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In vitro radioautographic studies of the biodistribution of radiopharmaceuticals on blood elements

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Abstract

In the present study we evaluated the binding of the radiopharmaceuticals sodium pertechnetate (Na $^{99m}TcO_4$), methylenediphosphonic acid (^{99m}Tc-MDP) and glucoheptonate acid (^{99m}Tc-GHA) to blood elements using centrifugation and radioautographic techniques. Heparinized blood was incubated with the labelled compounds for 0, 1, 2, 3, 4, 6 and 24 h. Plasma (P) and blood cells (BC) were isolated and precipitated with 5% trichloroacetic acid (TCA), and soluble (SF) and insoluble fractions (IF) were separated. Blood samples were prepared (0 and 24 h) and coated with LM-1 radioautographic emulsions and percent radioactivity (%rad) in P and BC was determined. The binding of Na $^{99m}TcO_4$ (%rad) to P was 61.2% (0 h) and 46.0% (24 h), and radioautography showed 63.7% (0 h) and 43.3% (24 h). The binding to BC was 38.8% (0 h) and 54.0% (24 h), and radioautography showed 36.3% (0 h) and 56.7% (24 h). ^{99m}Tc-MDP study presented 91.1% (0 h) to P and 87.2% (24 h), and radioautography showed 67.9% (0 h) and 67.4% (24 h). The binding to BC was 8.9% (0 h) and 12.8% (24 h), and radioautography showed 32.1% (0 h) and 32.6% (24 h). ^{99m}Tc-GHA study was 90.1% (0 h) to P and 79.9% (24 h), and radioautography showed 67.2% (0 h) and 60.1% (24 h). The binding to BC was 9.9% (0 h) and 20.1% (24 h), and radioautography showed 32.8% (0 h) and 39.9% (24 h). The comparison of the obtained results suggests that the binding to plasma and blood cells in the two techniques used (radioautography and centrifugation) is qualitatively in accordance.

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- Blood elements

The use of radionuclides is very important for clinical and laboratory evaluations as well as in research. ^{99m}Tc labels a variety of radiopharmaceuticals that are used in nuclear medicine (1-7). There is a growing interest in the biodistribution of radiopharmaceuticals at the cellular and subcellular level. There are two reasons for this: i) the need for knowledge of localization mechanisms, and ii) the need to consider microdosimetry for both diagnostic and therapeutic radionuclides. Several methods have been developed for the study of microscopic distribution of radiopharmaceuticals. Radioautography has the advantage of permitting simultaneous visualization of tissue and superimposed radioautography is possible. Radioautography studies in which beta particles and secondary

emissions such as Auger electrons are detected and localized is more commonly used (7-9). When a radiopharmaceutical is administered, part of it binds to blood elements. Differences in percent protein binding may be related to the different pharmacokinetics of each diagnostic agent. The importance of the present study is that it permits the localization of the ^{99m}Tc-products in the blood cells and plasma separately. Binding to plasma proteins may influence the radioactivity distribution of each agent (5,7,10-12). It is generally accepted that a variety of factors other than disease can alter the distribution of radiopharmaceuticals and one such factor is drug therapy (7,13-16). We studied the binding of the radiopharmaceutical sodium pertechnetate (Na $^{99m}TcO_4$) (1-7,17,18), used for thyroid and brain studies, and compared the results obtained using an *in vitro* model with reported data obtained with ^{99m}Tc-methylenediphosphonic acid (^{99m}Tc-MDP) (7,11,14) used for bone studies and ^{99m}Tc-glucoheptonate acid (^{99m}Tc-GHA) (7,12,17) used for brain and kidney studies.

These experiments were performed without sacrificing the animals. Na $^{99m}TcO_4$ was milked directly from a ^{99}Mo/^{99m}Tc generator (Instituto de Pesquisas Energéticas e Nucleares, SP, Brazil) and added to a kit (Laboratório de Radiofarmácia, INCa, Brazil) containing 1 mg $SnCl.2H_2O$ and 10 mg methylenediphosphonic acid to prepare ^{99m}Tc-MDP and 50 mg of glucoheptonate acid to prepare ^{99m}Tc-GHA. These radiopharmaceuticals were diluted approximately 1000 times with 0.9% NaCl. Heparinized blood samples (4.4 ml) from Wistar rats were incubated with radiopharmaceuticals. Samples were divided into aliquots of 0.7 ml and incubated for 0, 1, 2, 3, 4, 6 and 24 h with a 50-µl solution of the above radiopharmaceuticals. Then, 100 µCi/ml (3.7 MBq/ml) was added. Blood smears were prepared, dried and fixed (0 and 24 h). The preparations were treated with radioautographic emulsions (Amersham, Buckinghamshire, England), developed, fixed, dried and stained. Then, all samples were centrifuged after each time of incubation. Plasma (P) and blood cell (BC) samples (25 µl) were isolated and 25 µl of P and BC were also precipitated with 5% trichloroacetic acid (TCA) and soluble (SF) and insoluble fractions (IF) were isolated from P and BC. P, SF-P, IF-P, SF-BC and IF-BC samples were counted with a well counter and percent radioactivity (%rad) was calculated in relation to total radioactivity for P or BC in P + BC, for SF or IF of P in SF-P + IF-P, and for SF or IF of BC in SF-BC + IF-BC. The silver grains superimposed on the blood smears were observed under a light microscope (Olympus BH-2) coupled with a computer (IBM-PC). The visualized image was projected onto a video monitor and covered with the 2500-µm^2 image proplus software system grid. The silver grains were then located and counted.

Table 1 shows that in the control the amount of radioactivity in P (0 h) was slightly higher than in BC with Na $^{99m}TcO_4$. There was an increase of Na $^{99m}TcO_4$ binding to BC within 24 h both by the centrifugation technique (Table 1) and the radioautographic technique (Table 2). With ^{99m}Tc-MDP (Table 1) the %rad in P (0 and 24 h) was higher than in BC. The results obtained with centrifugation (Table 1) and radioautographic (Table 2) techniques showed that the %rad bound obtained with the two techniques was qualitatively similar. With ^{99m}Tc-GHA the %rad in P (0 h) was higher when compared with BC, and decreased with incubation time (24 h) (Tables 1 and 2). The percent of radioactivity binding to BC was lower at 0 h and increased within 24 h (Table 1). Both tested techniques showed that the results were qualitatively similar. This reduced binding to BC has been reported by several authors (6,7,12). The comparison of radioactivity by both techniques showed that in BC the activity bound to Na $^{99m}TcO_4$ was higher than the activity

Table 1 - Radiopharmaceutical distribution between plasma and blood cells determined by a centrifugation technique.

Na $^{99m}TcO_4$, ^{99m}Tc-MDP and ^{99m}Tc-GHA were incubated for 0 and 24 h with blood that was withdrawn from Wistar rats. Plasma and blood cells were isolated and centrifuged. The percent radioactivity was determined. Data are reported as means ± SD of 8 experiments.

Time (h)	Na $^{99m}TcO_4$ (%)		^{99m}Tc-MDP (%)		^{99m}Tc-GHA (%)	
	Plasma	Blood cells	Plasma	Blood cells	Plasma	Blood cells
0	61.2 ± 2.4	38.8 ± 2.4	91.1 ± 1.7	8.9 ± 1.7	90.1 ± 2.3	9.9 ± 2.3
24	46.0 ± 0.8	54.0 ± 0.8	87.2 ± 1.9	12.8 ± 1.9	79.9 ± 2.9	20.1 ± 2.9

Table 2 - Radiopharmaceutical distribution between plasma and blood cells determined by a radioautographic technique.

Na $^{99m}TcO_4$, ^{99m}Tc-MDP and ^{99m}Tc-GHA were incubated for 0 and 24 h with blood that was withdrawn from Wistar rats. The radioautographic technique was carried out. The silver grains were counted in the blood smears. The percent of radioactivity was determined. Data are reported as means ± SD of 8 experiments.

Time (h)	Na $^{99m}TcO_4$ (%)		^{99m}Tc-MDP (%)		^{99m}Tc-GHA (%)	
	Plasma	Blood cells	Plasma	Blood cells	Plasma	Blood cells
0	63.7 ± 4.2	36.3 ± 4.2	67.9 ± 5.4	32.1 ± 5.4	67.2 ± 1.0	32.8 ± 1.0
24	43.3 ± 1.3	56.7 ± 1.3	67.4 ± 0.1	32.6 ± 0.1	60.1 ± 1.9	39.9 ± 1.9

bound to ^{99m}Tc-MDP and ^{99m}Tc-GHA (Tables 1 and 2). This probably occurs due to the rapid and strong permeability of BC to Na $^{99m}TcO_4$ (19,20). Radioautography has been employed by other authors when studying the microscopic distribution of ^{111}In-radiopharmaceuticals in normal animal tissues. Linearity of the emulsion response to uniformly labeled tissue is related to the grain density in the radioactivity in the uppermost layers of the section (in immediate contact with the emulsion) (7-9). In comparison, the density of the radioactivity that was counted in BC with ^{99m}Tc-MDP and ^{99m}Tc-GHA (Table 2) was higher than that counted by the centrifugation technique (Table 1). The differences in the radioactivity of these radiopharmaceuticals may be explained by the location of the superimposed grain when observed on the plane of the light microscope without three-dimensional visualization (7). Then, some grains that were counted as if they adhered to the cellular membranes belonged, in fact, to P. Despite this limitation, radioautography is an attractive method for studying the biodistribution of radiopharmaceuticals at the microscope level. Computer image analysis is a useful, perhaps indispensable adjunct to this method which permits the processing of large grain numbers and accurately determining grain densities. In conclusion, ^{99m}Tc can be a good alternative radiopharmaceutical for qualitative radioautography studies in substitution of other coumpounds, because it is easily available and normally inexpensive (2,3,19) and permits good resolution.

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