Production Of Rhenium - Powder With A Jet Mill And Its Incorporation In Radioactive Microspheres For The Treatment Of Liver Tumors

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Abstract:

Metallic rhenium powder of 325 mesh is readily available, but well characterized powder of micrometer size is less easy to obtain. This paper describes the preparation of rhenium powder with an average particle size of 2 µm through the application of a jet mill to 325 mesh material. The particle size distribution was confirmed by light and scanning electron microscopy, and laser diffraction. The change in density and surface characteristics (porosity) was also analyzed.

Jet-milled Rhenium particles were then incorporated into biodegradable poly(lactic acid) microspheres using a solvent-evaporation method. After neutron-activation in a nuclear reactor, 186/188Re-microspheres were directly obtained with no need for further processing before their employment in radiotherapy. The usefulness of the rhenium microspheres for the treatment of liver tumors was tested by determining their biodistribution in rats. The result was a time-dependent, up to five-fold increase in Novikoff liver tumor uptake as compared to normal liver tissue.

We appreciate the help of Joe Talnagi and his crew at the OSU reactor in Columbus, Ohio.
Introduction:

Tumors of the liver are among the most common malignancies in the world. In the U.S., each year about 13,400 new primary tumors of the liver and biliary passages are diagnosed, with an estimated 12,300 deaths (1). This number increases dramatically if one includes the mortality from liver metastases which are responsible for more than 50% of breast, colorectal and prostate cancer deaths (2-4). The prognosis of the affected patients is clearly grave, and improvements in therapy are needed.

One approach to therapy targets hepatic tumors by direct injection of chemo- or radiotherapeutic agents into the hepatic artery, the main source of hepatic tumors' blood supply (5,6). The local potency of this treatment can be increased if these agents are first encapsulated in microspheres. Then, after injection, they become stuck in the tumor due to their size. This approach is called radioembolization therapy, if the cytotoxic agent incorporated into the microspheres is a radioactive drug (7-9).

The use of radioactive microspheres for tumor treatments has a number of advantages. In particular, such microspheres locally irradiate, in a defined, radioisotope dependent fashion, only the next few millimeters surrounding the microsphere while sparing the surrounding normal liver and more distant tissues from toxicity (10). In addition, they allow for the delivery of very high local radiation doses, are relatively easy to use and can be applied in an outpatient protocol in a single session.

The recognition of these advantages led to the development of glass microspheres containing Yttrium-90 (3,6,8). This radiopharmaceutical is approved for use in Canada and commercially available under the name Therasphere® [Nordion International, Kanata, Ontario]. The radioisotope Yttrium-90 has a maximum treatment range of 12 mm in tissue, although most of its energy is deposited within the first 2-3 mm. Since Yttrium-90 is a pure β-emitter, it is very difficult to image quantitatively, thus making the necessary online control during the application almost impossible (11).

The search for a radioisotope which would allow gamma-imaging, coupled with the wish to produce more biocompatible and biodegradable microspheres, led us to develop polymeric poly(lactic acid) microspheres containing metallic rhenium powder (≈ Re-MS) (12). These microspheres were designed to be stable when radioactive, and to biodegrade when non-radioactive, which occurs after a little more than a month. The microspheres are produced non-radioactively, and are then sterilized and stored in lyophilized form. Shortly before use, they are activated in a nuclear reactor thus converting the incorporated, naturally occurring Rhenium into the β-emitting radioisotopes Re-186 and Re-188, which have a maximum range in tissue of 5 and 11 mm, respectively. Additionally, their gamma emissions of 137.2 keV and 155.0 keV, respectively, can be used for gamma-imaging with a gamma-camera, since the diagnostic gold standard 186mTe has almost the same energy line of 140 keV.

This paper aims to characterize the preparation, analysis and properties of the Rhenium component of Re-MS and to determine the biodistribution of this novel radiopharmaceutical in rats with liver tumors.

Methods:

Preparation of the Rhenium-powder: Rhenium powder of 325 mesh size (Rhenium Alloys, Elyria, Ohio) was further reduced in size after drying (60 °C overnight) by four passes on a lined laboratory jet mill S/S (Glina Mills, Clifton, New Jersey) (see Figure 1). The jet mill was run with nitrogen at an input and output pressure of 105 psi and 100 psi, respectively, at a feeding speed of about 10 g Rhenium per minute. The Rhenium powder before and after size reduction was analyzed in dry form in the laser scattering particle size distribution analyzer LA910 (Horiba, Irvine, California), the particle size analyzer Aerosizer Mach 2 (API, Japan) and the surface area analyzer SA3100 (Coulter, Miami, Florida).

Figure 1: Schematic drawing of the fluid bed jet mill used for the pulverizing of Rhenium powder. Large particles were recirculated in the mill and hit, after returning via the down-leg and the O-tube, incoming feed particles in a head-on collision. The small, milled particles left the apparatus through the cyclone discharge hole (drawing with permission of Glina Mills).

Size distribution of the Rhenium-powder by image analysis: 5.3 mg of Re-powder was diluted with 100 µl of xylene and shortly sonicated. 5 µl were then immediately spread on a glass slide and covered with a cover glass. The powder was imaged under oil immersion in a light microscope at 100x magnification after all the xylene had evaporated. Images were taken with a black and white CCD camera (Javelin) connected to a computer and analyzed for particle size using the software Image Pro Plus (Media Cybernetics, Silver Spring, MD). At least 1000 particles were measured per sample. A typical picture seen during analysis with this imaging system is shown in Figure 2 with the unmilled Rhenium powder to the left and the milled one to the right. The particles were counted automatically, but each frame was manually checked and corrected. Automatic systems only work error free if the particles to be analyzed have a defined size and don’t clump.

Preparation of the microspheres: Rhenium microspheres (≈ Re-MS) were prepared by a solvent evaporation method: 225 mg of poly(lactide) (PLA) (Boehringer Ingelheim, Henley, NJ) with a molecular weight of 2000 was dissolved in 1 ml of chloroform and 95 mg of rhenium powder (Rhenium Alloys, Cleveland, Ohio) was added and sonicated. The suspension was then injected into a stirred solution of 6.5% polyvinyl alcohol with a Mw of 78,000 (Sigma, St. Louis, Missouri) and the Re-MS successively filtered through polyester filters of 30, 21, 15 and 10 µm (Spectrum Medical Industries, New Brunswick, NJ), washed with milliQ water and dried in a VirTis lyophilizer (FTS Systems) in weighed 1.5 ml
Pre- or post-removal. A 2.73 µm mesh platinum powder in the jet mill was found to be 2.65 + 1.29 pm in diameter, showing significant change after 4.5 cycles. The size distribution of the particles was measured by measuring the diameter of metal particles, as can be seen in Table 4.

Results:

Table 4: Surface area results of different size fractions of the platinum powder before and after jet milling.

<table>
<thead>
<tr>
<th>Particle size [µm]</th>
<th>Before milling</th>
<th>After 4 jet mill cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8 - 1.9 µm</td>
<td>2.5 ± 0.14 µm</td>
<td>2.5 ± 0.25 µm</td>
</tr>
<tr>
<td>1.9 - 2.0 µm</td>
<td>2.2 ± 0.2 µm</td>
<td>2.2 ± 0.16 µm</td>
</tr>
<tr>
<td>2.0 - 2.1 µm</td>
<td>1.9 ± 0.17 µm</td>
<td>1.9 ± 0.18 µm</td>
</tr>
<tr>
<td>2.1 - 2.2 µm</td>
<td>1.8 ± 0.19 µm</td>
<td>1.8 ± 0.19 µm</td>
</tr>
<tr>
<td>2.2 - 2.3 µm</td>
<td>1.6 ± 0.17 µm</td>
<td>1.6 ± 0.16 µm</td>
</tr>
<tr>
<td>2.3 - 2.5 µm</td>
<td>1.8 ± 0.16 µm</td>
<td>1.8 ± 0.16 µm</td>
</tr>
<tr>
<td>2.5 - 2.7 µm</td>
<td>1.9 ± 0.15 µm</td>
<td>1.9 ± 0.15 µm</td>
</tr>
<tr>
<td>2.7 - 3.0 µm</td>
<td>2.0 ± 0.18 µm</td>
<td>2.0 ± 0.18 µm</td>
</tr>
<tr>
<td>3.0 - 3.2 µm</td>
<td>2.2 ± 0.17 µm</td>
<td>2.2 ± 0.17 µm</td>
</tr>
<tr>
<td>3.2 - 3.5 µm</td>
<td>2.4 ± 0.16 µm</td>
<td>2.4 ± 0.16 µm</td>
</tr>
<tr>
<td>3.5 - 3.8 µm</td>
<td>2.5 ± 0.14 µm</td>
<td>2.5 ± 0.14 µm</td>
</tr>
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</table>

The microscopic analysis results were comparable to those obtained with laser scattering and image analysis (Figure 3). As expected, the size distribution showed a significant amount of nanoparticles, measured by microscopic image analysis of more than 100 particles each. Only the unmilled platinum contained particles above 30 microns.

Figure 3: Size distribution of the platinum particles measured by microscopic image analysis of more than 100 particles each. Only the unmilled platinum contained particles above 30 microns.
Jet milling was not able to completely break the Rhenium particles into small spheres during these 4 runs, as can be seen in the scanning electron microscopy pictures (Figure 4). It looks like the larger, unmilled Rhenium particles consist of tens of small particles fused together on their edges, and the impact of jet milling leads to some of them falling apart.

Figure 4: Scanning electron microscopy pictures of a) unmilled 325 mesh Rhenium powder and b) jet milled Rhenium powder after 4 cycles.

The preparation of Re-MS (Rhenium-microspheres) from unmilled Rhenium-powder and the polymer poly(lactic acid) resulted in irregularly shaped spheres, many of them containing no Rhenium at all. Such microspheres would not be useful for radiocemobilization therapy as they need to be round and well defined in size. Re-MS made with 30 weight% of the milled Rhenium (see Figure 5) looked almost identical to microspheres made only from poly(lactic acid). The surface was nice and smooth and the Rhenium particles seemed to be mainly located in the inside of the microspheres.

Figure 5: Scanning electron microscopy picture of Re-MS prepared with poly(lactic acid) and 30 weight% Rhenium powder (4 jet mill cycles) by a solvent evaporation method.

The neutron activation of the 30 mg of Re-MS for the in vivo biodistribution study resulted in the production of 133.2 MBq (3.6 mCi) of $^{186}$Re and 744 MBq (20.1 mCi) $^{188}$Re with no other radioactive contaminants. These radioactive $^{188}$Re-MS were delivered overnight and used in the experiment on the following day. After injection into the hepatic artery, always more than 95% of the injected radioactivity was found in the liver.

The biodistribution of $^{188}$Re-MS after intraarterial injection is time and organ dependent, as shown in Figure 6. Data from blood, heart, lung, spleen and femur are not shown, since they were all lower than 4 kBq (0.1 μCi)/g of tissue at any time point. The initial surge of radioactive Rhenium in the thyroid to 24 kBq (0.65 μCi)/g of tissue means that a small amount, less than 0.1% of the total injected radioactivity, is loosely bound to the microspheres and released immediately after injection. The thyroid is able to accumulate the oxidized form of Rhenium, the highly water-soluble perrenate $\text{ReO}_4^-$, almost as well as iodine (14). This means that any free circulating perrenate will be concentrated in the thyroid. But it also means that the thyroid uptake can be blocked by giving high doses of potassium iodide prior to radiotherapy. Within 6 hours, the thyroid levels fell to less than 4 kBq (0.1 μCi)/g of tissue, thus pointing to active secretion of perrenate.

Figure 6: Distribution of the radioactive Re-MS to different organs after injection of 7.4 MBq of $^{188}$Re-MS into the hepatic artery of groups of three rats each.

The radioactive $^{188}$Re-MS were homogeneously distributed over the right, left, median and caudate lobe of the liver, ranging from 111 to 259 kBq/g of tissue. The tumors with an average size of 2.14 g however contained up to 407 kBq/g of tissue immediately after injection. The tumor-to-normal tissue ratio was time dependent and peaked after 7 days when it reached 5.0 ± 1.4. The other tumor-to-liver ratios measured were 2.0 ± 0.8 just 1 hour after radioembolization and 1.1 ± 0.6 after 2 weeks.

A closer look at the location of radioactivity in the liver revealed a more heterogeneous picture. The distribution of radioactive microspheres, as detected by autoradiographs of 0.5 mm thick liver sections showed that not all areas contained radioactivity (Figure 7). The hot spots were generally near larger vessels indicating that the first microspheres to enter the smaller vessels were trapped and caused the accumulation of the microspheres that followed.

Discussion:

The jet-milling of Rhenium powder into particles of an average size of about 2 μm made it possible to prepare PLA microspheres containing Rhenium using the standard solvent-evaporation method. Previous attempts at preparation of such microspheres using unmilled Rhenium yielded irregularly shaped and inhomogeneously filled spheres. Some were empty while others contained Rhenium occupying half the sphere. In suspension, these imperfect spheres settled out at very different rates, thus not allowing for their reliable application in vivo. The use of the jet-milled Rhenium, however, made it possible to make homogeneous and round Rhenium-microspheres sized between 5-30 μm. Further sieving yielded 30-40 μm
microspheres ideal for radioembolization of the liver (15). These non-radioactive microspheres can be stored in lyophilized form and, just prior to use, directly neutron-activated in a reactor to yield *Re-MS.

Figure 7: A 0.5 mm thick liver section of a rat after injection of 7.4 MBq of Re-MS as seen a) by the CCD camera b) after film autoradiography and c) after image processing and dose calibration. The maximum width of the liver is 10 mm.

After injection of *Re-MS into the hepatic artery of rats, a biodistribution study showed higher concentrations of *Re-MS in cancerous regions than in normal liver tissue, as predicted. The large time dependency, with a maximum tumor-to-liver ratio of 5 after one week, however, is difficult to explain. Could it be that a re-distribution of the microspheres took place? Or do the biodegradable microspheres exposed to higher enzyme levels in the normal liver tissue break down faster than the *Re-MS in the tumor, and then release and excrete the radioactive Rhenium? After two weeks, the tumor-to-liver ratio had returned to almost unity. Does this mean that the radioactive microspheres in the tumor tissue underwent the same breakdown as the *Re-MS in the normal liver tissue, but just one week later? These explanations are very speculative, since the groups of animals at each time point were too small (between 1 and 3) to obtain statistical significance. Experiments with larger groups of animals as well as the histological exploration of the biodistribution of the Rhenium microspheres in vivo are necessary and planned. Additionally, the measurement of the rats' urine could bring to light currently undetected excretion pathways.

At a micro-level, autoradiography revealed the microsphere distribution in the liver to be highly inhomogeneous. This lack of homogeneity is not ideal for treatment and can be partially overcome through the use of higher, therapeutic activities which will lead to a "leveling" effect. The initial radiation dose delivered by the *Re-MS comes primarily from the beta-emitter *Re. It deposits most of its dose within 2-3 mm, which means that microsphere placed every 3 mm will completely irradiate an entire area or volume. A further enhancement of microsphere distribution into the tumor capillaries, however, is desirable and could be reached by the combined use of radioembolizing microspheres with a vasoactive agent such as angiotensin II (16). This would result in differential size adjustment of the liver and tumor capillaries since the immature tumor capillaries are not able to vasoconstrict as much as the capillaries of the liver, thus further enhancing the tumor’s microsphere uptake.

In summary, microspheres made with jet-milled Rhenium are a convenient new radiopharmaceutical and show promising attributes for application in radioembolization therapy. The observed time-dependence of the tumor-to-liver ratio *Re-MS,* however, necessitates further investigations into the biodegradation and behavior of the *Re-MS.

References: