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Original Article

Preclinical studies of ^{166}Ho-chitosan for treatment of hepatocellular carcinoma

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ABSTRACT

Introduction: Recently, due to the special characteristics of ^{166}Ho and chitosan, ^{166}Ho-chitosan complex was developed for treatment of tumors such as hepatocellular carcinoma. This complex has been lately prepared with high radiochemical purity in our lab. The preclinical studies of the complex however should be performed to evaluate the tracer concentration in target and normal tissues before human use.

Methods: In this study, ^{166}Ho-chitosan was prepared and its preclinical studies for treatment of hepatocellular carcinoma was carried out by injection of the radiopharmaceutical into the rabbit`s liver via two different methods, surgery and venography. Leakage of the injected activity from the injection site in the rabbit organs was investigated using SPECT and SPECT-CT imaging up to 24 hours.

Results: Both SPECT and SPECT-CT imaging of the rabbits showed that there was no significant leakage of the injected activity. Almost all the activity would remain in the injection site at least 24 h post injection.

Conclusion: Considering all of the excellent features of the complex, this radiopharmaceutical is suggestive for treatment of hepatocellular carcinoma by radioembolization method.

Key words: Chitosan; Holmium-166; Hepatocellular carcinoma; SPECT; SPECT/CT

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a malignant tumor of the hepatocyte. It is a common malignancy worldwide causing almost half a million deaths annually. Although surgery (hepatectomy or liver transplantation) is the principle form of treatment, the majority of patients may not eligible for surgery due to extent of tumor and dysfunction of liver [1].

Transarterial chemoembolization (TACE) is the most widely used modality in the treatment of unresectable HCC, and has been shown to improve survival in a meta-analysis [2] and a randomized controlled trial [3]. Internal radiation therapy with radioactive agents such as ^{90}Y and ^{131}I, as an alternative to chemotherapeutic drugs, has the advantage of targeting the liver tumor by the selective dose delivery to the tumor site [4, 5]. This type of therapy has been utilized for palliative treatment of inoperable HCC and for adjuvant therapy following curative resection of HCC. A randomized study has shown a markedly better tolerance for I-131 lipiodol than for TACE with equal long term outcome [5].

Recently, due to the suitable decay characteristics of ^{166}Ho (E_{β^-max} = 1.84 MeV, $T_{1/2}$ = 26.8 h) and special specifications of chitosan, which make it an excellent vehicle for retaining the radioactive material within the tumor, ^{166}Ho-chitosan complex has been developed for treatment of diseases such as HCC and rheumatoid arthritis [6, 7]. Furthermore, this complex has demonstrated effectiveness for malignancies such as gliomas [8] and prostate cancer [9].

Biodistribution and kinetics of ^{166}Ho-chitosan complex after intrahepatic administration into rats and mice was studied before in the Korea atomic energy institute. In that research, both ^{166}Ho-chitosan and ^{166}Ho were injected directly into the liver by a surgical technique using 27-gauge needle. The results indicated the retention of the most of ^{166}Ho-chitosan activity at the administration site for over 72 h. Intrahepatic administration of free unbound ^{166}Ho however, showed high radioactive concentrations in the blood as well as many other tissues [6].

In the other research, the effectiveness of ^{166}Ho-chitosan complex, for the possibility of its use in radiosynovectomy, was investigated after intra-articular injection into the rat knee joints. Biodistribution studies showed that the most of the injected activity would remain in the injection site even after 144 h. No detectable amounts of activity were observed in spleen and lung showing no leakage of the complex from the joints [10].

Nowadays, ^{166}Ho-chitosan is used for treatment of HCC in South Korea. In a phase I/II clinical trial, the complex was injected percutaneously into small HCC, resulting in complete necrosis in 77.5% of cases (31/40) with minimal toxicity [11]. In the other work, the response rate of 78% (42/54) was observed after transarterial administration of the complex in patients with single, large HCC [12]. Despite the promising result of the complex in patients with HCC, due to the short half-life of ^{166}Ho, the possibility of transmission to other countries is unlikely.

However, this radiopharmaceutical was lately prepared with high radiochemical purity in our lab for treatment of joints by radiosynovectomy [10], but the application of the complex for treatment of HCC has not been reported. Preclinical study for evaluation of the radioactivity distribution in target and normal tissues is carried out and previously reported [13]. Accordingly, ^{166}Ho-chitosan was prepared and its preclinical studies for treatment of HCC was carried out by injection of the radiopharmaceutical to male New Zealand rabbit's liver via two different method (surgery and venography).

METHODS

Production of ^{166}Ho was carried out at the Tehran Research Reactor (TRR) using ^{nat}Ho(n, gamma)^{166}Ho nuclear reaction. Natural holmium nitrate with purity of >99.99% was obtained from Aldrich Co. Chitosan (medium molecular weight, MW=400 kDa, DDA= %85) was obtained from Fluka (Bucks, Switzerland). Chromatography paper, 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) were used for counting the activity. 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 ± 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 edn.

Production and quality control of $^{166}HoCl_3$ solution

Holmium-166 was produced by neutron irradiation of 100 µg of natural $^{nat}Ho(NO_3)_3$ (^{165}Ho, 99.99%) according to reported procedures [14] in the Tehran Research Reactor at a thermal neutron flux of 4-5×10^{13} n.cm^{-2}.s^{-1}. The irradiated target was dissolved in 200 µL of 1.0 M HCl, to prepare $^{166}HoCl_3$ 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 $^{166}HoCl_3$ was checked using 2 solvent systems for ITLC (A: 10 mM DTPA pH.4 and B: ammonium acetate 10%:methanol (1:1)).

Preparation and quality control of ^{166}Ho-chitosan complex

^{166}Ho-chitosan complex was prepared according to the previously reported literature [10]. Briefly, 35 mg of a stock solution of chitosan (10 mg/mL in 1% acetic acid) was added to 2-5 mCi of $^{166}HoCl_3$ and maintained at room temperature for 30 minutes. Radiochemical purity of the complex was checked by ITLC method and using a mixture of methanol: water: acetic acid (4:4:2) as the mobile phase. The final complex was kept at room temperature for 24 h, while the radiochemical purity was checked using frequent ITLC analysis.

Injection of ^{166}Ho-chitosan to rabbit`s liver via surgery

Two male New Zealand white rabbits with 14 weeks of age and weighing about 2000 g were used in this study. The anesthesia was induced with intra-muscular (IM) injection of ketamine (50 mg/kg) and xylazine (5 mg/kg) and was maintained with isofluran (1-5%) after placement of an endotracheal tube. The ventral region was prepared for aseptic surgery. 200 µCi of ^{166}Ho-chitosan was injected in the portal vein after a 3 cm laparotomy incision. The incision site was closed by means of standard method (the linea alba, subcutaneous and skin were sutured separately). 25 mg/kg of cefazoline was injected (IV) as prophylactic antibiotic. Leakage of the injected activity from the injection site into the rabbit organs was investigated using SPECT imaging up to 24 hours post-injection. Vital signs of the animals were checked during the surgery and 72 h post-injection. The blood activities were measured by counting 2 mL blood samples with an HPGe detector at 1 and 24 h post-injection.

Injection of ^{166}Ho-chitosan to rabbit's liver via venography

Radioambolisation was performed in a male New Zealand rabbit with 14 weeks of age and weighing about 2000 g. The animal was anesthetized with IM injection of ketamine (50 mg/kg), xylazine (5 mg/kg) and atropine (0.4 mg/kg). A 4F catheter was introduced through the right femoral vein by cut down technique. Then a 0.014 guide wire (BMW abbott vascular, ca, USA) was placed into the right judkins catheter (cordis corporation, USA) and the both were entered into the sheet, followed by the guide wire in the vein under the fluoroscopy. To find the true path of the injection, contrast material (visipaque GE Health care, cork, Ireland) was used. After getting into the hepatic portal vein, catheter was removed and Micro catheter (2/3F) was placed into the venues and ^{166}Ho-chitosan (200 µCi) was injected to the left lobe of the liver. Leakage of the injected activity from the injection site into the rabbit organs was investigated using SPECT-CT imaging 20 min and 24 h post-injection. Vital signs of the animal were monitored during the venography and 72 h post-injection.

SPECT-CT imaging Procedure

The biodistribution of the complex was studied by planar and SPECT images after injection of ^{166}Ho-chitosan via venography. The imaging was performed by SPECT-CT system (Simbia T2, Siemens Healthcare, Germany) equipped with low energy-high resolution (LEHR) collimators. Planar imaging was performed in both anterior and posterior positions after injection of ^{166}Ho-chitosan. Matrix size was 256*256 (pixel size = 1.2 mm at zoom factor = 1.33). The total time for planar imaging was five minutes per acquisition. Symmetric energy window with photo-peak at 81± %15 keV of ^{166}Ho was selected.

SPECT imaging was acquired with 64 projections (30 seconds per view) and 360-degree detector rotation was orbited at step and shoot modes. Imaging matrix size was determined at 64 × 64 (pixel size = 4.8 mm) and the zoom factor was 1.33. All the data were reconstructed with filtered back-projection (FBP), using Butterwoth filter (cut off = 0.5, order = 5) at trans-axial, sagittal and coronal slices. Following SPECT examination, CT acquisition was performed for image fusion, using current intensity at 16 mAs, peak voltage of 130 kVp, and slice thickness of 5 mm. SPECT-CT images were fused automatically using the registered Siemens software (Syngo MI workplace) at trans-axial, sagittal and coronal slices.

RESULTS AND DISCUSSION

Production and quality control of ^{166}Ho

^{166}Ho was prepared in a research reactor with the specific activity of 3-5 GBq/mg. The radionuclidic purity was checked by counting the samples on the HPGe detector for 5 h, demonstrated the purity of more than 99.98%. Besides, the results of thin layer chromatograghy indicated the radiochemical purity of more than 98% (Figure 1).

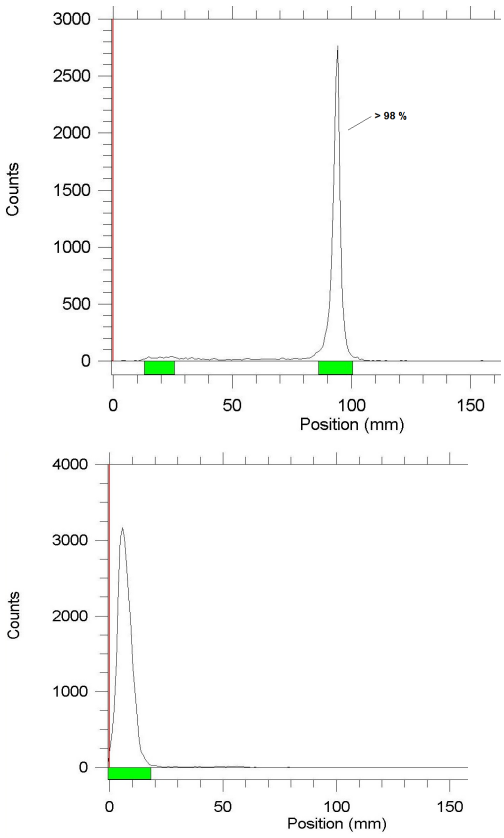

Fig 1. ITLC chromatograms of $^{166}HoCl_3$ solution in DTPA solution (pH.4) (above) and 10% ammonium acetate : methanol (1:1) solution (below) using Whatman No. 2.

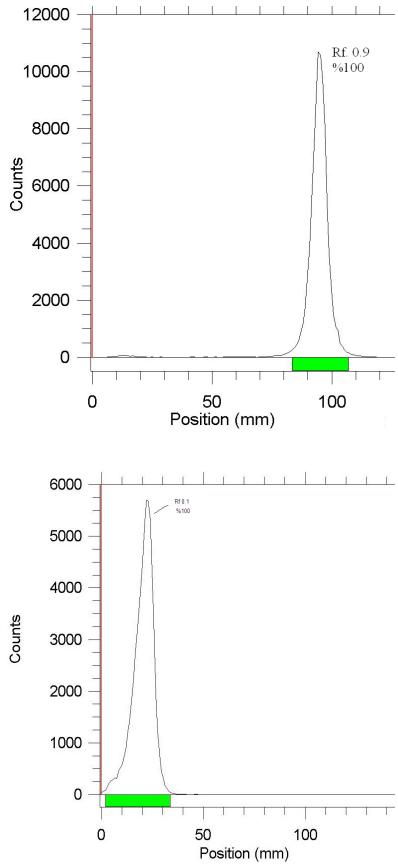

Fig 2. ITLC chromatograms of $^{166}HoCl_3$ (above) and ^{166}Ho-chitosan solution (below) on Whatman No. 2 paper using methanol: water: acetic acid (4:4:2) mixture.

Preparation and quality control of ^{166}Ho- chitosan complex

^{166}Ho-chitosan complex was prepared with the specific activity of approximately 35 GBq/mol. The results of thin layer chromatography showed that the complex is majorly prepared in 30 min with radiochemical purity of higher than 99% (Figure 2). The stability of the prepared complex under optimized reaction conditions was studied and excellent stability was observed at room temperature even after 24 h.

Imaging of ^{166}Ho-chitosan in male New Zealand rabbits

SPECT imaging of ^{166}Ho-chitosan after injection to the rabbit's liver via surgery showed the retention of radioactivity in the injection site after 24 h post injection (Figure 3). Region of interest analysis was performed for quantification (SD) of hepatic and background uptake after injection. Mean counts ± standard deviations were 256.89 ± 112.79 and 162 ± 60.38 in the anterior and inferior images, respectively.

Figures 4 and 5 show the SPECT-CT images of ^{166}Ho-chitosan complex after injection to the rabbit's liver via venography. This result also confirmed the retention of radioactivity in the injection site 24 h post injection. According to the analysis of the volume of the interest (VOI) in SPECT-CT imaging, the mean counts ± SDs of the liver were 2427±446.95 and 1404±292.05 at 20 min and 24 h post injection, respectively. Accordingly, SPECT-CT images were shown the stability of the radiolabeled complex in the rabbit's liver even 24 h post-injection. Also, the blood activity measurements were calculated as % ID/mL showed the values of 63×10^{-4} and 11×10^{-4} at 1 and 24 h post-injection, respectively.

Intra-arterial radionuclide therapy by means of beta-emitter radionuclides has been applied as a promising alternative for the treatment of liver cancer or liver metastases [15]. Various radionuclides such as ^{90}Y, ^{153}Sm, ^{165}Dy, ^{166}Ho and ^{177}Lu have been used for this purpose. Among these radionuclides, ^{166}Ho besides considerable decay characteristics can be easily produced by neutron bombardment of ^{165}Ho with natural abundance of 100% at a research reactor.

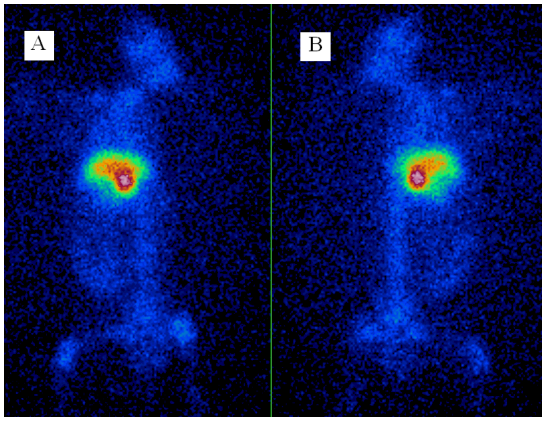

Fig 3. The anterior (A) and inferior (B) views of the radionuclide distribution in the rabbit's liver 24 h post-injection of ^{166}Ho-chitosan.

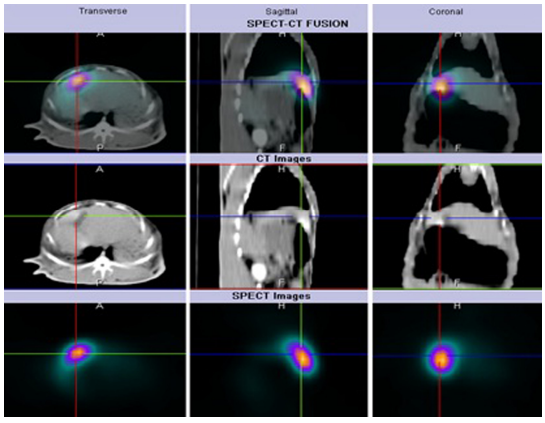

Fig. 4. The SPECT-CT images of the radiolabeled complex distribution in the rabbit's liver 20 min (early phase) post-injection, Trans-axial, sagittal and coronal slices images (left to right, respectively). Upper row: SPECT-CT fusion images, middle row: CT images and lower row: SPECT images.

Also, gamma rays emitted in the decay process of ^{166}Ho have suitable energies and low abundance that result in possible scintigraphic imaging without additional radiation dose to the patient.

Internal radiotherapy of liver cancer is often followed by the administration of radiolabeled complex with particulate carriers in specific size. However, distribution of the complex with particulate characteristics in the injection site is inhomogeneous and could lead to partial over-irradiation or under-irradiation of the tissue [7]. But chitosan is a natural, non-allergic biodegradable and biocompatible polysaccharide forming complexes with radionuclides in solution forms that result in a homogenous distribution. Furthermore, converting to a gel state at the neutral pH of organ is a unique property of chitosan which makes it as a suitable tracer for internal therapy. Due to these desirable characteristics, ^{166}Ho-chitosan has been widely used for treatment of HCC especially in some Far East Asian countries.

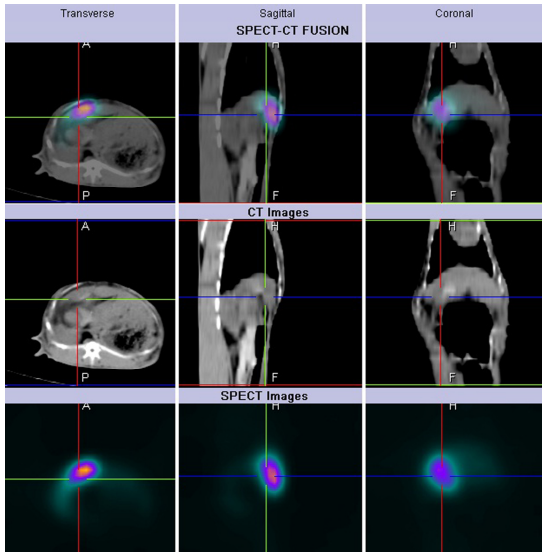

Fig 5. The SPECT-CT images of the radiolabeled complex distribution in the rabbit's liver 24 h (late phase) post-injection, Trans-axial, sagittal and coronal slices images (left to right, respectively). Upper row: SPECT-CT fusion images, middle row: CT images and lower row: SPECT images.

^{166}Ho-chitosan complex can be easily prepared with high radiochemical purity and high stability. It has been snown as an effective radiopharmaceutical for the treatment of the patients with HCC, but some difficulties have limited the application of this complex. For example, injection of this new radiopharmaceutical is an interventional procedure and needs multidisciplinary specialty cooperation namely nuclear physician, surgeon and radiologist.

In this research, this new complex was prepared and preclinical studies for the treatment of HCC were evaluated. In order to achieve the best route for the administration and to investigate the leakage of radionuclide to non-target organs, the complex was injected to the rabbit's liver via two different methods (surgery and venography).

The SPECT images indicated that the complex remained at the injection site even 24 h post-injection and no detectable amounts of activity were observed in the other organs. Also, the blood activity measurements showed the values of 63×10^{-4} and 11×10^{-4} at 1 and 24 h post-injection, respectively, which is consistent to the other reported values with the maximum rat's blood samples activity (% ID/mL) of about 90×10^{-4} which decrease to 10×10^{-4} within 48 h.

The prepared ^{166}Ho-chitosan complex showed almost the same characteristics as the other reports in the literature. The result of previous research on the biodistribution studies of the complex in the rats after intrahepatic administration has indicated retention of the most of the injected dose at the administration site with uneven radioactive distribution in the lungs [6]. The SPECT imaging showed stability and insolubility of the complex at the injection site with no activity seen in the lungs or other organs. The difference between the observed activities in the lung, as mentioned by Suzuki et al., can be related to the type of the under studies animals [6].

Also the results showed that both the surgery and venography methods were safe and practical. Since the biodistribution data did not depend on the type of the administration, due to the ease, the venography method can be preferred. According to the results, the complex can be used as an effective agent for the treatment of liver cancer in the country.

CONCLUSION

The ^{166}Ho-chitosan complex was prepared with high radiochemical yield (>99 %) in the optimized condition; 10 mg/mL of chitosan concentration in diluted acetic acid solution (pH=3). The prepared complex was stable in the final solution at room temperature and can be used even 24 hours after preparation. Injection of ^{166}Ho-chitosan to rabbit's liver was performed via two different methods and the leakage of the injected activity from the injection site in the rabbit organs was investigated using imaging procedures up to 24 hours demonstrating no significant leakage of the injected activity as evident by retained activity at the injection site. Considering all of the excellent features of the complex, this radiopharmaceutical could be an effective alternative for treatment of HCC by radioembolization method.

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