CATION EXCHANGE RESINS LABELED WITH HOLMIUM-166 FOR TREATMENT OF LIVER MALIGNANCE

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Abstract

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. Local radionuclide therapy using radioactive microspheres is a promising therapy for non-operable group of patients suffering from liver malignances. Many publications have shown the success of this technique. The emphasis in the present work is the resin-based microspheres labeled with ^{166}Ho. 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. Samples of Ho_2O_3 were irradiated in selected positions of the nuclear reactor IEA-R1 at IPEN/CNEN-SP. The neutron flux was 1.0×10^{13} $n.s^{-1}.cm^{-2}$ for 1 hour. The dissolution of Ho_2O_3 was studied with different volumes of 0.1M HCl and also varying the heating temperature. The AG50W-X8 200-400 mesh and CM Sephadex C-25 cation exchange resins were labeled with ^{166}Ho. The retention of ^{166}Ho in the resins was studied and also its stability. The results of the dissolution experiments of Ho_2O_3 showed that there is a direct relation between the increasing volumes needed to dissolve higher masses, and also the positive effect of raising the temperature. 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 containing the resins and its good stability towards saline solution, PBS solution and glucose.Although the resins employed in this work did not have the right particle size (20-50µm), the chemical behavior showed the very good labeling of the resins with ^{166}Ho, and its stability.

1 Introduction

The estimative of new cases of colorectal cancer for 2008 published by INCA (National Institute of Cancer), indicated 12.490 new cases in men and 15.500 new cases in women in Brazil. These values corresponded to 13 cases each 100.000 men and 15 cases each 100.000 women [1].

Liver malignances, primary as well as secondary, have a high incidence. Each year, worldwide over 600.000 people develop hepatocellular carcinoma (HCC) and cancer of the biliary tree (cholangiocarcinoma), while at least as much are diagnosed with liver metastases, often originating from tumors, arising in organs drained by the portal vein. The most commonly diagnosed of such tumors is colorectal carcinoma, with about one million new cases every year [2].

Few meaningful treatment options seem to be available for such patients. Hepatic resection is 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] radiofrequency ablation[5] and laser ablation[6]. However, these too are only applicable to a subgroup of those with colorectal liver metastases with a small number of relatively small lesions. [3]

Local radionuclide therapy using radioactive microspheres is a promising therapy for non-operable group of patients suffering from liver malignances [7]. In contrast to normal liver tissue, which

receives most of its blood flow from the portal vein, liver malignances are almost exclusively dependent on arterial blood supply[8]. Based on this difference in blood supply, it has been demonstrated that radioactive microspheres with a diameter between 20 and 50 ε m that are injected into the hepatic artery, selective lodge in and around the tumors and thereby irradiate the surrounding tissue [7,8]. Basically, 3 types of microspheres can be prepared with ^{166}Ho: resin, glass and polymer-based microspheres.

The treatment of cancer with holmium-166 (^{166}Ho) loaded microspheres has obvious advantages over the use of other radionuclides, because holmium is the only element which can be easily neutron-activated (it has a cross-section of 64 barn[9]) to a beta- and gama-emitter with a logistically favorable half-life (Table I), and it can also be visualized by magnetic resonance imaging (MRI) [10]. 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.

Table I ï Physical Characteristics of ^{165}Ho/^{166}Ho [11]

Natural Abundance	100% ^{165}Ho	
Production	^{165}Ho (n,ɔ)^{166}Ho	
Half-life	26.8h	
ɔ-Rays	Energy (keV)	Percentage (%)
	80.6	6.71
	1379.4	0.93
ɓ-Rays	1773.1	48.7
	1854.7	50.0
Tissue range (ɓ-rays)	8.6mm	

The emphasis in the present work is the resin- based microspheres. Chloride salts of holmium and yttrium can be added to cation exchange resins. Turner *et al*.[13] 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 labeling of cation exchange resins, and the preliminary results were shown.

2 Material and Methods

Irradiation of Holmium Oxide

The irradiation is done in a quartz ampoule 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 medium neutron flux was 1.0 x10^{13}n.s^{-1}.cm^{-2} for 1 hour. Samples of Holmium Oxide (Ho_2O_3 (III), Aldrich, 99.999%) were dissolved in different volumes of 0.1M HCl and also varying the heating temperature.

AG50W-X8 Labeled with ^{166}Ho

The AG50W-X8 cation exchange resin, purchased from BioRad, went first through the activation stage, being successively washed with hydrochloric acid, water and sodium hydroxide. 1g of resin were taken from the loading of the column with ^{166}Ho solution and from the elution with 0.1N HCl. The stability test was studied with 0.9% saline.

Sephadex Labeled with ^{166}Ho

The CM Sephadex C-25 cation exchange resin (1g), purchased from GE lifesciences, was first activated with deionized water. The dissolution of ^{166}Ho occurred in acid medium (HCl 0,1) and the resin maintains its properties in pH 4 to 10, therefore the pH of the target solution was adjusted to 6, the Sephadex was then percolated with 0.9% saline.

All 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 samples were maintained at 25ºC in different medium for the stability test.The particle size distribution of the resins used in this work was determined using a particle size counter (Cilas,France).

3 Results and Discussion

Irradiation of Ho_2O_3

The activity of the sample of Ho_2O_3 (100mg) irradiated with the neutron flux of $9.7x10^{12}n.s^{-1}.cm^{-2}$ during 1 hour was 1200 MBq (32.43mCi). Feasible conditions of irradiation in the IEA-R1 nuclear reactor were simulated and the results are shown in Table II.

Table II ï Activity of ^{166}Ho Produced at IEA-R1Nuclear Reactor

Irradiation Time (h)	Neutron Flux ($n.s^1.cm^{-2}$)	Specific Activity (Ci/g)	Specific Activity (Bq/g)	Note
1	$9,7 \times 10^{12}$	0.37	$1.37x10^{10}$	Experimental Value
1	$4,0 \times 10^{13}$	1.65	$6.1x10^{10}$	Best neutron flux in the present
60	$4,0 \times 10^{13}$	42.96	$158.95x10^{10}$	Best conditions in the present
1	$7,0 \times 10^{13}$	2.89	$10.37x10^{10}$	Highest neutron flux in the future*
60	$7,0 \times 10^{13}$	91.57	$338.8x10^{10}$	Best conditions in the future*

* If the nuclear reactor IEA-R1 operate at maximal power 5MW

Although the IEA-R1 nuclear reactor do not operate in the maximal power (5MW), it is possible to produce sufficient activity of ^{166}Ho for radioterapeutic dose (157mCi) for patients [13] using the best conditions of the reactor in the present days (60 hours, neutron flux of $4,0x10^{13}n.s^{-1}.cm^{-2}$).

Dissolution of Ho_2O_3

Table 3 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.

Table III. Dissolution of Ho_2O_3

Ho_2O_3 mass (mg)	0.1M HCl volume (ml)	Temperature (ºC)	Dissolution
1.8	1	60	+
4.5	8	60	+
6.0	1	90	-
6.0	2	90	+
8.1	2.5	90	+
10.7	2	90	-
10.7	3	90	+

AG50W-X8 and Sephadex Labeled with ^{166}Ho

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

Table IV. Labelling of Resins with ^{166}Ho

Resin	% ^{166}Ho Resin	% ^{166}Ho loading waste	% ^{166}Ho 0.1M HCl elutions
AG50W-X8	93.5	6.5	0
Sephadex	97.2	2.3	0.5*

*eluted with 0,9% saline solution

The results show very good retention of ^{166}Ho in both resins.

Stability Tests

Table V shows the results of the stability test, during 24h of contact of the labeled resin and the solvents of choice.

Table V. Stability Test of the resins labeled with ^{166}Ho

Resin	0.9%Saline	5% Glucose	PBS Solution
	% ^{166}Ho		
AG50W-X8	0	0	*
Sephadex	0	0	0

* not realized

Particle Size Determination

Table VI shows the results of the particle size determination of the resins used in this work.

Table VI. Nominal size distribution of cation exchange resins

Resin	Diameter at 10% (μm)	Diameter at 50% (μm)	Diameter at 90% (μm)
AG50W-X8	68.84	99.05	144.94
Sephadex	66.88	96.84	136.00

The nominal size of the resins used in this work are 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].

4. CONCLUSIONS

This work showed that is feasible to produce ^{166}Ho for radioterapeutic doses in the research nuclear reactor. The results of the preparation of resin-based microspheres labeled with ^{166}Ho showed a very good retention, and good stability. Further stability tests will be done with the resin AG50W-X8. Although resins in this work did not have the right particle size, Tomura *et al*.[14] suggested that particles of 100-450 ε m could be used in the treatment of head-and-neck tumours.

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