การพัฒนาไคโตซานไมโครสเฟียร์เป็นตัวนำส่งสารรังสี

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บทคัดย่อ

การทดลองนี้ได้ทำการเตรียมและศึกษาคุณลักษณะของไคโตซานไมโครสเฟียร์ หลังจากนั้นทำการติดฉลากด้วยเทคนิเชียม-99เอ็ม และศึกษาการกระจายตัวของสารติดฉลากดังกล่าวในสัตว์ทดลอง จากการทดลองพบว่าขนาดของไคโตซานไมโครสเฟียร์ที่เตรียมได้มีขนาด 77.26±1.96 µm มีประจุบนผิวเป็นประจุบวกโดยมีค่าซีต้าโพเทนเชียล 25.80 ± 0.46 mV มีประสิทธิภาพในการติดฉลากมากกว่า 95% และมีความคงตัวหลังการติดฉลากไม่น้อยกว่า 6 ชั่วโมง การศึกษาการกระจายตัวในสัตว์ทดลองพบว่ามีการสะสมของสารติดฉลากมากที่ปอด ม้ามและตับ

คำสำคัญ: ไคโตซาน เทคนิเชียม ไมโครสเฟียร์ การกระจายตัว

Chitosan Microspheres as Radiolabeled Delivery Devices

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Abstract

This study optimized conditions for preparing, characterizing, radiolabeled of chitosan microspheres and the biodistribution of ^{99m}Tc-Chitosan microspheres after intravenous administration. Particle size distribution of the microspheres was determined by light scattering. Zeta potential was studied by dynamic light scattering and electrophoresis technique. Biodistribution studies were performed by radiolabeling using ^{99m}Tc. The results shown that geometric mean diameter of the microspheres was found to be 77.26±1.96 µm. Microsphere surface charge of chitosan microspheres was positive charge and zeta potential was 25.80 ± 0.46 mV. The labeling efficiency for this condition was more than 95% and under this condition was stable for at least 6 h. Radioactivity

accumulated in different organs after intravenous administration showed a significant amount of radioactivity in the lung, spleen and liver.

Key words: Chitosan, technetium, microsphere, biodistribution

Introduction

Controlled drug delivery technology represents one of the frontier areas of science, which involves multidisciplinary scientific approach, contributing to human health care. These delivery systems offer numerous advantages compared to conventional dosage forms, which include improved efficacy, reduced toxicity, and improved patient compliance and convenience. Such systems often use macromolecules as carriers for the drugs. By doing so, treatments that would not otherwise be possible are now in conventional use. This field of pharmaceutical technology has grown and diversified rapidly in recent years. Understanding the derivation of the methods of controlled release and the range of new polymers can be a barrier to involvement from the nonspecialist.[1]

Chitosan is a linear polysaccharide comprised of two monosaccharides: *N*-acetyl-D-glucosamine and D-glucosamine linked together by glucosidic bonds. Chitosan has 1 primary amino and 2 free hydroxyl groups for each C_6 building unit. Due to the easy availability of free amino groups in chitosan, it carries a positive charge and thus, in turn, reacts with many negatively charged surfaces/polymers. Chitosan is produced by alkaline hydrolysis (deacetylation) of chitin obtained from crustacean shells and forms positively charged salts when dissolved in inorganic or organic acids. Chitosan is available in a wide range of molecular weights and degrees of deacetylation. It is non-toxic and easily biodegradable polymer[2]. Polymeric controlled delivery systems based on chitosan are now being used for a wide range of drug[3,4,5].

Materials and Methods

Materials

Chitosan (100 kGy irradiated at Office of Atoms for Peace, Thailand), 5% acetic acid prepared from glacial acetic acid (Sigma), paraffin oil (Carlo), span 80 (Fluka), 25% glutaric dialdehyde prepared from glutaric dialdehyde 50% wt. solution in water (Acros), petroleum ether

(analytical grade, boiling range 40-60 °C, Scharlau), stannous chloride ($SnCl_2.2H_20$, Merck), technetium-99m was purchased from Biogenetec. All other chemicals and solvents were of analytical grade and used without further purification.

Methods

1. Formulation of chitosan microspheres by water/oil method

Chitosan microspheres were prepared by using water/oil emulsion technique. The aqueous phase consisted of 2% chitosan, was prepared by using chitosan 1.0 g in 50 ml of 5% glacial acetic acid. The chitosan solution was filtrated before experiment. The oil phase consisted of 100 ml paraffin oil containing 1 ml of span 80. The oil phase was mixed by using magnetic stirrer at 1400 rpm. Chitosan solution was added to the oil phase drop by drop with syringe, and stirred at 1400 rpm for 5 min. After that, 2 ml of 25% glutaric dialdehyde was added into emulsion and stirred at the same speed for 5 min then decrease speed at 500 rpm for 1 h. Two ml of 25% glutaric dialdehyde were added again and stirred for1 h. The microspheres were collected by centrifugation for 10 min at 10,000 rpm. The precipitates were suspended and washed with petroleum ether and distilled water to remove paraffin and glutaric dialdehyde, respectively. The microspheres were then suspended in methanol and stored at 4-8 ^{0}C until further use.

2. Particle size

The particle size distribution of the microspheres was determined by light scattering on a Malvern particle size analyzer (Mastersizer X, Malvern, United Kingdom). The microspheres were added to the sample dispersion unit containing the stirrer and stirred to reduce the aggregation between the microspheres. Laser obscuration was maintained between 10% and 30%. All particle size measurements were repeated 3 times per sample and each sample was prepared in triplicate. The average values and standard deviations were calculated.

3. Surface charge

Electrophoretic mobility was measured using a Zetasizer Nano ZS (Malvern, United Kingdom).

4. Radiolabeling of chitosan microspheres

Chitosan microspheres were labeled with technetium-99m by direct labeling method. Briefly, 0.25 ml of chitosan solution was mixed with 1 ml distilled water and 20 µl of stannous chloride solution (freshly prepared in 10 mM hydrochloric acid at 1 mg/ml). To this mixture, 10 mCi of technetium-99m was added and incubated for 30 minutes at room temperature. The quality control was performed by instant thin layer chromatography using silica gel coated fiber sheets (ITLC-SG, Gelman Science) and 0.9% saline as the mobile phase. Labeling efficiency was consistently above 95% by using TLC scanner (Bioscan).

5. Biodistribution studies

Biodistribution studies were performed in ICR mouse (20-30 g). Each mouse received 100 µCi of 0.1 ml of labeled compound through the tail vein. The mice were killed at 1, 2, 4 and 24 h post-injection times and then were dissected. The amount of radioactivity in other tissues was determined by gamma counting. Three mice per time point were used. The results are expressed as percentage of injected dose per gram of tissue (% ID/g) by reference to standard prepared from dilution of the injected preparation.

Results and Discussion

1. Particle size and surface charge of chitosan microspheres

In this preparation technique, chitosan microspheres particle size was between 77.26 ± 1.96 µm. Microsphere surface charge of chitosan microspheres was positive charge and zeta potential was 25.80 ± 0.46 mV.

2. Labeling efficiency

Chitosan microspheres have been labeled with ^{99m}Tc with high efficiency by direct labeling technique. Data on radiochemical purity and stability of the labeled complex were obtained by ascending chromatography using saline as the mobile phase. The labeling efficiency for this condition was more than 95% and under this condition, this labeled compound was stable for at least 6 h.

3. Biodistribution studies

The Biodistribution of ^{99m}Tc-Chitosan microspheres after 1, 2, 4 and 24 h is shown in Fig 1. The percentage of radioactivity is expressed per gram of tissue. Data revealed that rapid clearance from the blood pool was observed and the labeled compound is excreted primarily via urine. Lung, spleen, and liver were three other organs with higher activity at 4 h postinjection and declined gradually at 24 h postinjection.

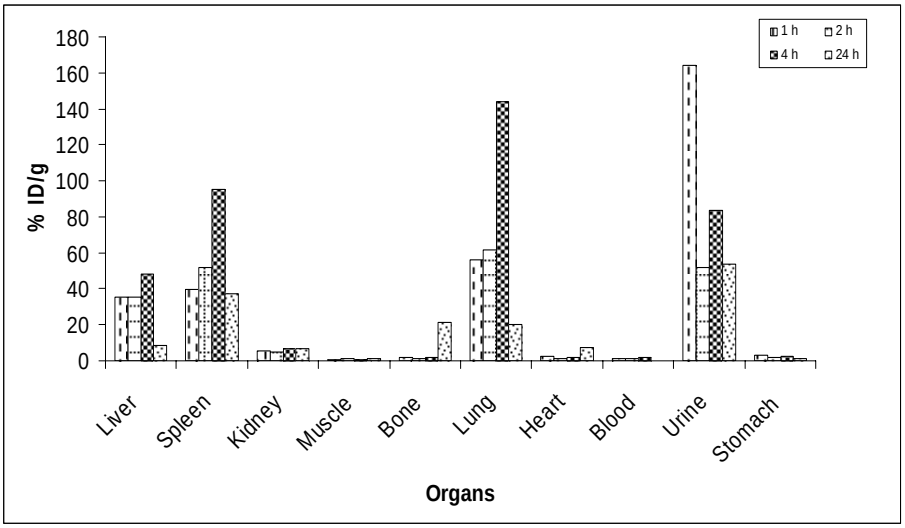

Fig 1. Biodistribution of ^{99m}Tc-chitosan microspheres in ICR mouse after intravenous administration.

Conclusions

Spherical chitosan microspheres were successfully formulated. Labeling efficiency and *in vitro* stability test at room temperature revealed that labeling of ^{99m}Tc-Chitosan microspheres was stable more than 95% over 6 h period. The labeled compound is extensively distributed in organs such as lung, spleen and liver. The concentration of radioactivity in other organs, such as kidney, muscle, bone, and heart, were quite low throughout the study.

Drug delivery system has rejuvenated interest in seemingly ineffective drugs by site specific drug targeting and obviating unwanted systemic side effects while simultaneously reducing the dose and toxicity. Biodegradable microsphere based on chitosan is one of the promising polymers that will be successful to convey a sufficient dose of drug to the lesion, suitable carriers of drugs and radiolabel compounds are needed.

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