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Influence of Methylxanthines on the Labeling of Blood Elements with ^{99m}Technetium

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Abstract: This work evaluated the effect of some methylxanthines on the fixation of ^{99m}Tc on blood elements. Blood was incubated with different concentrations of the drugs before ^{99m}Tc. Plasma (P), blood cells (BC), insoluble (IF-P, IF-BC) and soluble (SF-P, SF-BC) fractions were separated and percentage of radioactivity (%ATI) bound were determined. The %ATI in IF-P and SF-P was altered ($p<0.05$) by caffeine at the highest concentrations used. Data showed that methylxanthines in concentrations found in humans not modify the fixation of ^{99m}Tc.

Key words: Aminophylline, caffeine, plasma proteins, red blood cells, theophylline; technetium-99m

INTRODUCTION

Some drugs can modify the labeling of red blood cells (RBC) with ^{99m}Tc and alter the results obtained in the daily routine procedure in nuclear medicine laboratories[1-4]. High labeling yields and good *in vivo* stability of the *in vitro* labeling procedure gives superior images, while *in vivo* labeling is more convenient and thus, quite widely used. In addition to pool imaging and other uses in nuclear cardiology, applications of ^{99m}Tc -RBC have also included diagnosis of deep vein thrombosis, gastrointestinal bleeding, hepatic hemangionas and splenic reticuloendothelial system[5,6] (Bernardo-Filho *et al*., 1983; Bernardo-Filho *et al*., 1994).

The labeling of RBC with ^{99m}Tc has been influenced by patient medications[7] or labeling conditions[6,8]. Thus, the presence of disease may be missed and/or underestimated[7,9].

Aminophylline, caffeine and theophylline are methylxantines that share in common several pharmacological actions of therapeutics interest. Caffeine and theophylline are naturally occurring methylxantines and aminophylline is formed by complex between theophylline and ethylenediamine. Their actions include: (I) relax bronchial and cardiac muscles, (ii) stimulate central system and (iii) diuretic effect on the kidney[10]. Aminophylline and theophylline are used as bronchodilators to relieve the spasm of bronchial smooth muscle in conditions such as bronchial asthma, chronic bronchitis, emphysema and cystic fibrosis. Caffeine is present in human diet through various sources (coffee, chocolate, soft drinks) and it produces subjective and behavioral effects as such feelings well-being, motivation for work, energy, concentration, delays sleep and enhances vigilance performance on psychomotor tasks[11-14].

The aim of this work was to investigate the *in vitro* effects of aminophylline, caffeine and theophylline on the labeling of blood elements with ^{99m}Tc.

MATERIALS AND METHODS

Animals: Adult male Wistar naive rats (3-4 month of age, body weight 250-350 g) were housed, five per cage, in an environment controlled room with inverted light/dark cycle conditions (12 h light/12 h dark; lights on at 6:00 a.m.), for an acclimatization period of at least 3 weeks. Animals had free access to water and food and ambient temperature was kept at 25±2°C. Experiments were conducted in accordance with the Department Committee of Animal Care.

Drugs: Aminophylline and theophylline were purchased from Sanofi Wintrop Farmacêutica Ltda (Brazil) and caffeine was purchased from Sigma (USA).

Study protocol: An *in vitro* technique employed to label RBC described elsewhere[6] was used with minor modification.

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Heparinized whole blood was withdrawn from Wistar rats. Samples of 0.5 ml were incubated with 100 μl of aminophylline, or theophylline (50.0 μg ml^{-1}) or different caffeine concentrations (5.0, 50.0, 625.0, 1250.0, 2500.0, 5000.0 and 10000 μg ml^{-1}) for 1 h at room temperature. A sample of heparinized whole blood was incubated with NaCl 0.9% (Reagen, Rio de Janeiro, Brazil) as a control. Then, 0.5ml of stannous chloride (1.2 μg ml^{-1}) (Sigma Chemical Co., St Louis, USA) was added and the incubation continued for another 1h. After this period, ^{99m}Tc (0.1 ml), as sodium pertechnetate, recently milked from a ^{99}Molybdenium/^{99m}Technetium generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, Brazil), was added and the incubation continued for another 10 minutes. These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 μl) of P and BC were precipitated with 1 ml of trichloroacetic acid (TCA) 5% and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in P, BC, IF-P, SF-P, IF-BC and SF-BC were determined in a well counter (Clinigamma, gamma counter, LKB, Wallac, Finland). After that, the percentage of radioactivity (%ATI) was calculated, as previously described[6].

Statistical analysis: Data was reported as means±SE of %ATI were compared between the treated and control groups by One way analysis of variance - ANOVA, followed by Bonferroni post test with a $p<0.05$ as significant level. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California, USA).

RESULTS

The results in the Table 1 showed the distribution of the radioactivity on blood cells, insoluble fraction of blood cells (IF-BC) and insoluble fraction of the plasma (IF-P) from whole blood treated with aminophylline, caffeine or theophylline (50.0 μg ml^{-1}). The data presented the treatment with these drugs not significantly modify the uptake of ^{99m}Tc by blood cells, IF-BC or IF-P.

The Table 2 showed the fixation of the radioactivity on plasma and blood cells from whole blood treated with different concentrations of caffeine. The treatment with this drug not significantly modify the uptake of ^{99m}Tc by blood elements.

Table 3 showed the fixation of the radioactivity on plasma proteins from whole blood treated with the various concentrations of caffeine. The treatment with caffeine at highest concentrations (625 up to 10000 μg ml^{-1}) modify significantly ($p<0.05$) the fixation of ^{99m}Tc on insoluble (IF-P) and soluble (SF-P) fractions of plasma. The data suggest that caffeine at lowest concentrations (5 and 50 μg ml^{-1}) also may change the labeling of this plasma fractions. However, the difference with control is not significant ($p>0.05$).

The Table 4 showed the fixation of the radioactivity in blood cell proteins from whole blood treated with different concentrations of caffeine. The treatment with caffeine not modify significantly the fixation of the ^{99m}Tc on insoluble (IF-BC) and soluble (SF-BC) fractions of blood cells.

Table 1: Effect of aminophylline, caffeine and theophylline on the labeling of blood elements with ^{99m}Tc

	%ATI		
Drug	Blood Cells	IF-BC	IF-P
Saline	93.0±0.4	88.0±3.1	70.0±1.7
Aminophylline	92.7±1.9	94.6±3.8	78.2±2.9
Caffeine	98.3±0.2	92.5±1.0	52.1±5.2
Theophylline	97.2±0.2	85.3±1.8	65.2±2.9

Table 2: Effect of Caffeine on the labeling of plasma and blood cells with ^{99m}Tc

	%ATI	
Caffeine (μg ml^{-1})	Blood cells	Plasma
0.0	96.6±0.5	3.4±0.7
5.0	97.9±0.3	2.1±0.3
50.0	98.3±0.2	1.7±0.2
625.0	98.6±0.2	1.4±0.2
1250.0	96.8±0.9	3.2±0.9
2500.0	97.9±0.3	2.1±0.3
5000.0	97.5±0.8	2.5±0.8
10000.0	98.7±0.2	1.3±0.2

Table 3: Effect of Caffeine on the labeling of plasma proteins with ^{99m}Tc

	%ATI	
Caffeine (μg ml^{-1})	IF-P	SF-P
0.0	68.3±3.0	31.7±3.0
5.0	53.5±5.0	46.5±5.0
50.0	52.1±5.1	47.9±5.1
625.0	46.4±4.4	53.6±4.4
1250.0	39.2±7.3	60.8±7.3
2500.0	43.9±4.9	56.1±4.9
5000.0	45.0±4.1	55.0±4.1
10000.0	33.1±0.8	66.9±0.8

Table 4: Effect of Caffeine on the labeling of blood cells proteins with ^{99m}Tc

	%ATI	
Caffeine (μg ml^{-1})	IF-BC	SF-BC
0.0	92.8±1.6	7.2±1.6
5.0	89.1±2.2	10.9±2.2
50.0	92.5±1.0	7.5±1.0
625.0	89.2±2.0	10.8±2.0
1250.0	91.4±1.7	8.6±1.7
2500.0	95.0±0.5	5.0±0.5
5000.0	93.6±0.7	6.4±0.7
10000.0	94.6±0.6	5.4±0.6

DISCUSSION

The interaction of drugs with radiopharmaceuticals is of interest. However, the data from these studies are relatively scarce and the effects of pharmacologically active agents on the diagnostic radiopharmaceuticals can be evaluated. Thus, a therapeutic drug can modify the nature or amount of the ^{99m}Tc-radiopharmaceutical bound to blood elements and this may result in unexpected behavior of the radiopharmaceutical.

The analysis of data obtained in this work showed that the methylxanthines (aminophylline, caffeine and theophylline) at concentration of 50 μg ml^{-1} not modify the fixation of ^{99m}Tc in the blood elements (Table 1). Aminophylline, caffeine and theophylline in the plasma concentrations more than 8, 15 and 20 μg ml^{-1}, respectively, are associated with toxicity in human[10]. Thus, the results obtained in this work showed that methylxanthines in the plasma concentrations highest than founded in humans not alter the uptake of the ^{99m}Tc.

Caffeine is obtained in the diet through coffee, tea, chocolate and cola-flavored drinks. Its plasma concentrations in humans can be highly variable due to consuming habits and amounts ingested.

Caffeine at lowest concentration used not modify the fixation of the ^{99m}Tc on the blood cells and plasma as well as in insoluble and soluble fractions of blood cells proteins (IF-BC and SF-BC) (Table 2 and 4). However, in high concentrations (highest than 625 μg ml^{-1}), caffeine alters the fixation of ^{99m}Tc on plasma protein fractions (IF-P and SF-P).

The antioxidant ability of caffeine was demonstrated to be similar to that of the established biological antioxidant glutathione and significantly higher than ascorbic acid[15]. Others data shown that this compound competes with oxygen for electrons and inhibits lipid peroxidation and protein oxidation as a function of concentration in mitochondrial membranes exposed to the gamma-radiation[16]. Thus, the influence of caffeine on the fixation of ^{99m}Tc on plasma protein fractions observed at highest concentrations used may be related to a redox effect.

Another possibility that could explain the caffeine effect on fixation of ^{99m}Tc on plasma protein fractions is the competition between this drug and the radionuclide by the same binding sites. This hypothesis is based on other data that suggested that the labeling of blood elements with ^{99m}Tc can be altered by some drugs or extracts of plants[17-19].

In conclusion, the data presented in this work showed that methylxanthines (aminophylline, caffeine and theophylline) at concentrations similar to the concentrations usually found in the plasma of human beings not modify the labeling of blood elements with ^{99m}Tc in rats.

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