

Radionuclide Studies of Articular Cartilage in the Early Diagnosis of Arthritis in the Rabbit

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Abstract

The compound Se-75 bis[β-(N,N,N-trimethylamino)-ethyl]selenide diiodide (Se-75 BISTAES) has been synthesized and its biodistribution in rabbits studied. A high uptake of radioactivity is found in the knee cartilage. Good scans of the knee are obtained by nuclear scintigraphy at 15 minutes after the injection of Se-75 BZSTAES. The results of an equilibrium dialysis study show that Se-75 BISTAES binds to chondroitin sulfate and the binding is directly proportional to the chondroitin concentration. It appears that Se-75 BISTAES or its derivative should have potential as an articular imaging agent.

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Introduction

Osteoarthritis is a common joint disease that involves destruction of articular cartilage. Magnetic resonance imaging (MRI) provides a sensitive imaging modality for the diagnosis of osteoarthritis. Its main disadvantages include high cost and long scan acquisition time. Also, many patients cannot undergo MRI because of claustrophobia. Hence, a less expensive but sensitive and specific alternative would be helpful in the diagnosis and management of osteoarthritis.

Currently, there are no specific cartilage imaging agents. However, positively charged bisquaternary ammonium compounds have been shown to localize in articular cartilage. The localization is attributed to the electrostatic binding between the positively charged compounds and the negatively charged glycosaminoglycans such as chondroitin in the cartilage.¹⁻³ The radiolabelled bisquaternary compound, Se-75 bis[β-(N,N,N-trimethylamino)-ethyl]selenide diiodide (Se-75 BISTAES) has been synthesized and its biodistribution studied in normal rabbits and guinea pigs. The results show that the uptake of Se-75 BISTAES is significantly higher in articular cartilage than in surrounding tissues such as the femur, tibia and the blood.^{4,5} Since one of the earliest biochemical changes in osteoarthritis is the breakdown of proteoglycan, leading to a loss of chondroitin

and a decrease in negative surface charge of cartilage,⁶ it would appear that Se-75 BISTAES may have merit as an articular cartilage imaging agent. Nuclear scintigraphy studies and further *in vitro* work were conducted and are reported herein.

Materials and Methods

Synthesis of Se-75 BISTAES

Radioactive Se-75 BISTAES was synthesized and identified according to procedures previously described.^{3,7} The yield was 65% and the specific activity was 15 mCi/mg. Prior to animal studies, the Se-75 BISTAES was dissolved in normal saline, sterilized by filtration through a 0.22 μ millipore filter and stored at 4°C.

Biodistribution Study

A modified biodistribution study was conducted according to procedure^{4,5} described before in order to find the optimal time for nuclear scintigraphy. New Zealand white rabbits weighing 3 to 4 kg were injected with Se-75 BISTAES at a dose of 1.5 μg/kg (range 20 to 25 μCi). After the injection, each animal's pupillary response to light was observed by placing a pen light directly above the pupils. The animals were then sacrificed with carbon dioxide at 2, 15, and 30 minutes after injection. Four animals were sacrificed at each time period. The organs

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of interest, including articular cartilage from both knee, the femur, tibia, kidneys, and muscle from the hind limbs were removed and weighed. 1 ml of blood was also obtained by cardiac puncture immediately after the animals were sacrificed. The amount of radioactivity in each sample was counted in a NaI(Tl) radiation counter and was expressed as a percentage of injected dose per gram of sample.

Nuclear Imaging Study

Four New Zealand white rabbits weighing 3 to 4 kg were used in this study. The animals were anaesthetised using ketamine and pentobarbital, and then injected intravenously with Se-75 BISTAES at a dose of 250 μ Ci. Their pupillary response to light was observed. Nuclear scintigraphy of the lateral aspect of the right knee was conducted at 15 minutes and 25 minutes after injection using a Picker Dyna gamma camera and a pin-hole collimator. A total of 300,000 counts were accumulated for the scans. A posterior view of the kidneys and bladder at 15 minutes post-injection was also obtained. A total of 500,000 counts were accumulated for the scan.

Equilibrium Dialysis Study

The study was carried out to demonstrate the mechanism of localization of Se-75 BISTAES in articular cartilage. The binding between Se-75 BISTAES and chondroitin sulfate was studied using an equilibrium dialysis technique as described elsewhere.^{3,8,9}

Before the experiments, the dialysis membrane was soaked overnight in a 0.4 M phosphate buffer (pH = 7.4). The nonspecific binding of Se-75 and chondroitin sulfate to the membrane was determined as described.⁹ The dilution factor of chondroitin sulfate during dialysis was also determined.⁹ The membrane was then tied at one end to form a dialysis sac. 2 ml of chondroitin sulfate at concentrations of 0.2, 2, 10 and 20 mg/ml was added into the sac. The sac was then closed and suspended in a scintillation vial containing 10 ml of Se-75 BISTAES solution (5 μ g / 75 μ Ci Se-75 BISTAES in 10 ml 0.4 M phosphate buffer). The vial was placed in a water bath at 37°C and was agitated at 30 oscillation per minute for 24 hour. After the incubation aliquots of 0.5 ml of the chondroitin and Se-75 BISTAES were counted for radioactivity. The percentage of bound Se-75 BISTAES was calculated as described by Bowers:⁹

$$\text{Percentage of bound Se-75 BISTAES} = 100\% \left(\frac{C_B \times 100\%}{[(C_{CS} - C_B)DF + C_B]} \right)$$

where:

C_B = counts of the Se-75 BISTAES solution

C_{CS} = counts of the chondroitin sulfate

DF = dilution factor of the chondroitin sulfate, the average dilution factor determined is 1.25

A control study was conducted using Tc-99m MDP, a bone imaging agent, instead of Se-75 BISTAES.

Results

Biodistribution Study

After the injection of Se-75 BISTAES, no inhibition of pupillary response to light was observed in all rabbits. The results of the biodistribution study are shown in Table I. Throughout the study, there was a high level of radioactivity in kidneys, indicating that the compound is excreted rapidly through urine. The articular cartilage had the second highest concentration of radioactivity. The radioactivity in articular cartilage reached the maximum of 1.03% of injected dose at 15 minutes after injection and decreased thereafter. The radioactivity ratios of articular cartilage to various background tissue are shown in Table II. All ratios reached the highest at 15 minutes post-injection. According to these results, the optimal time for nuclear scintigraphy would be at 15 minutes post-injection of Se-75 BISTAES.

Nuclear Scintigraphy Study

The scintiscans are presented in Figures 1 and 2. In both scans, there was an increased uptake of radioactivity in the proximal end of tibia, the distal end of femur, and the bladder. The level of radioactivity in the background was low. The femur and tibia were not seen in the scans.

TABLE I: BIODISTRIBUTION OF SE-75 BISTAES IN RABBITS

	Percentage of injected dose*		
	5 min	15 min	30 min
Blood	0.15 (0.03)	0.07 (0.01)	0.07 (0.02)
Articular cartilage†	0.78 (0.09)	1.03 (0.17)	0.44 (0.04)
Femur‡	0.06 (0.01)	0.04 (0.01)	0.04 (0.01)
Tibia‡	0.05 (0.02)	0.02 (0.01)	0.03 (0.01)
Muscle‡	0.06 (0.02)	0.03 (0.01)	0.03 (0.02)
Kidney	0.38 (0.05)	0.27 (0.09)	0.18 (0.15)

* mean (SD) for 4 rabbits at each time interval; † pooled from both knee; ‡ pooled from both rear limbs

TABLE II: MEAN ARTICULAR CARTILAGE TO BACKGROUND RATIO*

	Time interval post injection		
	5 min	15 min	30 min
Articular cartilage / femur	13.0	25.8	11.0
Articular cartilage / blood	5.2	14.7	6.3
Articular cartilage / tibia	15.6	51.5	14.7
Articular cartilage / muscle	13.0	34.3	14.7

*Articular cartilage to background ratio = $\frac{\% \text{ dose / gram articular cartilage}}{\% \text{ dose / gram background tissue}}$

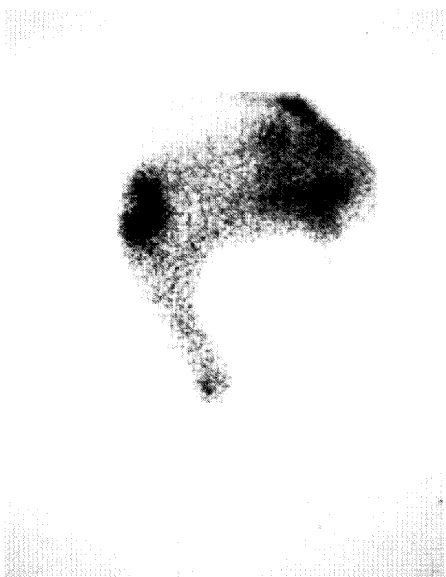


Fig. 1. Lateral view of the right knee of a rabbit at 15 minutes after the injection of Se-75 BISTAES.

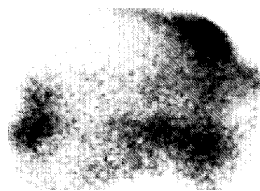


Fig. 2. Lateral view of the right knee of a rabbit at 25 minutes after the injection of Se-75 BISTAES.

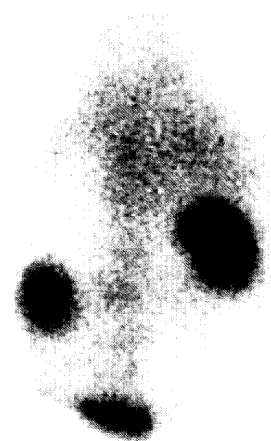


Fig. 3. Posterior view of kidneys of a rabbit at 15 minutes after the injection of Se-75 BISTAES.

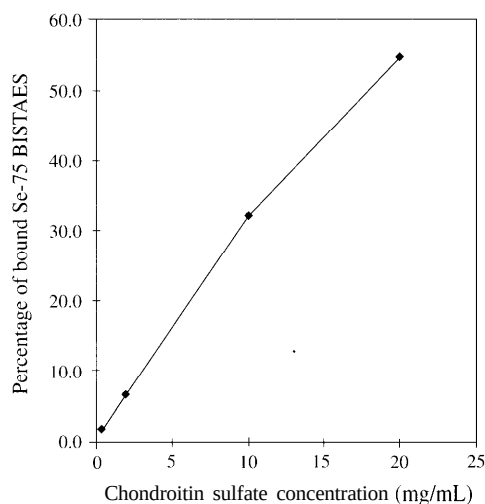


Fig. 4. The relationship between chondroitin sulfate concentration and Se-75 BISTAES binding.

When compared to the scan obtained at 15 minutes post-injection, the 25-minute scan demonstrated a lesser amount of radioactivity in the knee as well as in the background. Also, the femoral head was not identified in the 25-minute scan, but was clearly seen in the 15-minute scan. In addition, the knee was more clearly shown in the 15-minute scan than in the 25-minute scan. The nuclear scans concurred with the biodistribution data and the articular cartilage to background ratios, which predicted that the optimal time to conduct nuclear scintigraphy is 15 minutes after the injection of Se-75 BISTAES.

The scintiscan of the kidneys is shown in Figure 3. The kidneys and bladder contained a high level of radioac-

tivity. The liver and intervertebral disc of the spine could also be identified from the scan.

Equilibrium Dialysis Study

The results of the study are presented in Table III and Figure 4. The radioactivity in the chondroitin sulfate solution was higher than that in the Se-75 BISTAES solution, showing that the Se-75 BISTAES was bound to the chondroitin. The percentage of bound Se-75 BISTAES was 2.1, 7.0, 32.4, and 55.2 when the chondroitin sulfate concentration was 0.2, 2.0, 10.1, and 20.1 mg/ml respectively. This data indicate that there was a significant binding between Se-75 BISTAES and chondroitin sulfate and that the binding increased in accordance to the chondroitin sulfate concentration.

The results of the binding between Tc-99m MDP and chondroitin are shown in Table IV. The amount of radioactivity was the same in the chondroitin solution and in the Tc-99m MDP solution, suggesting that there was no binding between chondroitin and Tc-99m MDP.

Discussion

One of the many considerations in the development of radiopharmaceuticals is that no pharmacological actions be produced by the radiopharmaceuticals. However, bisquaternary ammonium compounds are well known for their ganglionic blocking actions resulting in side effects such as hypotension, tachycardia, and urinary retention. McIssac¹⁰ found that the inhibition of pupillary response to light is one of the earliest pharmacological actions of bisquaternary ammonium compounds. Hence, the animals' pupillary response to light was used as an indicator of any pharmacological actions produced by Se-75 BISTAES. Based on the observations

TABLE III: BINDING BETWEEN SE-75 BISTAES AND CHONDROITIN SULFATE DETERMINED BY EQUILIBRIUM DIALYSIS

Chondroitin sulfate concentration (mg/ml)	Radioactivity* in chondroitin solution	Radioactivity* in Se-75 BISTAES solution	Percentage† of bound Se-75 BISTAES
0.2 ± 0.0	58900 ± 2 1200	57900 ± 20900	2.1 ± 1.9
2.0 ± 0.1	30000 ± 16700	28100 ± 15800	7.0 ± 3.4
10.1 ± 0.1	22600 ± 2900	16500 ± 1900	32.4 ± 3.2
20.1 ± 0.1	28000 ± 2600	14500 ± 1800	55.2 ± 3.3

* Counts per minute per milliliter; † mean ± 1 SD for 5 samples per each chondroitin concentration

TABLE IV: BINDING BETWEEN TC-99M MDP AND CHONDROITIN SULFATE DETERMINED BY EQUILIBRIUM DIALYSIS

Chondroitin sulfate concentration (mg/ml)	Radioactivity* in chondroitin solution	Radioactivity* in Tc-99m MDP solution	Percentage† of bound Tc-99m MDP
0.2 ± 0.0	51500 ± 500	51700 ± 200	0.0
2.0 ± 0.1	49700 ± 2800	51500 ± 400	0.0
10.1 ± 0.1	51100 ± 500	51400 ± 500	0.0
20.1 ± 0.1	51100 ± 300	50900 ± 1200	0.0

* Counts per minute per milliliter; † mean ± 1 SD for 5 samples per each chondroitin concentration

in this study, no pharmacological actions occurred in the rabbits after the injections.

The results of the present biodistribution study concurred with those already published,^{3,4} although the animal models and dosages used are different. All studies show an increased uptake of radioactivity in the articular cartilage. As shown in Table I, the radioactivity in articular cartilage reached the maximum at 15 minutes post-injection. Since the articular cartilage of the knee is adjacent to bone and the synovial membrane which is high in vascularity, the radioactivity ratio of articular cartilage to blood, articular cartilage to bone, and articular cartilage to muscle are of particular importance. As shown in Table II, the ratios at 15 minutes post-injection were 14.7, 25.8, and 34.3 respectively. In nuclear scintigraphy, a target to background ratio of higher than 10 to 15 is required in order to clearly identify the target organ or tissue. Therefore, it appears that nuclear scintigraphy is best conducted at 15 minutes post-injection. Imaging at 30 minutes post-injection may produce a lesser quality scan since the radioactivity ratios are smaller.

The scintiscans support this prediction. The knee is more clearly shown in the 15-minutes scan. The high level of radioactivity in the kidneys also confirms the results of the biodistribution study that Se-75 BISTAES is excreted rapidly in urine.

The *in vivo* distribution of Se-75 BISTAES into the

articular cartilage is hypothesized to occur via the blood supply of the synovial membrane. The compound diffuses into the synovial fluid, and then into the articular cartilage. It may also diffuse into the articular cartilage through the subchondral bone, but to a much lesser extent.¹¹ The positively charged Se-75 BISTAES then binds to the negatively charged chondroitin sulfate and is retained in the articular cartilage.¹⁻³ The results of the equilibrium dialysis study support this hypothesis. The binding between Se-75 BISTAES and chondroitin increases as the chondroitin concentration increases. This finding may explain the previously published observation that the localization of Se-75 BISTAES in articular cartilage is greater than in the menisci.⁴ Articular cartilage is a form of hyaline cartilage, which contains more polyanionic glycosaminoglycan such as chondroitin sulfate when compared to the fibrocartilage such as the menisci. Thus a lesser amount of Se-75 BISTAES is found in the menisci.

It is well known that the primary site of destruction in osteoarthritis is the articular cartilage. One of the earliest biochemical changes is the depletion of chondroitin sulfate. As the disease progresses, the depletion becomes more substantial.¹²⁻¹⁴ Since Se-75 BISTAES localizes in articular cartilage and its binding to chondroitin sulfate is in accordance to the chondroitin concentration, it may be feasible to use a positively charged compound to monitor the course of the disease as well as results of the treatments.

However, Se-75 is not an ideal radioisotope for nuclear scintigraphy. Its high energy and long half-life is not suitable for gamma cameras, and it may increase the radiation exposure to patients. Future research efforts should be directed towards the modification of Se-75 BISTAES structure in order to decrease its toxicity and to allow labelling with Tc-99m. One of the many approaches is to synthesize a molecule with an iminodiacetic acid group at one end and a positively charged quarternary nitrogen at the other end. The iminodiacetic acid group is for labelling with Tc-99m while the positively charged quarternary nitrogen is for cartilage localization. Another approach is to use Se-73, a positron emitting isotope with a half-life of 7 hours. The higher sensitivity and resolution of positron emission tomography allows a smaller amount of BISTAES to be injected. Finally, whether it would be able to demonstrate osteonecrotic defects using BISTAES is an aspect worth studying. Further research efforts should also include the biodistribution of BISTAES in animal with articular defects.

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