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Binding Sites of ^{51}Chromium and ^{99m}Technetium to Red Blood Cells: A Comparative Study

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Summary. **This study investigated whether the binding sites of ^{51}Chromium (^{51}Cr) and ^{99m}Technetium (^{99m}Tc) to the beta chain on the hemoglobin were the same. Previous treatment of red blood cells (RBC) with ^{51}Cr did not interfere with the percentage of ^{99m}Tc-labeled RBC. When the previous treatment of RBC with ^{99m}Tc was carried out, the percentage of ^{51}Cr-labeled RBC was not altered. Previous treatment with large amounts of non-radioactive chromium (^{50}Cr) did not seem to alter the percentage of the ^{99m}Tc-labeled RBC, except for the highest concentration of ^{50}Cr. In this case RBC showed great morphological changes in optical microscopy. Our results show that, although ^{51}Cr and ^{99m}Tc bind to the beta chain of the hemoglobin, they probably bind at different target sites.**

INTRODUCTION

^{51}Chromium (^{51}Cr) and ^{99m}Technetium (^{99m}Tc) have been the most used radionuclides to label red blood cells (RBC). These labeled cells have permitted to perform various important investigations in nuclear medicine.[1,2]

The technique to label RBC with ^{51}Cr consists in the incubation of the blood with ^{51}Cr, as sodium chromate.[3,4]

Numerous methods for the preparation of ^{99m}Tc-RBC have been reported. A reducing agent, usually the stannous ion, is necessary in these procedures.[1,2] Three basic methods are used: *"in vitro"*,[5,6] *"in vivo"*[7,8] and *"in vivo/in vitro"*.[9,10] RBC treatment can be carried out with stannous ion and afterwards with ^{99m}Tc,[5,9,11] although the opposite can also be done.[11,12]

Similarities have been observed in the ^{99m}Tc and ^{51}Cr binding sites to RBC. It has been shown that these radionuclides attach to the hemoglobin, mainly, to the beta chain,[12-15] although ^{99m}Tc can also bind to the erytrocyte membrane.[12-14]

In order to contribute to the study of the ^{51}Cr and ^{99m}Tc binding sites on hemoglobin, experiments were performed to verify the possible interference of each radionuclide with the other in the RBC labeling.

MATERIALS AND METHODS

Whole blood from healthy male volunteers (25-40 years age) was anticoagulated with a mixture of dextrose, sodium citrate and citric acid (Don Baxter, Brazil) at a proportion of 4 : 1.

Stannous chloride, as $SnCl_2 \cdot 2H_2O$ (E. Merck, Darmstadt) ^{51}Cr, as sodium chromate and ^{99m}Tc, as sodium pertechnetate, recently milked, from a ^{99}Mo/^{99m}Tc generator (Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brazil) were used. The radioactivity was measured in a well counter with a NaI (Tl) crystal adjusted to ^{51}Cr and ^{99m}Tc photonic energies.

RBC treatment with ^{51}Cr before ^{99m}Tc-labeling: 9 ml of anticoagulated blood was washed three times with NaCl 0.9% solution. These re-suspended blood cells were put in 3 different vials. 1) The first vial was incubated with 1 ml of ^{51}Cr (370 kBq) solution for 30 min at 37°C. These ^{51}Cr-labeled RBC were washed three times and resuspended with NaCl 0.9% solution. Following this, 1 ml of 0.64 uM stannous chloride was added to this vial and incubated for 60 min at 37°C, then 0.5 ml of ^{99m}Tc (37 kBq) solution was added and the incubation continued for another 10 min. 2) The second vial was submitted to the same treatment, but the ^{51}Cr was changed for a 0.9% NaCl. 3) The third vial was treated only with ^{51}Cr solution.

After the different treatments the vials were

centrifuged, aliquots of supernatants and pellets were separated, and the radioactivity measured to determine the percentage of incorporation into RBC with each radionuclide.[5]

Another similar experiment was carried out using a ^{51}Cr solution with activity of 3,700 kBq.

RBC treatment with ^{99m}Tc before ^{51}Cr-labeling: Eighteen ml of blood with anticoagulant was washed three times with NaCl 0.9% solution and distributed into 6 different vials. Three vials were incubated with 1 ml of 0.64 uM stannous chloride for 60 min at 37°C. Following this, 0.5 ml of ^{99m}Tc (37 kBq) solution was put in the first vial, 0.25 ml of 0.9% NaCl solution plus 0.25 ml of ^{51}Cr solution (370 kBq) were put in the second vial, and 0.5 ml of 0.9% NaCl was put in the third vial. The other three vials received the same treatment, however, the stannous chloride solution was changed for 0.9% NaCl. All the vials were centrifuged and blood cells washed three times with NaCl 0.9% solution. One ml of ^{51}Cr solution (370 kBq) was added to the vials that were ^{51}Cr free, and 1 ml of NaCl 0.9% solution in the others. The vials were incubated for another 30 min at 37°C. After centrifuging, the supernatants and pellets were separated, counted in a well counter, and adjusted for ^{99m}Tc and ^{51}Cr. The percentages of radioactivity into red blood cells were determined for each radionuclide.

RBC treatment with non-radioactive chromium before ^{99m}Tc-labeling: Twenty-one ml of blood with anticoagulant was washed three times with 0.9% NaCl and distributed in 7 vials. One ml of a 100, 10, 1, 0.1, 0.01 and 0.001 mg/ml solution of non-radioactive sodium chromate (^{50}Cr) was put into 6 vials. One ml of 0.9% NaCl was put in the seventh vial, as a control. These vials were incubated for 30 min at 37°C and RBC were washed five times with 0.9% NaCl. One ml of 0.64 uM stannous chloride was added into the vials and the incubation continued for more 60 min. Then, 0.5 ml of ^{99m}Tc (37 kBq) was added to each tube and the incubation continued for more 10 min. The vials were centrifuged and aliquots of supernatants and pellets isolated and counted in a well counter to determine the percentage of radioactivity into RBC.

Experiments were also carried out without washing the RBC after the incubation with the non-radioactive ^{50}Cr.

Optical microscopy of treated RBC: Blood cells samples were separated, observed under an optical microscope and the morphologies evaluated at different steps of the various treatments.

The presented values are means plus standard deviations of at least four isolated experiments.

RESULTS

Table 1 shows that the percentage of ^{99m}Tc-labeling RBC was not modified when RBC were previously treated with ^{51}Cr. When the ^{51}Cr activity of 370 kBq was used, the percentage of ^{51}Cr uptake into RBC was 95.1±0.9 and the percentages of ^{99m}Tc uptake into RBC, with or without previous ^{51}Cr treatment,

Table 1. Effect of the RBC treatment with ^{51}Cr on the ^{99m}Tc-labeling.

^{51}Cr activity (kBq)	% of radioactivity into RBC	
	^{51}Cr	^{99m}Tc
0	—	96.5±2.1
370	95.1±0.9	—
370	94.0±1.1	95.7±1.8
0	—	97.4±1.1
3,700	94.1±1.9	—
3,700	94.3±0.7	96.1±2.4

The values presented correspond to the mean ± SD of four isolated experiments.

Table 2. Effect of the RBC treatment with ^{99m}Tc on the ^{51}Cr-labeling.

Treatment	% of radioactivity into RBC	
	^{99m}Tc	^{51}Cr
$SnCl_2+^{99m}Tc+^{51}Cr$	97.1±1.9	95.4±2.9
$SnCl_2+NaCl+^{51}Cr$	—	96.2±1.3
$SnCl_2+^{51}Cr$	—	96.9±0.7
$NaCl+^{99m}Tc+^{51}Cr$	54.1±4.1	95.8±2.2
$NaCl+NaCl+^{51}Cr$	—	94.7±3.1
$NaCl+^{51}Cr$	—	96.1±2.4

The values presented correspond to the mean ± SD of four isolated experiments.

Table 3. Effect of the RBC treatment with non-radioactive chromium on the ^{99m}Tc-labeling.

Sodium chromate (^{50}Cr)	% of ^{99m}Tc into RBC	
mg/ml	RBC not washed	RBC washed
0	97.1±1.1	97.4±1.9
0.001	96.9±0.7	97.1±0.3
0.01	97.5±1.9	96.7±1.3
0.1	96.8±0.7	97.3±1.2
1	79.9±1.9	97.6±0.1
10	56.7±2.3	98.1±0.8
100	53.1±1.9	57.8±3.1

The values correspond to the mean ± SD of five isolated experiments.

were 95.7±1.8 and 96.5±2.1, respectively. In the presence of ^{51}Cr activity of 3,700 kBq, the percentage of ^{99m}Tc uptake by RBC was the same 96.1±2.4.

Table 2 shows that the percentage of ^{51}Cr-labeled RBC was also not modified by the previous ^{99m}Tc treatment. As the ^{99m}Tc fixation on the hemoglobin is dependent on a reducing agent concentration,[5,16] the low incorporation of this radionuclide in the absence of stannous chloride can be explained by this fact, as already described.[1,2,5,9]

Due to the small amount of chromium atoms present in the radioactive samples, we performed experiments with non-radioactive chromium. Table 3 shows that when blood cells were incubated with ^{50}Cr before ^{99m}Tc-labeling, the percentage of this radionuclide into RBC was the same as the control in the presence of 0.001 to 0.1 mg/ml and was decreased from 1 to 100 mg/ml. However, when the blood cells treated with ^{50}Cr were washed five times before the ^{99m}Tc-labeling, the percentage of radioactivity into RBC was not changed for all ^{50}Cr concentrations, except for the concentration of 100 mg/ml.

Optical microscopy of RBC showed slight morphological alterations in the different treatments. However, when RBC were incubated with 100 mg/ml of non-radioactive sodium chromate, marked morphological changes were observed as anisocytosis, hypochromia and poikilocytosis (results not shown).

DISCUSSION

Previous studies carried out to identify the ^{51}Cr and ^{99m}Tc binding sites have demonstrated that these radionuclides bind mainly to the beta chain of the hemoglobin molecule.[12,13,15] Although bindings to the alfa chain are also possible, their importance is slight.[12,13,15]

^{99m}Tc has also been found to bind to erytrocyte membrane proteins.[12-14] It is supposed that this binding could be greater to the membrane proteins than to the hemoglobin if the membrane protein concentration was higher.[14]

Although ^{99m}Tc and ^{51}Cr could bind mainly to the same chain of the hemoglobin, the target where these bindings occur did not seem to be the same, because as the results here described showed, the previous ^{51}Cr or ^{99m}Tc treatment did not interfere with the RBC tagging with ^{99m}Tc or ^{51}Cr. As earlier described,[4] there is an inverse ratio between the amount of radioactive chromate used to label RBC and the radioactive percentage uptake by the RBC. When the quantity of radioactive chromate increases ten times, to the same volume of red cell suspension, the uptake to the erythrocytes decreases from 97.4 to 66.4%. These results suggest that there are limited sites to bind ^{51}Cr into RBC. In our experiments, we decided to treat RBC with a large amount of ^{50}Cr since the two ^{51}Cr activities (370 and 3,700 kBq) employed to label these cells did not seem to saturate all the binding sites to chromium. Furthermore, the treatment of blood cells with non-radioactive chromium did not influence the RBC labeling with ^{99m}Tc until concentrations as high as 10 μg/ml of sodium chromate. The decrease in the ^{99m}Tc-labeling of the non-washed RBC in presence of 1 and 10 μg/ml ^{50}Cr was not real since we did not observe any changes in the washed RBC at these ^{50}Cr concentrations. In this case the great amount of ^{50}Cr, as an ion, non-bound on the hemoglobin molecule, seemed to be responsible for the decrease in ^{99m}Tc-labeling with non-washed RBC.

The decrease in the radioactivity of ^{99m}Tc in the red blood cells observed when 100 μg/ml of sodium chromate was used, did not seem to indicate the saturation of the binding sites. The actual phenomenon could be the morphological modifications in the erytrocyte membranes as they were seen under optical microscopy. Another possibility could be the inhibition of the activity of enzymes, as glutation reductase, or by progressive inhibition of glycolise and cellular respiration observed at doses of sodium chromate higher than 10 μg/ml.[17,18]

We can conclude that, although ^{51}Cr and ^{99m}Tc bind mainly to the beta chain on the hemoglobin molecule, probably, there are different binding sites for each radionuclide.

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