THERAPEUTIC APPLICATION OF NEW HOLMIUM-166 CHITOSAN COMPLEX IN MALIGNANT AND BENIGN DISEASES

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Abstract

The new holmium-166 chitosan complex(^{166}Ho-CHICO, DW-^{166}HC) was prepared by reacting the aqueous acidic solutiom of chitosan with $^{166}Ho(NO_3)_3$ at room temperature with quantitative labelling yield. The progress of the reaction and labelling yield were determined by instant thin layer chromatography using silicic acid impregnated glass fiber (ITLC-SA) and developing solvent of MeOH:H_2O:HAC (49:49:2). The high labelling yield of more than 99% was obtained by reacting chitosan solution (35 mg/4 mℓ) with $^{166}Ho(NO_3)_3$ in which 7 mg of ^{165}Ho+^{166}Ho were contained as a maximum content. The labelling yield was highly dependent on the pH of the chitosan solution. The optimal labelling could be obtained at pH 2.5~3.5. The characteristics of ^{166}Ho-CHICO were similar to those of chitosan, which is biocompatible, biodegradable, non-toxic, soluble and viscous in acidic condition but geltatinuous at pH 6.0 and precipitating in alkaline conditions. ^{166}Ho-CHICO can be easily prepared by reconstituting freeze-dried chitosan(kit A) with $^{166}Ho(NO_3)_3$ solution(kit B) just prior to use. After intrahepatic administration of ^{166}Ho-CHICO to male rats, the radioactivity concentrations in blood were low and the cumulative urinary and fecal excretion over a period of 0 to 72 hours were 0.53% and 0.54%, respectively. The radioactivity concentration in tissues and the whole-body autoradiography images showed that most of the administered radioactivity was localized at the administered site, and only slight radioactivity was detected from the liver, spleen, lungs, and bones. An autoradiograph after intratumoral administration of ^{166}Ho-CHICO showed that radioactivity was localized at the administered site of the lesion without distribution to other organs and tissues. A biodistribution study in normal rabbits with ^{166}Ho-CHICO showed that most of the radioactivities were retained in the knee joint with negligible extra leakage at 72 hours after intra-articular administration to male rabbits. Biodistribution of ^{166}Ho-CHICO 48 hours after intraperitoneal administration to male mice showed most of the radioactivities were evenly distributed at the inner wall of the peritoneal cavity. The ease with which the ^{166}Ho-CHICO can be prepared as a kit form and its high *in-vitro* and *in-vivo* stability make it an attractive agent for radionuclide therapy in malignant and benign diseases.

1. INTRODUCTION

Radionuclides considered suitable for therapy may be divided into three categories, i.e. beta-particle emitters, alpha particle emitters, and Auger and Coster-Kronig electron emitters. The physical characteristics of the most commonly-used therapeutic radionuclides, along with others whose use is various stages of development, have been reported [1]. Most of the radionuclides in

routine use are beta emitters. Beta particles are of low LET and their RBE is unity. The path length is quite variable, ranging from 1.0 ㎜ for the beta particles of ^{169}Er, to 12.0 ㎜ for those of ^{90}Y. Nevertheless, the range of beta particles is considerably greater than the diameter of a typical cell (5~20 ㎛), and thus, the whole cell and surrounding cells are irradiated.

Many different radiopharmaceuticals have been applied clinically in the treatment of various malignant and benign conditions over the past 50 years. Only a small number of radiopharmaceuticals have been developed on a commercial basis and have become established as routine therapeutic agents. Falling into this category are ^{131}I-iodide for thyroid disorders ^{32}P-phosphonate for blood disorders, ^{89}Sr-Chloride for pain control in metastatic bone diseases, ^{131}I-MIBG for neuroendocrine tumors, ^{90}Y or ^{32}P-colloids for intracavitary therapy, such as the treatment of intra-peritoneal metastases and of malignant effusions, intracystic therapy, intrathecal therapy, radionuclide synovectomy, regional therapy of liver tumors, and radioimmunotherapy.

Among the various therapeutic applications, we focused on the intracavitary therapy and regional therapy of liver tumors. Direct intracavitary administration is a means of delivering radiopharmaceutical in high concentration to tumors which are spread out over the serosal linings of cavities and to tumor cells present in malignant effusions. In order to minimise leakage of the radionuclide from a cavity, it is usually given in the form of a radiocolloid or particulate. Even so, a proportion of the radionuclides will reach the blood stream by lymphatic drainage and by leakage of ionic radionuclides as the colloid is degraded. Intracavitary therapy is applied to the peritoneal, pleural and pericardial cavities as well as to cystic brain tumors and to the spinal canal. A non-cancer application of the method is the intra-articular injection of radiocolloids to treat inflammatory joint disorders.

In order to solve leakage problems in radiation synovectomy, the labelling of macroaggregates or microspheres of degradable particle s(1~10 ㎛) as carriers of radionuclides has been attempted. Ferric hydroxide macroaggregates(^{165}Dy-FHMA) [2], hydroxide macroaggregates(^{165}Dy-HMA) [3], ^{166}Ho-PLA microspheres [4], and particulate hydroxyapatites(^{153}Sm-PHYP) [5] have been reported. In the case of hepatic tumors, ^{90}Y-resin microspheres [6], ^{90}Y-glass microspheres (22 ㎛) [7], [8], ^{166}Ho-Poly (L-lactic acid, 10~45 ㎛) [9], and ^{90}Y-Lipiodol [10] have been reported.

Even though the extra-leakage problem of radioactivity from an administered site has been mostly solved by controlling the size of particulates, there are many limitations to be overcome in its practical use such as large scale production, appropriate size and density control. When possible, soluble radiopharmaceucicals rather than colloids or particulates are more desirable for the even distribution within lesions after administration. If so, such radiopharmaceuticals should be used in both intracavitary and regional therapy. To our knowledge, aqueous soluble radiopharmaceuticals for these therapies have not been reported until now.

In this context, continuous effort has been devoted to develop new biodegradable, biocompatible, lanthanide radionuclides such as ^{153}Sm, ^{165}Dy, ^{166}Ho, and ^{169}Er. Among the natural polymers, chitosan, which is deacetylated chitin (Poly-β(1-4)-N-acetyl-D-glucosamine) [11], and naturally abundant, especially in the cuticle of marine crustacean, was chosen. In this report, we established the optimal preparative conditions of holmium-166 chitosan complex (^{166}Ho-CHICO) and determined its *in-vitro* and *in-vivo* stability.

2. MATERIALS AND METHODS

Chemicals

A reagent grade (>99.99%) of $Ho(NO_3)_3 \cdot 5H_2O$, was supplied by the Aldrich Chem. Co. Inc. Chitosan (400,000-500,000 MW, degree of deacetylation, 90%) was purchased from the Samcheon Ri Pharm. Co. Instant thin layer chromatography plates (glass fiber impregnated with silicic acid, ITLC-SA) were supplied by

Gelman Science, Inc. All other chemicals were of analytical grade and obtained from commercial sources.

Preparation of Lanthanide Radionuclides (^{153}Sm, ^{165}Dy, ^{166}Ho, ^{169}Er)

For *in-vitro* and *in-vivo* analysis, 40-200 mg nitrate salt of lanthanide was irradiated in the HANARO Reactor at the Korea Atomic Energy Research Institute, Taejon, Korea (reactor power=30MW) for an appropriate time depending on the above experimental purposes. Radionuclidic purity was determined by a Multichannel Analyzer and the radioactivity of the irradiated samples was determined by a radioisotope calibrator (Model CRC-12, Capintec Inc.). Irradiated sample was dissolved in a dilute HCl solution (pH 3.0) and then filtered using a 0.2μm membrane filter for sterilization.

Preparation of Holmium-166 Chitosan Complex (^{166}Ho-CHICO)

$^{166}Ho(NO_3)_3$ was chosen as a typical radionuclide for the preparation of the chitosan complex. ^{166}Ho-CHICO was prepared by adding 0.1㎖ of 10% $^{166}Ho(NO_3)_3$ solution in dilute HCl(pH 3.0) to a sterile 1% chitosan solution in 1% acetic acid. The resulting solution was stirred thoroughly by a votex mixer or in a ultrasonic bath for 10 minutes. The reaction mixture was left standing for 30 min at room temperature and then the labelling yield and raidochemical purity of ^{166}Ho-CHICO was determined by Instant Thin Layer Chromatography [ITLC-SA, developing solvent, MeOH:H_2O:HAc(49:49:2)].

The Effect of pH on Formation of ^{166}Ho-CHICO

The chitosan solution, in which 40 mg of chitosan was dissolved in 4 ㎖ of 1% aqueous acetic acid solution, was prepared to give various solution (pH 1-5 range). A 0.1 ㎖ of 10% $^{166}Ho(NO_3)_3 \cdot 5H_2O$ solution was added into the resulting solutions at room temperature by stirring.

The Effect of an Amount of ^{166}Ho on Formation of ^{166}Ho-CHICO

At pH 3.0 and 35 mg/4 ㎖ chitosan solution fixed, the chitosan solution was reacted with the $^{166}Ho(NO_3)_3 \cdot 5H_2O$ soln. increasing the amount of holmium (3.74 mg, 7.48 mg, 11.22 mg, 14.96 mg, 22.44 mg, 29.92 mg).

***In-vitro* Analysis**

The Effect of Radioactivity on the Stability of ^{166}Ho-CHICO

To the chitosan solution (40 mg/4 ㎖) various $^{166}Ho(NO_3)_3$ solutions containing 5 mCi, 10 mCi, 30 mCi and 50 mCi was added and then the radiochemical purity was determined 1 hour after the addition of $^{166}Ho(NO_3)_3$.

The Effect of Ascrobic Acid on the Stability of ^{166}Ho-CHICO

To the chitosan solution containing various amounts of ascorbic acid (10 mg-40 mg) 50 mCi of $^{166}Ho(NO_3)_3$ solution was added and then the radiochemical purity was determined at appropriate time intervals after labelling.

***In-vivo* Studies**

For the *in vivo* retention study, two normal New Zealand White rabbits weighing 3.5-4.5 kg were used. After the preparation of ^{166}Ho - CHICO solution in aseptic conditions, about 5 mCi of ^{166}Ho-CHICO was administered into the knee joint of rabbits. Immediately after administration, the rabbits were imaged using a gamma carmera (Siemens Digitrac 37) fitted with a pinhole collimator. A 20% windows was centered arround the 81 keV γ-ray of ^{166}Ho. The background and decay-corrected counts per pixel at 2, 6, 24, and 48 hr, times of injection were determined and calculated as percents injected activity retained in the knee over time.

For biodistribution studies, ^{166}Ho-CHICO(1-2 mCi) was injected into one knee joint of a rabbit (n=3). Daily blood samples and the total urine excreted were obtained and counted for ^{166}Ho activity. It was assumed that the total blood volume for the rabbit was 57.7 ㎖/kg. Biodistribution data for ^{166}Ho were acquired by killing the rabbits at 48 and 120 hrs. after CHICO administration. The major organs were removed, weighed, and counted for activity in a NaI(Tl) well-counter. The percent injected dose in major organs per g was calculated. Extra-articular leakage was calculated as the sum of all activities in major organs, total urine excreted and the activity remaining in the circulating blood at the time of sacrifice.

Male Sprague Dowley rats (6 weeks old) and male ICR nude mice (5 weeks old) were purchased from Charles River Laboratories Japan, Inc. The animals were kept in stainless cages in a room with a maintained temperature of 22± 1°C, a relative humidity of 60±10% and an alternating 12 hr light/dark cycle. The animals were allowed free access to fresh tap water and laboratory animal chow, CE-2(Clea Japan, Tokyo). After acclimation for approximately a week, healthy rats and mice were used for the experiments. Tumor transplanted nude mice were prepared with transplantation of B16 melanoma into the liver lobe using a previously described method. The transplanted mice were kept for approximately 10 days and then used for the experiments.

In intrahepatic or intratumoral administration, ^{166}Ho-CHICO solution was directly injected into the liver or transplanted tumor by surgical techniques. In intravenous administration, ^{166}Ho-CHICO solution was administered via the caudal vein.

The animals were sacrificed by diethyl ether at the predetermined time. They were frozen in liquid nitrogen and then mounted for sectioning. From each animal, thin sections were prepared with the Cryomacrocut(Leica). The obtained sections were then freeze-dried at -20°C. The sections were exposed to the imaging plates (IP; Fuji Photo Film Co., Ltd.). After exposure, each autoradiography image was obtained by BAS 2000.

3. RESULTS

Preparation of ^{166}Ho - CHICO

Thin layer chromatography. Irradiation of 40 mg of $^{165}Ho(NO_3)_3 \cdot 5H_2O$ in a neutron flux of $1\times10^{13}n/cm^2 \cdot sec$ for 10 hr gave 100 mCi of $^{166}Ho(NO_3)_3 \cdot 5H_2O$ with radionuclidic purity (>99.9%). The progress of reaction and labelling yield were determined by ITLC-SA. The R_f of ^{166}Ho-CHICO and free $^{166}Ho(NO_3)_3$ were 0.3 and 0.9, respectively in the solvent system of MeOH:H_2O:HAc(49:49:2).

Labelling yield. The labelling yield was highly dependent on the pH of reaction mixture, chitosan concentration, the amount of $Ho(NO_3)_3$, and on the reaction time. A high labelling yield greater than 99% was obtained by reacting the chitosan solution (>35 mg/4 mℓ) with $^{166}Ho(NO_3)_3$ solution (<7 mg of $^{166}Ho+^{165}Ho$) in acidic conditions (pH 2.5-3.5) at room temperature for 20 min (Table I and II).

TABLE I. THE EFFECT OF PH ON LABELLING YIELD OF ^{166}HO-CHICO*

pH of Reaction Mixture	Labelling Yield**
1.47	20
2.00	30
2.78	95
3.53	95
4.03	30
5.00	20
6.00***	.

*Ten mg of $^{166}Ho(NO_3)_3 \cdot 5H_2O$ in 0.1 ml of distilled H_2O was reacted with 30 mg of chitosan dissolved in 4 ml of 1 % aq. HAc at r.t for 30 min.

**Determined by Instant Thin Layer Chromatography. (ITLC - SA, MeOH(49) : H_2O(49) : Acetic acid(2))

***Gel was formed up to the pH of 6.0.

TABLE II. THE EFFECT OF AN AMOUNT OF ^{166}HO ON LABELLING YIELD OF ^{166}HO-CHICO*

Volume of $^{166}Ho(NO_3)_3 \cdot 5H_2O$ (ml)***	Amount of $^{166}Ho \cdot ^{165}Ho$ contained (mg)	Labelling Yield (%)**
0.1	3.74	99
0.2	7.48	99
0.3	11.22	70
0.4	14.96	66
0.6	22.44	45
0.8	29.92	17

*Chitosan (35 mg/4 ml) was reacted with various amount of $^{166}Ho(NO_3)_3 \cdot 5H_2O$.
** See Table 1
***Stock solution of 200 mg of $^{166}Ho(NO_3)_3 \cdot 5H_2O$/2 ml water

TABLE III. PERCENTAGE OF ADMINISTERED DOSE OF ORGANS AND TISSUES AT 24HR AFTER INTRA-ARTICULAR ADMINISTRATION OF ^{166}HO-CHITOSAN COMPLEX TO MALE RABBITS

Organs and Tissue	% of injected dose				
	1	2	3	mean	±S.D.
Blood	N.D.	N.D.	N.D.	N.D.	-
Plasma	-	-	-	-	-
Brain	N.D.	N.D.	N.D.	N.D.	-
Pituitary	N.D.	N.D.	N.D.	N.D.	-
Eye	0.0D	N.D.	N.D.	N.D.	-
Hardarian gl.	N.D.	N.D.	N.D.	N.D.	-
Submandibular gl.	N.D.	N.D.	N.D.	N.D.	-
Thyroid	N.D.	N.D.	N.D.	N.D.	-
Thymus	N.D.	N.D.	N.D.	N.D.	-
Heart	N.D.	N.D.	N.D.	N.D.	-
Lung	0.00	N.D.	0.00	0.00	0.00
Liver	0.20	0.11	0.10	0.14	0.06
Kidney	0.01	0.01	0.01	0.01	0.00
Adrenal	N.D.	N.D.	N.D.	N.D.	-
Pancreas	N.D.	N.D.	N.D.	N.D.	-
Spleen	0.00	0.00	0.01	0.00	0.01
Carcass	N.D.	N.D.	N.D.	N.D.	-
Stomach	N.D.	N.D.	N.D.	N.D.	-
Administration site					
Synovia membrane	87.62	81.42	80.16	83.07	3.99
Synovia	0.82	1.76	1.75	1.44	0.54
*Others	6.82	17.02	17.87	13.90	6.15

*Others : Radioactive recovery from administration site except synovia membrane and synovia.

TABLE IV. PERCENTAGE OF ADMINISTERED DOSE IN ORGANS OR TISSUES AFTER INTRAHEPATIC ADMINISTRATION OF DW-166HC TO MALE RATS

Organ/tissue	% ID			
	2 hr	24 hr	72 hr	144 hr
Blood	0.06±0.04	0.02±0.01	N.D.	N.D.
Brain	0.00±0.00	N.D.	N.D.	N.D.
Thymus	0.00±0.00	0.00±0.00	0.00±0.00	N.D.
Heart	0.01±0.01	0.01±0.01	0.02±0.03	0.02±0.01
Lung	2.63±2.40	3.29±3.64	1.45±2.25	0.57±0.77
Liver	0.40±0.18	0.63±0.49	0.27±0.21	0.15±0.06
Kidney	0.04±0.04	0.03±0.02	0.02±0.01	N.D.
Adrenal	0.00±0.00	0.00±0.00	0.00±0.00	N.D.
Pancreas	0.01±0.01	0.01±0.01	0.00±0.01	N.D.
Spleen	0.02±0.02	0.02±0.02	0.01±0.01	0.02±0.01
Skin	0.10±0.08	0.08±0.05	N.D.	N.D.
Testis	0.00±0.00	0.00±0.00	N.D.	N.D.
Carcass	0.38±0.21	1.09±0.71	2.51±0.83	2.72±0.36
Stomach	0.00±0.01	0.01±0.01	0.02±0.01	N.D.
Small intestine	0.08±0.09	0.03±0.01	0.04±0.02	N.D.
Large intestine	0.01±0.01	0.03±0.02	0.04±0.03	N.D.
Administration site	92.39±2.73	90.45±4.25	91.78±3.50	93.31±2.20

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* All values were expressed as mean % ID±S.D. (n=3)

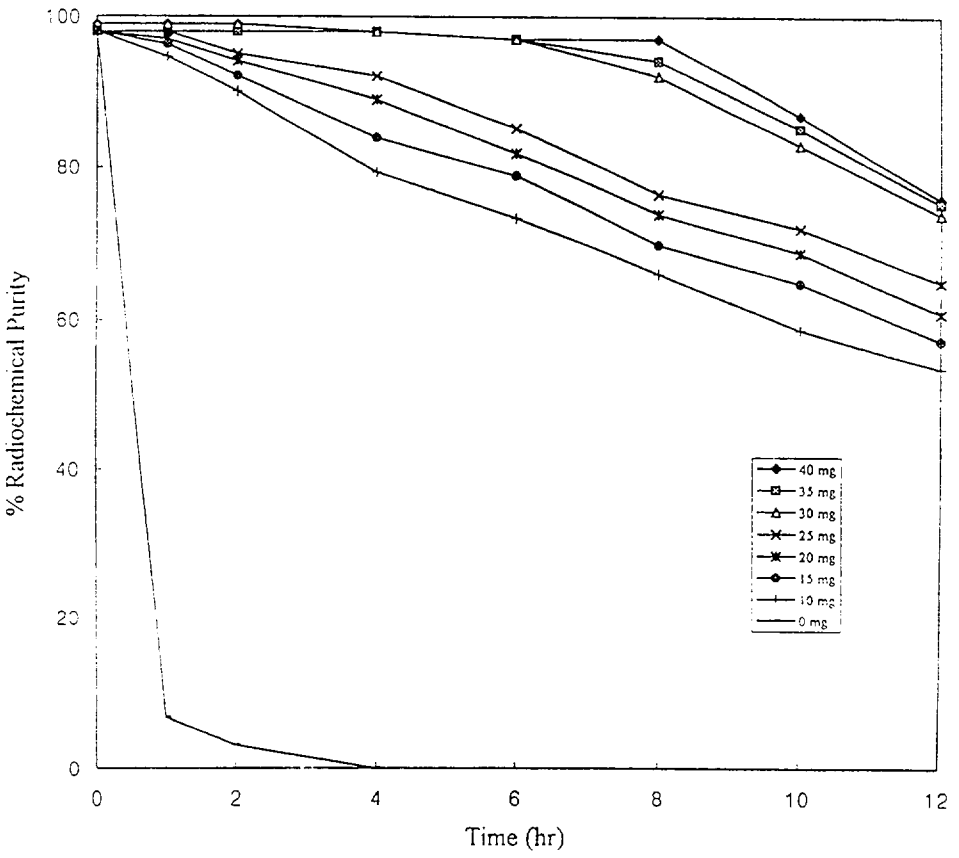

Figure 1. The Effect of ascorbic acid on *in-vitro* stability of ^{166}Ho-CHICO

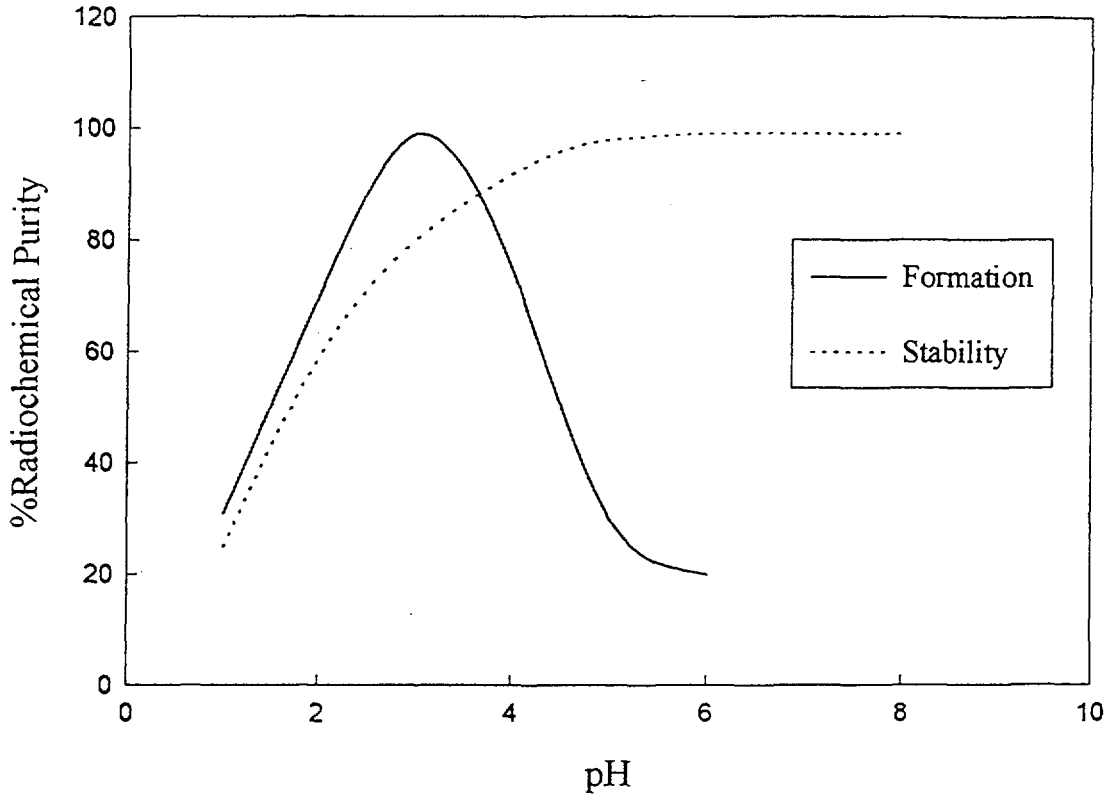

Figure 2. The Effect of pH on the formation and Stability of ^{166}Ho-CHICO

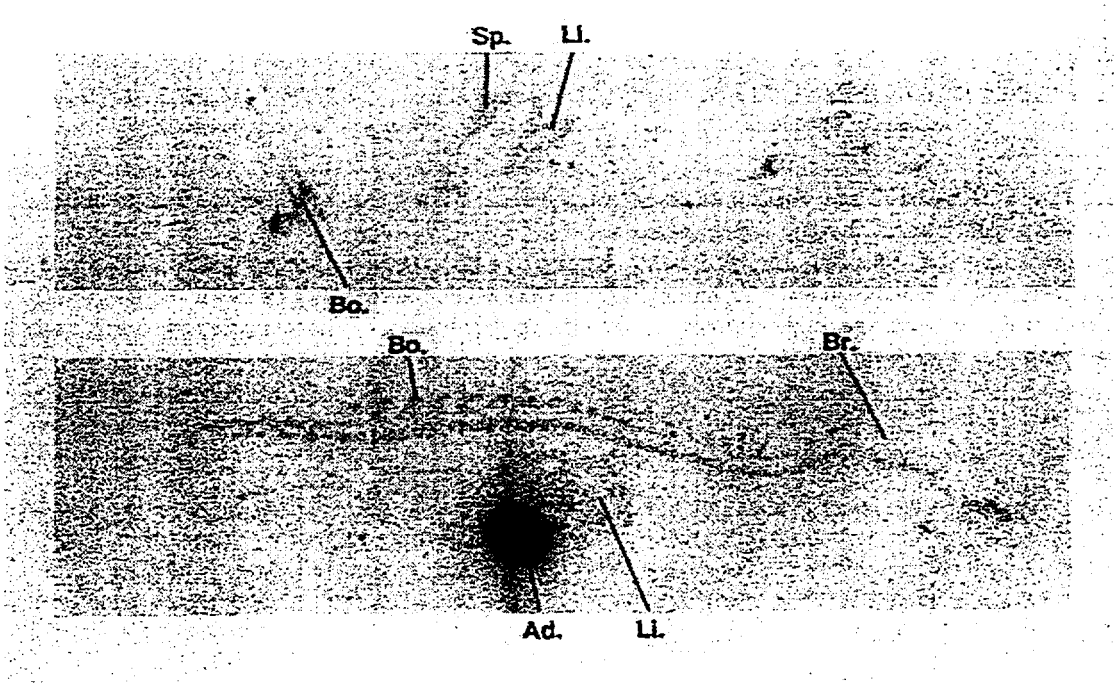

Figure 3. Whole-body autoradiographs at 72 hr after intrahepatic administration of ^{166}Ho-chitosan to a male rat.
Br.:Brain, Lu.:Lung, Li.:Liver, Sp.:Spleen, K.m.:Kidney medulla, K.c.:Kidney cortex, Bo.:Bone, U.b.:Urinary bladder, Ad.:Administration site,
Upper:dorso-ventral section including kidney, Lower:dorso-ventral mesion section

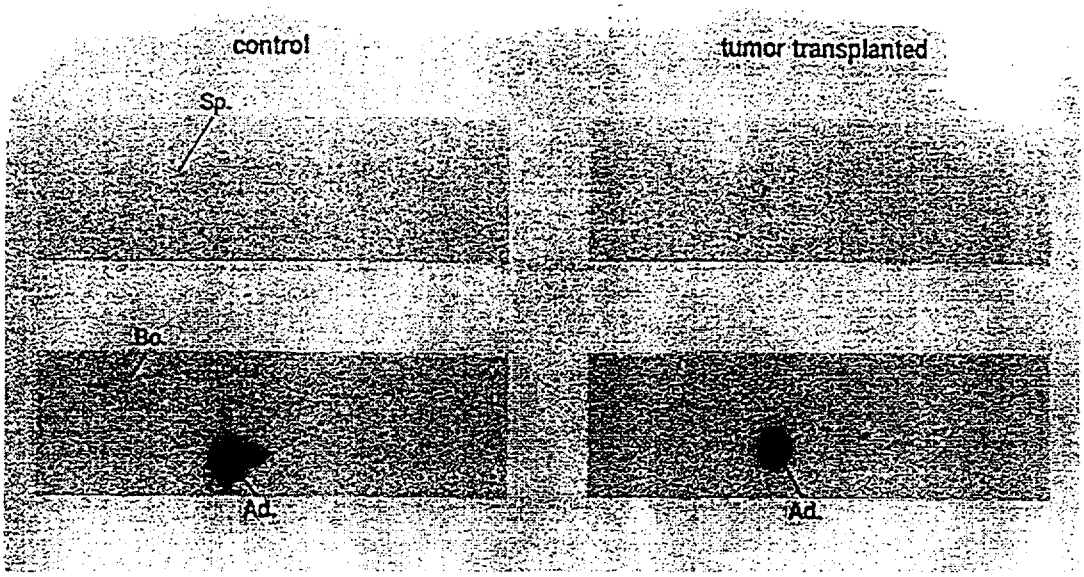

Figure 4. Whole-body autoradiographs at 72 hr after intrahepatic and intratumoral administration of ^{166}Ho-chitosan to a male control and tumor transplanted nude mouse, respectively.
Li.:Liver, Sp.:Spleen, Bo.:Bone, U.b.:Urinary bladder, Ad.:Administration site, Upper:dorso-ventral section including kidney, Lower:dorso-ventral mesion section

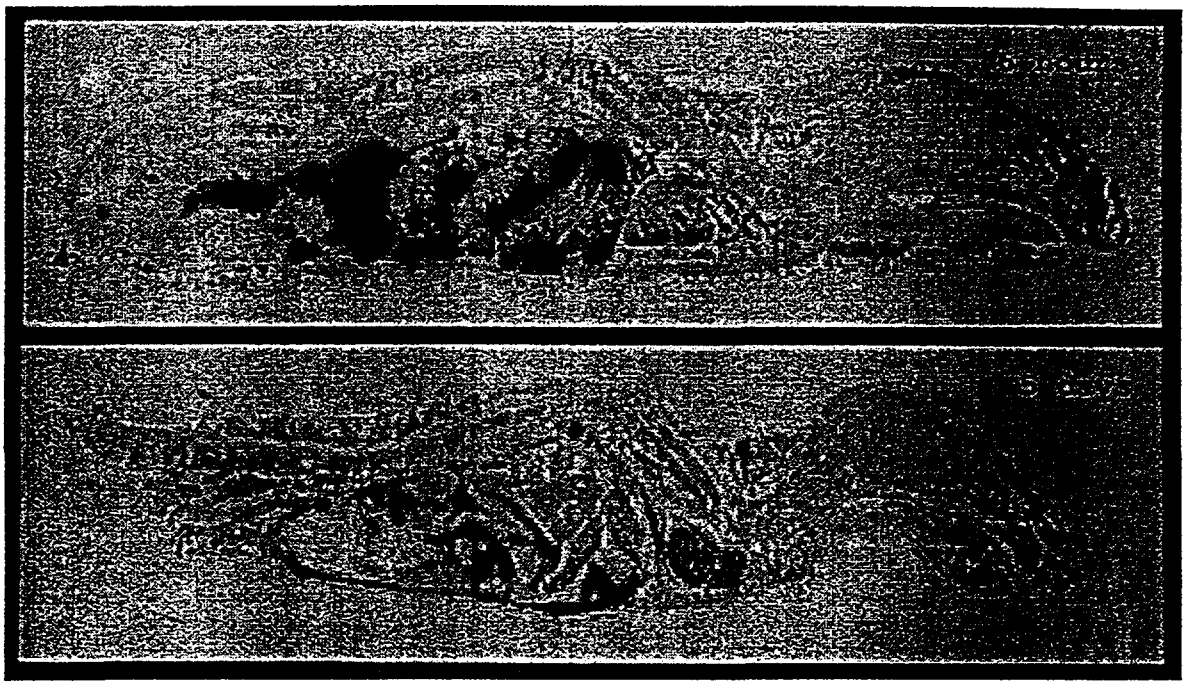

Figure 5. Whole-body autoradiographs at 6 hour and 5 days after intraperitoneal administration of ^{166}Ho-chitosan to rats.

In-vitro stability. Stability of ^{166}Ho-CHICO containing less than 2 mCi was examined in normal saline and in human plasma over four half-lives of each complex. In both the saline and plasma, no release of radioactivity from each complex was observed over the same period. The radiochemical purity(RCP) of ^{166}Ho-CHICO was highly dependent on the radioactivity of ^{166}Ho and the elapsed time after preparation. Its radiochemical purity decreased to 48% at one hour after preparation of ^{166}Ho-CHICO containing 10 mCi of ^{166}Ho. Similarly, in 50 mCi of ^{166}Ho, 5% of activity remained in ^{166}Ho-CHICO over the same time resulting in the high unstability of ^{166}Ho-CHICO under high activity conditions, probably due to the radiolysis and acid hydrolysis. On the other hand, in the presence of ascorbic acid (>35 mg), the stability of ^{166}Ho-CHICO, even though prepared from 50 mCi of ^{166}Ho, was dramaticaly increased, maintaining a high RCP (>98%) over 8 hours(Fig. 1). The ^{166}Ho-CHICO exhibited a high stability in neutral and alkaline conditions even in the absence of stabilizer(Fig. 2).

In-vivo stability and biodistribution study in rabbits with ^{166}Ho-CHICO showed most of the radioactivities were retained in knee joint with negligible extra leakage at 24 hr after intra-articular administration to normal male rabbits (Table III).

A biodistribution in tissues after intrahepatic administration of ^{166}Ho-CHICO was determined by radioactivity counting and autoradiography. The examination of organs and tissues showed that more than 90% of administered radioactivity was retained in the administered site for at least 144 hr after administration (Table IV). Autoradiography showed that most of the administered radioactivity was retained in the administered site of the liver lobe. Low levels of radioactivity were observed in the liver adjacent to the administered site, kidney, spleen and bones (Fig. 3).

An intratumoral administration study was performed using B16 melanoma transplanted nude mice as models. ^{166}Ho-CHICO was directly administered into tumors which had been transplanted into the liver lobe of mice and the radioactivity distribution was observed by autoradiography. In comparison, the tumor-transplanted mice showed a higher radioactivity retained at the administered site than in the control mice, and less radioactivity distributed to the body (Fig. 4).

Biodistribution of ^{166}Ho-CHICO at 48 hr after intraperitoneal administration to male mice showed most of the radioactivities were evenly distributed at the inner wall of the peritoneal cavity determined by autoradiography (Fig. 5).

4. DISCUSSION

^{166}Ho-CHICO was easily prepared by reacting the aqueous acidic solution of chitosan with $^{166}Ho(NO_3)_3$ or $^{166}HoCl_3$ at room temperature with a quantitative yield. The labelling yield and radiochemical purity were easily determined by instant thin layer chromatography.

The labelling yield was highly denpendent on the pH of the reaction mixture, chitosan concentration, and the amount of $Ho(NO_3)_3$ used. A labelling yield of more than 99% was obtained by reacting chitosan solution(35 mg/4 ml) with $^{166}Ho(NO_3)_3$ in which 7 mg(^{165}Ho+^{166}Ho) were contained as a maximum content. Three molecules (amine) of glucosamine in chitosan and one molecule of holmium are believed to participate in the comflex formation. ^{166}Ho is not a carrier free radionuclide because it is produced by the $^{165}Ho(n,\gamma)^{166}Ho$ reaction. Therefore, it is important to label the chitosan with as large amount of holmium as possible to get a high radioactivity for therapy. ^{166}Ho-CHICO was found to have incorporated about 18% of the Ho. Compared to ^{166}Ho-poly(L-lactic acid) microspheres(about 10%) [4], it contained a higher amount of holmium. From a practical point of view, it is important that ^{166}Ho-CHICO can be easily prepared by reconstituting freeze-dried chitosan(kit A) with ^{166}Ho solution(kit B) just prior to use, for instance, ^{99m}Tc instant labelling kits.

The characteristics of ^{166}Ho-CHICO were found to be similar to those of chitosan, which is biocompatible, biodegradable, non-toxic, soluble and viscous in acidic condition but gel-forming at pH 6.0 and precipitating in alkaline condition.

The *in-vitro* stability of ^{166}Ho-CHICO was highly dependent on the radioactivity of the ^{166}Ho used. In the case of <2 mCi, it was very stable at room temperature or at 37°C for one week maintaining high radiochemical purity (> 99%). On the contrary, it was very unstable even at room temperature with increased the radioactivity (>5 mCi). Radiochemical purity was decreased to 50% at 10 mCi, and 15% at 20 mCi one hour after preparation. It is apparent that radiolysis leads to degradation of the polymeric matrix, especially in acidic conditions, resulting in decreased viscosity of chitosan. This may be the result of oxidative or hydrolytic cleavage of the ether linkages [12]. However, it was stable at alkaline conditions (Fig. 3) in which hydrolysis is prevented. These results indicated that acid hydrolysis is promoted by radiolysis and is the main reason for the degradation of ^{166}Ho-CHICO. Fortunately, it was very stable in the presence of anti-oxidants such as ascorbic acid, maintaining its nearly original radiochemical purity (> 99%) even at 100 mCi for 8 hours. This stability in neutral or alkaline conditions hints that even though ^{166}Ho-CHICO in acidic conditions (pH 3.5-4.5) is administered into body, the pH is changed immediately to neutral conditions by body fluids and forms a gel, resulting in *in-vivo* stability and organ specificity.

A biodistribution study and gamma camera image in normal rabbits with ^{166}Ho-CHICO showed that most of the radioactivities were retained in the knee joint with negligible extra-articular leakage 72 hours after intra-articular administration. Even though ^{166}Ho-CHICO is a solution type which is completely different from other known colloid or particulate radiotherapeutic synovectomy agents such as ^{165}Dy-FHMA[2], ^{165}Dy-HMA[3], ^{166}Ho-PLA microspheres[4], and ^{153}Sm-PHYP[5], it exhibited lower extra-articular leakage than these agents.

After intrahepatic administration of ^{166}Ho-CHICO to male rats, the radioactivity concentrations in blood were low and the cumulative urinary and fecal excretion over a period of 0 to 72 hours were 0.53% and 0.54%, respectively. The radioactivity concentration in tissues and the whole-body autoradiography images showed that most of the administered radioactivity was localized at the administered site, and only slight radioactivity was detected from the liver, spleen, lungs, and bones. An autoradiograph after intratumoral administration of ^{166}Ho-CHICO showed that radioactivity was localized at the administered site of the lesion without distribution to the other organs and tissues.

The biodistribution of ^{131}I-lipiodol infused via the hepatic artery of patients with hepatic cancer has been reported[13]. The radioactive concentration in blood after administration had been kept as low as 10×10^{-4}% dose/ml for 8 days after administration. In our results, the radioactive concentration of ^{166}Ho-CHICO decreased rapidly and became 10×10^{-4} ID/ml level within 2 days. The concentrations were continuously decreased, whereas in the case of ^{131}I-lipiodol, the concentrations were gradually increased. The overall radioactivity in blood over a long period suggested that ^{166}Ho-CHICO is likely to be in the same level as ^{131}I-lipiodol.

There have been many reports of intra-arterial administration of ^{90}Y-microspheres to primary or secondary liver cancer[6,7,8,14,15]. Some of these reports showed a tumor:liver ratio of 45:1 or less[14]. Comparison of the biodistribution with other internal radiation therapeutic agents strongly suggests that ^{166}Ho-CHICO has an extremely high administration site : tissue ratio. The whole body autoradiographs after intratumoral administration of ^{166}Ho-CHICO into the melanoma(B16) showed that most of the administered radioactivity was localized at the administration site. As mentioned above, since ^{166}Ho-CHICO is a high viscous solution which displays gelation characteristics at a neutral pH in the body, it can be retained in the administration site in either tissue, if transfer into blood is avoided.

The whole body autoradiography after intraperitoneal administration of ^{166}Ho-CHICO into male mice showed most of the radioactivities were evenly distributed at the inner wall of the peritoneal cavity. Instillations of ^{32}P chromic phosphate(^{32}P-CP) have been used to treat intraperitoneal and pleural space malignancies, including ovarian cancer, for many years [16,17]. However, ^{32}P-CP has been shown that in animals about 85% of the colloidal ^{32}P was cleared from the blood in a single passage through the liver by phagocytosis in the reticuloendothelial system. A similar phenomina in which a large fraction of

the administered ^{32}P dose was absorbed by the liver was also observed [18]. In comparison with ^{32}P-CP, ^{166}Ho-CHICO showed a high radioactive concentration within the peritoneal cavity with relatively even distribution, which is rarely expected with radiocolloid or particulates.

5. CONCLUSION

The characteristics of ^{166}Ho-CHICO were similar to those of chitosan, which is well known to be biocompatible, biodegradable, non-toxic, soluble and viscous in acidic conditions but gel-forming in neutral conditions. The ease with which the ^{166}Ho-CHICO can be prepared as a kit form and its high *in-vitro* and *in-vivo* stability make it an attractive new agent for radionuclide therapy in malignant and benign diseases.

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