Development of ^{166}Ho -phytate Complex for Radiosynovectomy

Amir R. Jalilian · Akbar Anvari ·
Ali Bahrami-Samani · Mohammad Mazidi ·
Mohammad Ghannadi-Maragheh

Received: 25 May 2010 / Revised: 29 June 2010 / Accepted: 29 July 2010 / Published online: 17 February 2011
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Abstract

Background ^{66}Ho -chloride was obtained by bombardment of natural $\text{Ho}(\text{NO}_3)_3$ dissolved in acidic media using thermal neutron flux ($4\text{--}5 \times 10^{13} \text{ n.cm}^{-2}.\text{s}^{-1}$).

Methods ^{166}Ho -holmium chloride (185 MBq) was used successfully for preparation of ^{166}Ho -phytate complex with high radiochemical purity ($>99.9\%$, ITLC, $\text{MeOH}:\text{H}_2\text{O}$: acetic acid, 4: 4: 2, as mobile phase). The complex stability and viscosity were checked in the final solution up to 2 days. The prepared complex solution ($60 \mu\text{Ci}/100 \mu\text{l}$) was injected intraarticularly to male rat knee joints. Leakage of radioactivity from the injection site and its distribution in organs were investigated up to 2 days.

Results Approximately all of the injected dose had remained in the injection site 2 days after injection.

Conclusion The complex was proved to be a feasible agent for cavital radiotherapy in oncology and rheumatology.

Keywords Phytate · Radiosynovectomy · Holmium-166 · Biodistribution · SPECT

Introduction

With the aging of the human population around the world, the need for the management of diseases of the elderly,

such as rheumatoid arthritis and other joint problems, has emerged. Also the majority of diseases can cause arthropathy, such as spondylarthropathy, Lyme disease, Behcet's disease, persistent synovial effusion, hemophilic arthritis, calcium pyrophosphate dihydrate (CPPD) arthritis, pigmented villonodular synovitis (PVNS), persistent effusion after joint prosthesis, undifferentiated arthritis, etc., leading to pain, inflammation and also immobility of patients [1].

Radiosynovectomy (RSV) has been proposed as a potent palliative therapy around the world for the last 2 decades [1], and several radiopharmaceuticals have been developed for RSV, including ^{166}Ho -macroaggregates [2] and ^{166}Ho -phytate complex [3].

Many beta-emitters such as ^{153}Sm , ^{177}Lu and ^{166}Ho can be produced in reasonable amounts using (n, gamma) reactions, and holmium-166 ($E_{\beta^-} \text{ max}=1.84 \text{ MeV}$, $T_{1/2}=26.8 \text{ h}$) is one of the most interesting radionuclides for the development of therapeutic radiopharmaceuticals such as ^{166}Ho -DOTMP for bone palliation therapy [4], ^{166}Ho -DTPA in endovascular beta irradiation therapy [5], ^{166}Ho -oxine-lipiodol in liver cancer therapy [6] and also ^{166}Ho patches in the treatment of skin cancers [7].

In this research, ^{166}Ho -phytate complex production is described in detail, followed by the determination of complex radiochemical purity, stability and biodistribution (after intraarticular injection) in wild-type male rats.

Materials and Methods

Production of ^{166}Ho was performed at the Tehran Research Reactor (TRR) using a ^{nat}Ho (n, gamma) ^{166}Ho nuclear reaction. Natural holmium nitrate with purity $>99.99\%$ was obtained from Aldrich Co. Phytate complex was prepared

A. R. Jalilian (✉) · A. Bahrami-Samani · M. Mazidi ·
M. Ghannadi-Maragheh
Radiopharmaceutical Research and Development Laboratory (RRDL),
Nuclear Science and Technology Research Institute (NSTRI),
Tehran, Iran 14155-1339
e-mail: ajalili@aeoi.org.ir

A. Anvari
Faculty of Nuclear Engineering, Shahid Beheshti University,
P.O. Box: 19839-63113, Tehran, Iran

using a commercial phytate kit (Kavoshyar Co., Tehran, Iran, stannous chloride free). Chromatography paper, Whatman no. 1, was obtained from Whatman (Maidstone, UK). Radiochromatography was performed by using a bioscan AR-2000 radio TLC scanner instrument (Bioscan, Washington, DC). A high-purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-7500SL) multichannel analyzer and a dose calibrator ISOMED 1010 (Dresden, Germany) were used for counting distributed activity in rat organs. All other chemical reagents were purchased from Merck (Darmstadt, Germany). Calculations were based on the 81-keV peak for ^{166}Ho . All values were expressed as mean $\pm$ standard deviation (mean $\pm$ SD), and the data were compared using Student's t-test. Statistical significance was defined as $P < 0.05$. Animal studies were performed in accordance with the UK Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd edition. All of the rats were purchased from Pasteur Institute of Iran, weighed 180–220 g ($n=5$) and were kept at a routine day/night light program; they were fed common rodent diet pellets.

Production and Quality Control of $^{166}\text{HoCl}_3$ Solution

Holmium-166 was produced by neutron irradiation of 100 μg of natural $^{\text{nat}}\text{Ho}_2(\text{NO}_3)_3$ (^{165}Ho , 99.99%) according to reported procedures [8] in the Tehran Research Reactor at a thermal neutron flux of $4\text{--}5 \times 10^{13} \text{ n.cm}^{-2}.\text{s}^{-1}$. Specific activity of the produced ^{166}Ho was 5 GBq/mg after 20 h of irradiation. The irradiated target was dissolved in 200 μl of 1.0 M HCl to prepare $^{166}\text{HoCl}_3$ and diluted to the appropriate volume with ultra-pure water to produce a stock solution. The mixture was filtered through a 0.22- μm biological filter and sent for use in the radiolabeling step. The radionuclidic purity of the solution was tested for the presence of other radionuclides using beta spectroscopy as well as HPGe spectroscopy for the detection of various interfering beta and gamma emitting radionuclides. The radiochemical purity of the $^{166}\text{HoCl}_3$ was checked using two solvent systems for ITLC [A: 10 m M DTPA, pH 4; B: ammonium acetate 10%:methanol (1:1)].

Synthesis of ^{166}Ho -phytate Complex

Briefly, 5 mCi (60 μg , 0.50 μl) of [^{166}Ho]holmium chloride acidic solution prepared as described above was transferred to a sterile borosilicate vial, and the mixture was evaporated using a flow of N_2 gas and slight warming (50°C) for 5 min. Sterile normal saline solution (1 ml) was added to a commercial phytate kit (containing 10 mg phytic acid, no SnCl_2), followed by vigorous shaking for 30 s. The phytate mixture was then added in one portion to the activity-containing vial followed by stirring. The radiolabeling of the

kit was checked by ITLC every 10 min. After completion of the labeling, the mixture was filter-sterilized using a 0.22-micron membrane.

Quality Control

For measuring radiochemical purity and radiolabeling yield, a 1- μl sample of the [^{166}Ho]holmium phytate complex was spotted on a chromatography paper (Whatman no. 1) and developed in a mixture of methanol/water/acetic acid (4:4:2) as the mobile phase.

Stability Testing of the Radiolabeled Compound in Final Formulation

Stability of ^{166}Ho -phytate in final preparation was determined by storing the final solution at 4, 25 and 37°C for 2 days and performing frequent ITLC analysis to determine radiochemical purity. Also after subsequent ^{166}Ho -labeling of the 2 month-stored kit, both labeling efficiency and radiochemical purity were determined.

Biodistribution of $^{166}\text{HoCl}_3$ and [^{166}Ho]holmium Phytate in Male Wild-type Rats After Intravenous Injection

To determine the biodistribution of free $^{166}\text{HoCl}_3$ and [^{166}Ho]holmium phytate in case of any radioisotope/radiopharmaceutical leak from the injection site, the species dissolved in normal saline were administered to wild-type rats. The animals were killed by CO_2 asphyxiation at selected times after injection (2–48 h for free Ho^{3+}). Dissection began by drawing blood from the aorta followed by removing heart, spleen, muscle, brain, bone, kidneys, liver, intestine, stomach, lung and skin samples.

For each animal, the appropriate amount of $^{166}\text{HoCl}_3$ or [^{166}Ho]holmium phytate activity (125–150 $\pm 10 \mu\text{Ci}$, in 100 μl .) was injected intravenously into rats through their tail vein. The animals were killed at exact time intervals (3, 24 and 48 h), and the specific activity of different organs was calculated as percentage of injected dose per gram using an HPGe detector.

Biodistribution of $^{166}\text{HoCl}_3$ and [^{166}Ho]holmium Phytate Complex in Wild-type Rats After Intraarticular Administration

To determine the accumulation of $^{166}\text{HoCl}_3$ or [^{166}Ho]holmium phytate in the intraarticular cavity, their isotonic solutions were carefully administered to wild-type rats. A volume (100 μl) of final radiolabeled solution containing 120–150 μCi radioactivity was injected intraarticularly to rats. The animals were killed at exact time intervals (2, 24 and 48 h). The specific activity of different organs was

Fig. 1 Chemical formula for phytate

calculated as percentage of area under the curve of 80 keV peak per gram using an HPGe detector.

Results and Discussion

Production and Quality Control of ^{166}Ho

The radionuclide was prepared in a research reactor according to regular methods with a range of specific activity of 3–5 MBq/mg for radiolabeling use. After counting the samples on an HPGe detector for 5 h, two major photons (5.4% of 0.081 MeV and 0.9% of 1.38 MeV) were observed (Figs. 1 and 2).

The radioisotope was dissolved in acidic media as a starting sample and was further diluted and evaporated for obtaining the desired pH and volume followed by sterile filtration. The radiochemical purity of the ^{166}Ho solution was checked in two solvent systems; in 10 mM DTPA, free

Ho^{3+} cation is complexed to a more lipophilic [^{166}Ho]-DTPA form and migrates to higher R_f , whereas a small radioactive fraction remains at the origin, which could be related to other free Ho ionic species, such as HoCl_4^- , etc., and/or colloids.

On the other hand, a 10% ammonium acetate:methanol mixture (1:1) was also used for the determination of radiochemical purity. The fast-eluting species was possibly the ionic ^{166}Ho cations other than Ho^{3+} , and the remaining fraction at $R_f=0$ was a possible mixture of Ho^{3+} and/or colloids. Due to existence of 1% impurity in both cases, the existence of colloids is unlikely (Fig. 3).

Preparation of [^{166}Ho]holmium Phytate Complex

The effect of various factors on the labeling yield of [^{166}Ho]holmium phytate was studied. In higher concentration no significant difference exists in labeling yield for added [^{166}Ho]holmium chloride activity (30 mCi). The phytate that had a molecular weight of 400 kDa was used to investigate of effect of phytate concentration on labeling yield at pH=3.5.

Labeling yield increased with increasing phytate concentration and reached above 98% when the concentration reached 35 mg/3 ml. The highest labeling yield was achieved at pH=2.8–3.2, while it decreased beyond this range. A labeling yield of 99% was achieved after 30 min. The effect of absence and presence of ascorbic acid (at various concentrations) as a complex stabilizer was also studied.

Fig. 2 HPGe spectrum for Ho-166 chloride solution used in this study

Fig. 3 ITLC chromatograms of ^{166}Ho - HoCl_3 solution in DTPA solution (pH. 5) (left) and 10% ammonium acetate:methanol (1:1) solution using Whatman no. 1 paper

ITLC using a mixture of methanol, water and acetic acid showed that the complex is mostly prepared in 30 min with 99% radiochemical purity; the remaining 1% is possibly attributed to other Ho ionic species that cannot react with phytate (Fig. 4).

Based on the obtained results, the optimal procedure for the preparation of [^{166}Ho]holmium phytate complex with a high labeling yield is as follows: 35 mg of phytate (MW= 400 kDa) was dissolved in 3.5 ml of 1% acetic acid aqueous solution. The acidity of the obtained solution was adjusted to pH=3 by the addition of 0.5 M NaOH solution and followed by the addition of [^{166}Ho]holmium chloride solution. Finally, the total volume was adjusted to 4 ml by the addition of deionized water.

Stability Studies of [^{166}Ho]holmium Phytate Complex

The stability of prepared [^{166}Ho]holmium phytate complex was checked up to 48 h after preparation. The complex was

stable in acidic media (pH=3.5), and its radiochemical purity was above 99% even 48 h after preparation. Also the stability of the complex was determined at 4°, 25 and 37°C for 2 days, and the data were almost consistent with the final solution stability.

Biodistribution Studies for Free Ho^{3+} Cation in Rats

The tissue uptakes were calculated as the percent of area under the curve of the related photo peak per gram of tissue (% ID/g) (Fig. 5).

The biodistribution of Ho^{3+} cation was determined in wild-type animals for 2–48 h post-injection. The liver uptake of the cation is comparable to many other radio-lanthanide mimicking calcium cation accumulation; about 3.5% of the cation accumulates in the liver. The transferin metal uptake and final liver delivery look like the possible route of accumulation. The blood content is low at all time intervals, and this shows the rapid removal of activity in the

Fig. 4 ITLC chromatograms of ^{166}Ho - HoCl_3 (left) and ^{166}Ho -phytate solution (right) on Whatman no. 1 paper using a methanol: water: acetic acid (4:4:2) mixture

Fig. 5 Percentage of injected dose per gram (%ID/g) of $^{166}\text{HoCl}_3$ in wild-type rat tissues at 2, 3, 4, 24 and 48 h post injection

circulation. Brain, muscle and also skin did not demonstrate significant uptake. A 0.5–1% bone uptake was observed for the cation, which remains almost constant up to 24 h. Spleen also showed significant uptake possibly related to reticuloendothelial uptake.

Biodistribution Studies After Intraarticular Administration of Free Ho^{3+} Cation in Rats

The distribution of the injected dose in rat organs up to 144 h after intraarticular injection of ^{166}Ho holmium chloride (60 $\mu\text{Ci}/100\ \mu\text{l}$) solution was determined for control studies. Based on these results, it was concluded that most of the injected activity of ^{166}Ho holmium chloride was extracted to blood circulation and distributed in rat organs, which was consistent with free Ho^{3+} distribution while administered intravenously (data not shown).

Biodistribution Studies After Intraarticular Administration of ^{166}Ho -phytate Cation in Rats

Figure 6 presents the distribution of the injected dose in the rat organs at various time intervals after intratumoral injection of 60 $\mu\text{Ci}/100\ \mu\text{l}$ of ^{166}Ho holmium phytate complex as percentage of injected dose. In case of any leak from the joint, the complex would accumulate in the reticuloendothelial (RE) system because of the high molecular weight of the complex, unless the complex dissociated at serum pH and Ho^{3+} cation was formed.

Almost no detectable amounts of activity were observed in the spleen and lung, which are two important RE organs, showing no complex leak occurred. Very negligible liver and kidney uptakes were observed, which was possibly caused by ^{166}Ho cation release from the injected joint and not the radiolabeled complex uptake.

Figure 7 demonstrates the biodistribution of the compound among the tissues excluding the injected knee data in

Fig. 6 Distribution of ^{166}Ho -phytate in wild-type male rats, 4, 24, 48, 120 h and 144 h after intraarticular injection of 60 μCi of compound. % ID-percentage of injected dose. Each bar presents mean $\pm$ SD ($n=3$)

order to better understand the biodistribution of the leaks from the knee.

The distribution of the radioactivity among tissues after removing knee joint accumulation data demonstrated a typical Ho^{3+} cation biodistribution among the tissues. It is believed that free Ho cation is the only radiochemical species escaping from the knee joint, and no ^{166}Ho -phytate complex was found in circulation.

Conclusion

The ^{166}Ho holmium phytate complex was prepared with a high radiochemical yield (>99 %) in the optimized condition: 35 mg/3 ml of phytate concentration in diluted acetic acid solution (pH=3). The prepared complex was stable in the final solution at room temperature, 37°C and presence of human serum, and can be used even 24 h after preparation. Intraarticular injection of ^{166}Ho holmium phytate complex to male wild-type rats and investigation of leakage of activity in the body showed that most of

Fig. 7 Distribution of ^{166}Ho -phytate in wild-type male rats excluding injected knee data at 4, 24, 48, 120 h and 144 h after intraarticular injection of 60 μCi of compound. % ID-percentage of injected dose. Each bar presents mean $\pm$ SD ($n=3$)

injected dose remained in the injection site 144 h after injection.

Conflict of Interest The authors declare no conflict of interest.

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