Neutron-Activated Holmium-166-Poly (L-Lactic Acid) Microspheres: A Potential Agent for the Internal Radiation Therapy of Hepatic Tumors

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Biodegradable Poly(L-lactic acid) microspheres containing neutron-activable ^{165}Ho were designed for internal radiation therapy of hepatic tumors. Spheres composed of Poly(L-lactic acid) (PLA) were prepared with excellent reproducibility containing up to 36% of a holmium complex. The prepared spheres were irradiated in a high neutron flux converting ^{165}Ho to ^{166}Ho (Emax = 1.84 MeV, half-life = 26.9 hr). Thus, these microspheres can be prepared under conditions that do not require the handling of a hazardous radionuclide, and then irradiated just prior to administration. In vitro studies in plasma (n = 6) revealed 97.3% (±1.9) retention of ^{166}Ho in the microspheres after 240 hr. PLA spheres administered via the portal vein in rabbits (n = 6) show 94.5% (±3.4) retention of the original ^{166}Ho activity in the liver after 6 days.

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Despite the efforts of intensive therapeutic interventions the treatment of primary or metastatic liver cancer remains suboptimal based upon the average life expectancy following diagnosis. The average survival time for the untreated liver cancer metastases is 6 mo, with a median time of 1.5 mo (*1*). Internal radiation therapy using microspheres has the potential for overcoming the disadvantages of external beam radiation which is limited by the radiosensitivity of healthy tissue (*2*). Several investigators have considered the administration of therapeutic microspheres via the hepatic artery (*1–3*). In contrast to normal liver tissue, which receives 80% of its blood flow from the portal vein, hepatic malignancies derive their blood supply almost exclusively from the hepatic artery (*4*). Microspheres of the optimum size, administered in this manner, will lodge in the capillary bed of the liver. Highly ionizing negatrons emitted from the neutron-rich nuclides contained in the microspheres deposit their energy over a very short range, thereby selectively irradiating the tumors while sparing normal liver tissue. The application of radiotherapeutic microspheres delivered arterially has been found to have a 6-fold enhancement of the absorbed dose delivered (*5*). In 1987, Erhardt and Day reported on the preparation of activable ^{89}Y-glass microspheres (*1*). In their application, stable ^{89}Y was incorporated in glass microspheres and subsequently irradiated with neutrons to yield ^{90}Y. The glass spheres had several advantages, however, their high density (3.29 g/cc) (*6*) and apparent nonbiodegradability may be considered disadvantages.

The present report deals with the application of biodegradable PLA microspheres (density = 1.4 g/cc) containing ^{165}Ho which can be activated to ^{166}Ho. The PLA microspheres are designed to biodegrade shortly after the complete decay of the ^{166}Ho and, therefore, should present no long-term blood-flow barriers. Our choice of ^{165}Ho as the stable precursor was based on a number of chemical and nuclear properties of the stable isotope as well as the decay characteristics of the resultant radioactive isotope, ^{166}Ho.

MATERIALS AND METHODS

Chemicals

PLA (57,000 mol/wt) was obtained from the Henley Company, Montvale, NJ. Holmium chloride hexahydrate 99.9% was obtained from Rare Earth Products, Chesire, WA. Polyvinyl alcohol (88% hydrolyzed; 78,000 mol/wt) was purchased from Aldrich Chemical Company, Milwaukee, WI. All other chemicals were of analytic reagent grade and were obtained from commercial sources.

Preparation of the ^{165}Ho AcAc Complex

For incorporation into the PLA microspheres, ^{165}Ho acetylacetonate (AcAc) was prepared according to the method of Brown et al. (*7*). Briefly, NH_4OH was added to a holmium chloride solution containing the chelating agent, AcAc (mp = −23°C), until a pH of 7.36 was reached.

Preparation of Microspheres

Chloroform (30.0 ml) containing PLA (1.5 g) and Ho-AcAc (0.9 g) was added to a stirring continuous phase of 1% polyvinyl alcohol in deionized water. After 15 min the oil-in-water emulsion was transferred to a 2000-ml round bottom flask and diluted with 100 ml of deionized water. The solvent was removed by means

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of a rotoevaporator (Model R110 Brinkman; vacuum pressure 14.0 mmHg; water bath = 55–60°C; 25 min) and the precipitated spheres were sonicated and collected on a 20-μm Nylon filter. The spheres were suspended in 800 ml of 0.1 N HCl to remove the unincorporated Ho-AcAc, refiltered, and washed with deionized water. Desired diameter ranges of spheres were obtained by selective sizing using a mechanical microsiever (Model SS-5, Gilson Company, Inc., Worthington, OH) and a series of microsieves. The particle size distribution of the microspheres was verified by optical microscopy. (Nikkon Optiphot; Scientific Instruments, Carpentersville, IL). A Cf-252 neutron source (neutron flux = 10^6n/cm^{2}s) was used to determine the percent incorporation of ^{165}Ho.

Irradiation in High Neutron Flux

For producing high levels of ^{166}Ho activity, the prepared microsphere samples were irradiated in the TRIGA Reactor at the University of Illinois in Urbana. Samples consisting of 50 mg of microspheres (diameter range 10–45 microns) and 150 mg inositol were irradiated in the reactor (600 kW power) for up to 3 hr in a thermal neutron flux of 8.88×10^{12} n/cm^{2}s (with an added epithermal neutron flux of 7.10×10^{11} n/cm^{2}s) and yielded 30–35 mCi of activity.

In Vitro Analysis

The irradiated samples were placed in a four inch segment of pure regenerated natural cellulose dialysis membrane (Fisher, Spectra/Por 7 membranes 50,000 MW cut-off) and submerged in 25 ml human plasma contained in 50 ml polypropylene tubes. The tubes were incubated (37°C; 80 oscillations/min) and aliquots of plasma (100 μl) were taken at selected time points and counted for ^{166}Ho activity. At the end of the release study, the membrane was opened, washed with deionized water, and counted to determine the amount of released ^{166}Ho adsorbed to the membrane. Cumulative %^{166}Ho released was corrected for the ^{166}Ho adsorbed to the membrane.

In Vivo Distribution Studies

For the in vivo distribution studies, the spheres were administered into the portal vein of the six New Zealand white rabbits (rabbit hepatic arteries are too small for facile catheterization). Irradiated samples were suspended in 4.0 ml deionized water with five drops of Tween 80 and were administered into the portal veins via a 24-gauge quik-cath (1.6 cm) catheter. The rabbits were monitored with a gamma camera over the next 144 hr to determine the biodistribution of the spheres and to analyze for subsequent leaching of the ^{166}Ho.

In Vivo Degradation Studies

To evaluate sphere biodegradability, irradiated PLA spheres were administered to three rabbits. The rabbits were killed after 1 hr, 26 days, or 56 days and their livers were removed and fixed in formalin. Samples were stained with hematoxylin and eosin (H&E) and analyzed by light microscopy.

RESULTS

Analysis of the Prepared and Irradiated Microspheres

PLA microspheres (n = 6 batches), irradiated in the Cf-252 neutron source with HoAcAc as a standard, were found to have incorporated 27.7% (±4.9) of ^{165}Ho AcAc or 9.9% (±1.7) of the neutron-activable ^{165}Ho. Irradiation in the high neutron flux had no effect on microsphere size. Pre-irradiated microspheres (n = 6) had a particle diameter of 24.4 (±5.2) microns and irradiated spheres (n = 6) had a particle diameter of 23.6 (±4.9) microns. The addition of inositol, a high melting sugar, proved to be very effective in dispersing the internal heat produced during neutron irradiation and was easily dissolved by the suspending media before administration. A comparison of the infrared spectra of the microspheres before and after irradiation revealed complete maintenance of polymer structural integrity.

In Vitro Analysis

In Figure 1, the in vitro retention profiles of the PLA spheres are plotted as a function of time. The averaged (n = 6) results showed that freshly prepared spheres (shelf-time = 10–18 days) retained 97.3% (±1.9) of the ^{166}Ho after 240 hr. Aged PLA microspheres (shelf-time = 28 wk) exhibited a burst effect with 4% of the activity released in the first hour followed by stabilization over the next 5 days. At 120 hr, rapid release of ^{166}Ho from the microspheres was observed. Figure 2 compares extended retentive studies of two irradiated PLA sphere batches; one prepared using 1% PVA as the continuous phase and the other 3% PVA (more recently investigated). For both studies, no initial burst of ^{166}Ho was observed and >99.0% of the activity was retained after 191.5 hr (99.3% of the initial study activity had decayed). Spheres prepared with 1% PVA began rapidly degrading after 312 hr and had leached 49.4% of the encapsulated material by 406 hr. Spheres prepared with 3% PVA showed superior retention with only 2.6% of the ^{166}Ho released after 382.3 hr.

In Vivo Distribution Studies

PLA microspheres (with 310–950 μCi ^{166}Ho) were administered into six anesthetized rabbits at an injection rate

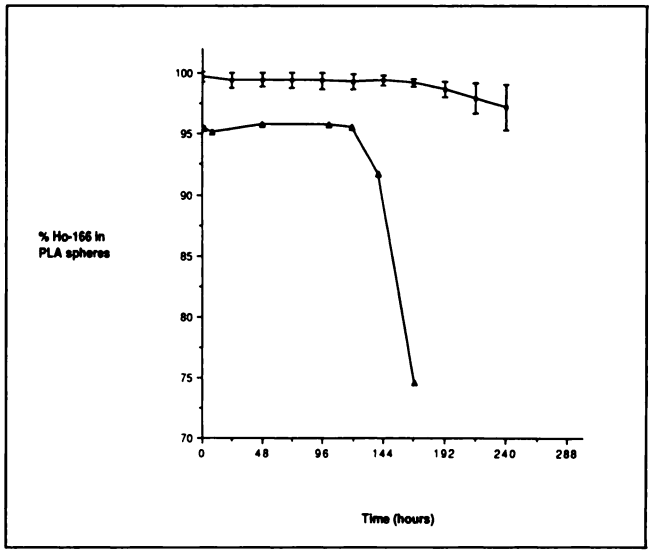

FIGURE 1. In vitro retention of ^{166}Ho in PLA spheres in human plasma. Key: (△) spheres with shelf time of 28 wk; (■) PLA spheres (n = 6) with shelf-time of 10–18 days.

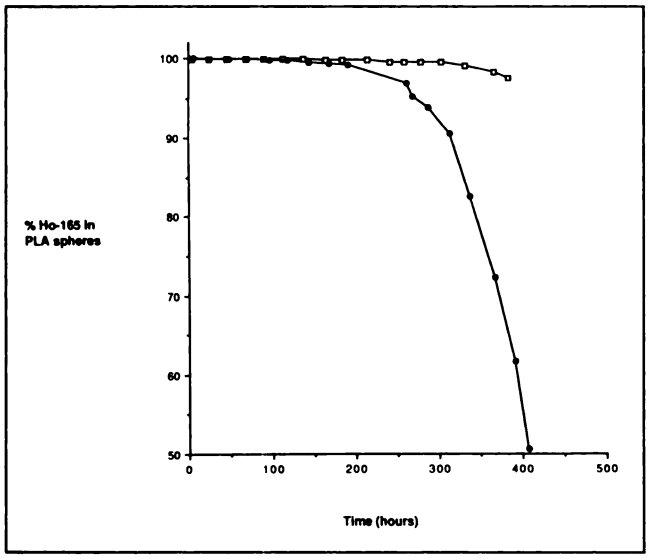

FIGURE 2. In vitro retention of ^{166}-Ho in PLA spheres in human plasma. Key: (●) spheres made from 1% PVA; (□) spheres made from 3% PVA.

of 4 ml/15 sec. Scintiphotos acquired at 24-hr intervals revealed uniform definition of the rabbit livers with little or no detectable leached ^{166}Ho activity. Blood samples acquired at various times during the studies showed only slightly higher than background activity. The average mass of spheres injected into the six rabbits was 24.2 mg (±7.1) corresponding to 3.2 million (±940,000) particles. The rabbits were killed after 144 hr and organs removed and counted, as well as bone marrow extracted from the rabbit femurs. The mean results are summarized in Table 1.

Feces samples collected daily and counted for activity reflected a similar trend for all six rabbits. Holmium-166 found in the feces decreased each day and by 100 hr 89.3% (±9.7) of the total activity in the feces had already been collected. With all rabbits, 100% of the activity in the feces was collected before 144 hr. To calculate radiation doses to the liver, the assumption was made that the activity found in the feces was due only to backflow of the spheres during administration with subsequent deposition in the gastrointestinal tract, and was not reflective of an elimination pathway of leached ^{166}Ho. This appears to be a good assumption since feces activity collected daily decreases rapidly to zero well before 144 hr. Adding the feces activity to the liver activity gives a better indication of the % ^{166}Ho retained in the liver. Table 2 summarizes this data if the previous assumption is made. Radiation doses were then calculated using the following equation: Dose (rads) = $E \times C \times T_{eff} \times (73.8)$; where E is the average beta energy of Ho-166 (0.61 MeV), C is the μCi of ^{166}Ho per gram of organ or tissue, T_{eff} is the effective half-life, and (73.8) is a conversion factor to rads. For radiation doses to the liver, it was assumed that T_{eff} equals T_{phy} and that the activity in the liver was the difference between the administered activity and the activity found in the feces.

TABLE 1
In Vivo Disposition of PLA Spheres in Rabbit (n = 6)

	% Total Dose at 144 hr postadministration
Liver	92.1 (±4.7)
Heart	0.1 (±0.0)
Spleen	0.2 (±0.2)
Kidneys	1.2 (±0.7)
Lungs	0.1 (±0.1)
Gallbladder	0.1 (±0.1)
Urine	3.1 (±2.1)
Feces	2.3 (±1.4)
Femur	0.8 (±0.5)
Bladder	0.0 (±0.0)

The dose to the rabbit liver was 1050.0 rads/mCi (±240.0). This radiation dose appears large due to the small weight of the rabbit liver. Radiation doses to the kidney are expected to be small since activity is rapidly excreted via the urine.

In Vivo Degradation Analysis

Figures 3–5 illustrate the in vivo degradation process of the irradiated PLA spheres. At 1 hr postadministration (Fig. 3), spherical particles with smooth, homogeneous surfaces can be seen embedded in a rabbit liver capillary. At 26 days postadministration (Fig. 4), a small foreign-body giant cell had engulfed the particles, which were noticeably fissured and reduced in size. At 56 days postadministration (Fig. 5), multinuclei large foreign-body giant cells had encapsulated the remaining sphere fragments. Large cavities in the particle matrices were evident. In addition, several void spaces and remnants of microspheres were seen where spheres had already eroded.

DISCUSSION

Holmium-165 (neutron capture cross-section = 64 barns) has a natural abundance of 100% and can be incorporated into PLA microspheres in high yields under non-hazardous conditions. Holmium-166, produced upon activation of ^{165}Ho, is a negatron emitter (Emax = 1.84 MeV) with a maximum soft-tissue range of 8.4 mm and a half-life of 26.9 hr. In addition, ^{166}Ho also emits gamma photons (0.081 MeV) that can be imaged with a gamma camera, but are of low enough photon yield (5.4%) to result in limited absorbed radiation dose to surrounding tissue. The absorbed radiation dose to the liver was cal-

TABLE 2
Retention of PLA Spheres in Rabbit Livers (n = 6) Assuming no Gastrointestinal Elimination of Leached ^{166}Ho

	% Total Dose at 144 hr postadministration
Liver + Feces	94.5 (±3.4)
Leached ^{166}Ho	5.5 (±3.4)
Kidneys + Urine + Bladder	4.3 (±2.7)

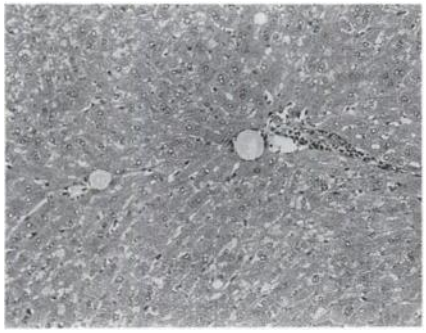

FIGURE 3. Irradiated PLA spheres in rabbit liver, one hour postadministration. (H&E stain analyzed by light microscope at 100×).

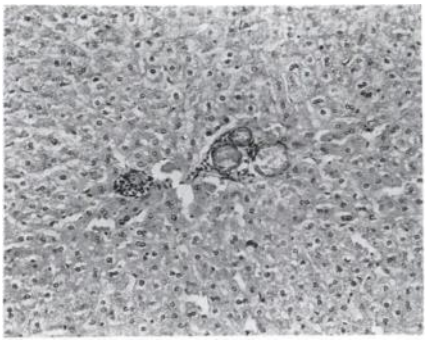

FIGURE 5. Irradiated PLA spheres in rabbit liver, 56 days postadministration. (H&E stain analyzed by light microscopy at 100×).

culated using a model in which 100.0 mCi of the therapeutic microspheres containing ^{90}Y or ^{166}Ho are administered to humans via hepatic artery catheter (Radiopharmaceutical Internal Dose Information Center, Oak Ridge, TN, *written communication*). Although ^{90}Y delivers a higher absorbed radiation dose (28 mGy/MBq) than ^{166}Ho (8.7 mGy/MBq), other factors such as the % natural abundance, neutron capture cross-section, saturation factor, and the isotopic mass of the element must be considered in determining the optimal stable isotope for incorporation into biodegradable PLA spheres. The irradiation time required to produce a therapeutically equivalent dose of ^{166}Ho is 17.6-fold less than that required for ^{90}Y. To deliver an absorbed radiation dose to the tumor equal to that provided by ^{90}Y, roughly three times the number of mCi of ^{166}Ho would have to be administered. However, since the half-life of ^{166}Ho is shorter, this dose would be delivered at a greater dose rate. The advantage of greater dose rates for therapeutic treatments is well documented (*8,9*).

It is apparent that increased shelf-time of PLA microspheres leads to degradation of the polymeric matrix resulting in decreased retentive ability of PLA spheres. This may be the result of oxidative or hydrolytic cleavage of the ester linkages, or the catalysis by small amounts of residual solvent or initiator remaining from polymer preparation (*10,11*). PLA spheres made from a 3% PVA solution provide the optimal retention of ^{166}Ho, which may be attributed to their non-porous and compact surface (*12*). In vivo studies of spheres made from the 3% PVA continuous phase are being initiated.

The in vivo biodistribution studies revealed that 94.5% (±3.4) of the activity deposited in the liver remained in the liver for 144 hr following administration. The loss of activity from the liver could have occurred by three mechanisms. First, smaller spheres not removed by microsieving could escape embolization in the capillary bed of the liver directly after administration, or break away over time. The data showed low bone marrow, lung and spleen activity, so this mechanism was not probable. Second, the microspheres could release ^{166}Ho due to erosion of the polymer matrix and/or solubilization of the ^{166}Ho complex. The higher kidney activity and excreted urine activity provide evidence that leaching of ^{166}Ho in a soluble form constituted the majority of ^{166}Ho not retained in the rabbit liver. Third, a small percentage of spheres could be lost during administration as a result of backflow. We assume that this loss of 2.3% (±1.4) of the initial activity is an administration consideration and should not reflect an elimination pathway of the leached ^{166}Ho. Therefore, if we assume that 94.5% of the ^{166}Ho was retained in the liver after 144 hr, then 5.5% (±3.4) of the ^{166}Ho was actually leached from the spheres. Of this percentage of the ^{166}Ho leached, it was found that 76.9% (±3.6) was excreted via the kidneys.

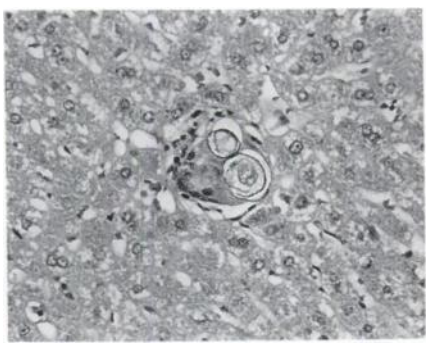

FIGURE 4. Irradiated PLA spheres in rabbit liver, 26 days postadministration. (H&E stain analyzed by light microscopy at 200×).

The novelty of the PLA microspheres extends from the fact that the particles are biodegradable. Ideally, after the ^{166}Ho has fully decayed, the polymer matrix of the spheres erodes with no permanent embolization of the liver. Although degradation of the spheres does not appear to be rapid, the most important goal (containment of ^{166}Ho until the isotope's complete decay) was attained. Similar degradation processes of PLA microspheres in-vivo have been reported (*13–15*). Thus, utilization of the biodegradable spheres as an internal radiotherapy agent (not for combined radiation and embolization) prevents the formation of anoxic foci for future radiotherapy or chemotherapy and reduces the possibility for neo-vascularization in the resistant tumor tissue.

The density of the microspheres is also an important determinant in the sphere distribution within the liver. Wagner et al. pointed out the problems in using glass microspheres for organ perfusion studies, and referred to their high density as their most limiting factor (*16*). Variable results using glass spheres were also reported by Hales (*17*). Carnauba wax and phosphoric acid ester micro-

spheres with a density nearly equal to blood were prepared by Emmenegger et al. and yielded a more accurate assessment of microsphere distribution within organs (*18*). To explain this variability in distribution of spheres with different densities, one may apply a governing parameter, the Reynolds number (N), which is the ratio of the inertia force to the viscous force for a fluid moving through a cylinder (*19*). N may also be expressed as: $N = (v \times d \times p)/n$; where v is the mean velocity of flow, d is the diameter of the tube or vessel, p is the density of the fluid, and n is viscosity of the fluid. The flow in a vessel becomes turbulent if a critical Reynolds number of 2300 is reached. In capillary blood vessels, the Reynolds number can be as low as 0.01. At these low Reynolds numbers the viscous force dominates and indicates a more laminar flow. Thus, the distribution of the higher density glass spheres in the capillary bed is limited by their rapid rate of sedimentation (*20*). This conclusion is supported by the apparent distribution differences with intra-arterially administered ^{90}Y-glass microspheres and ^{99m}Tc-MAA microspheres as reported by Herba et al. (*21*). The dimensionless Reynolds number can also be applied to determine the most appropriate rate to administer the spheres through the catheter. This may help to prevent backflow or enhanced sedimentation. The Reynolds number for blood flow through the hepatic artery is calculated to be 318 assuming the blood flow rate is 300 ml/min and the hepatic artery diameter is 6 mm. If an equivalent Reynolds number is to be achieved through a silastic catheter (internal diameter = 1.089 mm), a flow rate of approximately 0.35 ml/sec would be needed. Similar flow rates were utilized by Britten et al. for the arterial administration of low density biodegradable starch microspheres (*21*). In contrast, to prevent sedimentation of the glass microspheres in the catheter, a flow rate of 1 ml/sec was required (*23*). In a catheter with an inner diameter of 1.67 mm (5.5-high flow torque control cobra visceral catheter, Cook), this flow represents a Reynolds number of 761. This difference in Reynolds numbers may result in backflow during administration with deposition of spheres in the gastrointestinal tract. Due to the near plasma density of PLA spheres, the ideal catheter flow rate can easily be achieved.

CONCLUSION

Biodegradable PLA microspheres containing stable ^{165}Ho-AcAc were reproducibly prepared by the solvent evaporation technique and later irradiated in a high neutron flux to produce therapeutic amounts of ^{166}Ho. Holmium-165 was chosen as the most desirable isotope for incorporation into the biodegradable matrix since its %natural abundance and neutron capture cross-section lead to much shorter irradiation time. The activated spheres were of low density and were designed to maintain their integrity until decay of ^{166}Ho was complete. Due to the fact that the PLA spheres are easily suspended in aqueous media, compatible administration rates into the hepatic artery could be obtained allowing more uniform distribution within the tumor site.

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