Original Article

감염병소 영상을 위한 ^{99m}Tc-Transferrin 개발

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Development of ^{99m}Tc-Transferrin as an Imaging Agent of Infectious Foci

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Purpose: Purpose of this study is to synthesize ^{99m}Tc-labeled transferrin for infection imaging and to compare it with ^{67}Ga-citrate for the detection of infectious foci. **Materials and methods:** Succinimidyl 6-hydrazino-nicotinate hydrochloride-chitosan-transferrin (Transferrin) was synthesized and radiolabeled with ^{99m}Tc. Labeling efficiencies of ^{99m}Tc-Transferrin were determined at 10 min, 30 min, 1 hr, 2 hr, 4 hr and 8 hr. Biodistribution and imaging studies with ^{99m}Tc-Transferrin and ^{67}Ga-citrate were performed in a rat abscess model induced with approximately 2×10^8 colony forming unit of *Staphylococcus aureus* ATCC 25923. **Results:** Successful synthesis of Transferrin was confirmed by mass spectrometry. Labeling efficiency of ^{99m}Tc-Transferrin was 96.2±0.7%, 96.4±0.5%, 96.6±1.0%, 96.9±0.5%, 97.0±0.7% and 95.5±0.7% at 10 min, 30 min, 1 hr, 2 hr, 4 hr and 8 hr, respectively. The injected dose per tissue gram of ^{99m}Tc-Transferrin was 0.18±0.01 and 0.18±0.01 in the lesion and 0.05±0.01 and 0.04±0.01 in the normal muscle, and lesion-to-normal muscle uptake ratio was 3.7±0.6 and 4.7±0.4 at 30 min and 3 hr, respectively. On image, lesion-to-background ratio of ^{99m}Tc-Transferrin was 2.18±0.03, 2.56±0.11, 3.08±0.18, 3.77±0.17, 4.70±0.45 and 5.59±0.40 at 10 min, 30 min, 1 hr, 2 hr, 4 hr and 10 hr and those of ^{67}Ga-citrate was 3.06±0.84, 4.12±0.54 and 4.55±0.74 at 2 hr, 24 hr and 48 hr, respectively. **Conclusion:** Transferrin is successfully labeled with ^{99m}Tc, and its labeling efficiency was higher than 95% and stable for 8 hours. ^{99m}Tc-Transferrin scintigraphy showed higher image quality in shorter time compared to ^{67}Ga-citrate image. ^{99m}Tc-transferrin is supposed to be useful in the detection of the infectious foci. (Nucl Med Mol Imaging 2006;40(3):177-185)

Key Words: transferrin, Technetium-99m, animal model, infection

Introduction

Transferrin, a family of iron-binding proteins, is a key molecule in ^{67}Ga-citrate scintigraphy. Its fundamental role is to control the levels of free iron in body fluid by binding, sequestering and transporting Fe^{3+} ions. In infectious or inflammatory disease, transferrin presents in the inflammatory lesions due to leakage of plasma protein via increased vascular permeability.[1-5] *In vivo* studies using ^{67}Ga find that all gallium in blood is present in plasma (with traces in leukocytes) and is tightly bound to transferrin in the specific Fe^{3+} binding sites with similar affinity. This complex extravasates at the site of infection/inflammation owing to the locally enhanced vascular permeability. It is generally accepted that ^{67}Ga is transported mainly via transferrin-mediated mechanism.

^{67}Ga-citrate is the first gamma imaging agent for infectious and inflammatory lesions and has been widely used.[6,7] ^{67}Ga-citrate accumulates in not only the infectious and inflammatory lesions but also tumor. However, ^{67}Ga-citrate is not readily available in routine clinical setting because of several disadvantages such as delayed optimal imaging, poor image quality, high absorbed doses due to relatively long physical half-life, and unpredictable intestinal activity.[8-11]

Compared to other radiolabeled agents, ^{99m}Tc-labeled agents have favorable physical characteristics and several advantages for gamma camera imaging. ^{99m}Tc-labeled

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agents permit rapid imaging and additional delayed imaging after 2-4 hours, good image resolution and readily available in routine clinical setting for all laboratories of Nuclear Medicine, because of generator' product. Moreover, a short physical half-life ($T_{1/2}$ = 6 hr) of ^{99m}Tc allows additional studies to be performed after 2 days from the previous study.

Some investigators had performed studies using radiolabeled transferrin (^{131}I, ^{111}In, ^{113m}In, ^{68}Ga and ^{18}F) to evaluate bone marrow function,[12,13] pulmonary vascular permeability,[14,15] protein-losing enteropathy,[16] tumor[17,18] and transferrin receptor kinetics in the liver.[19] However, there has been no study with ^{99m}Tc-labeled transferrin for imaging infection or inflammation. ^{99m}Tc-labeled transferrin would be used to detect infectious or inflammatory foci more rapidly, and more easily than ^{67}Ga-citrate. It could have better image quality than ^{67}Ga-citrate.

The purpose of this study is to develop a new radiopharmaceutical, ^{99m}Tc-transferrin, and to compare it with ^{67}Ga-citrate for the detection of infectious foci in animal model.

Materials and Methods

1. Synthesis of HYNIC-chitosan-transferrin conjugate

Human apo-transferrin was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Succinimidyl 6-hydrazino-nicotinate hydrochloride (HYNIC) was synthesized as described by Abrams *et al.*[20] with minor modifications.[21,22] Low-molecular weight (approximately 5 kDa), water-soluble chitosan was supplied from Nah and Jo (Sunchon National University, Sunchon, Korea).

A chemical structure of designed HYNIC-chitosan-transferrin conjugate is presented in Figure 1. Transferrin is linked with chitosan and HYNIC consecutively. HYNIC makes it possible to be radiolabeled with ^{99m}Tc to HYNIC-chitosan-transferrin conjugate.

To synthesize HYNIC-chitosan-transferrin conjugate, two-step reaction (synthesis of chitosan-transferrin and synthesis of HYNIC-chitosan-transferrin) was used.

Synthesis of chitosan-transferrin was performed similarly as described previously for transferrin-PEI conjugates with some modifications.[23,24] A solution of chitosan as HCl salt in water was subjected to gel filtration on a Sephadex G-25 superfine column (Pharmacia, Uppsala, Sweden). A solution of transferrin in 30 mM sodium acetate buffer (pH5) was subjected to gel filtration on a Sephadex G-25 superfine column. The resulting solution was cooled to 0℃, and three equivalents of sodium periodate in 30 mM sodium acetate buffer (pH5) were added. The mixture was kept on ice, in the dark, for 90 minutes. For removal of the low molecular weight products an additional gel filtration (Sephadex G-25 superfine, 30 mM sodium acetate buffer pH5) was performed: this yielded a solution containing oxidized transferrin. The modified transferrin solution (30 mM sodium acetate pH5) was promptly added (within 15 minutes) to the chitosan solution (0.25 M sodium chloride) at a molar ratio of 1:1.2 and vigorously mixed at room temperature. The pH of the mixed solutions was adjusted to 7.3 by the addition of 2 M Hepes pH7.9. After 30 minutes, four portions of sodium cyanoborohydride (1 mg per 10 mg transferrin) were added at 1 hour-interval.

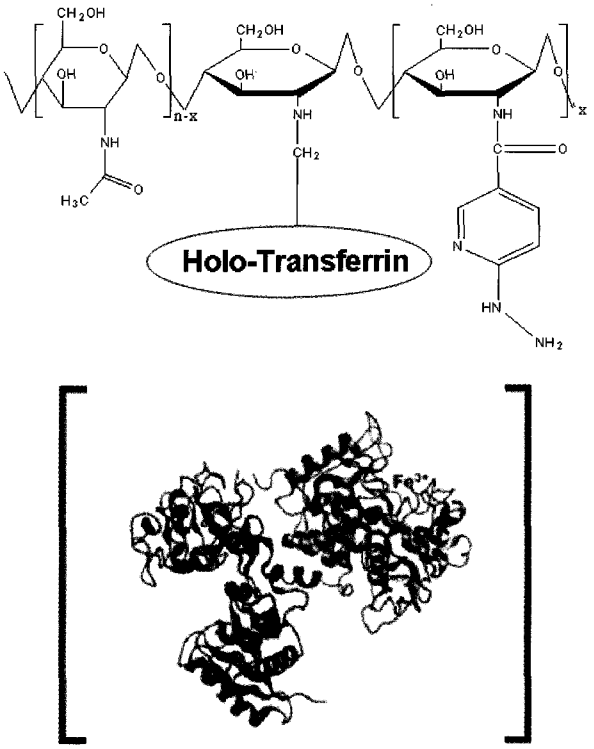

Fig. 1. Designed chemical structure of HYNIC-chitosan- transferrin conjugate. Human holo-transferrin is conjugated with chitosan-HYNIC.

After 19 hours, the salt concentration was adjusted to 0.5 M by addition of 3 M sodium chloride. The reaction mixture was loaded on to a cation-exchange column [Bio-Rad (Hercules, CA, USA) Macro-Prep high S HR 10/10] and fractionated with a salt gradient from 0.5 M to 3.0 M sodium chloride (with a constant content of 20 mM Hepes pH7.3). The major amount of conjugate eluted between 2.0 M and 3.0 M salt. After dialysis against 21 of HBS pH7.3 (150 mM sodium chloride, 20 mM Hepes pH7.3), the conjugate (designated as chitosan-transferrin) was obtained with a molar ratio of transferrin : chitosan = 1:1.0-1.1. Samples were diluted to 1 mg/ml chitosan concentration by addition of HBS pH7.3. Iron was incorporated by addition of 1.25 μl 10 mM iron (III) citrate buffer (containing 200 mM citrate and adjusted to pH7.8 by sodium bicarbonate addition) per milligram transferrin. The conjugates were aliquoted, shock-frozen in liquid nitrogen and kept at -80°C.

For synthesis of HYNIC-chitosan-transferrin, a 3 molar excess of freshly dissolved HYNIC (30 mM in DMF) was added drop-wise to a stirred solution of chitosan-transferrin. The solution was stirred gently for 24 hours at room temperature protected from light. This was followed by dialysis against 0.2 M, pH8.2 borate buffer at 4°C (six buffer changes over 48 hours). To verify successful conjugation of transferrin, HYNIC-chitosan-transferrin conjugates were performed mass spectrometry.[25]

2. Radiosynthesis of HYNIC-chitosan-transferrin with ^{99m}Tc-pertechnetate

A solution of HYNIC-chitosan-transferrin conjugate (200 μl, 2 μg) was obtained in a yielded vial. 15 μl $SnCl_2$ solution (containing 0.2 μg $SnCl_2$ and 0.03 *N* HCl) and 111 MBq ^{99m}Tc-pertechnetate were added to the solution of HYNIC-chitosan-transferrin conjugate in the yielded vial, respectively. Thereafter, total volume was fitted in 1 ml with normal saline. After room temperature incubation for 30 minutes, the mixture was filtered with 0.22 μm micropore. The resulting solutions were ^{99m}Tc-HYNIC-chitosan-transferrin (^{99m}Tc-transferrin). To determine labeling efficiency, all the produced ^{99m}Tc-transferrin was performed thin layer chromatography (TLC) using TLC scanner (AR-2000 TLC Imaging Scanner, BioScan, USA).

For labeling stability, labeling efficiency of ^{99m}Tc-transferrin was determined at 10 minute, 30 minute, 1 hour, 2 hour, 4 hour and 8 hour, respectively.

3. Generation of experimental rat abscess model

All the rats were purchased from Semtako Bio Korea (Osan, Korea). The study was performed according to the guidelines and the recommendations of the committee of animal research at the Chonnam National University Medical School. Rats were kept in cages with standardized conditions of light and free access to water and food.

Abscess was induced in the left calf muscles of young, male, randomly bred Wister rats (220~250 g) with approximately 2×10^8 colony forming unit of *Staphylococcus aureus* ATCC 25923 (*S. aureus*).

For bacterial suspension, the bacteria were incubated overnight on Tryptic Soy agar plates at 37°C, 5.0% CO_2 incubator. Colonies were diluted with sterile saline (API NaCl 0.85% Medium, bioMèrieux SA, France) and counted according to the McFerland turbidity standard by light spectrometry (ATB 1550, API SYSTEM SA, France).

Rats were anesthetized with mixture of a Ketamine/Rompun cocktail (Ketamine:Rompun = 4:1) by intraperitoneal injection. Bacterial suspension of 0.2 ml was injected to their left thigh muscle using 26 gauge needles, while the contralateral thigh muscle served as control.

To confirm the formation of abscess in the bacterial-inoculated thigh muscles, all the rats were sacrificed. Both thigh muscles obtained with razor blades, and fixed in 10% buffered formaldehyde solution for histopathology. The fixed tissues were embedded in paraffin and sectioned with a microtome. Sectioned fixed tissues were performed Hematoxylin and eosin (H-E) stain. One histopathologic expert reviewed all pathologic specimens.

4. Biodistribution of ^{99m}Tc-transferrin

Twenty-four hours after bacterial inoculation, when gross swelling was apparent in the left thigh muscles of the rats, ^{99m}Tc-transferrin (3~4 MBq) was injected with intravenously via tail veins and sacrificed at 30 minutes and 3 hours after intraperitoneal injection of 30 mg sodium Phenobarbital (each 4 rats). Blood sample was obtained by cardiac puncture. Following cervical dislocation, samples of

the infected left thigh muscle, uninfected right thigh muscle, liver, spleen, lung, heart, kidney and intestine were collected, weighed and counted in a shielded well-type gamma counter. To correct for physical decay and to permit calculation of the uptake of the ^{99m}Tc-transferrin, injected standard dose was counted simultaneously. The results were expressed as percentage injected dose per gram tissue (%ID/g).

5. Scintigraphic imaging with ^{99m}Tc-transferrin and ^{67}Ga-citrate

Scintigraphic imaging with ^{99m}Tc-transferrin and ^{67}Ga-citrate was obtained in abscess-induced eighteen rats, consecutively. In brief, all the rats were sedated by intraperitoneal injection of Ketamine/Rompun cocktail. After sedation, the rats were immobilized on a plate with an adhesive bandage and placed supine position under a single-headed gamma camera (DSX, Sophy, France) equipped with a 4 mm pinhole collimator. The energy windows were centered at 140 KeV photopeak of ^{99m}Tc and the triple photopeaks of ^{67}Ga (93 KeV, 184 KeV and 296 KeV) with 20% windows. After bacterial inoculation in the left thigh muscle at day 0, the rats were injected 18.5 MBq ^{99m}Tc-transferrin intravenously via tail vein at day 1. Scinitgraphic image was acquired at 10 minute, 30 minute, 1 hour, 2 hour, 4 hour and 10 hour post-injection. After ^{99m}Tc-transferrin imaging, image of ^{67}Ga-citrate (18.5 MBq) was obtained in the same rats at 2, 24 and 48 hours post-injection. Images (300 sec/image) were obtained and stored in a 256×256 matrix.

Image analysis was performed using region of interest (ROI) over the abscess and over the noninfected contralateral thigh muscles. Lesion-to-background ratios were calculated.

6. Statistical analysis

Statistical analysis was performed Students' *t*-test using the SPSS software package (Version 11.0, SPSS Inc, Chicago, USA). Descriptive data are presented as the mean ±standard deviation. The difference was considered to be significant if the *p* value was less than 0.05.

Results

1. Synthesis of HYNIC-chitosan-transferrin conjugate

A major mass peak of human apo-transferrin was noted on 80 kDa as known by mass spectrometry (Fig. 2A). In HYNIC-chitosan-transferrin, a major mass peak was apparent around 84 kDa (Fig. 2B). Compared to human apo-transferrin, the mass peak of HYNIC-chitosan-transferrin was broader and displaced to right side. This finding was caused by its increased molecular weight from the conjugation of transferrin with chitosan-HYNIC. This result implied that human apo-transferrin was successfully conjugated as a HYNIC-chitosan-transferrin.

2. Labeling efficiency and stability of ^{99m}Tc-transferrin

Labeling efficiency of ^{99m}Tc-transferrin was higher than 95% at 30 minute. The serial labeling efficiency was 96.2±0.7%, 96.4±0.5%, 96.6±1.0%, 96.9±0.5%, 97.0±0.7% and 95.5±0.7% at 10 minute, 30 minute, 1 hour, 2 hour, 4 hour and 8 hour, respectively. ^{99m}Tc-transferrin showed high and stable labeling efficiency (more than 95%) up to 8 hours.

3. Histopathology of the infected muscle

All the rats showed gross swelling and inflammatory mass in the left thigh muscle within 24 hours after bacterial inoculation. Histopathologic finding of the infected muscle was suitable for abscess formation within the muscle mass, which was necrotic cavity, infiltration of inflammatory cells between muscle fibers and destruction of the muscle fibers.

4. Biodistribution of ^{99m}Tc-transferrin

The biodistribution of ^{99m}Tc-transferrin determined at 30 minute and 3 hour after the injection is summarized in Table 1. The injected dose per tissue gram (%ID/g) of ^{99m}Tc-transferrin was 0.18±0.01% and 0.18±0.01% in the infected lesion and 0.05±0.01% and 0.04±0.01% in the normal muscle at 30 minute and 3 hour, respectively. Infected-to-normal muscle ratio was 3.7±0.6 and 4.7±0.4 at minute and 3 hour, respectively. The highest uptake was found in the kidney (4.39±0.65% and 3.79±0.53%,

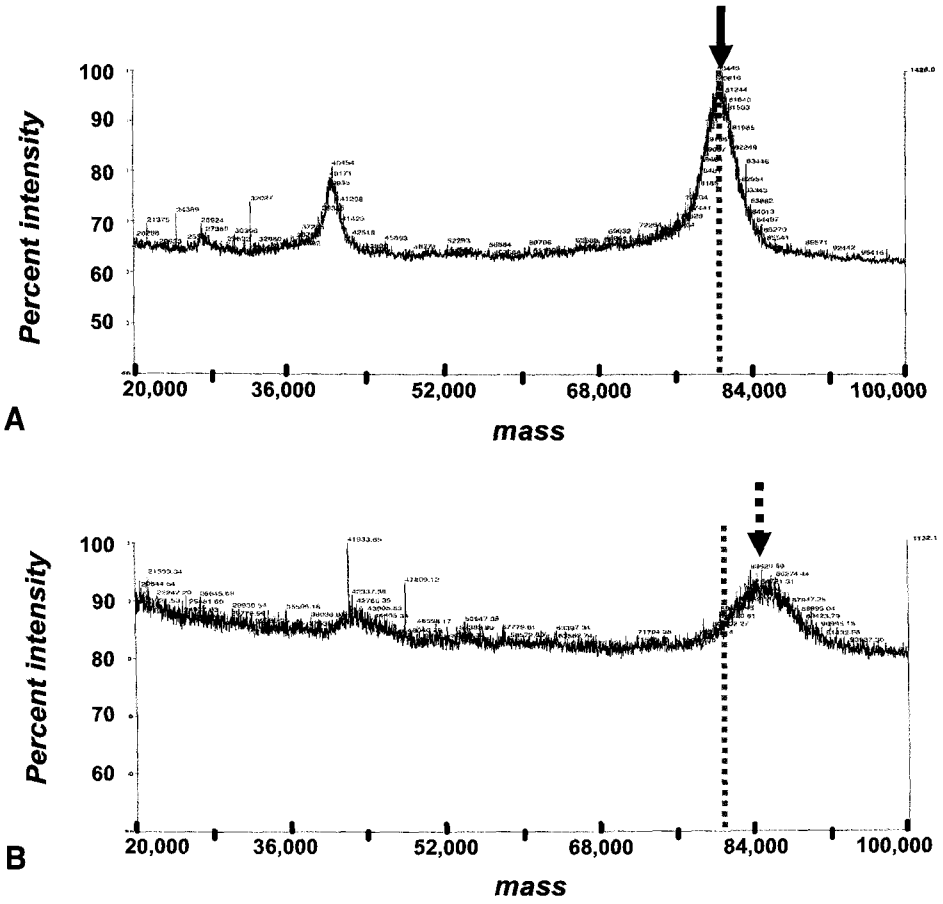

Fig. 2. Mass spectromety of the human apo-transferrin (A) and HYNIC-chitosan-transferrin conjugate (B). Mass peak of apo-transferrin was noted at the 80 kDa (A, line arrow), but that of HYNIC-chitosan-transferrin conjugate was around 84 kDa (B, dotted arrow). Mass peak of in HYNIC-chitosan-transferrin conjugate was wider, smaller and shifting to the right resulted from increasing molecular weight. This implies that human apo-transferrin was successfully conjugated with HYNIC-chitosan.

respectively). Liver, spleen and blood showed higher ^{99m}Tc-transferrin uptake than the infected muscle.

5. Scintigraphies of ^{99m}Tc-transferrin and ^{67}Ga-citrate

1) Visual analysis

In visual analysis, higher accumulation of ^{99m}Tc-transferrin was noted in the area of infected left thigh than the non-infected right thigh since 10 minute (Fig. 3A). Uptake of ^{99m}Tc-transferrin was gradually increased with time in the infected thigh, while decreased gradually in the normal thigh. Increased uptake was apparent definitely at 30 minutes in the infected thigh. But high activity was noted in the kidney and urinary bladder, liver and heart.

Uptake of ^{67}Ga-citrate in the infected muscle was noted (Fig. 3B). ^{67}Ga-citrate showed high hepatic and modest intestinal activity with poor image quality. But renal and urine activity was not noted on ^{67}Ga-citrate scintigraphy.

Table 1. Biodistribution of ^{99m}Tc-HYNIC-Chitosan-Transferrin

organ \ time	30 minutes (n=3)	3 hours (n=3)	
heart	0.27±0.06%	0.26±0.05%	
lung	0.39±0.04%	0.26±0.04%	
liver	1.95±0.20%	1.66±0.25%	
spleen	1.83±0.53%	1.45±0.45%	
small bowel	0.19±0.03%	0.27±0.04%	
large bowel	0.05±0.00%	0.07±0.03%	
kidney	4.39±0.65%	3.79±0.53%	
infected muscle	0.18±0.01%	0.18±0.01%	┐*
normal muscle	0.05±0.01%	0.04±0.01%	┘
blood	0.60±0.07%	0.32±0.02%	

Data expressed mean±standard deviation.
* P-value <0.05

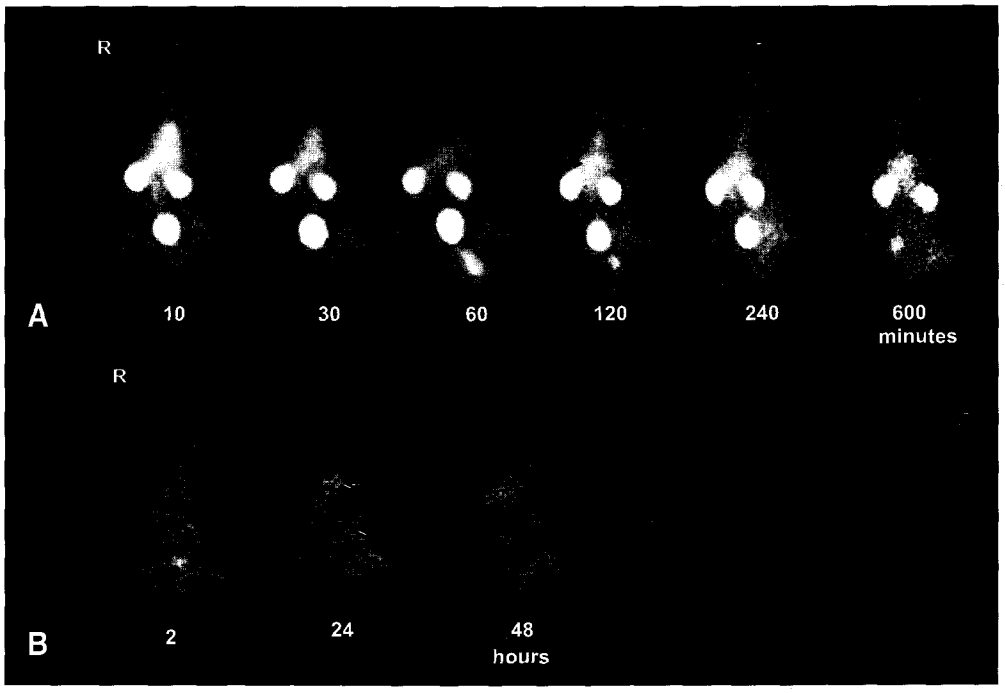

Fig. 3. Serial image of ^{99m}Tc-HYNIC-chitosan-transferrin (^{99m}Tc-transferrin) (A) and ^{67}Ga-citrate (B). Both ^{99m}Tc-transferrin and ^{67}Ga-citrate successfully showed infectious lesion in the left thigh of the abscess-induced rat model (red arrows), which were higher uptake than normal muscle (red arrow heads). ^{99m}Tc-transferrin showed infectious area as hot uptakes, which was visualized faster and more distinct than ^{67}Ga-citrate. But, ^{99m}Tc-transferrin image showed high uptake in liver, heart, kidney and urinary bladder (A, green arrow) as like liver and intestinal uptake on ^{67}Ga-citrate (B, yellow dotted arrow).

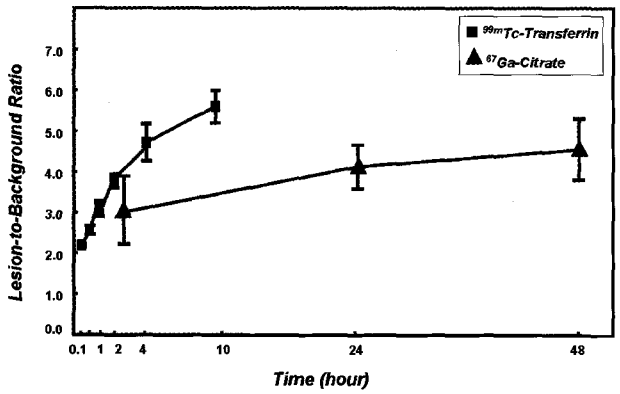

Fig. 4. Serial lesion-to-background (L/B) ratio of ^{99m}Tc-HYNIC-chitosan-transferrin (^{99m}Tc-transferrin) and ^{67}Ga-citrate. L/B ratio of ^{99m}Tc-transferrin was high and rapid increased with time as compared to that of ^{67}Ga-citrate.

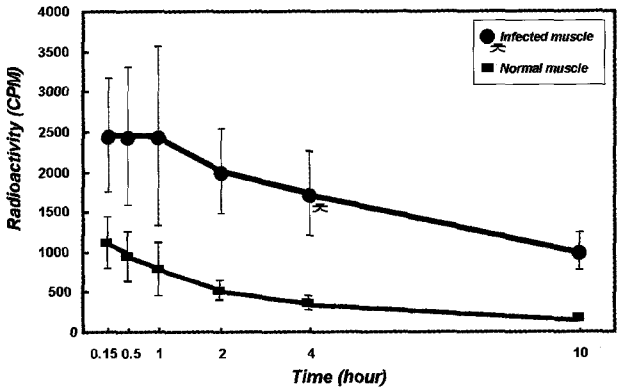

Fig. 5. Serial radioactivity of the normal and infected rat thigh muscles in scintigraphic image. Both radioactivity of the normal and infected muscles was decreased gradually with time. The slope of the infected muscle is lesser stiff than that of normal the muscle. That would be caused by different retention ratio between the normal and infected muscles.

Compared to ^{67}Ga-citrate image, ^{99m}Tc-transferrin image revealed the lesion more rapidly with better image quality.

2) Semiquantitative analysis

Lesion-to-background ratio (L/B ratio) was calculated on images with ^{99m}Tc-transferrin and ^{67}Ga-citrate (Fig. 4). On ^{99m}Tc-Transferrin image, L/B ratio was 2.18±0.03, 2.56 ±0.11, 3.08±0.18, 3.77±0.17, 4.70±0.45 and 5.59±0.40 at 10 minute, 30 minute, 1 hour, 2 hour, 4 hour and 10 hour, respectively. In ^{67}Ga-citrate, L/B ratio was 3.06±0.84, 4.12 ±0.54 and 4.55±0.74 at 2 hour, 24 hour and 48 hour,

respectively. ^{99m}Tc-transferrin image presented high L/B ratio since early phase and rapidly increased with time. But ^{67}Ga-citrate presented modest elevation of L/B ratio with time.

^{99m}Tc-transferrin showed higher L/B ratio than in ^{67}Ga-citrate at 2 hour. L/B ratio was higher in 4 hour image of ^{99m}Tc-transferrin than 48 hour image of ^{67}Ga-citrate. ^{99m}Tc-transferrin showed not only higher L/B ratio at early phase but also more rapid increase of L/B ratio than ^{67}Ga-citrate.

Comparing to serial counts, higher count was noted in the infected muscle than the normal muscle all the time, especially at early stage. And it was also noted that washout is slower in the infected muscle than in the normal muscle (Fig. 5). This retention may be associated with transferrin characteristics, as well as with the migration of inflammatory cells containing ^{99m}Tc-transferrin, which stay in the inflammatory lesion.

Discussion

In this study, the potential of ^{99m}Tc-transferrin for infectious imaging was explored in a rat abscess model. Transferrin can be labeled with ^{99m}Tc, using HYNIC-chitosan as a chelator, and the preparation allows rapid visualization of infectious lesions with reliable L/B ratio within several hours after intravenous injection. Compared to ^{67}Ga-citrate, ^{99m}Tc-transferrin image visualized infectious lesion more rapid and better image quality.

Transferrin is a monomeric glycoprotein with a single polypeptide chain of 670-700 amino acids and a molecular weight of about 80 kDa.[1,2] The well-known members of the transferrin family include serum transferrin, ovo-transferrin, lactoferrin, and melanotransferrin. Among these, serum transferrin is abundant in human body and can be found in the blood and other mammalian fluids including bile, amniotic fluid, cerebrospinal fluid, lymph and milk. Serum transferrin controls the levels of free iron in the body fluid by binding, sequestering and transporting Fe^{3+} ions. On ^{67}Ga-citrate scintigraphy, ^{67}Ga binds at the Fe^{3+} ions binding sites of circulating transferrin rapidly just after injection. Transferrin is rich in infectious or inflammatory lesions. Though some investigators had undergone several studies using ^{111}In-transferrin,[13,18] ^{113m}In-transferrin,[12,14] ^{68}Ga-transferrin,[15] ^{131}I-transferrin[17] and ^{18}F-transferrin,[19] ^{99m}Tc-transferrin has been rarely studied.

The possible mechanisms of ^{99m}Tc-transferrin to accumulate infectious and inflammatory lesions are extravasation and retention of transferrin at the sites of infectious and inflammatory lesions. It is known that transferrin presents in the inflammatory lesions due to leakage of plasma protein via enhanced vascular permeability.[13] In this study, uptake level of ^{99m}Tc-transferrin in infectious lesion is the highest at 10 minutes and maintained until 1 hour. After 1 hour, ^{99m}Tc-transferrin uptake in lesion diminished gradually with time. This result implies that mechanism of ^{99m}Tc-transferrin are extravasation due to increased vascular permeability in the early phase and retention in later at the sites of infectious and inflammatory lesions.

Large series of radiopharmaceuticals have been proposed for scintigraphic imaging for infection and inflammation. However, the limitations inherent in these agents warrant the search for an agent that can be prepared off shelf and that enable rapid and accurate visualization of inflammatory foci throughout the body.

^{67}Ga-citrate and radiolabeled leukocyte are commonly used to detect infectious/inflammatory lesions in field of Nuclear Medicine. ^{67}Ga-citrate shows infectious/inflammatory lesions usually 24-72 hours after injection and it also has the limitation of routine clinical use due to production from cyclotron.[8] Radiolabeled leukocyte also has some limitations in specific conditions. Moreover, a significant disadvantage is the need for *in vitro* isolation of blood cells which exposes both patient and technician to an infectious hazard and requires complex and time-consuming labeling procedure.[26,27]

Among several radiolabeled chemotactic peptides for infectious/inflammatory imaging study, some of these showed good results. But, radiolabeled chemotactic peptides required complex and time-consuming procedure, well-designed laboratory equipment, skillful worker, and expensive materials.

In field of imaging infection/inflammation, ^{99m}Tc-transferrin has several advantages for routine clinical use. First, ^{99m}Tc-transferrin allows rapid imaging with good image quality. In general, ^{99m}Tc-labeled agents permit rapid

imaging and additional delayed imaging at 2-4 hours, while ^{67}Ga-citrate and ^{111}In-labeled leukocyte imaging present optimal imaging in 24-72 hours. Additionally, ^{99m}Tc-labeled agents presents better image quality than ^{67}Ga-citrate, because ^{99m}Tc-labeled agents have favorable physical characteristics for gamma camera imaging and also because a high-dose of this agents could be administered to maximum safety dose. In this study, ^{99m}Tc-transferrin showed more rapid, higher and more distinct uptakes in infectious lesions than ^{67}Ga-citrate. Second, the preparation of ^{99m}Tc-transferrin is very simple and rapid, and its labeling efficiency is high and stable. ^{99m}Tc-transferrin can be prepared just to mix conjugates of transferrin, a reductant and ^{99m}Tc in the yielded vial and incubated 10~30 minutes at room temperature. Labeling efficiency of ^{99m}Tc-transferrin is higher than 95% and stable up to 8 hours at room temperature. This enables rapid approach to the detection of infectious lesions as possible as clinicians want. Third, ^{99m}Tc-transferrin can be used in any laboratories of Nuclear Medicine, if HYNIC-chitosan-transferrin is manufactured commercially as a cold kit. ^{99m}Tc-labeled agents are readily available in routine clinical setting because of a product of generator system, which is available in most ofthe laboratories of Nuclear Medicine.

Moreover, patients are exposed to lesser radiation than ^{67}Ga- and ^{111}In-labeled agents, which have long physical half-life and higher energy. And, additional studies would be able to be performed after 2 days from the previous study for short physical half-life ($T_{1/2}$ = 6 hr) of ^{99m}Tc.

Despite several advantages of ^{99m}Tc-transferrin, this study has several limitations. First, study population is small, and second, timing obtained for ^{99m}Tc-transferrin and that for ^{67}Ga-citrate image was different. ^{99m}Tc-transferrin image was acquired at the first day after bacterial inoculation, while ^{67}Ga-citrate image was acquired at the third day. It may make a chance of showing less uptake of ^{67}Ga-citrate in infectious lesions according to different activity of infection with different time. Third, ^{99m}Tc-transferrin image showed high renal, hepatic and splenic uptake. High renal uptake is common phenomenon for radiolabeled peptides.[28,29] The mechanism of renal uptake and retention is believed to involve glomerular filtration, subsequent reabsorption and catabolism in the proximal tubular cells. This process is dependent on molecular weight and charge of the proteins. In normal, transferrin is distributed rich in liver and spleen. Further experiments will be needed for reducing organic uptake of ^{99m}Tc-transferrin such as kidney, liver and spleen by modulating the size and charge of the proteins.

In conclusion, ^{99m}Tc-transferrin, a newly developed radiopharmaceutical, showed high labeling efficiency and high uptake in infectious foci of a rat abscess model. ^{99m}Tc-transferrin imaging also has potential to detect infectious lesions in a shorter time with better image quality compared to ^{67}Ga-citrate. Therefore, ^{99m}Tc-transferrin is supposed to be useful in the detection of the infectious foci.

요 약

목적: 본 연구의 목적은 ^{99m}Tc-방사성표지 트렌스페린(^{99m}Tc-transferrin)을 개발하여 감염/염증병소의 진단에 이용할 수 있는지 알아보고, 이를 ^{67}Ga-citrate 영상과 비교하고자 하였다. **대상 및 방법:** Succinimidyl 6-hydrazino-nicotinate hydrochloride-chitosan-transferrin (Transferrin)을 합성하고, 여기에 ^{99m}Tc 방사성 표지를 시행하였다. ^{99m}Tc-transferrin의 방사성표지효율은 표지 후 10분, 30분, 1시간, 2시간, 4시간, 8시간에 측정하였다. 포도상구균(ATCC 25923, 2×10^8 colony forming unite, 0.2 ml)을 접종한 쥐농양모델에서 ^{99m}Tc-transferrin과 ^{67}Ga-citrate의 생체내 분포를 평가하고 영상 검사를 실시하였다. **결과:** 질량분석계를 이용하여 Transferrin이 성공적으로 제조되었음을 알 수 있었다. ^{99m}Tc-transferrin의 방사성표지효율은 10분, 30분, 1시간, 2시간, 4시간, 8시간에 각각 96.2±0.7%, 96.4±0.5%, 96.6±1.0%, 96.9±0.5%, 97.0±0.7%, 95.5±0.7% 이었다. ^{99m}Tc-transferrin의 단위섭취량은 감염병소에서 0.18±0.01%, 0.18±0.01%, 정상근육에서 0.05±0.01%, 0.04±0.01%이었고, 감염병소 대 정상근육 섭취비는 30분과 3시간에 각각 3.7±0.6, 4.7±0.4이었다. ^{99m}Tc-transferrin 영상에서 10분, 30분, 1시간, 2시간, 4시간 그리고 10시간에서의 병소/배후방사능비는 각각 2.18±0.03, 2.56±0.11, 3.08±0.18, 3.77±0.17, 4.70±0.45 그리고 5.59±0.40이었고, ^{67}Ga-citrate의 경우 2시간, 24시간, 48시간에 3.06±0.84, 4.12±0.54, 4.55±0.74 이었다. **결론:** Transferrin에 ^{99m}Tc을 이용한 방사성표지가 성공적으로 이루어졌고, ^{99m}Tc-transferrin의 표지효율은 8시간까지 95% 이상의 안정된 방사성표지효율을 보였다. ^{99m}Tc-transferrin을

이용한 감염영상을 성공적으로 얻을 수 있었으며, ^{67}Ga-citrate 영상과 비교하여 더 빠른 시간 안에 우수한 영상을 얻을 수 있었다. 그러므로 ^{99m}Tc-transferrin이 감염 병소의 영상진단에 사용될 수 있을 것으로 기대된다.

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