HEPATIC TUMOR RADIOEMBOLIZATION IN A RAT MODEL USING RADIOACTIVE RHENIUM (\textsuperscript{186}Re/\textsuperscript{188}Re) GLASS MICROSPHERES

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Purpose: The aim of this study was to fully characterize newly developed radioactive rhenium glass microspheres in vivo by determining their biodistribution, stability, antitumor effect, and toxicity after hepatic arterial injection in a syngeneic rat hepatoma model. The dose response of the tumors to increasing amounts of radioactive \textsuperscript{186}Re and \textsuperscript{188}Re microspheres was also determined.

Methods and Materials: Rhenium glass microspheres were made radioactive by neutron activation and then injected into the hepatic artery of Sprague–Dawley rats containing 1-week-old Novikoff hepatomas. The biodistribution of the radioactivity and tumor growth were determined 1 h and 14 days after injection.

Results: Examination of the biodistribution indicated a time-dependent, up to 7-fold increase in Novikoff hepatoma uptake as compared to healthy liver tissue uptake. After 14 days, the average T:L ratio was 1.97. Tumor growth in the rats receiving radioactive microspheres was significantly lower than in the group receiving nonradioactive microspheres (142% vs. 4824%, \( p = 0.048 \)). Immediately after injection, 0.065% of the injected radioactivity was measured in the thyroid; it decreased to background levels within 24 h.

Conclusion: Radioactive rhenium microspheres are effective in diminishing tumor growth without altering hepatic enzyme levels. The microspheres are safe with respect to their radiation dose to healthy tissue and radiation release in vivo and can be directly imaged in the body with a gamma camera. Furthermore, rhenium microspheres have an advantage over pure beta-emitting microspheres in terms of preparation and neutron-activation time. In sum, this novel radiopharmaceutical may provide an innovative and cost-effective approach for the treatment of nonresectable liver cancer. © 1999 Elsevier Science Inc.

Rhenium radioisotope, \textsuperscript{186}Re, \textsuperscript{188}Re, Microspheres, Liver tumor, Hepatoma, Animal model, Radioembolization

INTRODUCTION

Malignant neoplasms of the liver, both primary and metastatic, are some of the most common tumors worldwide. In the USA, 18,500 new primary tumors of the hepatic and biliary tree are diagnosed each year, with an estimated 12,300 deaths annually (1). In addition, 150,000 cases of metastatic liver tumors develop yearly, mostly from adenocarcinomas of the colon, rectum, stomach, and pancreas (2). Because complete curative surgical resection is often not possible, current treatments for the majority of these lesions are limited to chemotherapy and radiation (3). Unfortunately, none of these regimens has clearly improved patient survival (4).

The regional administration of antineoplastic agents through the hepatic artery is one strategy that has been developed to improve tumor response because both primary and metastatic liver tumors receive the majority of their blood supply from this vessel (5–7). Injection into the hepatic artery selectively delivers these drugs to the tumor, thus maximizing the antineoplastic effect while sparing toxicity to the surrounding healthy liver. One of the more promising groups of agents is the beta-emitting particulate radiopharmaceuticals, namely microspheres (ceramic, plastic, polymer, resin, or glass) labeled with \textsuperscript{90}Yttrium (\textsuperscript{90}Y). Several reports of human trials, in which both primary and metastatic liver tumors have been treated with \textsuperscript{90}Y-microspheres, are encouraging (8, 9). The clinical use of \textsuperscript{90}Y-glass microspheres (Theraspheres\textsuperscript{®}) is approved in Canada, and further approvals in the USA and Europe are expected shortly. Unfortunately, \textsuperscript{90}Y-glass microspheres have two major shortcomings. First, they require several weeks of neutron irradiation before radiotherapeutic amounts are produced. Second, \textsuperscript{90}Y is a pure beta-emitter, and the microspheres, therefore, cannot be directly imaged to verify dose distribution and retention (10).

To address these shortcomings, we recently developed novel rhenium-containing poly (lactic acid) microspheres.
that contain metallic rhenium particles. The two naturally occurring rhenium isotopes, $^{185}\text{Re}$ and $^{187}\text{Re}$, have large cross-sections for neutrons, and these microspheres can easily be neutron-activated in a reactor within a few h, yielding therapeutic amounts of the beta-emitters $^{186}\text{Re}$ and $^{188}\text{Re}$. However, these biodegradable microspheres are unable to withstand the high neutron fluxes in a nuclear reactor that are necessary to achieve the high specific activity required to treat liver tumors. This problem has been resolved by incorporating the rhenium metal into magnesium alumino borate glass microspheres that can withstand high neutron fluxes. These glass microspheres (Fig. 1) can incorporate more than 50 wt% rhenium oxide ($\text{ReO}_2$) and are chemically stable in vitro (12). The beta-emitting $^{186}\text{Re}$ and $^{188}\text{Re}$ microspheres emit electrons with a maximal energy of 1.1 and 2.1 MeV, respectively. These energies translate into a treatment range of a maximum of 6 and 11 mm in soft tissue, respectively. Further, the two rhenium radioisotopes emit 9.5% ($^{186}\text{Re}$) and 15.0% ($^{188}\text{Re}$) of their radiation as $\gamma$-rays, at energies of 137 and 155 keV, respectively. This allows the use of a gamma camera for semiquantitative, real-time dose titration and distribution control during injection into the patient.

The aim of this study was to fully characterize these newly developed rhenium glass microspheres in vivo by determining their biodistribution, stability, antitumor effect, and toxicity to healthy tissue after hepatic arterial injection in a syngeneic rat hepatoma model. Furthermore, the dose response of the tumors to increasing amounts of radioactive $^{186}\text{Re}$ and $^{188}\text{Re}$ microspheres was determined.

**MATERIALS AND METHODS**

**Microsphere preparation**

Rhenium glass microspheres were prepared by first making a magnesium-alumino-borate glass (12). A 40 mol% $\text{B}_2\text{O}_3$, 40 mol% $\text{MgO}$ and 20 mol% $\text{Al}_2\text{O}_3$ (Alfa Aesar, Ward Hill, MA) glass was melted in a platinum crucible at 1350°C. The melt was poured onto a stainless-steel plate, allowed to harden, and then crushed using a porcelain mortar and pestle into particles smaller than 44 μm. Fifteen wt% $\text{ReO}_2$ powder (Aldrich, St. Louis, MO) was mixed with the crushed glass by wet-ball milling in ethanol for approximately 12 h. The slurry was then dried at 50°C, placed in a covered platinum crucible, and heated in a furnace at 1050°C for 10 min. After quenching the sintered powders, a foamy glass mixture formed. The glass mixture was crushed to particles smaller than 44 μm. These particles were spheroidized by melting them in a propane/air flame (13). The spheres were wet-sieved using acetone so that the final microspheres were 25 to 32 μm in diameter (Fig. 1).

The final washes were done using acetone and methanol.

**Neutron activation of the microspheres**

For the biodistribution study, 30 mg of microspheres were weighed into a quartz vial, sent to the Ohio State University reactor in Columbus, Ohio, and placed directly in the neutron beam for 2 h at a neutron flux of $1.5 \times 10^{13}$ n/cm$^2$/s. The amount of $^{186}\text{Re}$ and $^{188}\text{Re}$ radioisotopes and possible contaminants were determined using a high-purity germanium detector coupled to a multichannel analyzer (EG&G Ortec, Oak Ridge, TN). The radioactive microspheres were delivered overnight and used the following day.

**Animal model and microsphere injection procedure**

The Novikoff hepatoma is a highly chemo- and radioreistant tumor induced by administration of 4-dimethylaminoazobenzene to a male Sprague–Dawley rat (14). This chemoresistance mimics the resistance often seen in human liver neoplasms. In addition, the hepatoma can be cultured easily in minimal essential media supplemented with 4 mM glutamine and 10% fetal bovine serum, retains its potency over many passages, and grows into localized, round tumors about 5 mm in diameter within a week of injection.

All animals received humane care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health. Male Sprague–Dawley rats ($n = 21$), about 300 g each, received an injection of 500,000 Novikoff hepatoma cells in 100 μl media directly below the capsule of the right hepatic lobe under ketamine and xylazine anesthesia (15).

One week later, a laparotomy was performed and, with the aid of an operating microscope, a PE-50 catheter (Intramedic, Becton Dickinson, Sparks, MD) was inserted into the proper hepatic artery via the gastroduodenal artery. This Catheter 1 (Fig. 2) had been stretched on one end so that the
Final wall thickness was equal to that of a PE-10 catheter, to allow direct insertion into the artery. The unaltered end of Catheter 1 was connected to a saline-filled 1-ml Syringe A with a 22-ga needle. To prepare for the microsphere injection, a 30-cm long PE-50 Catheter 2 was connected to a saline-filled 1-ml Syringe B. A polished 1-cm piece of 22-ga needle was inserted into the free end of Catheter 2 for use as a connector between Catheters 1 and 2. The microspheres in 100 µl saline were then taken up and were entirely contained in Catheter 2, not in Syringe B, which only served as reservoir for the 1 ml of rinsing solution (saline). Immediately before microsphere injection, Catheter 1 was crimped, and Syringe A exchanged for Catheter 2 containing the microspheres. The microspheres were injected slowly, taking about a minute for completion. This method made the injection of virtually 100% of the radioactive microspheres possible, with no contamination.

**General study design**

We examined 1. the antitumor effect of the radioactive microspheres, 2. the biodistribution of the radioactive microspheres, 3. microsphere stability by analyzing the pharmacokinetics of free perrhenate uptake in the thyroid, 4. distribution in the liver and tumor, 5. toxicity, and 6. ease in imaging the liver with a γ-camera. In rats receiving radioactive microspheres, 2 mg of microspheres with varying amounts of radioactivity were injected into the hepatic artery. The tumor was measured with calipers at the time of microsphere injection, and the biodistribution determined in 3 animals each. After sacrifice, rat blood was spun down at 2500 rpm for 10 min and the plasma immediately frozen and stored until complete decay from the treatment group receiving 50.4, 59.2, and 78.1 MBq that were sacrificed at 14 days after injection. The ratio of radioactivity (i.e., microspheres) in tumor compared to surrounding healthy liver (the T:L ratio) was determined in all rats receiving radioactive microspheres.

**In vitro studies** with the radioactive microspheres have shown that a small amount of rhenium is released during the first day. Rhenium is known to be taken up by the thyroid (16) and we, therefore, determined in a separate experiment using the same methods, but a different microsphere batch, the uptake kinetics of rhenium into the thyroid. Thirteen rats were sacrificed at 1 h (n = 5), 8 h (n = 1), 1 day (n = 1), 3 days (n = 1), 4 days (n = 1), 6 days (n = 3), and 13 days (n = 1) after the injection of up to 1.85 MBq of radioactive microspheres into the hepatic artery. The decay-corrected radioactivity was then graphed as thyroid activity after the injection of 1 MBq of radioactivity.

**Tumor size determination**

The tumor volume was used to calculate tumor growth. The tumor was measured with calipers at the time of microsphere injection and again 14 days later; the difference was expressed as the percentage change. For the baseline measurement, it was only possible to measure width a and length b, and the volume was then calculated according to $V = \frac{1}{6}abc$ with the depth being c. Both formulas give the most accurate volumes according to Tomayko and Reynold’s review (17).

**Biodistribution study of the radioactive microspheres**

Three rats each were sacrificed at 1 h and 14 days after microsphere injection, and the biodistribution determined in a γ-counter by counting activity in the total liver, right, left, median, and caudate lobes of the liver, thyroid, blood, heart, lungs, small intestine, stomach, spleen, kidney, right femur, and tumor. The whole organs were weighed and the biodistribution was expressed as activity per gram of tissue (kBq/g).

**Microscopic determination of the rhenium microsphere distribution**

The microscopic distribution of radioactive microspheres in tumor and peritumor regions was determined by cutting 0.5-mm thick, fresh liver tissue sections with a razor blade at room temperature, placing each on a glass slide, and photographing them. An unusually thick slice was used so that the microspheres would not be ejected from the tissue during sectioning because the microspheres are much harder than tissue.

**Toxicity evaluation of the radioactive microspheres**

Short-term (1 h) and intermediate-term (14 days) toxic effects of radioembolization to the liver were evaluated by analyzing hepatic enzymes in 3 animals each. After sacrifice, rat blood was spun down at 2500 g for 10 min and the plasma immediately frozen and stored until complete decay.
of the rhenium radioisotopes. The plasma was then analyzed for alkaline phosphatase as an indicator of biliary toxicity, for the alanine aminotransferase as an indicator of hepatocellular toxicity, and for amylase as an indicator of pancreatic toxicity. For control purposes, the blood of untreated rats (n = 3) and of rats just receiving tumor cells (n = 1) or nonradioactive microspheres plus tumor cells (n = 1) was also analyzed for the same hepatic enzymes.

**Gamma camera imaging**

A rat was injected, in the same way as described under general study design, with 0.5 mg microspheres containing 31.1 MBq of $^{186}$Re. The activity was chosen to reflect an intermediate amount given to the treatment group, which received both tumor cells and radioactive microspheres. One h later, the rat was placed on its stomach on a Searle $\gamma$-camera with a circular measurement field of 38 cm in diameter. A 150-$\mu$l calibration vial containing 5.4 MBq of $^{186}$Re in 20 $\mu$l saline was placed to the right of the animal. The rat was imaged for the total of 400,000 counts (3.5 min).

**Statistical analysis**

To compare dose with tumor volume, tumor growth, and the T:L ratio, Pearson’s correlation coefficient was calculated after transforming the data logarithmically (log base 10). Two animals from the treatment group were excluded: In the first rat, which had received 78.1 MBq, the tumor grew outside the liver and attached to the abdominal wall. In the second rat, which had received 36.3 MBq, the tumor was hard and larger than 1 cm in diameter at the time of injection. This second rat was the only one that died (postinjection Day 8), and its left lobe was greatly taken over by the tumor. Also, groups receiving no radiation treatment were compared to treated groups on tumor volume and growth. The untransformed values for tumor volume and tumor growth were compared using the nonparametric Wilcoxon rank sum test. A 1-sample $t$ test was used to determine if the T:L ratio differed significantly from 1 at either time-point and overall. A T:L ratio greater than 1 indicates that tumor uptake is greater than liver uptake.

The $t$ test was used to compare the biodistribution data and enzyme levels between the two time-points. If the $t$ tests underlying assumption of equal variances between time points was violated, then the Cochran and Cox adjustment to the $t$ test was used.

Data were considered significant if $p < 0.05$, and all tests were 2-tailed. All analyses were performed using the SAS® system.

**RESULTS**

**Microsphere application**

The rhenium glass microspheres were perfectly spherical (Fig. 1). Such spheres are mechanically rigid, can be sieved easily and reliably to very narrow size distributions, and are chemically stable, as described in detail by Conzone et al. (12). Neutron activation of 30 mg of rhenium glass microspheres yielded 92.5 MBq (2.5 mCi) $^{186}$Re and 544 MBq (14.7 mCi) $^{188}$Re within 2 h at a neutron flux of $1.5 \times 10^{13}$ n/cm$^2$/s, with no other radioactive contaminants. Increasing the rhenium fraction of the microspheres up to 3-fold, using higher neutron fluxes or using a longer activation time may be used to produce higher amounts of radioactivity.

A slow and complete injection of the radioactive microspheres in the necessary mg amounts was successfully achieved by using the lumen of a PE-50 catheter as the reservoir for the microspheres (Fig. 2). Previous attempts to transfer the glass microspheres (density $\sim$2.9 g/cm$^3$) into a microsyringe always led to a 30% to 60% loss of the radioactive microspheres. This was mainly due to liquid currents at the edges inside the syringe and syringe tips, large dead volumes in the catheters, as well as microsphere adhesion and liquid flow that was too slow at various points of the injection system. The radioactive catheter was easily shielded with a piece of acrylic at least 6-mm thick, and only low-energy $\gamma$-radiation similar to that of $^{99m}$Tc was measured outside the animal after injection. After injection into the hepatic artery, more than 95% of the injected radioactivity was always found in the rat livers.

**Pharmacokinetics and microsphere in vivo stability**

One of the aims of this study was to confirm the good *in vitro* radiochemical stability of the rhenium glass microspheres (12) in an *in vivo* system. In aqueous solution, any radiochemical instability of the glass-embedded rhenium will result in oxidation to Re$^{7+}$ in the form of ReO$_4^-$ This anion, perrhenate, is highly water-soluble and can, therefore, leak from the microspheres as soon as it is produced. During *in vitro* studies, a small amount of perrhenate was released immediately after the microspheres were suspended in water, in a so-called “burst effect,” but no further activity was released in the following 4 weeks, during which the radioactivity decays completely (12). The present *in vivo* biodistribution study confirmed these *in vitro* stability data. A small radioactive peak of 46.2 kBq/g was seen in thyroid activity decays completely (12). The present *in vivo* biodistribution study confirmed these *in vitro* stability data. 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tissue 1 h after microsphere injection (Fig. 3). The pharmacokinetics of perrhenate is such that it is almost completely excreted within 24 h. The biological half-life in the thyroid may, thus, be estimated from the pharmacokinetic curve as 6 h.

**Biodistribution data**

The biodistribution of the radioactive microspheres was time- and organ-dependent (Table 1). Most nontarget organs took up amounts of radioactive rhenium that were only slightly higher than twice background (1 kBq/g) at the 1-h time-point, and most showed background activity by the 14-day time-point.

Importantly, the only redistribution was to the lung tissue. Although the difference between the two time-points was not statistically significant (owing to the large standard deviation at 14 days), apparently some microspheres escape from the liver and then become trapped in the lungs.

### Table 1. Comparison between the biodistribution data collected at 1 h and 14 days after injection of radioactive microspheres (3 rats per time point)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time-point</th>
<th>Mean (kBq/g)</th>
<th>SD (kBq/g)</th>
<th>Range (kBq/g)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Hour 1</td>
<td>0.466</td>
<td>0.019</td>
<td>0.451–0.485</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>0.000</td>
<td>0.000</td>
<td>0–0.063</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>Hour 1</td>
<td>0.614</td>
<td>0.725</td>
<td>0.122–1.447</td>
<td>0.30 (C)</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>0.037</td>
<td>0.033</td>
<td>0–0.063</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>Hour 1</td>
<td>0.300</td>
<td>0.081</td>
<td>0.244–0.396</td>
<td>0.26 (C)</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>104.669</td>
<td>117.475</td>
<td>12.965–237.085</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>Hour 1</td>
<td>0.159</td>
<td>0.022</td>
<td>0.137–0.181</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>0.022</td>
<td>0.037</td>
<td>0–0.063</td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>Hour 1</td>
<td>0.185</td>
<td>0.093</td>
<td>0.200–1.721</td>
<td>0.33 (C)</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>0.792</td>
<td>0.814</td>
<td>0.200–1.721</td>
<td></td>
</tr>
<tr>
<td>Right kidney</td>
<td>Hour 1</td>
<td>2.712</td>
<td>0.056</td>
<td>0.211–0.318</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>0.007</td>
<td>0.011</td>
<td>0–0.019</td>
<td></td>
</tr>
<tr>
<td>Left kidney</td>
<td>Hour 1</td>
<td>0.263</td>
<td>0.074</td>
<td>0.178–0.318</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>0.022</td>
<td>0.041</td>
<td>0–0.070</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>Hour 1</td>
<td>0.540</td>
<td>0.407</td>
<td>0.152–0.966</td>
<td>0.48 (C)</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>3.611</td>
<td>6.231</td>
<td>0–10.804</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>Hour 1</td>
<td>46.187</td>
<td>6.175</td>
<td>39.912–52.259</td>
<td>0.006† (C)</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>0.411</td>
<td>0.710</td>
<td>0–1.228</td>
<td></td>
</tr>
<tr>
<td>Femur</td>
<td>Hour 1</td>
<td>0.148</td>
<td>0.026</td>
<td>0.122–0.174</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>0.063</td>
<td>0.107</td>
<td>0–0.185</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>Hour 1</td>
<td>9.909</td>
<td>3.434</td>
<td>7.245–13.783</td>
<td>0.39 (C)</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>34.702</td>
<td>39.246</td>
<td>7.030–79.617</td>
<td></td>
</tr>
<tr>
<td>Liver Total</td>
<td>Hour 1</td>
<td>2519.700</td>
<td>140.600</td>
<td>884.300–2823.100</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>1387.500</td>
<td>114.700</td>
<td>1298.700–1517.000</td>
<td></td>
</tr>
<tr>
<td>Right lobe</td>
<td>Hour 1</td>
<td>2083.100</td>
<td>1047.100</td>
<td>884.300–2823.100</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>1472.600</td>
<td>477.300</td>
<td>925.000–1772.300</td>
<td></td>
</tr>
<tr>
<td>Left lobe</td>
<td>Hour 1</td>
<td>2445.700</td>
<td>214.600</td>
<td>2257.000–2675.100</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>1224.700</td>
<td>307.100</td>
<td>891.700–1502.200</td>
<td></td>
</tr>
<tr>
<td>Median lobe</td>
<td>Hour 1</td>
<td>2654.500</td>
<td>414.400</td>
<td>2216.300–3045.100</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>1502.200</td>
<td>392.200</td>
<td>1073.000–1846.300</td>
<td></td>
</tr>
<tr>
<td>Caudate lobe</td>
<td>Hour 1</td>
<td>2771.300</td>
<td>1013.800</td>
<td>1602.100–3367.000</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>1309.800</td>
<td>370.000</td>
<td>888.000–1572.500</td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>Hour 1</td>
<td>3226.400</td>
<td>1291.300</td>
<td>1912.900–4499.200</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>3873.900</td>
<td>876.900</td>
<td>3252.300–4495.500</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different between 1 h and 14 days by t test (p < 0.05); (C) = Cochran and Cox adjustment to t test.
† All data are decay corrected, given in kBq/g tissue and normalized to the injection of 37 MBq of $^{186/188}$Re.

**Macroscopic and microscopic distribution of microspheres**

The macroscopic microsphere distribution throughout the liver was relatively homogeneous, with $2.52 \pm 0.14$ MBq/g tissue 1 h after the injection of 37 MBq of the rhenium microspheres, when compared with the microsphere concentrations in the different lobes (Table 1). The tumor uptake at the same time-point was $3.23 \pm 1.29$ MBq/g tissue, resulting in a mean T:L ratio of 1.28 (Table 2). By the 14-day time-point, the difference between liver and tumor distribution had widened to $1.39 \pm 0.11$ and $2.74 \pm 0.87$ MBq/g tissue, respectively. When the treated rats ($n = 13$; 12 plus the 3 in the biodistribution study minus the 2 excluded from analysis) were analyzed, the T:L ratio was 1.97 at 14 days after microsphere injection. Although the T:L ratio tends to be larger than 1, the difference from the measured T:L ratio is not statistically different from 1 (Table 2). However, the ratio of almost 2 (twice as many microspheres per gram of tumor than per gram of healthy liver tissue) might be clinically relevant.
Microscopic examination showed that microsphere distribution was unequal and much less homogeneous in the liver sections than the macroscopic analysis revealed. Single microspheres (Fig. 4A), as well as small chains of microspheres (Fig. 4B) were typically dispersed throughout the liver tissue. It seems that many of the microspheres do not reach the capillaries, but remain in the arterioles. “Hot spots” were generally near larger vessels, indicating that the first microspheres to enter the smaller vessels were trapped and caused the subsequent microsphere accumulations.

Another finding is that the microspheres often accumulated near the tumor borders. The new capillaries produced by tumor-growth–related angiogenesis seem to be too small to disperse the microspheres deep into the tumor. The use of smaller sized microspheres might, thus, be more effective, at least in rats.

**Hepatic toxicity**

No significant differences ($p < 0.05$) were found between the liver enzyme activity in treated rats at 1 h and 14 days after microsphere injection (Table 3). In addition, enzyme levels did not significantly differ between radiation-treated rats and untreated rats ($p = 0.18, 0.94,$ and $0.12$ for alkaline phosphatase, alanine aminotransferase, and amylase) (Fig. 5). The hepatic toxicity of the treatment, therefore, appears to be low, as might be expected from patient studies in which doses of up to 100 Gy were directed to the liver using the beta-emitter $^{90}\text{Y}$ (18).

**Tumor treatment results**

The tumor sizes at the time of injection were comparable for the rats receiving radioactive microspheres ($330 \pm 159 \text{ mm}^3$; min. 87, max. 607 mm$^3$) and for the rats receiving nonradioactive microspheres ($155 \pm 103 \text{ mm}^3$; min. 87, max. 355 mm$^3$).

Tumor growth was suppressed in treated rats during the 14-day study period (Table 4). However, dose and tumor volume were not significantly correlated (Table 5), although there is a trend toward less growth, and even shrinkage, at higher activities. The tumor growth difference between an-

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>3</td>
<td>1.30</td>
<td>0.59</td>
<td>1.25</td>
<td>0.74 to 1.91</td>
<td>−0.16 to 2.76</td>
<td>0.47</td>
</tr>
<tr>
<td>14 days</td>
<td>10</td>
<td>1.97</td>
<td>2.31</td>
<td>1.20</td>
<td>0.05 to 7.76</td>
<td>0.32 to 3.62</td>
<td>0.22</td>
</tr>
<tr>
<td>All*</td>
<td>13</td>
<td>1.81</td>
<td>2.04</td>
<td>1.25</td>
<td>0.05 to 7.76</td>
<td>0.58 to 3.04</td>
<td>0.18</td>
</tr>
</tbody>
</table>

The statistical analysis determines if the tumor-to–liver ratio is statistically different from 1. SD = standard deviation; CI = confidence interval.

* 1 h plus 14-day groups.

![Fig. 4](image_url) Light microscopic picture of a 0.5-mm thick section of the caudate liver. (A) Typical microsphere distribution showing single microspheres (arrow) and chains of microspheres (*). The top right quarter of the picture is tumor tissue. (B) A closeup of a chain of 16 rhenium glass microspheres that accumulated at an arterial branching site.
imals that received radioactive or nonradioactive microspheres, however, is significant ($p = 0.048$). The average growth in the Control Group was 4842% whereas growth in the Treatment Group was 142% (Table 4).

**DISCUSSION**

Magnesium alumino borate glass microspheres containing nonradioactive rhenium were successfully created to treat liver tumors by radioembolization. The microspheres, which for this purpose were 25 μm to 32 μm in diameter, can be weighed into quartz ampoules, sterilized, and stored in dry form until the day before use, when the ampoule is neutron-activated in a nuclear reactor to yield radioactive $^{186}$Re/$^{188}$Re microspheres.

The $^{188}$Re and $^{186}$Re radioisotopes are not the only candidates for radioembolization therapy. Another beta-emitter, radioactive $^{90}$Y, is currently used in Canada and has a similar range in tissue (12 mm, as compared to 11 and 6 mm for the two rhenium isotopes, respectively). However, the larger cross-section for neutrons makes activating rhenium microspheres possible within a few h, whereas activating yttrium takes several days to weeks, depending on the reactor used. The activation of naturally occurring rhenium produces a mixture of $^{188}$Re and $^{186}$Re at a ratio of typically 3 to 1, at the time of injection into the patient on the day after neutron activation. A high initial concentration of $^{188}$Re, with a relatively short half-life of 17 h, is radiobiologically preferable because the radiation is given at a high initial dose rate. Many tumor cell lines have been shown to be much more resistant to radiation if the dose rate falls below about 0.40 Gy per h (19). The use of $^{188}$Re will, thus, decrease the cell’s ability to repair sublethal damage and lead to decreased cell survival on a Gy by Gy basis, compared with $^{90}$Y.

Although the low-energy γ-rays of the two rhenium radioisotopes add only a few percent to the therapeutic dose of the β-radiation, they might be useful for diagnostic purposes. The amount and location of radioactive microspheres can, thus, be followed during microsphere infusion, at least semiquantitatively. For dose calculations, the target volume can be estimated from geometric patterns, as observed in true scale scans. Diagnostic embolization to localize the tumor can be done as a separate procedure or just before the therapeutic embolization using either small amounts of the radioactive rhenium microspheres or $^{99m}$Tc-macroaggregated albumin microspheres, as described in detail by Rößler et al. (20, 21). This will allow one to immediately adjust the number of microspheres to be injected, depending on their distribution in the liver. Determination of whether the injected dose is sufficient, therefore, would not have to be based solely on body surface, patient weight, or previous liver scans, but on the actual distribution of rhenium glass microspheres in the liver. Our imaging test using a γ-camera to track radioactive glass microspheres showed that obtain-

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**Table 3. Comparison between the enzyme levels collected 1 h and 14 days after radioactive microsphere injection (3 rats per time point)**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Time-point</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase</td>
<td>Hour 1</td>
<td>139.0</td>
<td>7.2</td>
<td>131 to 145</td>
<td>0.29 (C)</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>252.7</td>
<td>136.6</td>
<td>141 to 405</td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>Hour 1</td>
<td>62.7</td>
<td>27.0</td>
<td>39 to 92</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>109.0</td>
<td>44.4</td>
<td>79 to 160</td>
<td></td>
</tr>
<tr>
<td>Amylase</td>
<td>Hour 1</td>
<td>2046.3</td>
<td>287.5</td>
<td>1715 to 2230</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>2561.7</td>
<td>291.5</td>
<td>2273 to 2856</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different between 1 h and 14 days by $t$ test ($p < 0.05$); (C) = Cochran and Cox adjustment to $t$ test.

SD = standard deviation.
ing images with good resolution and short scanning times is possible with rhenium radioisotopes (Fig. 6).

The intrahepatic injection of rats with rhenium microspheres worked well, and more than 95% of the radiopharmaceutical ended up in the liver and tumor. The T:L ratio of 1.97 at 14 days after injection agrees with published data that the intrahepatic injection of microspheres results in an up to 3-fold enhancement of uptake in the tumor over the healthy liver tissue uptake (22). Higher doses of radioactivity can, thus, be delivered to tumors without unduly toxic effects. Low toxicity is especially important in the liver because an external radiation dose of only 30 Gy leads to liver damage and necrosis. If using radioactive rhenium microspheres permits the dose limit to be doubled, the liver tumor may be completely destroyed. Further, the T:L ratio can be improved in other ways. The two most promising approaches are 1. to use vasoconstrictor drugs to shunt blood away from the normal liver and to the tumor via its differential effects on the normal hepatic arterioles, or 2. to use advanced intrahepatic application techniques (i.e., selective catheterization).

Using vasoactive agents to restrict blood flow to healthy liver tissue will direct the microspheres to the tumor capillaries. Such an approach could be achieved by combining radioembolizing microspheres with a vasoactive agent such as angiotensin II (23–25) or vasopressin (26). Such vasoactive agents have not been used yet with the radioactive rhenium microspheres, but we are planning such tests.

An advanced technique to increase tumor uptake, especially in nonmetastatic solid tumors or primary tumors, is to use superselective catheterization (20). In this procedure, the microspheres are not released into the appropriate hepatic artery, but into an artery that directly leads to a tumor.

Ultrasound-guided delivery of the microspheres also resulted in better target uptake and has been reported to yield a higher cure rate without severe side effects (27). The patients who would benefit the most would, therefore, be those in whom the tumor is limited to one hepatic segment but is unresectable due to size or proximity to major vascular structures. This approach must be tested in a larger animal model because the arteries supplying hepatic tumors in rats invariably are too small for such experiments.

We characterized the hepatic microsphere distribution both macroscopically (whole lobes) and microscopically. Macroscopically, overall twice as much activity was found in the tumor than in the liver parenchyma. All liver segments received similar amounts when distribution was analyzed as activity per gram of tissue. However, microscopic examination revealed that the microsphere distribution was not so homogeneous. Chains of microspheres could be seen at many arterial branching sites, and larger concentrations of microspheres were deposited near the tumor surface. This corresponds to the description of a peripheral “rim” of

Table 4. Comparison of tumor volume and tumor growth between groups receiving radioactive microspheres and those receiving nonradioactive microspheres

<table>
<thead>
<tr>
<th>Variable</th>
<th>Radiation (n = 13)</th>
<th>No radiation (n = 6)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor volume (ml)</td>
<td>0.77 ± 0.65</td>
<td>2.76 ± 3.22</td>
<td>0.17</td>
</tr>
<tr>
<td>Tumor growth (% change in volume)</td>
<td>142.5 ± 83.8</td>
<td>4842.9 ± 10487.4</td>
<td>0.048*</td>
</tr>
</tbody>
</table>

* Significantly different between groups (p < 0.05) by Wilcoxon rank sum test.

Table 5. Correlation of dose with log-transformed tumor growth, tumor volume, and tumor-to-liver ratio

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Pearson correlation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor volume</td>
<td>19*</td>
<td>-0.181</td>
<td>0.46</td>
</tr>
<tr>
<td>Tumor growth (%)</td>
<td>19*</td>
<td>-0.464</td>
<td>0.045†</td>
</tr>
<tr>
<td>Tumor-to–liver ratio</td>
<td>13†</td>
<td>0.401</td>
<td>0.17</td>
</tr>
</tbody>
</table>

* All animals growing tumors after the exclusion of 2 (see statistical analysis).
† Correlation is significantly different from zero (p < 0.05).
‡ All animals receiving radioactivity after the exclusion of 2 (see statistical analysis).

Fig. 6. Gamma-camera image of a nontumor-bearing rat liver after the injection of 31.1 MBq of 186Re glass microspheres into the hepatic artery. The photograph of the rat was merged with the radiographic image to show the imaging setup.
Radioembolization with radioactive rhenium microspheres  • U. HAFELI et al

Thus, greater amounts of radioactivity lead to significantly less tumor growth and even shrinkage.

The only significant change of microsphere location between the 1-h and 14-day time-points was seen in the lungs. This microsphere movement to the lungs is a known side effect that has led, in a few reported clinical cases, to pneumonitis, lung fibrosis, and death (28). It seems to depend highly on the individual’s microvascular anatomy and the microsphere size. Radioactive rhenium microspheres are easily tracked and it is possible to detect any migration to the lungs by using a γ-camera during microsphere injection. Small amounts of the microspheres should, thus, be injected before treatment to determine the expected distribution. Unlike albumin microspheres labeled with 99mTc, our microspheres have a much narrower, controlled size distribution with none of them being smaller than 25 μm or larger than 32 μm. Also, the production of microspheres with nearly any specified size range is possible. No clinical trial comparing differently sized microspheres has ever been done. The microspheres currently used have a nominal diameter of 20- to 30-μm (Theraspheres®), and their use is based on animal experiments (29) and may not be optimal.

One aim of this study was to determine the fate of the small amount of radioactivity leaking from the microspheres. In vitro studies have shown that less than 1.2% of the rhenium is released until complete decay after 32 days (12). The compound released from the rhenium glass microspheres, perrhenate, accumulates in the thyroid, as was expected from an earlier report (16). Perrhenate is then quickly excreted with a biological half-life of about 6 h (Fig. 3). The maximum release from an injection of 18.5 GBq of 186Re/188Re microspheres, which is the maximum treatment dose for an adult with liver tumor (liver size > 5 kg), would, thus, result in 24.5 mGy to the thyroid [for dose calculations using the MIRD scheme, see Conzone et al. (12)]. Such a small dose is comparable to the dose received by the thyroid during a diagnostic 99mTc-pertechnetate scan (20 mGy).

No toxic effects were seen in the rats after the application of up to 78.1 MBq of 186Re/188Re. The radioactivity did not induce radiation hepatitis in the rats, as indicated by the liver enzyme activity levels. Radioactive rhenium microspheres, thus, behave very similarly to 90Y-microspheres, where minimal or no liver function changes had been reported in a clinical trial (30). In addition, no pathological radiation damage, such as hepatic fibrosis or necrosis, was detected. Longer term studies, however, are necessary to determine if such effects may occur. It is important to note that radiation damage takes different forms, depending on the species observed. Lethal veno-occlusive disease, for example, occurs in dogs, pigs, and humans at doses above 30 Gy. Rats, on the other hand, never show this effect (31).

**CONCLUSION**

We found radioactive rhenium microspheres to be effective in slowing down, or even diminishing, liver tumor growth and even shrinkage.
growth without altering hepatic enzyme levels in an extended fashion. These microspheres are safe in regard to biodistribution and radiation release in vivo, and able to achieve almost 2 times higher tumor concentrations than liver concentrations. They can also be directly imaged in the body with a γ-camera. Further, the rhenium microspheres have an advantage over pure beta-emitting microspheres in terms of preparation and neutron-activation time. This novel radiopharmaceutical may, thus, be a good and cost-effective approach for the treatment of liver cancer. Additional potential applications include local use for treating incompletely resected tumors immediately after surgery (tumor-bed application), the radioembolization of other organs, such as the spleen (32), or the use of smaller rhenium microspheres for the radioembolization of small joints (33, 34).

REFERENCES

by combined external-beam irradiation and radioimmuno-
32. Becker CD, Roesler H, Biasiutti FD, Baer HU. Congestive
hypersplenism: Treatment by means of radioembolization of
33. Chinol M, Vallabhajosula S, Goldsmith SJ, Klein MJ, Deutsch
KF, Chinen LK, Brodack JW, Deutsch EA, Watson BA, Tofe
AJ. Chemistry and biological behavior of Samarium-153 and
Rhenium-186-labeled hydroxyapatite particles: Potential ra-
diopharmaceuticals for radiation synovectomy. *J Nucl Med*
1993;34:1536–1542.
34. Johnson LS, Yanch JC. Calculation of beta dosimetry in
radiation synovectomy using Monte Carlo simulation (EGS4).