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ORIGINAL PAPER

Preparation and primary evaluation of ^{66}Ga-DTPA-chitosan in fibrosarcoma bearing mice

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Abstract. Chitosan was chemically modified by diethylenetetraaminepentaacetic acid (DTPA) in different degrees of modification (DM = 6.1, 10.3, 15.7 and 20.9%). DTPA-chitosans were radiolabeled with gallium-66 radionuclide. The effect of several factors on labeling yield such as degree of modification, acidity and concentration of DTPA-chitosan solution, contact time and radioactivity was investigated. Radiolabeled DTPA chitosans were intratumorally injected to fibrosarcoma bearing mice and the leakage of radioactivity from the injection site was evaluated. In comparison with chitosan, all DTPA chitosans showed better efficiency in preventing the leakage of radioactivity from tumor lesion and DTPA-chitosan (DM = 10.3%) was the best which led to remaining 97% of injected dose in the injection site after 54 h of injection. The highest leaked radioactivity from the injection site was in the lungs, liver, spleen and the kidneys. Our results indicated that the DTPA modified chitosan can be an effective carrier for therapeutic radionuclides for tumor treatment by the intratumoral injection technique.

Key words: DTPA-chitosan • degree of modification (DM) • gallium-66 • radiolabeled • intratumoral injection

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Introduction

Though intravenous injection is the conventional injection mode for cancer radiotherapy using liquid radiotherapeutic pharmaceuticals, the ratio of radiopharmaceuticals reaching the target tumoral tissue to the non-target normal tissues is low [19, 26, 34]. Thus, their side effects are notable because of their systemic toxicities while remaining for a long period in blood circulation and/or accumulating in normal tissues. To deliver high concentration of radiotherapeutic reagents and minimize their side effects on the non-target tissues, intratumoral injection is an attractive approach. Intratumoral injection of several non-removable radiolabeled agents such as holmium-165 loaded poly-L-lactide acid (PLLA) nanoparticles [9], ^{90}Y particles embedded in an alginate gel [10], ^{90}Y-glass microspheres [4, 18, 28], ^{32}P glass microspheres suspended in gelatin [32], rhenium-188 sulfide suspension [14–16], ^{153}Sm-chitosan [25] and ^{166}Ho-chitosan [21, 27] has already been reported and they have shown suitable efficiency in preventing the leakage of radioactivity from the injection site.

In our previous work [22], we prepared a ^{66}Ga-chitosan complex and investigated the effect of several factors on its labeling yields. The highest labeling yield was achieved at pH = 3.1–3.9, while the yield decreased out of this range. The ^{66}Ga-chitosan complex with radiochemical purity higher than 99% was directly injected to tumoral lesion of fibrosarcoma bearing mice. However, only 85.4% of injected dose remained

in the injection site after 54 h of injection. The leakage of radioactivity might be caused by changing in pH of the injected solution after intratumoral injection which leads to deprotonating of amino groups of chitosan and weakening their interaction with anionic form of Ga(III) ions ($[Ga(OAc)_n]^{3-n}$) [8, 29, 33].

However, the less the leakage of radioactivity from the injection site, the less the side effects of leaked radioactivity on normal tissues. In order to reach better efficiency, chitosan, a polysaccharide containing a large amount of active primary amino groups, can be chemically modified by complexing agents of metal ions such as DTPA and ethylenediaminetetraacetic acid (EDTA) which can absorb metal ions in a wide range of acidity [3, 11, 31].

In this research, chitosan was chemically modified by DTPA in various DM. DTPA modified chitosans were radiolabeled using [^{66}Ga]gallium acetate solution. The effect of several parameters such as: the DM of chitosan, acidity and concentration of DTPA-chitosans solutions, radioactivity and contact time, was investigated on radiolabeling yield. Finally, ^{66}Ga-DTPA-chitosans were intratumorally administered to fibrosarcoma bearing mice and the leakage of radioactivity from the injection site was investigated.

Materials and methods

Materials

Chitosan (molecular weight, MW = 400 kDa, degree of deacetylation, DDS = 85%, Cat. no.: 22742) was obtained from Fluka, Switzerland. DTPA-dianhydride, trinitrobenzene sulfonic acid (TNBSA) and dialysis tube (MWCO = 12 500) were purchased from Sigma-Aldrich. All other chemicals used in this study were of analytical grade and were obtained from Merck, Germany. Deionized water used in this study was obtained using a Direct Q 3 water purification system, Millipore, USA.

Synthesis of DTPA-modified chitosan

To improve its reactivity, chitosan was functionalized with DTPA in a way slightly different from previously described methods [20, 23]. 8 g of chitosan, corresponding to 40.6 mmol of glucosamine units, was dissolved in 400 ml of 10% (v/v) aqueous acetic acid solution. The solution was diluted five times with methanol and its acidity was adjusted to pH = 4.5 by using 5 N NaOH solution. The solution was divided into four parts in a series of 1000 ml Erlenmeyer flasks. Afterward, various amounts (5, 10, 20 and 30 g corresponding to 14, 28, 56 and 84 mmol) of DTPA-dianhydride dissolved in dimethyl sulphoxide (DMSO) were added to flasks and the mixtures were stirred vigorously for 48 h at room temperature. After filtration, the precipitates were mixed with methanol and stirred for a further 12 h. After filtering again, the precipitates were mixed with a dilute NaOH solution (pH = 11) and stirred for 12 h to remove unreacted DTPA. After filtering once again, to achieve high purity products, the precipitates were dissolved in 1% (v/v) aqueous acetic acid solution and were put into a series of dialysis tubes and dialyzed against deionized water for 12 h, diluted NaOH solution for 12 h and again deionized water for 12 h. The final products were obtained by freeze drying.

Characterization of DTPA modified chitosan

The formation of additional functional groups on chitosan after modification with DTPA was studied using a Fourier transform infrared spectroscopy (FTIR) spectroscope, type MB-100, ABB Bomem Inc., (Canada). The DM was determined by measuring the free amino groups of unmodified and modified chitosan according to previously described method [1, 2], employing a UV-Vis spectrophotometer at 344 nm wavelength and 5% TNBSA regent solution as colorimetric reagent. Briefly 1.5 mg of each modified chitosans were swelled in 200 µl of demineralized water and incubated with 200 µl of 4% $NaHCO_3$ and 200 µl of 5% TNBSA at 37°C for 2 h. Thereafter, 200 µl of 2 N HCl was added before absorbance. The amount of remaining free amino groups was calculated using a standard curve obtained by the amino-group determination of a series of solutions with increasing amounts of unmodified chitosan.

To determine their kinetic viscosity, chitosan and DTPA-modified chitosans were dissolved in 1% (v/v) aqueous acetic solution and then acidity of solutions was adjusted to pH = 5.5 using 1 N NaOH solution. A series of calibrated glassware viscosimeters were used to measure kinetic viscosity of solutions.

Preparation and quality control of ^{66}Ga-DTPA-chitosan

Gallium-66, a carrier free radioisotope ($T_{1/2}$ = 9.49 h, total β^+ abundance = 56.5%, E_{mean} = 1.7 MeV, mean range of 28 mm in tissue and the most β^+ abundance = 51.5%, E_{max} = 4.2 MeV) and as a candidate for cancer radiotherapy [7, 17, 30] was produced based on the ^{66}Zn(p, n)^{66}Ga nuclear reaction according to previously described method [6, 22, 24, 35]. To prepare ^{66}Ga-DTPA-chitosan complex, desired amounts of DTPA-chitosan was dissolved in 2 ml of 1% (v/v) aqueous acetic acid solution and [^{66}Ga]gallium acetate solution was added. The acidity of solutions was adjusted to desired pH using 0.5 N NaOH and 0.5 N HCl followed by adjusting the volume to 3 ml using deionized water. Afterward, the solution was vigorously stirred for 5 min and kept for 30 min at room temperature. The effect of several factors on radiolabeling yield such as: acidity and concentration of DTPA-chitosan solution, contact time and radioactivity, was investigated by measuring radiochemical purity for various DM of DTPA-chitosans. For determining radiochemical purity, a 1 µL sample of the ^{66}Ga-DTPA-chitosan solution 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. The R_f values of free Ga-66 and ^{66}Ga-DTPA-chitosan complex were 0.9 and 0.0–0.1, respectively. A radio thin-layer chromatography (TLC) scanner, type AR-2000, Bioscan (USA) was used to measure the area under curve of radio-peaks.

Stability tests

The stability of prepared ^{66}Ga-DTPA-chitosan complexes was examined by measuring radiochemical purity by paper chromatography at 1, 2, 4, 8, 16, 24, 36 and 48 h after preparation. In order to investigate *in vitro* stability of ^{66}Ga-DTPA-chitosan complex, 200 µl of complex solution was dispersed into 500 µl of 50 mM phosphate buffer solution (PBS), pH = 7. The mixture was incubated at 37°C for 2 days and radiochemical purity was checked by paper chromatography during incubation.

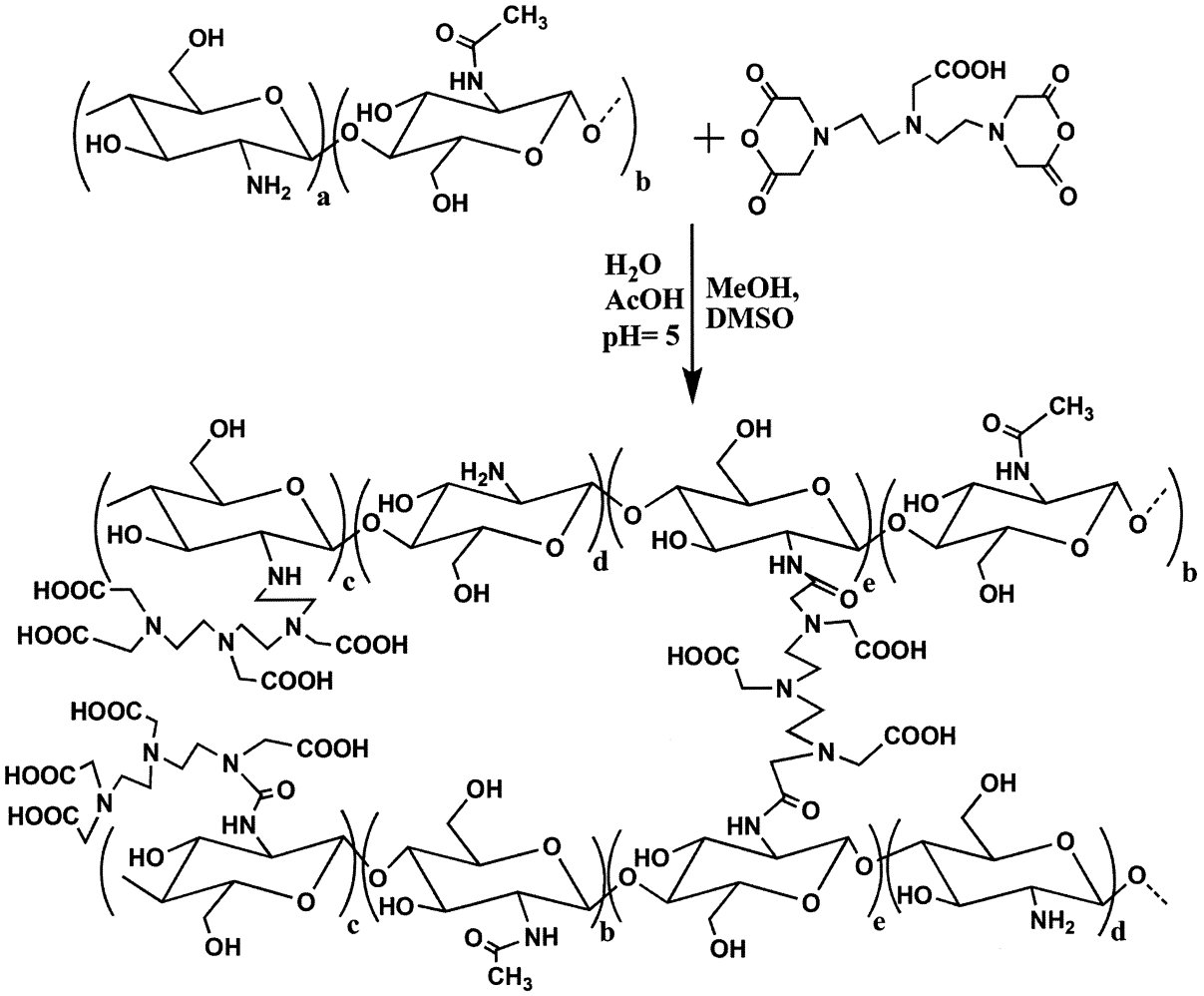
Animal studies

Tumor induction was performed by the use of polyaromatic hydrocarbon injection in rodents as reported previously [6, 12, 13]. For tumor model preparation, 10 µl of 3-methyl cholanthrene solution in extra-virgin olive oil (4 mg/ml) was injected subcutaneously (SC) to the dorsal area of the mice. After 14–16 weeks, the tumor weighed 0.2–0.4 g and was not grossly necrotic. Tumor tissues of some random animals were sent for pathological tests and were diagnosed as fibrosarcoma. In order to perform *in vivo* studies, 100 µL (600 µCi) samples of ^{66}Ga-DTPA-chitosan solution were injected directly to tumoral lesion of mice. Moreover, 100 µL (600 µCi) of [^{66}Ga]gallium chloride solution was injected directly into tumoral lesion of another group of mice for control study. To investigate the leakage of radioactivity, the animals (n = 3) were sacrificed by ether asphyxation at selected times after injection (3, 9, 18, 27 and 54 h) and percentage of injected dose in the tissues (brain, heart, liver, kidneys, testis, spleen, lung, stomach, bladder, large and small intestine, carcass and tumor) were determined with a high purity germanium (HPGe) γ-ray spectrometer or an ISOMED 1010 dose calibrator.

Results and discussion

Synthesis and characterization of DTPA-modified chitosans

DTPA modified chitosans were prepared by the formation of covalent amide bonds between carboxyl groups of the DTPA and primary amino groups of the polymer (Scheme 1). DTPA modified chitosans with various degrees of modification; DM = 6.1, 10.3, 15.7 and 20.9% were respectively synthesized when chitosan reacted with DTPA anhydride in 1:1.38, 1:2.76, 1:5.52, 1:8.27 molar ratios. Figure 1 shows the FTIR spectrograms of chitosan and modified chitosan which absorbance peaks at 1628 and 1532 cm^{-1} are respectively related to carboxylate groups of DTPA and new formed amide



Scheme 1. Synthesis of DTPA-chitosan from chitosan and DTPA-dianhydride.

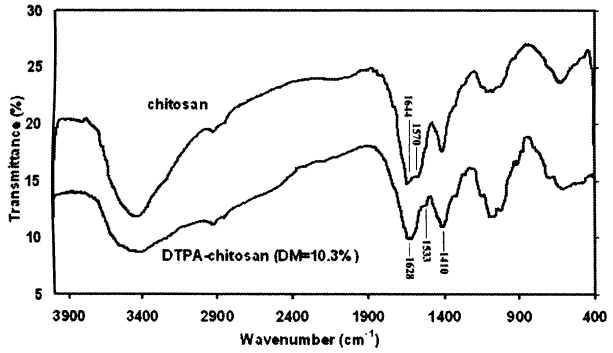


Fig. 1. FTIR spectrograms of chitosan and DTPA modified chitosan.

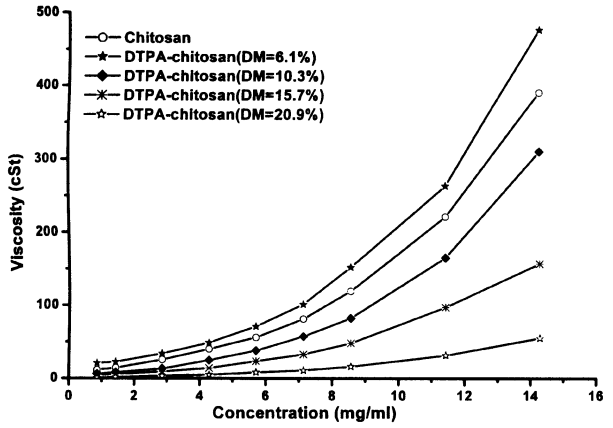


Fig. 2. Viscosity diagrams of chitosan and DTPA modified chitosans as a function of their concentration.

bonds [5]. Figure 2 shows the viscosity of chitosan and modified chitosans in acetate buffer solution (pH = 5.5) as a function of their concentrations. DTPA chitosan (DM = 6.1%) has higher viscosity than chitosan. However, other DTPA chitosans have lower viscosity. Viscosity changing behavior is probably because of two factors; cross linking caused by reacting two amino groups of chitosan with one DTPA molecule and increasing solubility due to the presence of carboxyl groups. The cross linking leads to increase in viscosity. In contrast, increasing of solubility leads to decrease in viscosity [20, 23].

Preparation of ^{66}Ga-DTPA-chitosan

DTPA modified chitosans were radiolabeled using carrier free gallium-66 acetate solution and the effect of several parameters was investigated on radiolabeling yield. Figure 3 shows the radiolabeling yield as a function of pH. In comparison with chitosan, which has highest radiolabeling yield at pH = 3.1–3.9, DTPA chitosans showed the highest radiolabeling yields at pH > 5. Furthermore, their radiolabeling yields increased at pH = 1–2.5. In contrast, their radiolabeling yields decreased at pH = 2.8–4. The decrease in radiolabeling yields is probably the result of inter and/or intramolecular ionic interaction between negatively charged carboxylate groups of DTPA moiety and positively charged amino groups of glucosamine units. This can prevent the interaction between DTPA chitosans

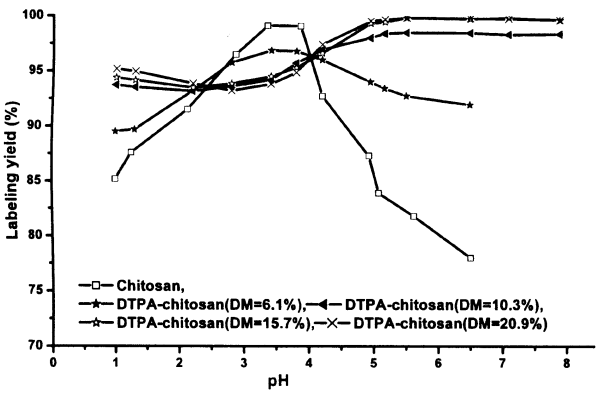


Fig. 3. Effect of pH on labeling yields of chitosan and DTPA--chitosans. Tests conditions: 60 mCi samples of gallium-66 were added to 3 ml samples of 10 mg/ml chitosan and modified chitosans solutions. Radiochemical purity was examined after 30 min. Each point presents average of three points.

and low concentrated radioactive Ga(III) ions [3, 11, 20, 23, 31]. However, by raising pH, the concentration of negatively charged carboxylate groups of DTPA increases. In contrast, the concentration of positively charged amino groups of glucosamine units decreases. Consequently, the interaction between Ga(III) ions and negatively charged carboxylate groups of DTPA is strengthened which leads to increase in the radiolabeling yields of DTPA-modified chitosans at pH > 5. In general, modification of chitosan by metal ion complexing agent has improved radiolabeling yield of DTPA-chitosans in a wider range of pH. However, the higher DM, the higher radiolabeling yield achieved in acidic (pH = 1–2.5) and neutral solutions (pH = 5–7.5). It seems that formation of a gallium DTPA-chitosan complex is the outcome of several possible ways. First, chelating of ionic form of Ga(III) ($[Ga(OAc)_n]^{3-n}$) by unmodified protonated glucosamine units which has the main role at pH = 3–4 [8, 29, 33]. Secondly, chelating of free Ga^{3+} by carboxyl groups of DTPA modified glucosamine units at pH = 1–2.5 and finally chelating of gallium ions by DTPA modified glucosamine units of polymer via changing in Ga(III) solvation groups. The last one has a significant role at pH > 5. Our results are slightly different from previous researches which have presented the pH = 2.1 as the best acidity to achieve highest efficiency in absorption of metal cations from aqueous sulfuric acid solution by DTPA-chitosan. This difference is probably due to counter-ion effects [3, 11, 20, 23, 31].

All DTPA chitosans presented the same behavior for the influence of concentration and contact time on radiolabeling yield at applied acidity (pH = 5.5). The concentration and contact time to achieve a radiolabeling yield higher than 99% were 11.6 mg/ml and 30 min, respectively. The radiolabeling yield for all ^{66}Ga--DTPA-chitosans decreased only by 2%, after addition of 4 times of radiogallium to DTPA-chitosan solutions at a concentration of 11.6 mg/ml.

Stability of ^{66}Ga-DTPA chitosan complex

The stability of the prepared ^{66}Ga-DTPA-chitosan complexes was checked with time up to 48 h after

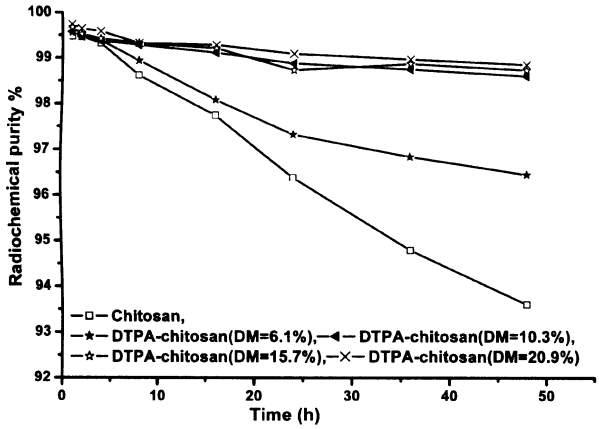


Fig. 4. Stability of ^{66}Ga-chitosan and ^{66}Ga-DTPA-chitosans. Each point presents average of three points.

preparation (Fig. 4). The complexes were completely stable at applied acidity (pH = 5.5). Investigation of *in vitro* stability in PBS (pH = 7) showed that ^{66}Ga-DTPA--chitosans are more stable than ^{66}Ga-chitosan. The radiochemical purities of ^{66}Ga-DTPA-chitosans (DM = 10.3, 15.7 and 20.9%) remained higher than 98.5%. Only the radiochemical purity of ^{66}Ga-DTPA-chitosan (DM = 6.1%) decreased to 96.45% after 48 h.

Animal studies

To investigate efficiency of the modified chitosans in preventing the leakage of radioactivity, 600 µCi samples of ^{66}Ga-DTPA-chitosans were intratumorally injected to a series of fibrosarcoma bearing mice. Figure 5 shows the distribution of radioactivity in mice organs. The accumulated radioactivities in small and large intestines, brain, testis, heart and stomach were less than 0.05% and are not shown in this figure. In comparison with chitosan [22], DTPA chitosan (DM = 6.1%) showed a slight increase of efficiency (87.3 vs. 85.4% for chitosan) in preventing the leakage of radioactivity from the injection site after 54 h of injection. Whereas, DTPA chitosan (DM = 10.3%) showed a significant increase and reached 97.3%. However, the efficiencies of DTPA chitosans (DM = 15.7 and 20.9%) decreased to 92.5 and 86.77%, respectively. Although, all the DTPA modified chitosans had better efficiency than unmodified chitosan, DTPA-chitosan (DM = 10.3%) had the best efficiency. Unexpected decrease in efficiency for DTPA-chitosans (DM = 15.7 and 20.9%) might be the result of their aqueous solutions viscosity declining. When gallium-66 chloride solution was injected intratumorally, the leaked radioactivity was accumulated in all organs of mice [22]. While, after intratumoral injection of radiolabeled DTPA-chitosans, the leaked radioactivity was accumulated just in special organs like lung, liver, blood and kidneys. These results show that radioactivity is probably leaked in the form of radiolabeled macromolecules.

Conclusion

In our previous work [22], we produced a gallium-66 chitosan complex. The produced complex was injected

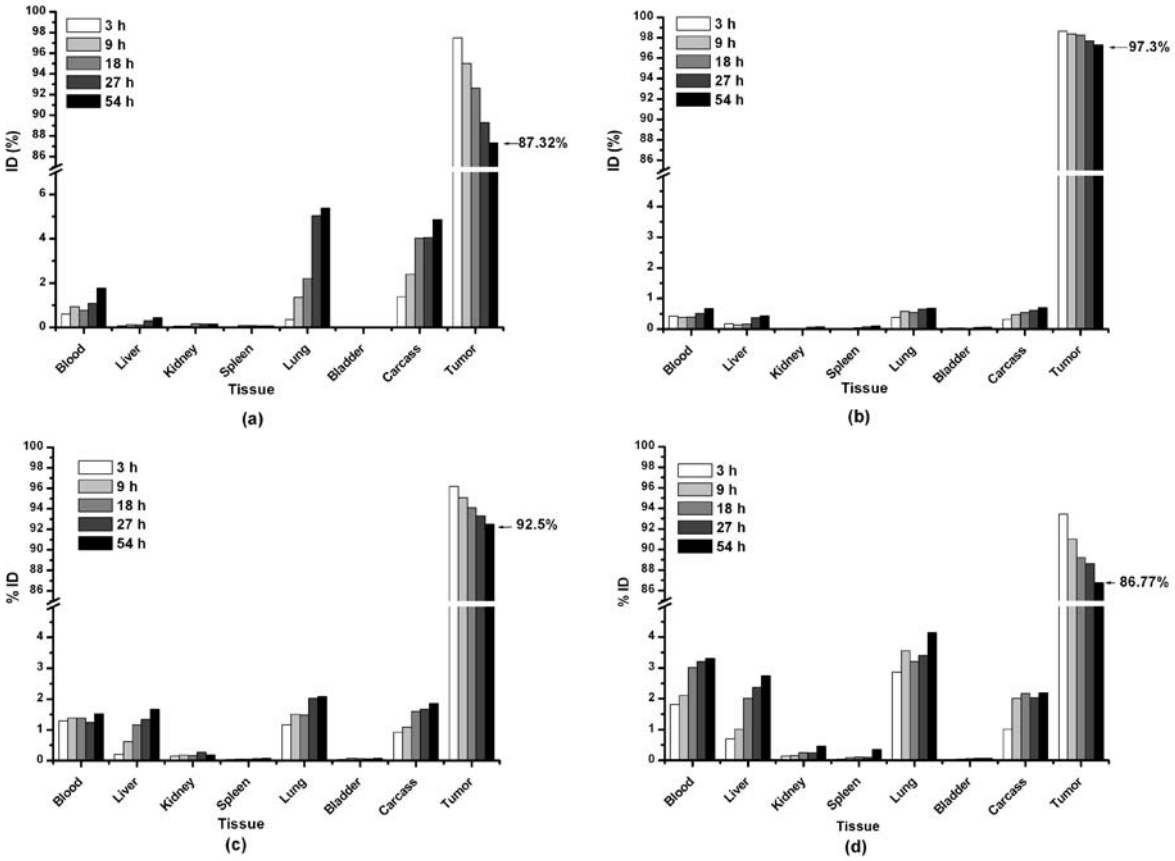


Fig. 5. Distribution of radioactivity after intratumoral injection of 600 µCi (100 µl) of radiolabeled DTPA chitosans; a – DM = 6.1%; b – DM = 10.3%; c – DM = 15.7%; d – DM = 20.9%. Each point presents average of three points.

intratumorally to fibrosarcoma bearing mice and showed an efficiency of 85.4% in preventing the leakage of radioactivity from the injection site. To increase its efficiency, chitosan was modified by DTPA as a metal ion complexing agent in several degrees of modification (DM = 6.1, 10.3, 15.7 and 20.9%). DTPA modified chitosans were radiolabeled with gallium-66 radionuclide and then were injected intratumorally to fibrosarcoma bearing mice. In comparison with chitosan, DTPA--chitosans showed higher radiolabeling yields in acidic and neutral solutions, demonstrating better efficiencies in preventing the leakage of radioactivity from the injection site. DTPA chitosan (DM = 10.3%) showed the highest efficiency (97.3%) after 54 h of injection. The efficiencies of DTPA chitosans (DM = 15.7 and 20.9%) decreased to 92.5 and 86.77%, respectively which can be the result of their viscosity decreasing at the applied acidity (pH = 5.5). Our results indicated that DTPA modified chitosan can be an effective carrier for radiotherapeutic radionuclides for tumor treatment by the intratumor injection technique.

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References

1. Bernkop-Schnürch A, Krajicek ME (1998) Mucoadhesive polymers as platforms for peroral peptide delivery and absorption: synthesis and evaluation of different chitosan--EDTA conjugates. J Control Release 50:215–223
2. Bernkop-Schnürch A, Paikl C, Valenta C (1997) Novel bioadhesive chitosan-EDTA conjugate protects leucine enkephalin from degradation by aminopeptidase N. Pharmaceut Res 14:917–922
3. Chassary P, Vincent T, Guibal E (2004) Metal anion sorption on chitosan and derivative materials: a strategy for polymer modification and optimum use. React Funct Polym 60:137–149
4. Chen SD, Hsieh JF, Tsai SC, Lin WY, Cheng KY, Wang SJ (2001) Intra-tumoural injection of ^{90}Y microspheres into an animal model of hepatoma. Nucl Med Commun 22:121–125
5. Darras V, Nelea M, Winnik FM, Buschmann MD (2010) Chitosan modified with gadolinium diethylenetriaminepentaacetic acid for magnetic resonance imaging of DNA/chitosan nanoparticles. Carbohyd Polym 80:1137–1146
6. DiGiovanni J, Rymer J, Slaga TJ, Boutwell RK (1982) Anticarcinogenic and cocarcinogenic effects of benzo[e]pyrene and dibenz[a,c]anthracene on skin tumor initiation by polycyclic hydrocarbons. Carcinogenesis 3:371–375
7. Graham MC, Pentlow KS, Mawlawi O, Finn RD, Daghighian F, Larson SM (1997) An investigation of the physical characteristics of ^{66}Ga as an isotope for PET imaging and quantification. Med Phys 24:317–326
8. Guibal E (2003) Interactions of metal ions with chitosan--based sorbents. Sep Purif Technol 38:43–74
9. Hamoudeh M, Fessi H, Salim H, Barbos D (2008) Holmium-loaded PLLA nanoparticles for intratumoral radiotherapy via the TMT technique: preparation, characterization, and stability evaluation after neutron irradiation. Drug Dev Ind Pharm 34:796–806
10. Holte O, Skretting A, Bach-Gansmo T *et al*. (2006) Localized internal radiotherapy with ^{90}Y particles embedded in a new thermosetting alginate gel: a feasibility study in pigs. Nucl Med Commun 27:185–190
11. Inoue K, Yoshizuka K, Ohto K (1999) Adsorptive separation of some metal ions by complexing agent types of chemically modified chitosan. Anal Chim Acta 388:209–218
12. Jalilian AR, Akhlaghi M, Shirazi B *et al*. (2006) [^{201}Tl](III)-Bleomycin for tumor imaging. Radiochim Acta 94:453–459
13. Jalilian AR, Rowshanfarzad P, Yari-Kamrani Y, Shafaii K (2007) Production and tumor uptake of [^{64}Cu]Pyruvaldehyde-bis(N4-methylthiosemicarbazone) for PET and/or therapeutic purposes. Nucl Med Rev 10:6–11
14. Junfeng Y, Duanzhi Y, Xiaofeng M *et al*. (1999) [^{188}Re]Rhenium sulfide suspension: a potential radiopharmaceutical for tumor treatment following intra-tumor injection. Nucl Med Biol 26:573–579
15. Junfeng Y, Duanzhi Y, Xiaofeng M *et al*. (1999) Preparation of [^{188}Re]rhenium sulfide suspension and its biodistribution following intra-tumor injection in mice. J Labelled Compd Rad 42:233–243
16. Junfeng Y, Ruping Z, Xinlan D *et al*. (2000) Intratumoral injection with [^{188}Re]rhenium sulfide suspension for treatment of transplanted human liver carcinoma in nude mice. Nucl Med Biol 27:347–352
17. Lewis MR, Reichert DE, Laforest R *et al*. (2002) Production and purification of gallium-66 for preparation of tumor-targeting radiopharmaceuticals. Nucl Med Biol 29:701–706
18. Lin WY, Tsai SC, Hsieh JF, Wang SJ (2000) Effects of ^{90}Y-microspheres on liver tumors: comparison of intratumoral injection method and intra-arterial injection method. J Nucl Med 41:1892–1897
19. Liu S (2008) Bifunctional coupling agents for radiolabeling of biomolecules and target-specific delivery of metallic radionuclides. Adv Drug Deliv Rev 60:1347–1370
20. Nagib S, Inoue K, Yamaguchi T, Tamaru T (1999) Recovery of Ni from a large excess of Al generated from spent hydrodesulfurization catalyst using picolylamine type chelating resin and complexane types of chemically modified chitosan. Hydrometallurgy 51:73–85
21. Park KB, Kim YM, Shin BC, Kim JR, Ryu JM, Lim SM (1998) Therapeutic application of new holmium-166 chitosan complex in malignant and benign diseases. In: Modern trends in radiopharmaceuticals for diagnosis and therapy. Proceedings of a Symposium held in Lisbon, Portugal, 30 March – 3 April 1998. IAEA-TECDOC-1029. IAEA, Vienna, pp 569–580
22. Pourjavadi A, Akhlaghi M, Jalilian AR (2011) Preparation and evaluation of [^{66}Ga]gallium chitosan complex in fibrosarcoma bearing animal models. Nukleonika 56;1:35–40
23. Repo E, Warchol JK, Kurniawan TA, Sillanpaa MET (2010) Adsorption of Co(II) and Ni(II) by EDTA- and/or DTPA-modified chitosan: kinetic and equilibrium modeling. Chem Eng J 161:73–82
24. Sabet M, Rowshanfarzad P, Jalilian AR, Ensaf MR, Rajamand AA (2006) Production and quality control of ^{66}Ga radionuclide. Nukleonika 51;3:147–154
25. Shin BC, Park KB, Jang BS, Lim SM, Shim CK (2001) Preparation of ^{153}Sm-chitosan complex for radiation synovectomy. Nucl Med Biol 28:719–725
26. Sofou S (2008) Radionuclide carriers for targeting of cancer. Int J Nanomed 3:181–191
27. Suzuki YS, Momose Y, Higashi N *et al*. (1998) Biodistribution and kinetics of holmium-166-chitosan complex (DW-166HC) in rats and mice. J Nucl Med 39:2161–2166

28. Tian JH, Xu BX, Zhang JM, Dong BW, Liang P, Wang XD (1996) Ultrasound-guided internal radiotherapy using yttrium-90-glass microspheres for liver malignancies. J Nucl Med 37:958–963
29. Tuck DG (1993) The coordination and solution chemistry of aluminium, gallium, indium and thallium. In: Downs AJ (ed) Chemistry of aluminum, gallium, indium and thallium. Blackie Academic and Professional, London, pp 430–473
30. Ugur O, Kothari PJ, Finn RD *et al*. (2002) Ga-66 labeled somatostatin analogue DOTA-DPhe1-Tyr3-octreotide as a potential agent for positron emission tomography imaging and receptor mediated internal radiotherapy of somatostatin receptor positive tumors. Nucl Med Biol 29:147–157
31. Varma AJ, Deshpande SV, Kennedy JF (2004) Metal complexation by chitosan and its derivatives. Carbohyd Polym 55:77–93
32. Wang XM, Yin ZY, Yu RX, Peng YY, Liu PG, Wu GY (2008) Preventive effect of regional radiotherapy with phosphorus-32 glass microspheres in hepatocellular carcinoma recurrence after hepatectomy. World J Gastroenterol 14:518–523
33. Weiner RE, Thakur ML (2003) Chemistry of gallium and indium radiopharmaceuticals. In: Welch MJ, Redvanly CS (eds) Handbook of radiopharmaceuticals: radiochemistry and applications. John Wiley and Sons, Chichester, pp 363–400
34. Williams LE, DeNardo GL, Meredith RF (2008) Targeted radionuclide therapy. Med Phys 35:3062–3068
35. Zweit J, Sharma H, Downey S (1987) Production of gallium-66, a short-lived positron emitting radionuclide. Int J Rad Appl Instrum A 38:499–501