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Evaluation of 99mTechnetium-Radiopharmaceutical Binding to Blood Elements using Different Trichloroacetic Acid Concentrations

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Secure determination of the binding of 99mTc-radiopharmaceuticals to plasma (P) and blood cell (BC) constituents can help to understand the biodistribution of radiophamaceuticals. The reported precipitation studies of blood with radiopharmaceuticals have shown that the results can not be easily compared between studies. We decided to determine the "gold standard" concentration of trichloroacetic acid (TCA) to evaluate the binding to blood elements for several radiopharmaceuticals used in routine nuclear medicine. We have studied phytic (99mTc-PHY), diethylenetriaminepentaacetic (99mTc-DTPA), glucoheptonic (99mTc-GHA) and dimercaptosuccinic (99mTc-DMSA) acids. Blood was incubated with radiopharmaceuticals, centrifuged and P and BC separated. Samples of P and BC were also precipitated with TCA concentrations (20.0, 10.0, 5.0, 1.0, 0.5 and 0.1 percent) and soluble (SF) and insoluble fractions (IF) were isolated. The percent radioactivity (percent rad) in IF-P depends on TCA concentration. It varied from 36.4 to 65.0 (99mTc-PHY), from 17.9 to 32.0 (99mTc-DTPA), from 11.5 to 38.8 (99mTc-GHA) and from 52.8 to 66.2 (99mTc-DMSA). The results for the binding of 99mTc-PHY to IF-P show that there was no differences in the percent rad when TCA concentrations of 0.1 to 1.0 percent were used. For 99mTc-DTPA, 5.0 percent is the best TCA concentration. For 99mTc-GHA, low values of percent rad bound to IF-P is found with TCA concentrations of 0.1, 0.5 and 1.0. Interestingly, with 99mTc-DMSA, high values of bound radioactivity are not dependent on TCA concentrations (0.1 to 10.0). Radioactivity in IF-BC depends on TCA concentration and it varied for 99mTc-PHY (80.1 to 54.1) and for 99mTc-GHA (85.5 to 61.7). With 99mTc-DTPA and with 99mTc-DMSA the percent rad in IF-BC seems independent of TCA concentration. We suggest that the evaluation of the binding of the various 99mTc-radiopharmaceuticals to blood constituents, using only one TCA concentration, should be avoided.

INTRODUCTION

Secure determination of the binding of 99mTc-radiopharmaceuticals to plasma proteins and blood cell constituents can help to understand the uptake of radiopharmaceuticals in targets in human beings. Many factors can affect the biodistribution of radiopharmaceuticals. If unknown, such factors may lead to poor organ visualization, requiring repeat examinations and increasing the radiation dose to the patient [1-3].

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[b] *Abbreviations:* BC, blood cells; P, plasma; 99mTc, technetium-99m; 99Mo, molybdenum-99; DTPA, diethylenetriaminepentaacetic acid; DMSA, dimercaptosuccinic acid; GHA, glucoheptonic acid; PHY, phytic acid; TCA, trichloroacetic acid; SF, soluble fraction; IF, insoluble fraction; percent rad, percentage of radioactivity.

There is considerable evidence that the biodistribution or radiopharmacokinetics of radiopharmaceuticals may be altered by a variety of drugs. One reason for this observation is the effect of the drug in interfering with the binding of the radiopharmaceuticals to plasma proteins and/or blood cell constituents. Accurate, precise and reproducible determination of the radioactivity bound to blood elements is worthwhile (i) to understand how a drug is capable of modifying the biodistribution of radiopharmaceuticals and (ii) to evaluate the specific characteristics of the binding of each radiopharmaceutical to their targets in the blood. There are different methods to quantitate the binding to blood elements, however it is known that 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, is dependent on the association-dissociation equilibrium between the 99mTc-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 [4-8]. If the values obtained depend on the short-term stability of the 99mTc-radiopharmaceutical-protein complex, these methods do not provide clear data.

Several 99mTc-radiopharmaceuticals are employed in different static and dynamic procedures in nuclear medicine in various organs and systems. Phytic acid (99mTc-PHY) (liver and spleen), glucoheptonic acid (99mTc-GHA) and diethylenetriaminepentaacetic acid (99mTc-DTPA) (kidneys and brain), dimercaptosuccinic acid (99mTc-DMSA) (kidneys), iminodiacetic acid analogs (99mTc-IDA) (liver and biliary function), methylenediphosphonic acid (99mTc-MDP) (bone), hexamethylpropyleneamineoxime (99mTc-HMPAO) (brain and inflammatory processes) and 2-methoxyisobutylisonitrile (99mTc-MIBI) (heart and oncologic processes), besides others are usually utilized [1, 2].

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 blood cells (BC) have not been adequately evaluated and the results can not be easily compared. Thus, we decided to determine the "gold standard" concentration of trichloroacetic acid (TCA) for each radiopharmaceutical in order to determine the radioactivity present in precipitation of P and BC. In this study, we compare the results obtained with 99mTc-radiopharmaceuticals: 99mTc-PHY, 99mTc-GHA, 99mTc-DMSA and 99mTc-DTPA.

MATERIAL AND METHODS

The experiments were carried out with heparinized whole blood withdrawn from Wistar rats (sex: male, weight: 120 g, age: four months). The employed kits (PHY, GHA, DMSA and DTPA) were obtained from the Radiopharmacy Department, Instituto Nacional de Câncer, Rio de Janeiro, Brazil. 99mTc, as sodium pertechnetate was obtained from a 99Mo/99mTc generator purchased from Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, Brazil and was added to the kits to prepare 99mTc-PHY, 99mTc-GHA, 99mTc-DMSA and 99mTc-DTPA.

Fresh whole blood (3 ml) was isolated and incubated with recently prepared samples (1 ml) of the 99mTc-radiopharmaceuticals (99mTc-PHY, 99mTc-GHA, 99mTc-DMSA and 99mTc-DTPA) (3.7 MBq) for 60 min at room temperature. After these incubations, the blood preparations were centrifuged and plasma (P) and blood cells (BC) were isolated. Aliquots (20 µl) of P and BC were precipitated with 1 ml of solutions of trichloroacetic acid (Reagen, Brazil) in various concentrations (20.0, 10.0, 5.0, 1.0, 0.5, 0.1 percent) 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 (percent rad) in P was determined dividing the counts in P by the sum of the counts in P plus BC, percent rad in BC was determined dividing the counts in BC by the sum of the counts in P plus BC, percent rad in IF-P was determined dividing the counts in IF-P by the sum of the counts in IF-P plus SF-P and percent rad 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 were multiplied by 100.

RESULTS

Table 1 shows the distribution on plasma and blood cells of the 99mTc-radiopharmaceuticals that were incubated with whole blood for 60 min. As expected, the radiopharmaceuticals that are used for renal evaluations (99mTc-DTPA, 99mTc-GHA and 99mTc-DMSA) are mainly found in plasma. However, most of the 99mTc-PHY is found in the blood cell fraction.

Table 2 shows the percent rad in insoluble fractions obtained from plasma samples precipitated with different trichloroacetic concentrations. These samples of plasma were obtained from whole blood that had been incubated for 60 min with the radiopharmaceu-

Table 1. Distribution of the radioactivity of different radiopharmaceuticals in plasma and blood cell fractions.

Radiopharmaceutical	Plasma (P)	Blood cells (BC)
99mTc-DTPA	88.6 ± 5.8	11.4 ± 5.8
99mTc-GHA	76.5 ± 5.4	23.5 ± 5.4
99mTc-DMSA	83.5 ± 4.8	16.5 ± 4.8
99mTc-PHY	27.5 ± 5.5	72.5 ± 5.5

Fresh whole blood was incubated with samples of the 99mTc-radiopharmaceuticals (99mTc-PHY, 99mTc-GHA, 99mTc-DMSA and 99mTc-DTPA) for 60 min at room temperature. After these incubations, the blood preparations were centrifuged and plasma (P) and blood cells (BC) were isolated. The several samples (P and BC) were counted in a well counter with NaI(Tl) crystal. Percentage of radioactivity (percent rad) in P was determined dividing the counts in P by the sum of the counts in P plus BC and percent rad in BC was determined dividing the counts in BC by the sum of the counts in P plus BC. The values found were multiplied by 100. The values are averages ± standard deviations of 8 experiments.

ticals. The percent rad in IF-P depends on TCA concentration and it varied from 36.4 to 65.0 (99mTc-PHY), from 17.9 to 32.0 (99mTc-DTPA), from 11.5 to 38.3 (99mTc-GHA) and from 52.8 to 66.2 (99mTc-DMSA). The results obtained for the fixation of 99mTc-PHY in the IF-P show that there is no differences in the percent rad when TCA concentrations of 0.1 to 1.0 percent were used for precipitation. For 99mTc-DTPA, 5.0 percent TCA concentration is the best one to precipitate the bound radiopharmaceutical. For 99mTc-GHA, lower values of percent rad bound to insoluble fraction of plasma is found for the TCA concentrations of 0.1, 0.5 and 1.0. However, for 99mTc-DMSA the highest values of bound radioactivity are not dependent on TCA concentration in the range of 0.1 to 10.0 percent.

Table 3 shows the percent rad in insoluble fractions obtained from blood samples precipitated with different trichloroacetic concentrations. These samples of blood cells were obtained from whole blood that was incubated for 60 min with the given radiopharmaceutical. The percent rad in IF-BC depends on TCA concentration and it varied for 99mTc-PHY (80.1 to 54.1) and for 99mTc-GHA (85.5 to 61.7). However, to 99mTc-DMSA and

Table 2. Distribution of the percent of radioactivity in insoluble fractions obtained with the precipitation of samples of plasma with different trichloroacetic acid (TCA) concentrations.

TCA conc. (%)	Insoluble fraction			
	99mTc-PHY	99mTc-DTPA	99mTc-GHA	99mTc-DMSA
0.1	64.3 ± 1.6	17.9 ± 4.1	15.2 ± 5.0	63.6 ± 7.6
0.5	62.4 ± 9.4	23.8 ± 11.5	11.5 ± 3.0	66.2 ± 2.7
1.0	65.0 ± 6.4	24.9 ± 9.1	19.3 ± 5.1	65.2 ± 9.1
5.0	45.6 ± 9.5	32.0 ± 0.7	38.3 ± 2.5	64.8 ± 4.8
10.0	38.1 ± 9.9	26.9 ± 2.8	36.8 ± 4.6	60.7 ± 4.8
20.0	36.4 ± 4.9	26.0 ± 6.8	35.8 ± 5.7	52.8 ± 4.7

Fresh whole blood was incubated with 99mTc-radiopharmaceuticals (99mTc-PHY, 99mTc-GHA, 99mTc-DMSA and 99mTc-DTPA) for 60 min at room temperature. After these incubations, the blood preparations were centrifuged and plasma (P) and blood cells (BC) were isolated. Aliquots of P and BC were precipitated with solutions of trichloroacetic acid at various concentrations (20.0, 10.0, 5.0, 1.0, 0.5, 0.1 percent) and soluble (SF) and insoluble (IF) fractions from plasma were separated. The samples were counted in a well counter with NaI(Tl) crystal and the percentage of radioactivity (percent rad) in IF-P was determined dividing the counts in IF-P by the sum of the counts in IF-P plus SF-P. The values found were multiplied by 100. The values are averages ± standard deviations of 8 experiments.

99mTc-DTPA, the percent rad in the insoluble fraction seems to be independent of the TCA concentration.

DISCUSSION

The distribution of radioactivity of the radiopharmaceuticals in plasma and blood cell compartments has been studied by several authors. The results described here (Table 1) for 99mTc-PHY, 99mTc-DTPA, 99mTc-GHA and 99mTc-DMSA are in agreement with

Table 3. Distribution of the percent of radioactivity in insoluble fractions obtained with the precipitation of samples of blood cells with different TCA concentrations.

TCA conc. (%)	Insoluble fraction			
	99mTc-PHY	99mTc-DTPA	99mTc-GHA	99mTc-DMSA
0.1	80.1 ± 1.3	63.5 ± 8.7	61.7 ± 8.7	82.8 ± 8.7
0.5	80.0 ± 10.1	71.6 ± 8.5	77.5 ± 5.6	85.0 ± 6.7
1.0	76.0 ± 2.1	70.7 ± 6.0	75.5 ± 6.7	84.4 ± 6.5
5.0	80.1 ± 3.7	75.3 ± 10.0	85.5 ± 7.3	96.0 ± 1.6
10.0	70.5 ± 3.4	70.5 ± 7.8	85.5 ± 2.5	91.6 ± 4.2
20.0	54.1 ± 8.1	74.1 ± 9.0	77.1 ± 9.5	87.8 ± 5.8

Fresh whole blood was incubated with 99mTc-radiopharmaceuticals (99mTc-PHY, 99mTc-GHA, 99mTc-DMSA and 99mTc-DTPA) for 60 min at room temperature. After these incubations, the blood preparations were centrifuged and plasma (P) and blood cells (BC) were isolated. Aliquots of BC were precipitated with solutions of trichloroacetic acid at various concentrations (20.0, 10.0, 5.0, 1.0, 0.5, 0.1 percent) and soluble (SF) and insoluble (IF) fractions from plasma were separated. The samples were counted in a well counter with NaI(Tl) crystal and the percentage of radioactivity (percent rad) 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 averages ± standard deviations of 8 experiments.

the literature data. The 99mTc-PHY radioactivity is lower and the other radiopharmaceuticals are higher in the plasma [1-2]. However, there are conflicting results described in the literature about the binding of the radiopharmaceuticals to the blood elements [4-8]. We agree with De Ligny et al. [6] that the value found for the protein binding of 99mTc-radiopharmaceuticals appears to be dependent on the method used and on the experimental conditions. Nevertheless, our results indicate that other factors should be considered to better evaluate the binding of the radiopharmaceuticals to blood elements. The radiopharmaceutical uptake in organs may depend on its biochemical characteristics besides the binding to blood elements. De Lingny et al. [6] have reported that protein binding to 99mTc-MDP does not occur, but Vanlic-Razumenic et al. [8] disagreed with this conclusion and suggested that dissociation of the 99mTc-MDP-protein complex was a consequence of excess dilution in their experiments.

Concerning 99mTc-PHY, Vanlic-Razumenic et al. [8] reported, using 10 percent TCA, a percent rad in the IF-P of 35.1. In our study, we found 38.1, however, if we choose another TCA concentration, this value can vary between 36.4 and 65.0 percent.

Although the comparison of our 99mTc-DMSA results (if we consider the TCA concentrations between 0.1 and 10.0 percent) are in agreement with the reported one [5, 8], they are more similar to Gano et al. [5].

Savelkouk et al. [7] have shown that precipitation with TCA sometimes yields high values, presumably by decomposition of the 99mTc-diphosphonate complexes in the strongly acid solution and subsequent binding of the 99mTc to the denatured proteins. Decomposition can also occur in gel chromatography and dialysis.

The general comparison with other results shows that for the fixation of the 99mTc-radiopharmaceuticals to IF-BC there is a dependence on the TCA concentration: 99mTc-PHY (0.1 to 5.0), 99mTc-DTPA (0.1 to 20.0), 99mTc-GHA (0.5 to 20.0) and 99mTc-DMSA (5.0 to 10.0). If we compare the radiopharmaceuticals, they can present the same capability to bind to the blood cells with 1.0 percent TCA, but if we employ 10.0 percent TCA, 99mTc-DMSA=99mTc-GHA>99mTc-DTPA=99mTc-PHY.

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 [4, 10] and/or (iv) different formulations of the kits [11].

We can speculate that the binding of the radiopharmaceuticals to 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 in their capability to bind to protein complexes in the blood should be carefully carried out. The comparison of the binding of 99mTc-radiopharmaceuticals using only one TCA concentration should be avoided. Thus, depending on the specific characteristic of each radiopharmaceutical, it is possible that the TCA could (i) precipitate more completely the proteins (to which the radiopharmaceutical is bound), (ii) affect the affinity of the radiopharmaceutical for plasma and blood cell proteins, or (iii) dissociate the 99mTc from the radiopharmaceutical.

The correct determination of the binding of radioactivity on blood elements is worthwhile (i) to understand how a drug is capable of modifying the biodistribution of radiopharmaceuticals, (ii) to evaluate the specific characteristics of the binding of each radiopharmaceutical to their targets in the blood, (iii) to avoid misdiagnosis, (iv) to avoid the repetition of examinations, (v) to avoid poor organ visualization and (vi) to reduce the radiation dose to the patients [1].

In conclusion, the complexity of radiopharmaceutical-protein binding may be explained by the variety of proteins present in P and BC as well as the mechanisms

involved in the labeling uptake and the type of each radiopharmaceutical. The study of other radiopharmaceutials is now in progress

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