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STUDY OF THE BINDING OF ^{99m}TECHNETIUM-RADIOPHARMACEUTICALS ON BLOOD CELLS AND PLASMA PROTEINS: EVALUATION USING PRECIPITATION WITH TRICHLOROACETIC ACID.

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SUMMARY

Nuclear medicine uses radioactive tracers called radiopharmaceuticals to study the bloodflow, metabolism and morphology of an organ. Sodium pertechnetate ($^{99m}TcO_4Na$) and many ^{99m}Tc products are the most frequently radiopharmaceuticals used in nuclear medicine. Secure determination of the binding of ^{99m}Tc-radiopharmaceuticals to plasma (P) and blood cell (BC) constituents can help to understand the biodistribution of radiopharmaceuticals. The reported evaluations about the binding of radiopharmaceuticals on blood elements have shown that the results can not be easily compared. We decided to determine the gold standard concentration of trichloroacetic acid (TCA) to study the binding of radiopharmaceuticals on blood proteins: ^{99m}Tc-stannous colloid (^{99m}Tc-Sn-Colloid), sodium pertechnetate ($^{99m}TcO_4Na$), methylenediphosphonic acid (^{99m}Tc-MDP) and diisopropyliminodiacetic

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acid (^{99m}Tc-DISIDA). Blood of *Wistar* rats, was incubated with the radiopharmaceuticals for 5 minutes at room temperature, centrifuged and plasma (P) and blood cells (BC) were isolated. Samples of P and BC were also precipitated with TCA concentrations (0.1, 0.5, 1.0, 5.0, 10.0 and 20.0%) and soluble (SF) and insoluble fractions (IF) were isolated and counted. The percent radioactivity (%ATI) in IF-P depends on TCA concentration. It varied from 23.7 to 75.7 (^{99m}Tc-Sn-Colloid), from 7.8 to 26.2 ($^{99m}TcO_4Na$), from 10.7 to 40.4 (^{99m} Tc-MDP), from 52.2 to 60.7 (^{99m}Tc-DISIDA). The gold concentrations of TCA to study the binding of the studied radiopharmaceuticals in blood elements were revealed from the obtained results: (i) for ^{99m}Tc-Sn-Colloid in the IF-P is shown that there is no differences in the percent of radioactivity when TCA concentrations of 20 to 5.0 percent were used for precipitation, (ii) for $^{99m}TcO_4Na$, 0.5 percent TCA concentration is the best one to precipitate the bound radiopharmaceutical, (iii) for ^{99m}Tc-MDP the % ATI increased from 10.7 to 40.4 with TCA concentrations from 0.1 to 5.0 percent and decreased from 40.4 to 23.8 with TCA concentrations from 5.0 to 20.0 percent and (iv) for ^{99m} Tc-DISIDA, the values of bound radioactivity are not dependent on TCA concentration in the range of 0.1 to 5.0 percent. The %ATI in IF-BC depends on TCA concentration and it varied for $^{99m}TcO_4Na$ (28.8 to 77.9), for ^{99m}Tc-MDP (68.8 to 83.7), for ^{99m}Tc-DISIDA(69.3 to 92.8). However, for ^{99m}Tc-Sn-Colloid, the %ATI in the insoluble fraction seems to be independent of the TCA concentration. The analysis of these results will contribute to understand the involved mechanisms on the binding of radiopharmaceuticals on blood elements.

Key words: radiopharmaceuticals, precipitation, blood elements, technetium-99m , trichloroacetic acid, binding.

INTRODUCTION

Nuclear medicine uses radioactive tracers called radiopharmaceuticals to study the bloodflow, metabolism and morphology of an organ. Sodium pertechnetate ($^{99m}TcO_4Na$) and many ^{99m}Tc products are the most frequently radiopharmaceuticals

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used in nuclear medicine (1). Secure determination of the binding of ^{99m}Tc-radiopharmaceuticals to plasma (P) and blood cell (BC) constituents can help to understand the biodistribution of radiopharmaceuticals. There are different methods to determine the quantity of the binding radioactivities to blood elements, however, it is known that the determination of protein binding involves many problems and the results normally are not unequivocal. Precipitation methods are generally reliable, while the dialysis method, which is widely used was found to be dependent on the association-dissociation equilibrium between the ^{99m}Tc-radiopharmaceutical and the protein that complexes with it. By application of dialysis or gel chromatography, only the technetium that is irreversibly bound to proteins is observed, in many studies (2,3,4, 5, 6). Once the obtained values depend on the stability of the technetium-99m (^{99m}Tc)-radiopharmaceutical-protein complex, these methods do not provide clear data. The elucidation of the binding of radiopharmaceuticals to blood is worthwhile and theoretical and practical aspects of this binding with plasma (P) have been studied, although with blood cells (BC) have not been adequately evaluated and the results can not be easily compared. Previously, we have demonstrated that the percent of radioatictivity (%ATI) in IF-P depends on TCA solutions and it is modified from 36.4 to 65.0 (^{99m}Tc-PHY), from 17.9 to 32.0 (^{99m}Tc-DTPA), from 11.5 to 38.8 (^{99m}Tc-GHA), and from 52.8 to 66.2 (^{99m}Tc-DMSA) (7, 8). In this study, we have compared the results obtained with radiopharmaceuticals ^{99m}Tc-stannous colloid (^{99m}Tc-Sn-Colloid), sodium pertechnetate ($^{99m}TcC_4Na$), methylenediphosphonic acid (^{99m}Tc-MDP) and diisopropyliminodiacetic acid (^{99m}Tc-DISIDA).

MATERIAL AND METHODS

The experiments were carried out with heparinized whole blood withdrawn from *Wistar* rats. The employed kits (^{99m}Tc-Sn-Colloid, ^{99m}Tc-MDP and ^{99m}Tc-DISIDA), from the Radiopharmacy Department, Instituto Nacional de Cancer, Rio de Janeiro, Brazil. ^{99m}Tc, as sodium pertechnetate was obtained from 99Mo/99mTc generator, Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, Brazil and was added to the kits to prepare ^{99m}Tc-Sn-Colloid, ^{99m}Tc-MDP and ^{99m}Tc-DISIDA. Fresh whole blood (3 ml) was incubated with samples (1 ml) of

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(^{99m}Tc Sn-Colloid, ^{99m}Tc-MDP, $^{99m}TcO_4Na$ and ^{99m}Tc-DISIDA) (3.7 MBq) for 5 min, at room temperature. After these incubations, the blood preparations were centrifuged and plasma (P) and blood cells (BC) were isolated. Aliquots (25 µl) of P and BC were precipitated with 1 ml of solution of trichloroacetic acid (TCA) in various concentrations (0.1, 0.5, 1.0, 5.0, 10.0, 20.0 %) and soluble (SF) and insoluble (IF) fractions from plasma and blood cells were separated. The several samples (P, BC, IF-P, SF-P, IF-BC and SF-BC) were counted in a well counter with NaI(Tl) crystal (1272 Clinigamma Gamma Counter, LKB Wallac, Finland). The percentage of radioactivity (% ATI) in P was determined dividing the counts in P by the sum of the counts in P plus BC, %ATI in BC was determined dividing the counts in P by the sum of the counts in P plus BC, %ATI in IF-P was determined dividing the counts in IF-P by the sum of the counts in IF-P plus SF-P and % ATI in IF-BC was determined dividing the counts in IF-BC by the sum of the counts in IF-BC plus SF-BC. The values found were multiplied by 100. The values are means of 8 isolated experiments. Statistical analysis were performed with F test ($p<0.01$) and Benferroni test ($p<0.05$) to compare the %ATI of each radiopharmaceutical in the blood constituents and the various TCA concentrations.

RESULTS

Table 1 shows the distribution on plasma and blood cells of the ^{99m}Tc-radiopharmaceuticals that were incubated with whole blood for 5 min. The %ATI for the $^{99m}TcO_4Na$, ^{99m}Tc-MDP and ^{99m}Tc-DISIDA were mainly found in the plasma. However, most of the ^{99m}Tc-Sn-Colloidal was found in the blood cells compartment.

Table 1 Distribution of the % of radioactivity of samples of plasma and blood cells

Radiopharmaceuticals	Blood Cells	Plasma
^{99m}Tc-Sn-Colloid	72.7 ± 2.4	27.3 ± 2.4
^{99m}Tc-DISIDA	19.2 ± 2.5	80.8 ± 2.5
^{99m}Tc-MDP	15.0 ± 2.3	85.0 ± 2.3
$^{99m}TcO_4$ Na	37.8 ± 8.7	62.2 ± 8.7

The values are means ± standard deviations of 8 isolated experiments.

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Figure 1 shows the %ATI in the insoluble fractions obtained from plasma samples precipitated with different TCA concentrations. The statistical analysis with F test ($p<0.01$) and Benferroni test ($p<0.05$) indicated that the %ATI in IF-P depends on TCA concentrations and it varied from 23.7 to 75.7($p<0.05$) (^{99m}Tc-Sn-Colloid), from 7.8 to 26.2 ($p<0.05$) ($^{99m}TcO_4Na$), from 10.7 to 40.4($p<0.05$) (^{99m}Tc-MDP) and from 52.2 to 60.7 ($p<0.05$) (^{99m}Tc-DISIDA). The results obtained for the fixation of ^{99m}Tc-Sn-Colloid in the IF-P show that there is no differences in the percent of radioactivity when TCA concentrations from 5.0 to 20.0 percent were used. For $^{99m}TcO_4Na$, 0.5% TCA concentration is the best one to precipitate the bound radiopharmaceutical. For ^{99m} Tc-MDP the % ATI increased from 10.7 to 40.4 with TCA concentrations from 0.1 to 5.0% and decreased from 40.4 to 23.8 with TCA concentrations from 5.0 to 20.0%. For ^{99m} Tc-DISIDA values of bound radioactivity are not dependent on TCA concentration in the range of 0.1 to 5.0 percent.

Figure 1. Distribution of the % of radioactivity in insoluble fractions obtained with the precipitation of samples of plasma with trichloroacetic acid (TCA)

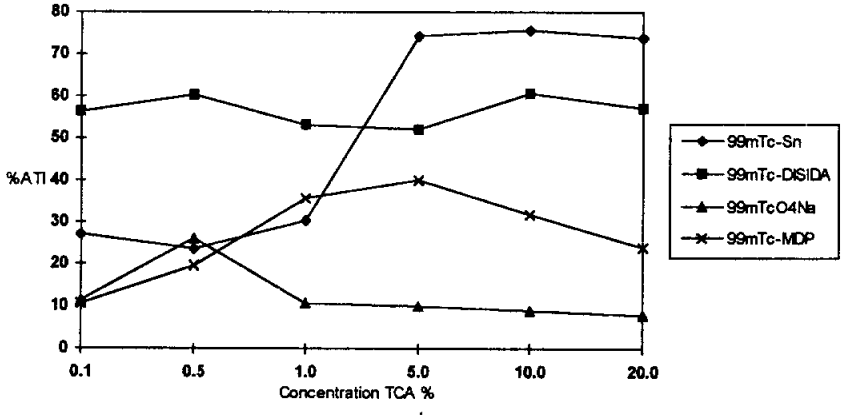

Figure 2 shows the %ATI in insoluble fractions obtained from blood cells samples precipitated with different TCA concentrations. The statistical analysis with F test ($p<0.01$) and Benferroni test ($p<0.05$) indicated that the %ATI in IF-BC depends on TCA concentration and it varied for $^{99m}TcO_4Na$ (28.8 to 77.9), for ^{99m}Tc-MDP (68.8 to 83.7), for ^{99m}Tc-DISIDA(69.3 to 92.8). However, for ^{99m}Tc-Sn-Colloid, the %ATI in the insoluble fraction seems to be independent of the TCA concentration.

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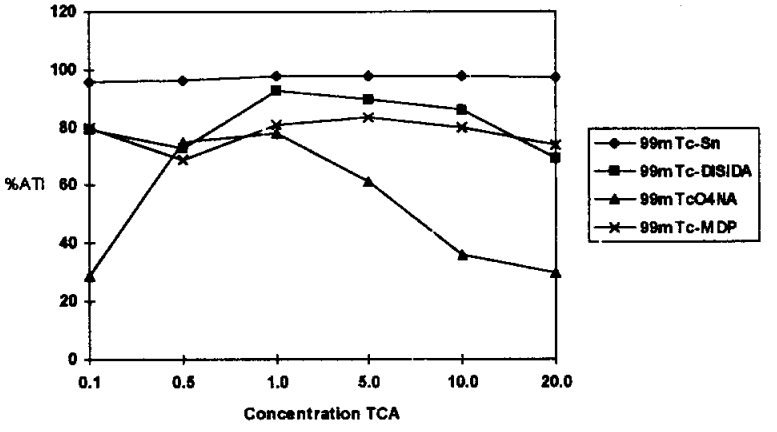
Figure 2. Distribution of the % of radioactivity in insoluble fractions obtained with the precipitation of samples of blood cells with trichloroacetic acid (TCA).



DISCUSSION

The distribution of radioactivity of the radiopharmaceuticals in plasma and blood cells compartments has been studied by several authors (4,9). The results described here (table 1) for ^{99m}Tc-Sn-Colloid, for $^{99m}TcO_4Na$,for ^{99m}Tc-MDP and ^{99m}Tc-DISIDA are in agreement with the literature data. The ^{99m}Tc-Sn-Colloid radioactivity is lower than the other radiopharmaceuticals in the plasma (1,10). However, there are conflicting results described in the literature about the binding of the radiopharmaceuticals to the blood elements (2,3,4,5,6). We agree with De Ligny *et al.*(3) that the value found for the protein binding of ^{99m}Tc-radiopharmaceuticals appears to be dependent on the used method and on the experimental conditions. Gano et al (4), have found for $^{99m}TcO_4Na$ 33.5% of the radioactivity binding to plasma proteins when precipitated with TCA 20.0% while our studies revealed 7.8%. Nevertheless, our results indicate that other factors should be considered to better to evaluate the binding of radiopharmaceuticals to blood elements. The radiopharmaceutical uptake in organs may depend on its biochemical characteristics besides the binding to blood elements. De Ligny et al (3) have reported, that protein binding to ^{99m}Tc-MDP does not occur, but Vanlic-Razumenic et al (6) disagreed with this conclusion and suggested that dissociation of the ^{99m}Tc-MDP-protein complex was

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a consequence of excess dilution in their experiments. Savelkouk et al (5) have shown that precipitation with TCA sometimes yields high values, presumably by decomposition of the ^{99m}Tc-diphosphonate complexes in the strongly acid solution and subsequent binding of the ^{99m}Tc to the denatured proteins. Decomposition can also occur in gel chromatography and dialysis.

The general comparison with other results shows that for the best study fixation of the ^{99m}Tc-radiopharmaceuticals on IF-P there is a dependence on the chosen TCA concentration to the ^{99m}Tc-Sn-Colloid (0.5 to 10), for $^{99m}TcO_4Na$, for (0.5 to 20), for ^{99m}Tc-MDP (0.1 to 5.0) and for ^{99m}Tc-DISIDA (5.0 to 20). Possibly this fact can be explained by (i) different affinity of the radiopharmaceuticals to specific proteins on plasma and blood cells, (ii) different binding sites, (iii) presence of different stannous chloride concentrations, (2, 12) and/or (iv) different formulations of the kits (11).

We can speculate that the binding of the radiopharmaceuticals on blood elements and the precipitation effect may depend on the characteristics of each radiopharmaceutical. We suggest that the direct comparison among the various radiopharmaceuticals and the differences on their capability to bind to protein complexes in the blood, should be carefully carried out. The comparison of the bindings of ^{99m}Tc-radiopharmaceuticals using only one TCA concentration should be avoided.

Therefore, the gold concentrations of TCA to study the binding of the studied radiopharmaceuticals in blood elements were revealed from the obtained results: (i) to ^{99m}Tc-Sn-Colloid in the IF-P is shown that there is no differences in the percent of radioactivity when TCA concentrations of 20 to 5.0 percent were used for precipitation, (ii) to $^{99m}TcO_4Na$, 0.5 percent TCA concentration is the best one to precipitate the bound radiopharmaceutical, (iii) to ^{99m}Tc-MDP the % ATI increased from 10.7 to 40.4 with TCA concentrations from 0.1 to 5.0 percent and decreased from 40.4 to 23.8 with TCA concentrations from 5.0 to 20.0 percent and (iv) to ^{99m}Tc-DISIDA, the values of bound radioactivity are not dependent on TCA concentration in the range of 0.1 to 5.0 percent. The %ATI in IF-BC depends on TCA concentration and the gold concentrations varied for $^{99m}TcO_4Na$ (28.8 to 77.9), for ^{99m}Tc-MDP (68.8 to 83.7), for ^{99m}Tc-DISIDA (69.3 to 92.8). However, ^{99m}Tc-Sn-Colloid, the %ATI in

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the insoluble fraction seems to be independent of the TCA concentration. in conclusion, the radiopharmaceutical-protein binding complexity could be explained by the variety of proteins present in P and BC, besides the mechanisms involved in the labeling uptake and the type of each radiopharmaceutical.

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