HPLC were used as quality control techniques to determine complexation yields which were estimated to be >95% by both the methods. In TLC studies using acetone as the solvent, reduced hydrolyzed rhenium/technetium along with the complexes remained at the point of application and free perrhenate/pertechnetate migrated to the solvent front. In TLC using saline, the complexes migrated with the solvent front along with free perrhenate/pertechnetate and reduced hydrolyzed rhenium/technetium remained at the point of application. Free perrhenate content was found to be <2%, while the impurity due

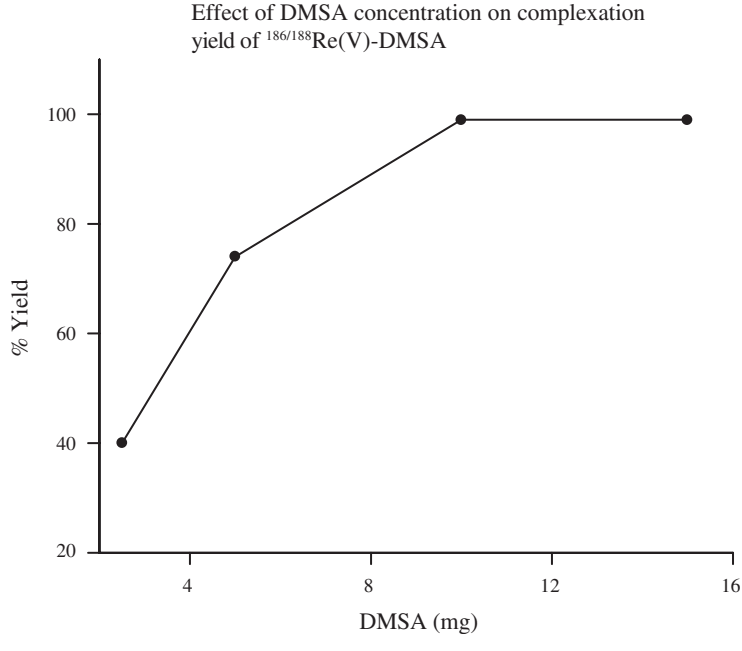

Figure 2. Effect of DMSA concentration on complexation yield of $^{186/188}$Re(V)-DMSA

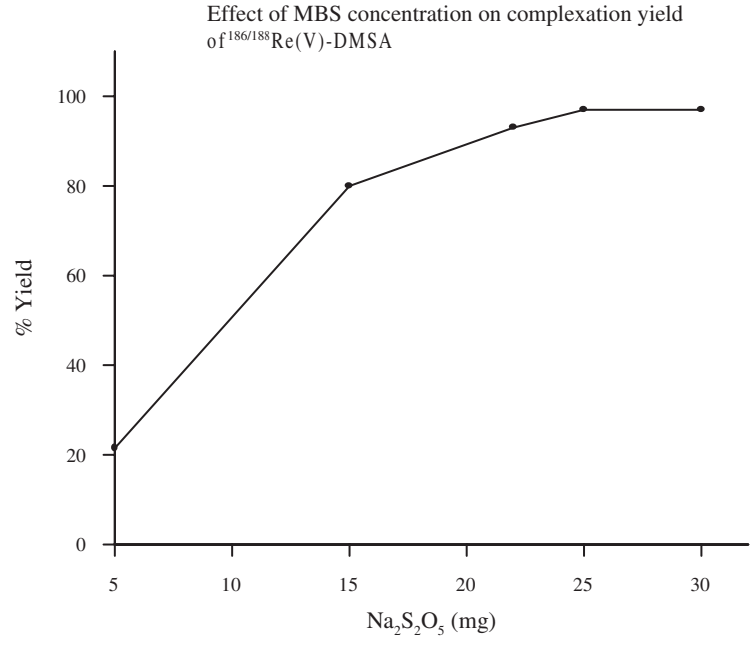

Figure 3. Effect of MBS concentration on complexation yield of $^{186/188}$Re(V)-DMSA

to reduced hydrolyzed rhenium was ~2%. The HPLC pattern of the $^{186/188}$Re(V)-DMSA ($SnCl_2$) with isocratic system using water:acetonitrile (90:10) mobile phase is shown in Figure 4. About 95–97% of the injected activity was recovered after passing through the column. The chromatogram indicated the presence of three main isomers of the radioactive complex with retention times of 6.7 ± 0.3, 13.7 ± 1 and 29.4 ± 1 (min). From the elution pattern, the different isomers appear to be anti, syn-endo and syn-exo, respectively, with traces of a fourth species (5%) of unknown structure which eluted between the anti and syn-endo isomers (retention time 8.7 ± 0.3 min) as reported earlier.[21,22] HPLC chromatogram of the $^{186/188}$Re (V)-DMSA (MBS) (Figure 5) revealed a similar pattern but the yield of the fourth species which eluted between the anti and syn-endo isomer was significantly higher (~ 20%). The composition of each species formed with complexes prepared with the two different reductants mentioned above is given in Table 1. In the case of $^{186/188}$Re(V)-DMSA ($SnCl_2$), the 'anti' isomer was predominant (~43–46%), while in case of $^{186/188}$Re(V)-DMSA (MBS), the yield of 'anti' isomer decreased to 28–30% with a corresponding increase in the

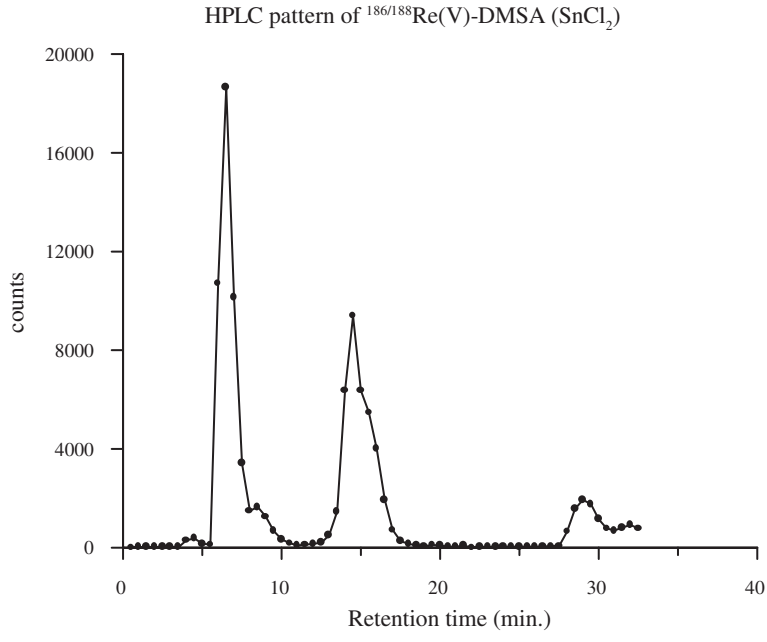

Figure 4. HPLC pattern of $^{186/188}$Re(V)-DMSA ($SnCl_2$)

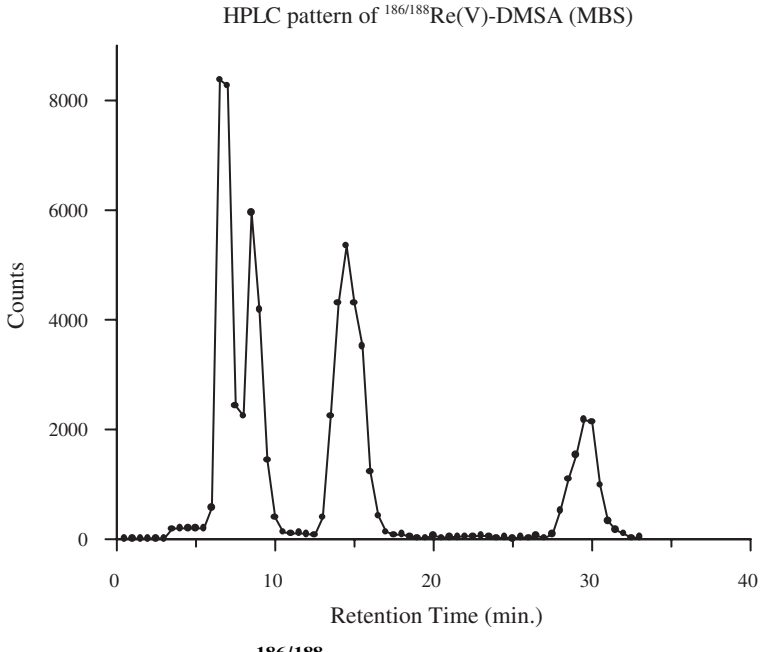

Figure 5. HPLC pattern of $^{186/188}$Re(V)-DMSA (MBS)

Table 1. Yield of different species of $^{186/188}$Re(V)-DMSA ($SnCl_2$) and $^{186/188}$Re(V)-DMSA (MBS) separated by HPLC

	Peak 1 (anti) [a]6.9 ± 0.25 (%)	Peak 2 (—) [a]8.6 ± 0.47 (%)	Peak 3 (syn–endo) [a]14 ± 0.83 (%)	Peak 4 (syn-exo) [a]29.2 ± 1.3 (%)
^{186}Re(V)-DMSA ($SnCl_2$)				
Batch 1	46	3.0	36.5	12
Batch 2	43	5.5	36	11
Batch 3	44	5.7	37	8
^{186}Re(V)-DMSA (MBS)				
Batch 1	28.5	21.5	35	8
Batch 2	29	23	29	10.4
Batch 3	30	22	33	13.5

[a] Retention time in minutes with S.D., $n = 3$.

fourth species between anti and syn-endo isomers. The free perrhenate associated with the complex was $<2\%$ (retention time 4.7 ± 0.2 min).

The stability of the complex prepared under optimum conditions was studied for 1 week at room temperature. The complex was found to be stable with radiochemical purity $>95\%$ at the end of 1 week. Individual yields of the different species eluted over HPLC remained unchanged with time.

Biodistribution studies

Results of the biodistribution studies of $^{186/188}$Re(V)-DMSA (MBS) and $^{186/188}$Re(V) DMSA ($SnCl_2$) are given in Table 2. No significant difference in the blood clearance as well as uptake in major organs was noticed except in the kidneys with both the complexes. The kidney retention of $^{186/188}$Re(V)-DMSA ($SnCl_2$) was 2.93 ($\pm$ 0.93)%/g after 24 h. In case of $^{186/188}$Re(V)-DMSA (MBS) the corresponding kidney uptake value was 0.68 ($\pm$ 0.06)%/g. The amount of $^{186/188}$Re(V)-DMSA injected in both the cases was nearly identical. Another interesting observation was that the bone uptake in mice with $^{186/188}$Re(V)-DMSA ($SnCl_2$) was 5.57 ($\pm$ 1.15)%/g at 1 h p.i. The retention did not decrease with time up to 24 h p.i. but was observed to be slightly increased to 7.45($\pm$ 2.17)%/g. In the case of $^{186/188}$Re(V)-DMSA (MBS), although the femur activity was similar at 1 h pi. 5.54 ($\pm$ 1.7)%/g, it decreased considerably after 24 h p.i. to 1.93 ($\pm$ 0.57)%/g.

Table 2. Biodistribution of $^{186/188}$Re(V)-DMSA in 6–8 week old BALB/c mice (% injected dose/g of organ/tissue)[a]

Organ/ tissue	$^{186/188}$Re(V)-DMSA (SnCl2)			$^{186/188}$Re(V)-DMSA (MBS)		
	1 h p.i.	3 h p.i.	24 h p.i.	1 h p.i.	3 h p.i.	24 h p.i.
Blood	0.70 (0.18)	0.31 (0.12)	0.13 (0.05)	1.01 (0.21)	0.25 (0.15)	0.04 (0.02)
Liver	0.81 (0.14)	0.74 (0.13)	0.52 (0.13)	1.11 (0.11)	1.25 (0.40)	0.40 (0.11)
Kidneys	5.12 (1.64)	6.29 (3.4)	2.93 (0.93)	2.59 (0.41)	2.68 (0.24)	0.68 (0.06)
Stomach	0.36 (0.05)	0.80 (0.19)	0.34 (0.38)	0.45 (0.34)	0.60 (0.08)	0.08 (0.06)
Femur	5.57 (1.15)	6.55 (1.6)	7.45 (2.17)	5.54 (1.7)	4.37 (1.54)	1.93 (0.57)
Intestine	1.13 (0.55)	1.0 (0.57)	1.14 (0.95)	0.38 (0.07)	0.51 (0.29)	0.09 (0.02)
Lungs	2.48 (0.98)	0.46 (0.30)	0.27 (0.16)	1.09 (0.12)	0.61 (0.27)	0.36 (0.13)
Heart	1.19 (0.16)	0.35 (0.26)	0.22 (0.06)	0.40 (0.19)	0.13 (0.13)	0.15 (0.04)
Muscles	0.54 (0.02)	0.23 (0.04)	0.15 (0.05)	0.70 (0.10)	0.09 (0.08)	0.06 (0.04)
Spleen	0.82 (0.66)	0.28 (0.08)	0.54 (0.14)	1.25 (0.21)	—	0.16 (0.03)

[a] Each value is mean ($\pm$ S.D.) $n = 3$.

In vitro cell uptake studies

Cell uptake of $^{186/188}$Re(V)-DMSA was found to be maximum in acidic pH (pH 5.5) and after 10 min incubation time. Cell uptake increased with increase in dilution of the complexes. In case of $^{186/188}$Re(V)-DMSA (MBS) cellular uptake was found to be 14% at optimum dilution (1:300). Similarly, uptake of $^{186/188}$Re(V)-DMSA ($SnCl_2$) was 15% at optimum dilution (1:200) (Figure 6) while the uptake with control ($^{186/188}ReO_4^-$) was 0.8%. Similar studies were also carried out with ^{99m}Tc(V)-DMSA complex following the reported procedure.[23] The maximum cell uptake with diluted complex was found to be 19%. The cell viability was $\sim$98%. After addition of cold DMSA into incubation media there was no change in cell viability.

Experimental

The radioisotope used in this study was a mixture of ^{186}Re and ^{188}Re ($^{186/188}$Re) which was produced by irradiating 5 mg of natural rhenium in the Dhruva reactor at a flux of 6×10^{13} n/cm^2/s for 7 days followed by 4 days cooling. The sample was dissolved in 5 ml of 2 M HNO_3 and processed as ammonium perrhenate by the method reported earlier.[24] The perrhenate obtained had $>$99% radiochemical purity, 34 mCi/mg (1.26 GBq/mg) of specific activity and a radioactive concentration of

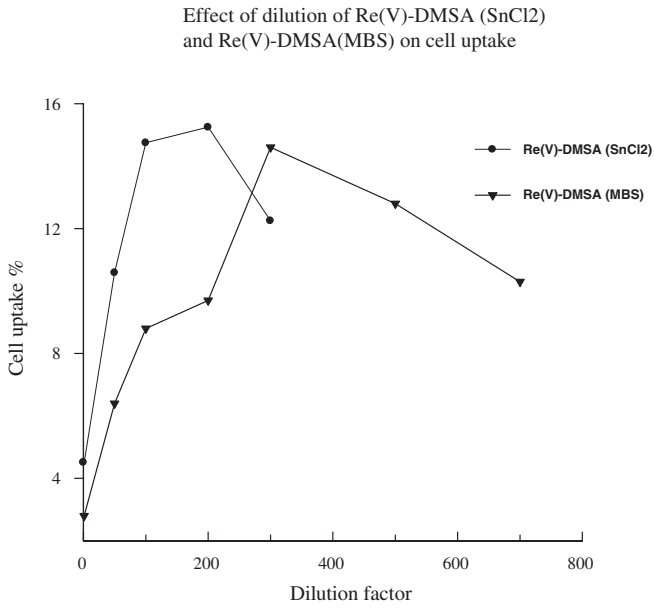

Fig. 6. Effect of dilution of Re(V)-DMSA ($SnCl_2$) and Re(V)-DMSA (MBS) on cell uptake

16 mCi/ml (0.59 GBq/ml).[14] ^{99m}Tc-(V)-DMSA kit was procured from the Board of Radiation and Isotope Technology, Vashi, New Mumbai. All the chemicals were obtained from Sigma Chemical Co. TLC plates ($7.5 \times 2.5\,cm^2$, coating thickness 0.25 mm) were from M/s J.T. Baker. NaI (Tl) scintillation counter was used for measuring ^{99m}Tc activity; the same was used without any further adjustments for measuring radioactivity of $^{186/188}$Re. Ehrlich Ascites Tumor Cell (EATC) lines for cell uptake studies were procured from the National Center for Cell Sciences (NCCS), Pune.

Preparation of $^{186/188}$Re(V)- DMSA (MBS)

About 30 mg of $Na_2S_2O_5$ (0.15 mM) was dissolved in 0.3 ml of normal saline; 0.2 ml (100 μg, 0.54 μM) of ReO_4^- solution was added to $Na_2S_2O_5$ solution and vortexed. The solution was mixed with a suspension of 10 mg of DMSA (0.05 mM) in 0.1 ml of double distilled water. The reaction mixture appeared turbid. Turbidity disappeared after purging the reaction mixture with nitrogen for 10 min. The purged reaction mixture was heated in a boiling water bath for 1 h after adjusting the pH to 3.5 with 1 M HCl.

Preparation of $^{186/188}Re(V)$-DMSA ($SnCl_2$)

Preparation of ^{186}Re(V)-DMSA ($SnCl_2$) was carried out as per the reported procedure.[14] DMSA (2 mg, 11 μM) dissolved in 0.1 ml of bicarbonate buffer (0.5 M, pH 9), 0.7 ml of normal saline and 0.2 ml (100 μg, 0.54 μM, 4 mCi) of $^{186/188}ReO_4^-$ solution were mixed in a 10 ml vial. To this, 0.02 ml of stannous chloride (20 mg/ml) dissolved in concentrated HCl was added. The reaction mixture was purged with nitrogen and heated in a boiling water bath for 30 min and allowed to cool down to room temperature. The complexes $^{186/188}$Re(V)-DMSA (MBS) and $^{186/188}$Re(V)-DMSA ($SnCl_2$) were characterized by TLC, paper electrophoresis and HPLC.

Characterization of the complexes

Thin layer chromatography

TLC was performed using flexible silica gel plates. About 5 μl portions of the test solutions were applied at 1.5 cm from the lower end of the TLC plate. The strips were developed in two different solvents (acetone and normal saline). The strips were dried, cut into eight equal segments and the radioactivity was measured.

High performance liquid chromatography

Waters dual pump HPLC unit with a PRP reverse phase column (15 cm) was used. The mobile phase used was a mixture of water and acetonitrile (90:10) containing 0.1% TFA. The flow rate was adjusted to 1 ml/min. About 20–30 μl of the sample was injected. Fractions of 0.5 ml were collected and counted in an NaI (Tl) scintillation detector.

Biodistribution studies

Biodistribution studies of $^{186/188}$Re(V)-DMSA (MBS) and $^{186/188}$Re(V)-DMSA ($SnCl_2$) were performed in male albino mice weighing 22–25 g. The complexes were diluted before injection and 80–100 μCi (3–3.7 MBq) of the diluted complexes in 0.1 ml volume was injected through a lateral tail vein and the mice were sacrificed after different time intervals (1, 3 and 24 h) by cervical dislocation. Three mice were used for each time point. The tissues and organs were excised and

counted with an NaI (Tl) scintillation detector with flat geometry (15 cm diameter). Distribution of activity in different organs was calculated as percent injected dose/g. Blood activity was calculated by assuming blood volume to be as 7% of the body weight. (All the biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.)

In vitro cell studies

Cell uptake studies were carried out with $^{186/188}$Re(V)-DMSA (MBS) and $^{186/188}$Re(V)-DMSA ($SnCl_2$) using EATCs. The studies were also carried out with ^{99m}Tc(V)-DMSA for comparative evaluation. Preparation of ^{99m}Tc(V)-DMSA was carried out as per the reported procedure.[15]

Conclusion

A simple method for the preparation of $^{186/188}$Re(V)-DMSA (MBS) is described. Biodistribution studies with the complex exhibited lower kidney uptake as compared to $^{186/188}$Re(V)-DMSA ($SnCl_2$). Cell uptake studies of the complexes with EATCs showed similar uptake (15%), indicating the possibility of exploring $^{186/188}$Re(V)-DMSA (MBS) as a potential therapeutic agent for medullary thyroid carcinoma as well as for bone pain palliation.

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