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PRELIMINARY STUDIES FOR PREPARATION OF MICROSPHERES LABELED WITH HOLMIUM-166

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ABSTRACT

The research activity is expanding the applications for therapeutic radiopharmaceuticals, employing more sophisticated targeting methodologies and more appropriate therapeutic isotopes for the tumors being treated. These new agents will reduce treatment time and accelerate recovery for many patients. The increasing interest in new therapeutic radiopharmaceuticals is prompting investigators to utilize isotopes with more focused capabilities for treating various tumors, reducing the negative effects on neighboring healthy cells. A number of radioisotopes are in development employing ^{177}Lu, ^{90}Y, ^{188}Re, ^{186}Re and ^{166}Ho. Liver metastases cause the majority of deaths from colorectal cancer, and response to chemotherapy and external radiotheraphy is poor. An alternative is an internal radionuclide therapy using microspheres labeled with ^{166}Ho, it is a beta minus emitter (E_{max}=1.84 MeV), maximum tissue range 8.4 mm,also emits photons (81keV,6.2%) suitable for imaging. Holmium has a natural abundance of 100% and thus only one radioisotope. ^{166}Ho is formed by neutron bombardment of holmium oxide targets. The aim of this work is the development of methods for the preparation of microspheres labeled with ^{166}Ho. Holmium was produced by neutron bombardment of Holmium Oxide targets at suitable positions in the IEA-R1 Reactor at IPEN-CNEN/SP. The initial experiments involved the labelling of cation exchange resins. The results of the labelling of these resins with ^{166}Ho were presented, together with its stability, retention capacity and physical properties.

1. INTRODUCTION

The estimative of new cases for 2006 published by INCA (National Institute of Cancer), indicated colorectal cancer as the fifth most common cancer in men (11,390 new cases) and the fourth most common cancer in women (13,970 new cases)[1] in Brazil. The following estimative applies to the U.S.A. only: in 2007, 112,340 new cases of colon cancer and 41,420 new cases of rectal cancer will arise. The estimative is for 52,180 deaths of colon and rectal cancer combined [2].The development of metastatic tumors within the liver occurs in nearly 50% of patients with colorectal carcinoma and is the major cause of death in those with this disease[3].

Few meaningful treatment options seem to be available for such patients. Hepatic resection in possible in up to 15% of cases and may be curative with reported 5-year survival rates of 20% to 50%. However the majority of patients are not suitable for hepatic resection because of size, number and location of their lesions or the presence of extra hepatic disease[3].The goal of palliation is to improve survival with minimal morbidity and an acceptable quality of life. The efficacy of palliative therapy can only be judged against the natural progression of the disease process. A number of palliative treatments have gained attention in recent years, and evidence is accumulating to suggest that these may extend life. Such treatments include

focally destructive modalities such as cryotherapy[4-6] radiofrequency ablation[7] and laser ablation[8]. However, these too are only applicable to a subgroup of those with colorectal liver metastases with a small number of relatively small lesions. [3]

The ideal properties for radiolabeled microspheres or particle for SIRT (Selective Internal Radiation Therapy) are summarized in Table 1. Treatment with radioactive microspheres is based on this principle. Microspheres with a diameter between 20 and 50μm will lodge in the vascular bed of the liver [9-12]. The spheres are administered via catheter through the hepatic artery, after administration of a vasoactive drug. Owing to the high selectivity of this technique, the radiation is mainly restricted to the tumor, with absorbed radiation doses to the tumor varying between 50 and 150 Gy[13].

Table 1. Ideal properties of radiolabeled microspheres for intra-arterial therapy[14]

High mechanical stability to resist breakdown and passage through the capillary network
High chemical stability to resist elution of radioactive label, macrophage removal, or radiolysis;
Uniform size;
Unit density to prevent settling or streaming;
Relative ease of labeling;
Radionuclide labeling with high-energy beta particle, low photofraction and intermediate (days) half-life.

Two different types of ^{90}Y microspheres are commercially available at the present time. The first, SIR-spheres® (Sirtex Medical Ltd, Sydney, Australia), was fully approved by FDA in the USA for use in colorectal cancer liver metastases in March 2002 and has more recently been similarly approved for use throughout Europe. They are resin microspheres, and a typical dose involves administration of 20-40 million microspheres. The second, Therasphere® (MDS Nordion, Toronto, Canada), is not fully FDA-approved but has a humanitarian device exemption (HDE) for the treatment of hepatocellular carcinoma (HCC). They are glass microspheres and a typical dose involves administration of 5-8 million microspheres[15].

Holmium-165 (neutron capture cross-section =64 barns) has a natural abundance of 100%. ^{166}Ho produced upon neutron activation of ^{165}Ho, is a β^- emitter (E_{max}=1.84 MeV) with maximum soft-tissue range of 8.4mm and a half-life of 26.8 hours. 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 production of ^{166}Ho is feasible in the IEA-R1 Reactor at IPEN-CNEN/SP, because it does not need high power and high neutron fluxes. Basically, 3 types of microspheres can be prepared with ^{166}Ho: resin, glass and polymer- based microspheres.

The emphasis in the present work is the resin- based microspheres. Microspheres based on ion exchange resins are favored for radioembolization due to their lower density compared with glass and their commercial availability. Chloride salts of holmium and yttrium can be added to cation exchange resins. Turner *et al.*[16] prepared microspheres by addition of ^{166}Ho-chloride to the cation exchange resin Aminex A-5, which has sulphonic acid functional groups attached to styrene divinylbenzene copolymer lattices. A reproducible, non-uniform distribution of the ^{166}Ho-microspheres throughout the liver was observed on scintigraphic images, following intrahepatic arterial administration in pigs. This predictable distribution allowed these investigators to determine the radiation absorbed dose from a tracer activity of ^{166}Ho-microspheres, and to define the administered activity required to provide a therapeutic dose.

The aim of this work is the development of methods for the preparation of microspheres labeled with ^{166}Ho. The initial experiments involved the labelling of cation exchange resins, and the preliminary results were shown.

2. MATERIAL AND METHODS

High purity Ho_2O_3 was employed as target material in all the experiments. The AG50W-X2 100-200 mesh and AG50W-X8 200-400 cation exchange resins in the H^+ form were purchased from Bio-Rad. Samples of Ho_2O_3 were irradiated inside sealed aluminum containers (7 cm height, 2.1 cm diameter) in selected positions of the nuclear reactor IEA-R1 at IPEN/CNEN-SP. The neutron flux was 1.0 x10^{13} n .s^{-1}.cm^{-2} for 1 hour. After the irradiation the samples were sent to the Radiopharmacy Center for the processing.

The AG50W-X8 cation exchange resin went first through the activation stage, being successively washed with hydrochloric acid, water and sodium hydroxide. The proper amount of resin was then poured inside a 1 mL plastic syringe and further conditioned with 5 mL of 0.1M HCl.

The dissolution of Ho_2O_3 was studied with different volumes of 0.1M HCl and also varying the heating temperature. The irradiated target was dissolved with 0.1M HCl and loaded into the small column. A known volume of 0.1M HCl was percolated through the column followed by a solution of 5% glucose.

Samples were taken from the loading of the column, and from the elution with 0.1M HCl and 5% glucose. These samples were then analysed by gamma ray spectroscopy using a HP Ge detector, from Canberra, in order to measure the presence of ^{166}Ho. The particle size distribution of the resins used in this work was determined using a particle size counter (Cilas,France).

3- RESULTS AND DISCUSSION

3.1 Dissolution of Ho_2O_3

Table 2 shows the results of the dissolution experiments of Ho_2O_3. It can be seen a direct relation between the increasing volumes needed to dissolve higher masses, and also the positive effect of raising the temperature.

Table2. Dissolution of Ho_2O_3

Ho_2O_3 mass (mg)	0.1M HCl volume (mL)	Temperature (ºC)	Dissolution
1.8	1	60	Yes
4.5	8	60	Yes
6.0	1	90	No
6.0	2	90	Yes
8.1	2.5	90	Yes
10.7	2	90	No
10.7	3	90	Yes

3.2 Resin labeling with ^{166}Ho

The results of the incorporation of ^{166}Ho in the resins can be seen in Table 3.

Table 3. Labelling of resins with ^{166}Ho

Resin	% ^{166}Ho resin	% ^{166}Ho loading waste	% ^{166}Ho 0.1M HCl elutions
AG50W-X2 100-200 mesh	95.4	3.9	0.7
AG50W-X8 200-400 mesh	93.5	6.5	0

The results show very good retention of ^{166}Ho in both columns, even when high volumes of 0.1M HCl are passed through the column. Even the glucose solution did not remove ^{166}Ho from the resin, as expected.

3.3 Particle size determination

The nominal size range of the resins (see Table 4) used in this work are is bigger than the range of sizes reported in the literature, with diameters varying from 13-75μm[16]. Microspheres of around 30μm are the optimum size for hepatic radionuclide therapy, as they are most evenly distributed within the normal liver tissue[10].

Table 4. Nominal size distribution of cation exchange resins

Resin	Particle size (μm)
BioRad analytical grade cation exchange resin **AG50W-X2** 100-200 mesh	106-300
BioRad analytical grade cation exchange resin **AG50W-X8** 200-400 mesh	63-150

Table 5 shows the results of the particle size determination of the AG50W-X2 100-200 mesh resin used in this work.

Table 5. Particle size determination of AG50W-X2 100-200 mesh resin

Amount (%)	Size article (μm)
90	221.09
50	159.45
10	117.48

These last results showed clearly that the particle size of the resin is not appropriate for in *vivo* studies.

4. CONCLUSIONS

This work showed the preliminary results of the preparation of resin-based microspheres labeled with ^{166}Ho. Although the resins employed in this work did not have the right particle size, the chemical behavior showed a very good labeling of the resins with ^{166}Ho, and its stability. Further work will be done with resins with the appropriate particle size.

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