^{166}Ho-EDTMP IN DETECTING BONE METASTASES: PRELIMINARY RESULTS

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ABSTRACT

Holmium-166-EDTMP (ethylenediminetetramethylenephosphonic acid) due to its promising biological properties, has proved to be a palliating therapeutic agent for bone cancer in human beings. In a basic medium, ^{166}Ho-EDTMP can be readly prepared with a complexing molar ratio EDTMP/Ho = 4.34. The radiochemical purity of the complex was higher than 98%. The biodistribution in rats and mice, showed a high skeletal uptake, a fast blood clearance and a low soft tissue upatake and a lesion to normal bone ratio equal to 2.

INTRODUCTION

Almost half a century after the first use of iodine-131, phosphorus-32 and strontium-89 for therapy in Nuclear Medicine, target radiotherapy using radiopharmaceuticals has become increasingly popular in the last decade, particularly in the fields of oncology, endocrinology and rheumatology.

Of the several phosphonates complexes with beta emitters radionuclides that have been described in the literature as potential therapeutic agents, Re, Sm and Ho show to be ones most likely for treatment of metastatic bone lesions. These compounds have been applied for the palliation of extreme skeletal pain in patients with lung, breast and prostate cancer.

The reported use of ^{166}Ho as a therapeutic radionuclide has increased in the last few years due to its favorable nuclear properties. Its half-life of 26.8 hr is long enough to eliminate logistic problems encountered with the short-lived ^{165}Dy, but is sufficient to provide a high radiation dose rate. The maximum soft-tissue penetration of a beta particle emitted from ^{166}Ho is 8.4 mm with an average of 3.3 mm.

The purpose of this study is to produce a radiopharmaceutical labelled with EDTMP, which is a chelate that can be labelled with ^{166}Ho with great in vitro stability. The labelled compound preferentially localizes in bone metastases and is rapidly cleared from the blood by the kidneys. It is used in terminal patient, as a palliative therapy in treatment of bone metastases, and so improving his life quality.

MATERIALS AND METHODS

A solution of holmium chloride (containning 222MBq ^{166}Ho/ml) was prepared by irradiating 6 mg of holmium oxide (Ho_2O_3 - Aldrich Chem. Co.) in IEA-R1 reactor of IPEN/CNEN-SP, for 8 hours in a neutron thermal flux of 1 x 10^{12} $n.cm^{-2}.s^{-1}$ and converted to holmium chloride by dissolving it by heating in 0.3 ml of 1N hydrocloric acid and completed to 1.0 ml volume 0.1N hydrochloric acid. .

1-Preparation of kits EDTMP: Ethylenediaminotetramethylenephosphonic acid (EDTMP) - ICN Biochemicals Cleveland Ohio 44128 PM = 436.14. The formulation of the kit solution is: EDTMP (60 mg), 1N NaOH (0.5 ml), bidestiled H_2O (0.5 ml). The vial were liophylized for 12 hours. The kit consist of 60 mg of EDTMP titrated with base to pH = 10.5.

2-Preparation of ^{166}Ho-EDTMP: 0.21 ml (1.26 mCi) of $^{166}HoCl_3$ solution were added to a lyophylized kit containning 60 mg of EDTMP (EDTMP/Ho molar ratio = 4.34) and the volume was completed with 0.1N HCl and sufficient 1N NaOH was added to bring the final product to a pH of 7.5 - 8.0. The chelate preparation can be stored in the formulation vial at room temperature.

3-Radiochemical purity: For determination of the purity of the ^{166}Ho-EDTMP complex a paper chromatographic method was studied using different mobile phases: a) pyridine:ethanol:water in the proportion of 1:2:4 v/v/v, b) ammonium hydroxide:methanol:water in the proportion 0.2:2:4 v/v/v, c) ammonium hydroxide:ethanol:water in the proportion 0.1:2:4 v/v/v. Whatman 3 MM chromatographic paper (8.0 cm x 1.0 cm) and the miniaturized system as supporting medium were used. The Rf value is 0.0 for free Ho ion and 0.8 for the complex.

4-Biodistribution: The biodistribution of ^{166}Ho-EDTMP was studied in mice weighting 25-30 g and male Wistar rats weighting 250-300 g. Thirty and one hundred microliters of the ligand complex were injected into the tail vein of unanesthetized mice and rats, respectively. They killed in groups of three animal by decapitation at 1,3 and 24 hr postinjection. One milliter samples of blood were drawn and the following organs removed: heart, kidney, lung, liver, spleen, stomach, muscles,

marrow, intestine, normal fermur, lesion femur and spinal cord. The radioactivity of the samples were counted in NaI (Tl) well detector (Abbott Gamma).

RESULTS AND DISCUSSION

The influence of reaction time and complexing yields is given in Table 1.

TABLE 1 - Radiochemical purity : The influence of reaction time on labelling yield.

TIME	15min	30min	1hr	24hr	MOBILE PHASE
	95.51 ± 0.81 #	98.0 ± 0.92	98.02 ± 0.68	98.36 ± 0.80	**pyridine**
	98.46 ± 0.67	99.21 ± 0.29	99.25 ± 0.08	99.21 ± 0.09	**methanol**
	97.64 ± 0.67	98.46 ± 0.67	98.37 ± 0.46	98.18 ± 0.31	**ethanol**

X ± SD

The labelling yields increase of the reaction time and they are less than 98%, when pH is over 7.5. The complex showed to be stable for at last three day after reconstitution of the kits.

TABLE 2 - Nuclear properties of ^{166}Ho

RADIONUCLÍDE	Ho-166
Half-life	1.12 (27hr)
Beta energy	1.8 MeV (51%)
Gamma energy	81 KeV (6.33%)
	1380 KeV (0.9%)

TABLE 3- Biodistribution of ^{166}Ho-EDTMP (% dose/g) after administered dose in mice (N=3)

TIME ORGAN	1hr	3hr	24hr
Heart	0.03±0.01#	0.01±0.00	0.01±0.002
Lung	0.04±0.03	0.04±0.01	0.03±0.01
Kidneys	0.49±0.07	0.41±0.09	0.30±0.11
Liver	0.11±0.07	0.05±0.00	0.16±0.06
Spleen	0.04±0.01	0.01±0.00	0.03±0.01
Muscle	0.06±0.03	0.01±0.01	0.01±0.004
Stomach	0.11±0.09	0.04±0.04	0.03±0.004
Intestine	0.04±0.01	0.02±0.01	0.03±0.02
N.Femur	2.61±0.21	3.11±0.00	1.74±0.00
L.Femur	2.96±0.41	4.25±0.00	2.44±0.33
Spinal cord	0.13±0.07	----------	0.14±0.05
Blood	0.05±0.01	0.01±0.00	0.02±0.01
Skeleton *	7.83±0.38	9.33±0.00	5.22±0.00

* 10% animal weight, N. Normal, L. Lesion, # X ± SD

TABLE 4 - Biodistribution of ^{166}Ho-EDTMP (% dose/g) after administered dose in rats

TIME / Organ	3hr
Heart #	0.015±0.007
Lung	0.02±0.000
Kidneys	0.24±0.042
Liver	0.025±0.021
Spleen	0.015±0.007
Muscle	0.036±0.04
Stomach	0.025±0.07
Intestine	0.01±0.001
N.Femur	1.16±0.49
L.Femur	2.12±0.02
Spinal Cord	0.07±0.035
Blood	0.05±0.014
Skeleton *	38.36±16.22

* 10% animal weight, N. Normal, L. Lesion, # X ± SD

Phosphonate complexes when labelled with radionuclides can be used for effective and safe palliative therapy for patients with bone metastases. Researchers in the field of oncology have tried to find a standard fractioned series of radioisotope doses which would be capable of providing pain palliation for patients with osseous metastases of varied malignant degree. (Tong D. et al. 1982). There is a great interest in searching a radiopharmaceutical which presents high affinity for certain organ cells. With base on the mentioned factors, phosphonate compounds have been widely studied and the data collected from these studies were compared to those of MDP-^{99m}Tc,since MDP-^{99m}Tc belongs to the same pharmacological class and it has been sucessfully used in nuclear medicine. Experiments in rats and mice have shown that EDTMP-^{166}Ho is a stable compound and that it presents a rapid blood clearance and a biodistribution similar to that of MDP-^{99m}Tc. The labelled compound, ^{166}Ho-EDTMP, has shown a high affinity with osseous tissues. The maximum uptake occurs in 3 hr (3.11 ± 0.00 and 4.25 ± 0.00 for normal and lesion femur, respectively) and then decreases slowly until 24 hr (1,74 ± 0.00 and 2.44 ± 0.33 for normal and lesion femur, respectively). Uptake in other organs was significantly reduced what is good in terms of radiation dosimetry. As ^{166} Ho is a beta emitting radioisotope of short half-life and as the complex ^{166}Ho-EDTMP shows a good selective skeletal uptake, one can suggest that it should be useful in nuclear medicine for metastatic carcinoma patients, after considering the radiotoxicity as a consequence of radiotherapy treatments.

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