

Biodistribution of ^{99m}technetium-labeled creatinine in healthy rats

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Abstract

The distribution of creatinine, one of the toxic guanidine compounds, in various tissues has not been studied in detail by using radiolabeled creatinine. Our objective was to investigate the biodistribution of creatinine labeled with ^{99m}technetium (^{99m}Tc) by the stannous (II) chloride method in healthy male Wistar rats. Quality controls were carried out by radio thin layer chromatography, high-performance liquid chromatography, and paper electrophoresis. The labeling yield was 85 ± 2% under optimum conditions (pH 7 and 100 µg stannous chloride). Rats (N = 12) were injected intravenously with ^{99m}Tc-creatinine and their blood and visceral organs were evaluated for ^{99m}Tc-creatinine uptake as percent of the injected dose per gram wet weight of each tissue (%ID/g). The lowest amount of uptake was detected in the brain and testis. When the rate of uptake was evaluated, only the kidney showed increasing rates of uptake of ^{99m}Tc-creatinine throughout the study. Kidneys showed the highest amount of uptake throughout the study (P < 0.001 compared to all other organs), followed by liver, spleen and lung tissue.

Key words

- Biodistribution
- Creatinine
- ^{99m}Technetium
- ^{99m}Tc-creatinine
- Rat

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Creatinine, the anhydride of creatine, is mainly formed in muscle by non-enzymatic dehydration of creatine phosphate (1). Since creatinine is not protein bound in plasma and has a low-molecular weight (113 kDa) it is totally cleared by the glomeruli. In addition, a small degree of gastrointestinal clearance does exist (2). The serum level of creatinine is kept within a fairly stable range since daily production and renal excretion are constant in healthy mammals (3). Thus, serum creatinine remains the most widely used laboratory test to estimate renal function both in asymptomatic persons and in patients suspected of having renal disease (2). To maxi-

mize its utility, serum creatinine must be interpreted in light of clinical information such as age, gender, weight, stability of renal function, muscle mass, and degree of catabolism. Serum creatinine concentration increases when more than 50% of renal function has been lost (2). Although creatinine, like urea, is not generally considered to be an important uremic toxin, both compounds have been shown experimentally to be toxic in acutely uremic rats (4). Moreover, creatinine is one of the guanidine compounds contributing to uremic encephalopathy. The level of guanidine compounds including creatinine is highly increased in serum, cere-

brospinal fluid and brain of uremic patients. Uremic guanidine compounds have excitatory effects on the central nervous system (5).

Labeled compounds are frequently used in metabolic studies, with their use having an advantage over quantitative assays and being comparatively simple. Labeling with a radioisotope provides compounds of high specific activity. Thus, a labeled compound is of great advantage for the purposes of pharmacokinetic and metabolic studies, being commonly used as a radiotracer in life sciences research as a result of the abundance of hydrogen in molecules of biological importance. One of the best ways to study how a chemical is distributed and altered in the body is to label it with a trace amount of a radioactive element. In this case, a low-energy β^- emitter (such as ^3H or ^{14}C) is usually chosen for laboratory manipulation. In addition, radioiodine complexes are also used in pharmacokinetic studies, representing methods of high sensitivity, accuracy and simplicity (6). The metabolism, reabsorption and transcellular transport of creatinine in renal tubular cells have been studied using (^{14}C)-creatinine (7-10). Boroujerdi and Mattocks (11) investigated the amount of creatinine and its metabolites in various rabbit tissues using two forms of radiolabeled creatinine ((amidino- ^{14}C)-creatinine and (carbonyl- ^{14}C)-creatinine).

Thus, a labeled compound is of great advantage for the purposes of pharmacokinetic and metabolic studies. $^{99\text{m}}\text{Tc}$ Technetium ($^{99\text{m}}\text{Tc}$) has been widely used as a radiotracer in nuclear medicine and in biomedical research to label molecular and cellular structures due to its many desirable characteristics: it emits a 140-keV γ -ray with 89% abundance, which is suitable for imaging with gamma cameras, and its half-life (6 h) is sufficiently long to synthesize and use the $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals. Stannous chloride (SnCl_2) is frequently used as a reducing agent in the $^{99\text{m}}\text{Tc}$ -labeling process (12).

A radioactively labeled form of creatinine has been used in the past to examine creatinine exchange between mother, fetus and amniotic fluid in rhesus monkeys (13). However, the distribution of radiolabeled creatinine in various tissues and the factors affecting this biodistribution have not been studied in detail.

In the present study, we labeled creatinine with $^{99\text{m}}\text{Tc}$ by the SnCl_2 method (14) in order to evaluate the biodistribution of the labeled compound in healthy rats.

Creatinine labeling with $^{99\text{m}}\text{Tc}$. Creatinine and other chemicals were purchased from Merck (Whitehouse Station, NJ, USA). $\text{Na}^{99\text{m}}\text{TcO}_4$ was supplied by the Department of Nuclear Medicine of Ege University. Creatinine was labeled with $^{99\text{m}}\text{Tc}$ by the SnCl_2 method. One milligram of creatinine was dissolved in 1 mL distilled water in a vial and a $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ solution (100 $\mu\text{g}/100 \mu\text{L}$) was freshly prepared and added to the vial. Labeling was carried out at different pH values by adjusting the medium pH with 0.1 N ammonium hydroxide or 0.1 N NaOH. $\text{Na}^{99\text{m}}\text{TcO}_4$ (37-74 MBq) was added to the mixture, which was incubated for 20 min at room temperature (14). Radiolabeling was completed in the presence of ascorbic acid (1 mg/0.5 mL) (15) and a sample was taken from the mixture for quality control by radio thin layer chromatography (RTLC), paper electrophoresis and radio high-performance liquid chromatography (RHPLC) (16). For RTLC, two different solvent systems were used for development, i.e., RTLC1: saline (0.9% sodium-chloride solution) and RTLC2: ACD (citrate-dextrose buffer solution; Sigma-Aldrich, St. Louis, MO, USA). Rf and labeling yield were determined from RTLC chromatogram data. The buffer solution, standing time and applied voltage for paper electrophoresis were ACD, 1 h and 250 volts, respectively. The activity was counted using a Cd(Te) detector equipped with a RAD 501 single-channel analyzer and the labeling yields were obtained from the chromato-

grams. To obtain the optimum labeling yield of ^{99m}Tc-creatinine, the effects of pH of the reaction mixture, SnCl₂ concentration, and stability over time were investigated. RHPLC: A low-pressure gradient HPLC system with an LC-10 ATvp quaternary pump, UV detector (Shimadzu SPD-10ATvp Exsil 100 x 5SAX column; 250 x 4.0 mm ID; Hichrom) with a Cd(Te) detector equipped with a RAD-501 single-channel analyzer, were used. The mobile phase was acetonitrile and water (60/40). The flow rate of the eluent was set at 1.0 mL/min and absorbance was measured at 239 nm. Chromatograms obtained for creatinine and ^{99m}Tc-creatinine are shown in Figure 1.

Evaluation of the biodistribution of ^{99m}Tc-creatinine. Male albino Wistar rats (N = 16, 312 ± 17 g) were used. The study was carried out in compliance with national laws for the conduct of animal experimentation. ^{99m}Tc-creatinine, specific activity 37.0 MBq/mg (0.5 mg creatinine/rat), was injected into the tail vein of the rats and groups of 4 animals were sacrificed under ether anesthesia at 1, 2, 3, and 4 h after injection. Samples of muscle, fat, brain, heart, lung, liver, spleen, pancreas, kidney, testis, stomach, small and large bowel, and blood samples were obtained and weighed and the specific activity of ^{99m}Tc-creatinine was determined. Then, the activity of ^{99m}Tc-creatinine per gram tissue wet weight was calculated. Results are reported as percent of the injected dose per gram (%ID/g) of each tissue. The uptake of ^{99m}Tc-creatinine over time in blood and in the other tissues was evaluated. In addition, various tissues were compared with respect to %ID/g of ^{99m}Tc-creatinine at the 1st, 2nd, 3rd, and 4th hours.

Statistical analyses were performed by univariate analysis of variance, with the level of significance set at P < 0.05.

The RTLC, R_f values of ^{99m}Tc-creatinine, ^{99m}TcO₄⁻, and R-^{99m}Tc were 0.21 ± 0.02, 0.78 ± 0.01, and 0.06 ± 0.01, respectively, in saline solution and 0.11 ± 0.06,

0.76 ± 0.04, and 0.77 ± 0.01, respectively, in ACD solution (16). Electrophoresis experiments indicated that the complex has a negative charge. The ^{99m}Tc-creatinine complex was labeled with a yield of 85 ± 2.0%.

The effect of pH on the radiolabeling yields was examined at pH 2, 5, 7, and 9, with yields of 24 ± 3, 72 ± 2, 85 ± 1, and 17 ± 3% being obtained at pH 2, 5, 7, and 9, respectively. The highest labeling yields were obtained at pH 7. The effect of stannous chloride concentration on radiolabeling was studied in the range 50-200 µg/100 µL of stannous chloride concentrations, with labeling yields of 25 ± 1, 85 ± 2, 46 ± 1, and 33 ± 2 for 50, 100, 150, and 200 µg stannous chloride, respectively. The data show that the radiolabeling yield increased with increasing of stannous chloride concentration from 50 to 100 µg, reaching a maximum yield at 100 µg. The stability of the radiolabeled compound over time was also investigated. The labeling yields of ^{99m}Tc-creatinine were 85 ± 2, 81 ± 3, 81 ± 2, 81 ± 2, 81 ± 1%, at 15, 30, 60, 120, 180 min, respectively. ^{99m}Tc-creatinine is stable for up to 3 h after labeling. The labeling yield of the complex was 85% after 15 min, decreasing to 81% after 30 min and remaining at this level

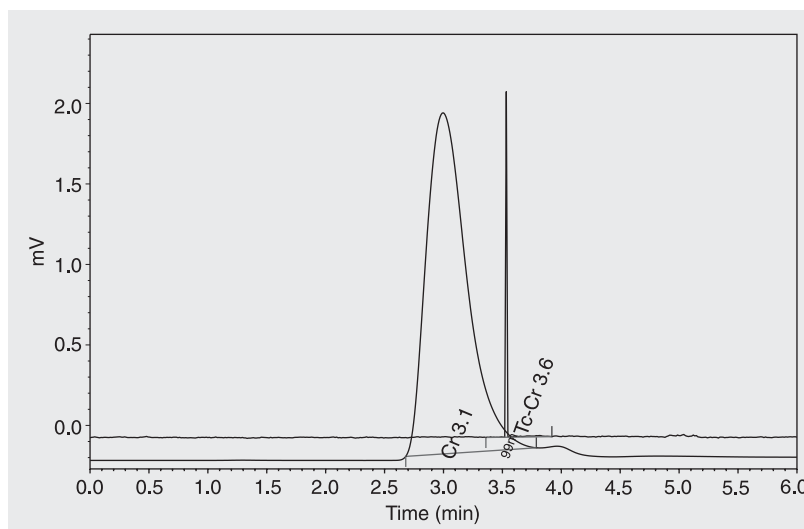


Figure 1. RHPLC chromatogram of ^{99m}Tc-creatinine and creatinine detected at 239 nm.

for up to 3 h.

The RHPLC retention time was 3.6 min for ^{99m}Tc -creatinine. The retention time of creatinine was 3.1 min on HPLC chromatogram. Figure 1 shows the composition of both chromatograms. The labeling of ^{99m}Tc -creatinine was optimum under the following reaction conditions: 1 mg creatinine/mL, pH 7, 100 $\mu\text{g}/100\ \mu\text{L}$ SnCl_2 , 1 mg/0.5 mL ascorbic acid, and reaction time of 20 min at room temperature.

^{99m}Tc -creatinine uptake by the organs of healthy rats. The change of ^{99m}Tc -creatinine uptake in various organs over time is shown in Figure 2. Most of the activity was increasingly confined to renal tissue throughout the study (%ID/g value in kidney from 0.21 at 1 h to 0.44 at 4 h). Renal tissue had a significantly greater uptake than all other tissues ($P < 0.001$), followed by liver, which had a ^{99m}Tc creatinine uptake similar to that of blood, and finally by spleen and lung. The lowest uptake was detected in the brain and testis. When the change in uptake over time was evaluated, only the kidney showed in-

creasing uptake of ^{99m}Tc -creatinine throughout the study, with significant values ($P = 0.001$ for the 2nd, 3rd, and 4th hours vs the 1st hour, and $P = 0.002$ for 4th vs the 2nd and 3rd hours). ^{99m}Tc -creatinine uptake increased in the 2nd and/or 3rd hours of the study and decreased thereafter in muscle, fat, heart, lung, spleen, testis, stomach, and large bowel. The increase in uptake was significant for muscle (2nd vs 1st hour), fat (2nd vs 1st hour), lung (2nd vs 1st hour) and spleen (2nd and 3rd vs 1st, and 3rd vs 2nd hour; $P < 0.05$). In contrast, ^{99m}Tc -creatinine uptake in blood and liver tissue remained almost constant throughout the study. The activity in brain tissue reached its maximum by the 1st hour and decreased significantly thereafter ($P < 0.05$).

Serum creatinine level is used as a marker of renal function and, in addition, creatinine is also considered to be one of the uremic toxins. Although serum creatinine is increased in renal failure, it is unclear whether creatinine is elevated intracellularly in uremia. Creatinine content was measured in

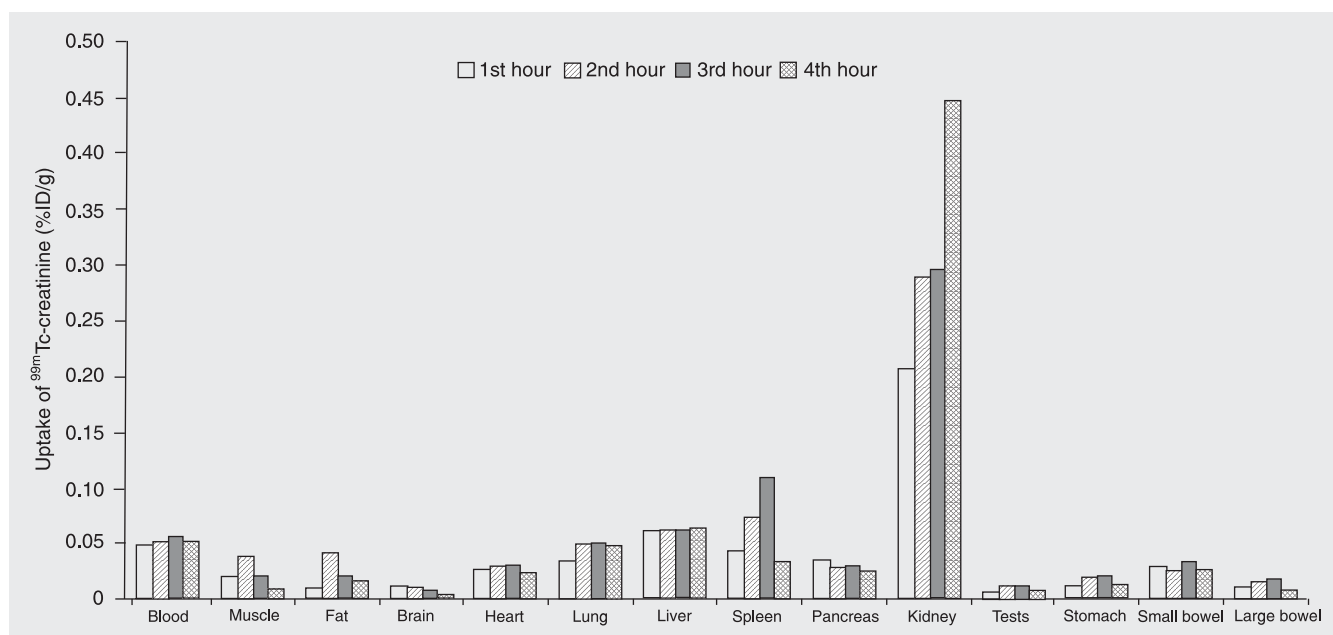


Figure 2. Uptake of ^{99m}Tc -creatinine by various rat organs. Data are reported as percent of injected dose per gram tissue wet weight (%ID/g) 1, 2, 3, and 4 h after injection.

various tissues of uremic rats and found to be normal in myocardium and skeletal muscle (3). Although the uptake of ^{99m}Tc-creatinine in blood did not change considerably for 4 h following *iv* injection, there were striking alterations of activity at the tissue level in healthy rats. In general, while uptake of ^{99m}Tc-creatinine decreased in many tissues, renal uptake increased over time. After reaching the kidney, creatinine is excreted into urine mostly by glomerular filtration. Some creatinine is secreted into the tubular fluid by tubular cells. We did not measure the activity in the urine of rats, but measured the activity within the renal tissue. Thus, what we measured in renal tissue was ^{99m}Tc-creatinine activity in tubular cells. Significantly higher levels in renal tubular cells might be due to an extra creatinine load (1 mg/mL) in the rats, since tubular secretion of creatinine increases with increasing serum creatinine levels (17). ^{99m}Tc-creatinine activity was higher in liver, lung and spleen than in the other tissues, except the kidney. Boroujerdi and Mattocks (11) studied the amount of creatinine and its metabolites in various rabbit tissues using (amidino-¹⁴C)-creatinine and reported high uptake of radioactivity in the kidney, brain and liver. Although in our study a low-radioactivity uptake was observed in brain compared to the cited study, the data concerning the distribution of ^{99m}Tc-creatinine in the kidney and liver of rats

were in good agreement (11). On the other hand, ^{99m}Tc-creatinine uptake was highest at 1st hour and decreased rapidly almost to zero at 4th hour in brain tissue. This shows that creatinine concentration in brain tissue increases rapidly following an acute creatinine load, but decreases progressively in healthy rats with normal renal function. It seems that the normal brain content of creatinine is very low but increases considerably in the presence of an extra creatinine load. Creatinine levels have been found to be increased in the brain of both uremic persons and experimental animals, with the latter suffering convulsions after intraperitoneal injection of creatinine (18,19). The uptake of ^{99m}Tc-creatinine in blood did not change significantly during the present study, possibly due to a balanced exchange of creatinine between blood and tissues. Since creatinine from many tissues passed into circulation, it tended to accumulate in the kidney, possibly resulting in stable blood levels at least for the first 4 h of intravenous creatinine load.

Creatinine can be labeled with ^{99m}Tc with fairly high yields and ^{99m}Tc-creatinine is stable for up to 3 h at room temperature. We observed that the uptake of ^{99m}Tc-creatinine was high in liver, lung, spleen, and kidney in normal rats. This approach could also be used in pathological conditions such as renal failure to determine the organs that might be most affected by uremic toxicity.

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