Preparation and quality control of ^{177}Lu-chitosan for radiosynovectomy

Original Article

Hassan Yousefnia[1], Amir Reza Jalilian[1], Fereydoun Abbasi-Davani[2], Samaneh Zolghadri[1], Ali Bahrami-Samani[1], Mohammad Ghannadi-Maragheh[1], Mohammad Mazidi [1]

[1]Radiopharmaceutical Research and Development Lab, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran (AEOI), Tehran, Iran
[2]Radiation Application Group, Faculty of Nuclear Engineering, Shahid Beheshti University, Tehran, Iran

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is the most common autoimmune disease, leading to the destruction of the joints and causing pain, disability, and immobility in the patients. Radiosynovectomy (RSV) has been applied as an effective treatment for the patients with resistant synovitis after failure of long-term pharmacotherapy and intra-articular steroid injection for more than 50 years. Several radiopharmaceuticals have been developed for RSV so far, but still development of new radiophamaceuticals is of crucial interest. In this research, the ^{177}Lu-chitosan complex (^{177}Lu-CHITO) was introduced as a new agent for RSV.

Methods: ^{177}Lu was produced by irradiation of a natural Lu_2O_3 target at a thermal neutron flux of approximately 4×10^{13} n/cm^2·s. ^{177}Lu-CHITO was prepared in the diluted acetic acid solution. The radiochemical yield was checked by ITLC method. The biodistribution of the complex was investigated by intra-articular injection to rabbits' and rats' knee joints. The leakage of injected dose from the injection site in the rabbit organs was investigated using SPECT imaging up to 48 hours.

Results: ^{177}Lu was prepared with a specific activity of 2.6-3 GBq.mg^{-1} and radionuclide purity of 99.98%. ^{177}Lu-CHITO was prepared successfully with high radiochemical purity (95%) and specific activity of 888 TBq/mmol. Both the biodistribution data in rats and SPECT imaging of the rabbit showed that there was no significant leakage of the injected activity even after 192 h.

Conclusion: Considering all of the excellent features of the complex, this radiopharmaceutical can be used for effective management of synovial inflammation.

Key words: Chitosan; Radiosynovectomy; Lu-177; Biodistribution; SPECT

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Corresponding author: Dr Amir Reza Jalilian, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, Tehran, Iran E-mail: ajalili@aeoi.org.ir

INTRODUCTION

Rheumatoid arthritis (RA) is the most common autoimmune disease, leading to the destruction of the joints causing pain, disability, and immobility in the patients [1]. It affects approximately 1% of the population worldwide, with female to male ratio ranging from 2:1 to 4:1 [2].

For the majority of the patients, RA can be controlled by the administration of various drugs; but in the cases where pharmacotherapy and intra-articular steroid injection are unsuccessful, radiosynovectomy can be applied as an effective treatment [3]. RSV is an intra-articular injection of β^--emitting radionuclides in colloidal form or radiolabeled particulates used for the reduction of pain and swelling.

Selection of the radionuclide depends on the size of the joint. The energy of β^- particles should be sufficient for penetration to the inflamed synovium, without causing any damage to the underlying bone and cartilage [4]. Nowadays, three radionuclides ^{90}Y, ^{186}Re and ^{169}Er are utilized in many countries for RSV of large, medium and small sized joints respectively [3, 5]. Other radionuclides such as ^{153}Sm, ^{188}Re, ^{166}Ho and ^{165}Dy have also been reported for RSV [6-9].

Among the therapeutic radionuclides, it seems that ^{177}Lu with suitable decay characteristics [$T_{1/2}$ = 6.73 d, $E_\beta$max = 497 keV, E_γ = 113 keV (6.4%), 208 keV (11%)] as well as the feasibility of large-scale production in adequate specific activity together with ^{131}I and ^{90}Y could cover the most needs of the systematic therapy in future [10] and can be a good choice for RSV of the small sized joints.

^{177}Lu labeled hydroxyapatite (HA) particles has been used for RSV of digital joints and therapy of the liver metastases [11, 12]. However side effects as well as the quality control limitations (due to the colloidal nature of the product) are the major concerns for the application of this product.

The particles used for radiosynovectomy should be small enough to be phagocytized by synoviocytes and distributed throughout the joint but large enough to be retained in the joint. The appropriate particle size is 1-20 µm [7]. Also an ideal agent should be biodegradable showing excellent binding with the radionuclide [3, 13]. Chitosan, is a natural, non-allergic and biodegradable polysaccharide (Figure 1) with a wide range of applications in biopharmaceutics, agriculture, water treatment purposes and RSV [14, 15]. ^{166}Ho-chitosan and ^{153}Sm-chitosan complexes are previously reported for internal radiation therapy [16, 17]. These complexes showed good retention at injection site.

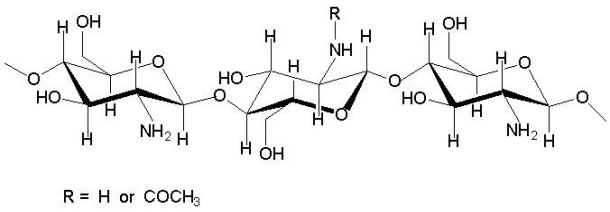

Fig 1. Chemical formula for chitosan.

Direct labeling of chitosan with various metals is based on the existence of various amino groups among the chitosan polymer which is repeatedly reported by authors. In this research, ^{177}Lu-CHITO complex, as a biodegradable/biocompatible radiopharmaceutical for RSV applications, was prepared and the effects of several preparation parameters were studied. Various quality control experiments for ^{177}Lu-CHITO complex were performed and the final product was injected intra-articularly into the rabbits' and rats' knee joints. A kit formulation was also developed for in-situ preparation of the product.

METHODS

^{177}Lu was produced by irradiation of natural Lu_2O_3 target at a thermal neutron flux of approximately 4 × 10^{13} n/cm^2.s for 5 days at Tehran Research Reactor (TRR). Chitosan (medium molecular weight, MW=400 kDa, DDA= %85) was obtained from Fluka (Bucks, Switzerland). Whatman No. 1 was obtained from Whatman (Maidstone, UK). Radio-chromatography was performed by using a bioscan AR-2000 radio TLC scanner instrument (Bioscan, Washington, DC, USA). A high purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-7500SL) multichannel analyzer and a dose calibrator ISOMED 1010 (Dresden, Germany) was used for counting distributed activity in the mice organs. All other chemical reagents were purchased from Merck (Darmstadt, Germany). Calculations were based on the 112 keV peak for ^{177}Lu. All values were expressed as mean ± standard deviation (Mean± SD) and the data were compared using student T-test. Statistical significance was defined as $P<0.05$. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd ed. The male healthy rabbits and rats were purchased from Pasteur Institute, Tehran, Iran.

Production and quality control of $^{177}LuCl_3$ solution

Lutetium-177 was produced by neutron irradiation of 1 mg of natural Lu_2O_3 (99.999% from Aldrich Co.

UK) according to the reported procedures [18] in Tehran Research Reactor at a thermal neutron flux of 4×10^{13} n.cm^{-2}.s^{-1} for 5 days. The irradiated target was dissolved in 200 µl of 1.0 M HCl, to prepare $^{177}LuCl_3$ and diluted to the appropriate volume with ultra pure water, to produce a stock solution of final volume of 5 ml. The mixture was filtered through a 0.22 µm biological filter and sent for use in the radiolableing 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 $^{177}LuCl_3$ was checked by means of 2 solvent systems for ITLC [A: 10mM DTPA pH.5 and B: ammonium acetate 10%:methanol (1:1)].

Synthesis of ^{177}Lu-CHITO complex

The acidic solution (0.5 ml) of $^{177}LuCl_3$ (111 MBq, 3 mCi) was transferred to a 5 ml-borosilicate vial and heated dry utilizing a flow of N_2 gas at 50-60 °C. Fifty microliters of chitosan in acetic acid (10 mg/ml) was added to the activity-containing vial and the mixture was diluted by the addition of normal saline (450 µl) and vortexed at 25°C for 30-60 min. The active solution was checked for radiochemical purity by ITLC. The final solution was then passed through a 0.22 µm filter and the pH was adjusted to 5.5-7.

Quality control

For measuring radiochemical purity and radiolabeling yield, 1 µl sample of the ^{177}Lu-CHITO complex was spotted on a chromatography paper (Whatman No. 1), and developed in a mixture of methanol/water/acetic acid (4.5/4.5/1) as the mobile phase.

Stability testing of the radiolabeled compound in final formulation

Stability of ^{177}Lu-CHITO in final preparation was determined by storing the final solution at 25 ºC for 48 hours and performing frequent ITLC analysis to determine radiochemical purity.

Biodistribution of radiolabeled chitosan in wild-type rats after intra-articular and intravenous administration

50 µl of the radiolabeled chitosan solution (containing 3.7 MBq radioactivity) was carefully administered intra-articularly to wild-type rats. The animals were sacrificed at the fixed time intervals (2, 4, 24, 48 and 192 h). The specific activity of different organs was calculated as the percentage of area under the curve of 112 keV peak per gram using an HPGe detector.

Also in order to determine the pharmacokinetic behaviuor of ^{177}Lu-chitosan transferred into the blood flow from the administration site, diluted ^{177}Lu-CHITO (100 µl, 1.85 MBq) was administered intravenously to the rats via their tail veins.

Imaging studies

7.4 MBq of lutetium-177 chitosan (MW=400 kDa, 10 mg/ml) solution was injected directly to a rabbit's knee joint followed by 2, 24 and 48 h imaging studies using an animal SPECT system.

RESULTS

Production and quality control of ^{177}Lu

The radionuclide was prepared in a research reactor according to the regular methods in a range of specific activity 2.6-3 GBq/mg for radiolabeling use, after counting the samples on an HPGe detector for 5 h, two major photons (6.4% of 0.112 MeV and 11% of 0.208 MeV) were observed.

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 filtering. The radiochemical purity of the ^{177}Lu solution was checked in the two solvent systems, in 10mM DTPA, free Lu^{3+} cation was complexed to more lipophilic LuDTPA form and migrated to higher R_f, while small radioactive fraction remained at the origin which could be related to the other Lu ionic species, not forming LuDTPA complex, such as $LuCl_4^-$, and/or colloids. On the other hand, 10% ammonium acetate:methanol mixture (1:1) (solvent 2) was also used for the determination of radiochemical purity. Due to existence of impurity in both cases, the existence of colloids was unlikely.

Preparation of ^{177}Lu-CHITO complex

The effect of various factors on the labeling yield of ^{177}Lu-CHITO was studied. The chitosan which had a molecular weight of 400 kDa was used to investigate the effect of chitosan concentration on labeling yield at pH=3.

Labeling yield increased with increasing chitosan concentration and reached above 95% when the concentration reached 10 mg/ml. In higher concentrations, no significant difference was noted on labeling yield. The highest labeling yield was achieved at pH=2.8-3.2 while decreased beyond this range. The effect of the absence and presence of ascorbic acid (at various concentrations) as a complex stabilizer was also studied.

ITLC using a mixture of methanol, water and acetic acid showed that the complex was prepared in 30 min

with 95% radiochemical purity; the remaining 5 % was possibly attributed to the other Lu ionic species which could not react with chitosan (Figure 2).

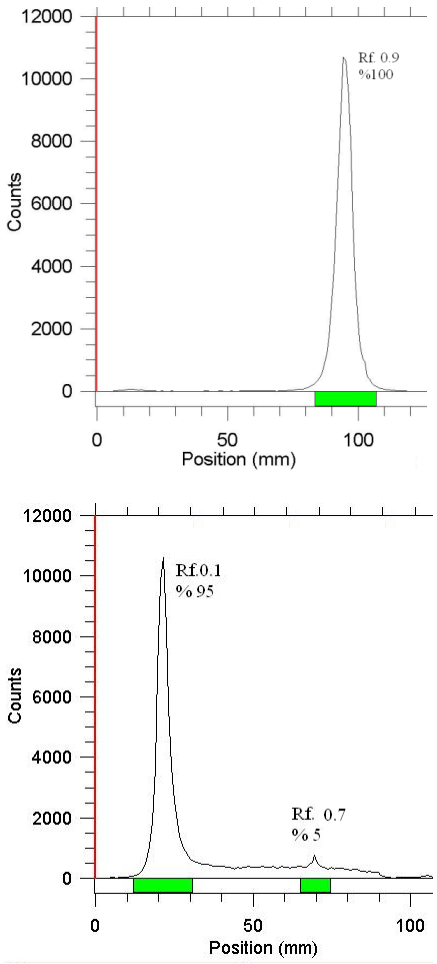

Fig 2. ITLC chromatograms of $^{177}LuCl_3$ (above) and ^{177}Lu-CHITO solution (below) on Whatman No. 1 paper using methanol: water: acetic acid (4.5:4.5:1) mixture.

Based on the obtained results, the optimal procedure for the preparation of ^{177}Lu-CHITO complex with a high labeling yield is as follows: 35 mg of chitosan (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 ^{177}Lu-chloride solution.

Stability studies of ^{177}Lu-CHITO complex

The stability of the prepared ^{177}Lu-CHITO complex was checked to 48 hours after preparation at room temperature. The complex was stable in acidic media (pH=3) and its radiochemical purity was above 95% even 48 hours after preparation.

Biodistribution of radiolabeled chitosan in wild-type rats after intra-articular and intravenous administration

The distribution of the injected dose in the rat organs up to 192 h after intra-articular injection of ^{177}Lu-chitosan (%ID/g) is shown in Figure 3.

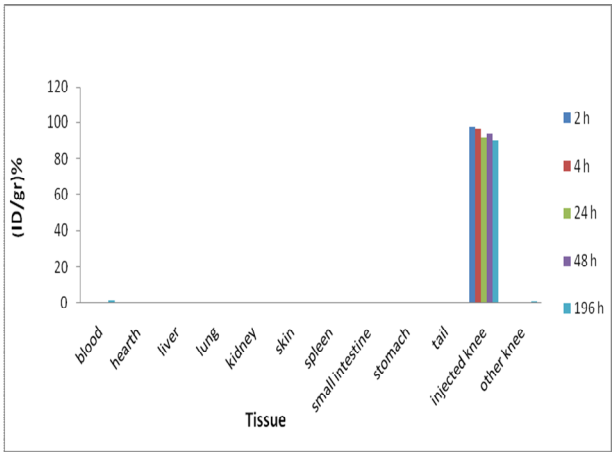

Fig 3. Percentage of injected dose per gram of tissues (ID/g %) at 2, 4, 24, 48, 192 h after intra- articular injection of ^{177}Lu-CHITO in wild-type rats.

No significant leakage of the injected activity was observed even after 192 h. Also the distribution of the injected dose in the rat organs after the intravenous injection of ^{177}Lu-chitosan (%ID/g) was determined (not shown). The data showed that, if the complex leak from the injection site, the most of the activity will accumulate in the bone, liver and spleen.

Imaging of ^{177}Lu-CHITO in wild-type male rabbits

SPECT imaging after intra-articular injection to rabbit's knee joint showed the retention of radioactivity in the injection site after 48 h post injection (Figure 4).

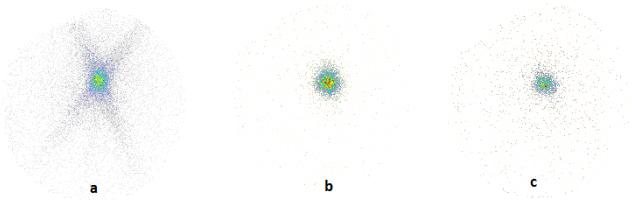

Fig 4. The SPECT images captured after intra-articular injection of 7.4 MBq ^{177}Lu-CHITO into the rabbit knee joint. The rabbits anesthetized by xylazine/ketamine injection.

Table 1: Nuclear decay characteristics of the radionuclides.

Radionuclide	Half life (days)	E_β max (keV)	Average tissue range (mm)	Eγ (keV)	Thermal neutron capture cross section (b)
^{169}Er	9.4	351 (55%)	0.3	110 (0.0013)	1.95
^{175}Yb	4.1	470 (86%)	0.34	396 (6.40%) 282 (3.01%) 113 (1.90%)	69
^{177}Lu	6.7	497 (78%)	0.35	208 (11.0%) 113 (6.40%)	2100

DISCUSSION

As reported previously, ^{169}Er citrate is accepted for RSV of small sized joints in many countries. Lately, ^{175}Yb-HA as a viable alternative to ^{169}Er has been reported and its efficacy for RSY was studied [19]. In this research, ^{177}Lu- CHITO complex for the RSV of small joints was prepared and biodistribution of the complex after intra-articular injection was determined. The nuclear decay characteristics of the mentioned radionuclides are given in Table 1.

All these radionuclides are produced via (n, γ) reaction in reactor. The thermal neutron capture cross section of ^{177}Lu is much higher compared with those of ^{174}Yb and ^{169}Er (Table 1). Therefore, ^{177}Lu can be produced with high specific activity without needing the use of enriched target. However, the production of ^{175}Yb and ^{169}Er, due to low cross section of these reactions, requires enriched targets with higher costs.

Leakage from the treated joint is a drawback of RSV. There are several ways for the reduction of leakage from the joints: utilization of particles with appropriate size and also a radionuclide with short half life [20, 21]. Since leakage from the joint requires time, the radiopharmaceuticals which leak before decay lead to radiation of non-target organs [22]. Therefore, from this point of view, ^{177}Lu and ^{175}Yb are better in comparison to ^{169}Er.

It is important to obtain images to check adequate intra-articular isotope distribution and to predict therapy efficacy. As ^{169}Er has gamma rays with low branching ratio, it is not suitable for scintigraphical scanning. ^{177}Lu has gamma rays with suitable energy for imaging.

Not withholding the excellent results of ^{169}Er utilization in RSV [23-26], the production of the new radiopharmaceuticals with better characteristics (ease of production, economical aspect,_improvement of retention in the joints suitable gamma-rays for imaging) are all of considerable potential and of interest.

^{175}Yb-HA injected intra-articularly in the knee joint of wistar rats showed complete retention of activity in the joint till 144 h post injection [19]. However, the general reported method of preparation of HA particle is time consuming, needing crucial control for obtaining the particles of desired sizes. These limitations restrict the wide and extensive utility of HA particles [27].

In this research, considering the favorable characteristics of ^{177}Lu and also the good properties of chitosan for RSV, the complex of ^{177}Lu-CHITO was prepared. No significant leakage from the joint was observed even after 192 h.

CONCLUSION

The ^{177}Lu-CHITO complex was prepared with high radiochemical yield (>95 %) in the optimized condition; 10 mg/ml of chitosan concentration in the diluted acetic acid solution (pH=3). The prepared complex was stable in the final solution at room temperature and could be used even 48 hours after preparation. Both the biodistribution data in rats and SPECT imaging of the rabbit showed that there was no significant leakage of the injected activity even after 192 h. Considering all of these features, this product can be used to manipulate synovial inflammation effectively. Finally, a kit formulation was developed for the in-situ preparation of the radiopharmaceutical in the remote clinical centers.

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