



Preparation and biodistribution of rhenium-188 labeled albumin microspheres B 20: a promising new agent for radiotherapy

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Abstract

Intra-arterial infusion of labeled particles is an effective method for endoradiotherapy of tumors. In this study, we radiolabeled biodegradable HSA microspheres (mean diameter = 25 μm) with the short-lived beta-emitter ^{188}Re available from the alumina-based $^{188}\text{W}/^{188}\text{Re}$ generator system. After 1 h 35–40% of the relative large amount of Sn(II) chloride required for effective reduction of Re(VII) for efficient attachment to the particles is precipitated as an amorphous coat of tin hydroxide colloid on the particle surface. The final ^{188}Re bound to the particles was found to be stable in vitro. The radiolabelling yield was >90%. The biological half-life was >250 h and demonstrated sufficient in vivo stability after i.v. injection in Wistar rats. Because of the attractive properties of ^{188}Re and the uniform particle size and stability, in vivo, this new agent is an attractive candidate for endoradiotherapy of tumors after selective catheterization. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The endoradiotherapy of tumors by selective catheterization has been proven to be an effective local treatment for cancer. The radionuclide should be retained completely in the endotumoral microvascular system during the first passage. This method allows to achieve a high radionuclide concentration in the tumor after intraarterial (i.a.) injection, thus overcoming the disadvantages of systemic application which is limited

by the radiosensitivity of non-target tissues. Radionuclides used over the last decade are most ^{90}Y , and in some cases ^{166}Ho (Andrews et al., 1994; Lau et al., 1998; Hafeli et al., 1995; Turner et al., 1994; Mumper et al., 1991; Harpert, 1996). The transport away from the vascular bed is restricted for particles with a mean diameter larger than about 10 μm . So far particles labeled with a beta-emitter are not approved as radiopharmaceuticals in Europe.

Rhenium-188 is a promising short-lived beta-emitter (physical half-life = 17 h, maximum beta-energy = 2.11 MeV) for in-house production of radiopharmaceuticals for therapeutic purposes (Knapp et al., 1995). The con-

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venient on-demand availability of carrier-free ^{188}Re from a $^{188}\text{W}/^{188}\text{Re}$ generator (Knapp et al., 1997; Callahan et al., 1989) allows a prompt response to clinical demands. Gamma emission (155 keV) makes distribution studies possible with common nuclear medicine techniques.

Radioactively labeled human serum albumin (HSA) microspheres have been widely used in clinical nuclear medicine especially for lung scanning since 1969 (Rhodes et al., 1969). One advantage of HSA microspheres compared to other labeled particles (e.g. human macroalbumin) is the uniformity of their size with a mean particle diameter of 25 μm . Another advantage is the biocompatibility and in vivo degradability. In contrast to preparations of ^{188}Re proposed for synovectomy (Wang et al., 1995, 1998) (styrene divinylbenzene copolymer resin, rhenium sulfur colloid) the occlusion of blood vessels following i.a. application is only temporary because sulfur colloids are too small to cause permanent occlusion (mean diameter 1–5 μm). Our own experiences showed a good digestibility of astatine (^{211}At) labeled HSA microspheres (Wunderlich et al., 1986). Finally, basic HSA microspheres B20 are a radiopharmaceutical commonly used for $^{99\text{m}}\text{Tc}$ labeling (Mallinckrodt medical, Germany).

We have succeeded in labeling HSA microspheres with ^{188}Re in an advantageous manner. The yield depends considerably on the reaction conditions. In this paper we present studies on the efficient processing of ^{188}Re HSA microspheres and on the stability of the ^{188}Re bond to the microspheres.

2. Material and methods

2.1. Labeling of the microspheres

Highly pure carrier free $[\text{}^{188}\text{Re}]\text{NaReO}_4$ was obtained in 20 ml of normal saline from an alumina-based $^{188}\text{W}/^{188}\text{Re}$ generator (Oak Ridge National Laboratory, TN, USA) (Knapp et al., 1997; Callahan et al., 1989).

The labeling process was carried out in the presence of tin chloride. For example, 1 ml of ^{188}Re sodium perrhenate eluate (185 MBq) in 0.9% saline was mixed in a glass vial with 3 mg of gentisic acid (20 μmol , Sigma), and 3.9 mg of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (17 μmol , Sigma), and 2.5 mg HSA microspheres B20 (300 000–500 000 particles, the content of the original kit vial version for $^{99\text{m}}\text{Tc}$ labeling, Mallinckrodt medical, Germany) by sonication. Then, the vial was heated to 95 °C and shaken for 1 h. After centrifugation, the sediment was washed with 0.1 N HCl, twice with water, and resuspended in 50% (v/v) Ultravist 30 (Nycomed, Germany) in 0.9% saline.

We tested the effects of incubation time (0–90 min),

the concentration of the reductant $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (0–40 $\mu\text{mol}/\text{ml}$), and the specific radioactivity available per particle.

Tin precipitated on the particle surface was determined by Isotope Dilution Analysis (IDA) with $[\text{}^{113}\text{Sn}]\text{SnCl}_2$ (Polatom, Swierk, Poland) measuring the gamma-emission of the daughter nuclide $^{113\text{m}}\text{In}$ (392 keV). For IDA 1 MBq $[\text{}^{113}\text{Sn}]\text{SnCl}_2$ was mixed with $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ solved in saline with gentisic acid (concentrations see above). After labeling (0–90 min) the radioactivity on the particles was determined as usual.

The microspheres were investigated by light microscopy and digital scanning electron microscopy (DSM 982 Gemini, Zeiss, Oberkochen, Germany). After labeling, the grainsize distribution of the microspheres was measured with a particle measurement system (PMS) based on single particle light extinction (TU Dresden, Germany).

To estimate the in vitro stability, samples of the final preparation of the particles were incubated with human plasma for 48 h. Aliquots of the suspension were removed after 3, 24 and 48 h, respectively, centrifuged and counted in a well-type scintillation counter (Cobra II, Packard, USA).

Instant thin layer chromatography (ITLC) (Gelman Sciences, USA) on glass fiber sheets was performed to characterize the radioactivity in the supernatant. Free perrhenate and reduced hydrolysed rhenium were determined in two separate systems using acetone and 0.9% saline as solvent, respectively.

2.2. Biodistribution studies

The biodistribution of ^{188}Re labeled albumin microspheres B20 was studied after intravenous injection in the tail vein of 3 Wistar rats for each time point (0.33, 4, 24, 48, 96 h) using the lungs as a model for a well-perfused tumor. For injection of the radiopharmaceutical and to immobilize the rats during the scintigraphic examination the animals were anesthetized using xylazine and ketamine. The animals were kept in plastic boxes and fed and watered ad libitum. They were sacrificed by inhalation of carbon dioxide. Various organs and tissues were removed, and radioactivity content was counted in a well-type gamma counter. Tissue concentrations of radioactivity were calculated as percent of injected dose per gram and injected dose per organ. Permission of the local ethics committee was obtained and all animal experiments were performed according to German government legislation and accepted international standards in biomedical research.

Whole body scans were performed 10 min to 96 h p.i. using a single head gamma camera (CX 250, Picker, USA) with a high-energy general-purpose collimator.

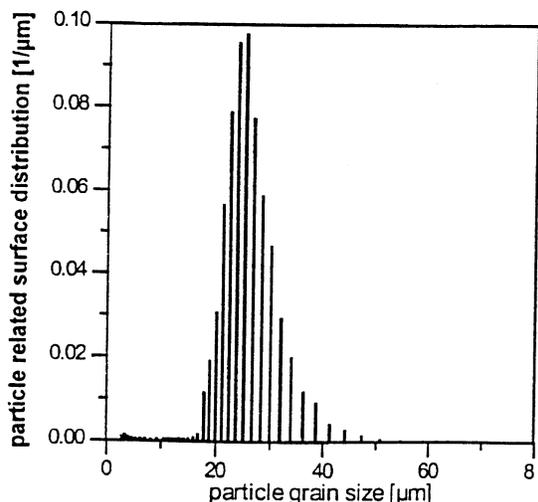


Fig. 1. Particle diameter related surface distribution of the microspheres B20 after labelling with ^{188}Re .

3. Results and discussion

In our studies we tried to characterize the microspheres after labeling, we estimated the effects of reaction time and reductant concentration on the yield, and determined the accessible specific radioactivity of the particles.

After labeling the microspheres remained in a narrow grainsize distribution. 95% of the surface area to be labeled belonged to particles with a diameter between 17 and 40 μm , (modal value 25 μm) (Fig. 1).

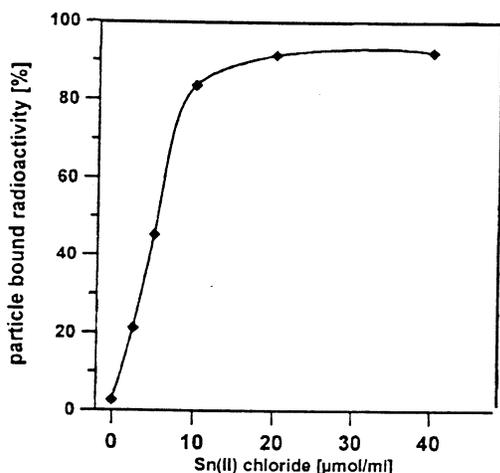


Fig. 2. Effect of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ on particle bound radioactivity, 2.5 mg microspheres B20 and 20 μmol gentisic acid per ml reaction suspension, reaction time 90 min.

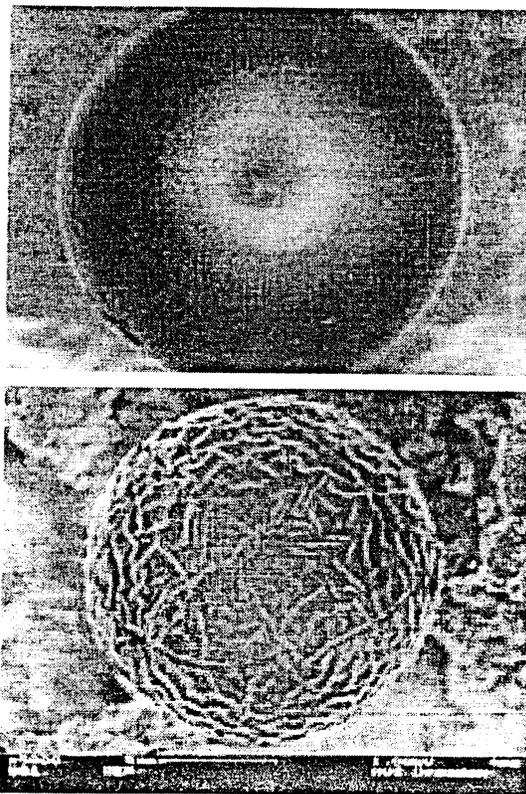


Fig. 3. Scanning electron micrographs of HSA microspheres B20 before (above) and after (bottom) labeling reaction.

The particle labeling process is time-consuming and depends on $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ concentration at pH 2 determined by the gentisic acid concentration (Fig. 2). Above the optimum concentration of 20 μmol $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ per ml the solution becomes increasingly cloudy and the product is visibly mixed with tin hydroxid colloid. Other groups applied even much more tin chlorid (up to 200 mg vs 3.9 mg per ml reaction solution) and lower pH for labeling of radiation synovectomy agent (Wang et al., 1998).

One reason of such a relatively high amount of tin for the labeling reaction on a carrier free level was shown by the electron micrographs of the labeled particles. Scanning electron micrographs showed that after the labeling the originally smooth glossy particle surface is coated with an amorphous shell of about 1 μm thickness, probably a tin hydroxid precipitate. After drying under vacuum, a shrunken and very structured surface is observed (Fig. 3). Light microscopy showed no differences between coated and uncoated particles. The radioactivity in the supernatant was defined by ITLC after 30 min reaction time as 30% $\text{ReO}_2 \cdot x\text{H}_2\text{O}$ (reduced, hydrolyzed rhenium) and 70% ReO_4^- and

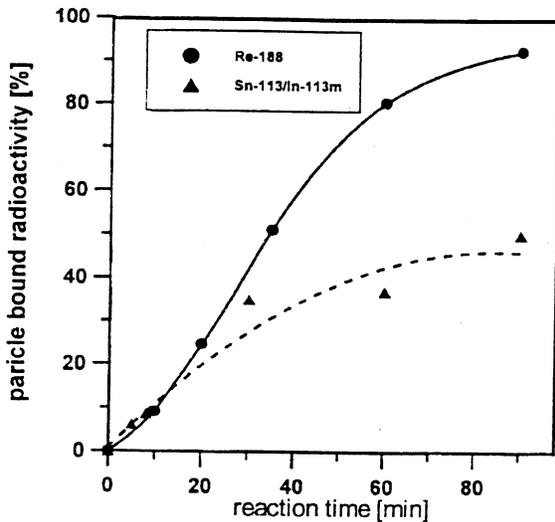


Fig. 4. Effect of reaction time on particle bound rhenium (^{188}Re) and particle bound tin ($^{113}\text{Sn}/^{113\text{m}}\text{In}$), 2.5 mg microspheres B20, 20 μmol gentisic acid and 17 μmol $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ per ml reaction suspension.

after 90 min reaction time as 48% $\text{ReO}_2 \cdot x\text{H}_2\text{O}$ and 52% ReO_4^- . This means that the rhenium reduction is time-consuming and result probably in $\text{ReO}_2 \cdot x\text{H}_2\text{O}$.

Therefore, the particle labeling (coating) may be achieved by a combination of the reduction reaction of Re(VII) with Sn(II) and a particle surface-related coprecipitation effect of tin hydroxid colloid with high adsorption capacity and reduced, hydrolyzed rhenium.

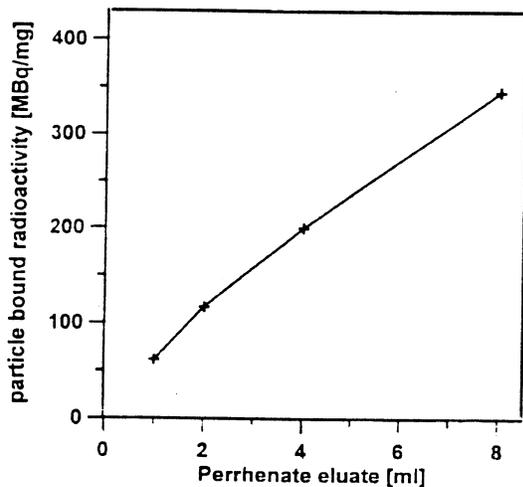


Fig. 5. Specific radioactivity of the HSA microspheres B20 depending on the generator eluate volume (concentration of Sn and gentisic acid per ml = const.).

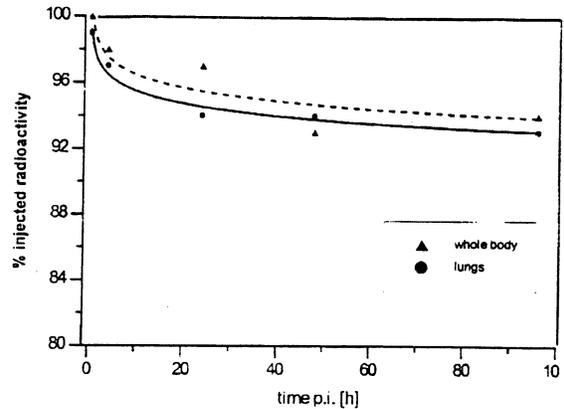


Fig. 6. Retention of ^{188}Re in the lungs and in the whole body of Wistar rats after i.v. injection of [^{188}Re]HSA microspheres B20 (time corrected).

Depending on the reaction time, yields up to 90% for ^{188}Re are achievable. Usually, after 1 h incubation under optimal reaction conditions (see above) the yield is approx. 80% (Fig. 4). The quantity of tin precipitated on the particle surface is time-dependent and was determined by IDA with ^{113}Sn . After a reaction time of 60 min and washing as usually 35–40% of the tin was found in the particle sediment.

Fig. 5 shows that ^{188}Re on microspheres can be concentrated to a high specific radioactivity, but the concentration of tin and gentisic acid per ml solution must kept constant. Maximum labeling was found to be 500 MBq/mg HSA microspheres (2.5–4.2 kBq/particle). Thus for therapeutic purposes, radiation dose per particle is adjustable over a wide range. The particles were found to be stable in vitro in human plasma as 87% of the ^{188}Re was bound to the microspheres after 48 h of incubation.

The biodistribution results revealed excellent in vivo stability with low uptake in the non-target tissues (Table 1). After i.v. injection, the microspheres were effectively trapped in the pre-capillary arterioles of the lungs. Small microspheres ($< 10 \mu\text{m}$) were collected in the RES of liver and spleen. There was only a very slow decrease of radioactivity in the lungs. 96 h p.i. about 90% of the total ^{188}Re was found in the lungs (Fig. 6). The effective half-life of the ^{188}Re labeled particles was 15.9 h, based on a biological half-life of more than 250 h in the lungs. Since after five physical half-lives only about 3% of the administered radioactivity is left to be effective for the endoradiotherapy, the achieved labeling stability is considered sufficient. On the contrary, a small increase of radioactivity could be observed outside the lungs in the gastrointestinal tract ($< 1\%$ of administered dose) and in the kidneys ($< 2\%$ of administered dose). Probably, the ^{188}Re

Table 1

Biodistribution of ^{188}Re HSA microspheres B20 (% of injected dose/whole organ) in selected organs and whole body (gamma camera) after i.v. injection in Wistar rats, mean of 3 animals for each time; in addition the biodistribution of perrhenate (all corrected to the time of injection), $T_{b1,2}$ —biological half-life in the lungs (h)

Time (h)	Lungs	Liver	Spleen	Kidneys	Stomach	Femur	Bowel	Blood	Thyroid	Body	$T_{b1,2}$
0.33	98.6	0.42	0.02	0.28	0.05	< 0.01	0.08	0.15	< 0.01	100.0	–
4	98.3	2.40	0.29	0.35	0.37	0.01	0.23	0.24	0.02	98.0	–
24	94.6	1.15	0.10	0.85	0.11	0.01	0.21	0.08	< 0.01	97.0	–
48	92.9	0.81	0.07	1.36	0.12	0.01	0.17	0.09	< 0.01	90.8	> 250
96	92.2	0.89	0.10	1.23	0.05	0.01	0.12	0.04	< 0.01	93.5	–
$^{188}\text{ReO}_4^-$, 48 h	0.08	0.03	< 0.01	0.01	0.30	< 0.01	0.06	0.06	< 0.01	16.1	11

released during the slow degradation of the particles was reoxidized rapidly and excreted predominantly by the kidneys and was characterized as perrhenate by ITLC.

The biodistribution and biological half-life of [^{188}Re]perrhenate are clearly different from rhenium microspheres (Table 1). Perrhenate was rapidly excreted by the kidneys and 48 h p.i. only a small accumulation in the stomach wall and lumen of about 1% was counted. Thus, perrhenate released from the labeled microspheres will not be measured in the particle retaining tissues.

4. Conclusion

We conclude that HSA microspheres B20 are useful carriers for ^{188}Re because of their uniform size, their biokinetics and excellent in vivo stability. The preparation procedure is not very difficult. During the labeling process an amorphous coat of tin hydroxide is precipitated on the particle surface. Total time needed for preparation is < 2 h, and more than 70% of the original ^{188}Re is labeled to the particles. These are the radiopharmaceutical prerequisites that allow selective intraarterial therapy potentially using [^{188}Re]HSA microspheres B20 as an approved radiopharmaceutical. The particles should be degradable in vivo, thus repeated application through the same artery seems possible.

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