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A Simple Technique for Obtaining ^{99m}Tc-Labeled Leukocytes and Their Organ Distribution in Rabbits

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Summary. **This paper presents a simple and reproducible method for the ^{99m}Tc-labeling of leukocytes, with high labeling efficiency, viability, low cost and short preparation time. Mononuclear cells were incubated with different stannous chloride concentrations for the reduction of $^{99m}TcO_4^-$ and labeling of the cells. In healthy rabbits, ^{99m}Tc-leukocytes are located primarily in the lungs, liver and spleen. The distribution of ^{99m}Tc labeled cells correlated well with existing data, suggesting that this method is applicable as a mononuclear label for diagnostic gamma-imaging procedures.**

INTRODUCTION

In the past years, a considerable amount of work has been published describing various methods for preparing labeled compounds in order to detect pyogenic abscesses. Most of these methods have used gallium-67 as citrate, or indium-111-labeled leukocytes.[1-3)]

Technetium-99m (^{99m}Tc) is perhaps the most promising radionuclide for labeling white blood cells for detection by gamma camera imaging. ^{99m}Tc is readily available and inexpensive.[4)]

Many authors prefer *in vitro* methods for the ^{99m}Tc labeling of leukocytes in whole blood, based on phagocytosis. For instance, the SnF_2 colloid method is applicable to granulocytes and monocytes.[5)] However, the machanism of labeling is shown to be surface adherence rather than phagocytosis.[6)] Others have developed a method of ^{99m}Tc labeling of leukocytes starting with a commercial albumin colloid kit, but 30% of the labeled colloid remains unbound to cells.[7)] The glucoheptonate method has had clinical success in Chile, although *in vivo* recovery and distribution in dogs were found to be low and variable.[8)]

Since 1986, most work for ^{99m}Tc labeling of leukocytes has been centered around the hexamethylpropyleneamine oxine, usually known as HMPAO.[8,9)] The high cost, the low availability and labeling efficiency are the main disadvantages of this method, in addition to the inability to label mononuclear cells and the large amount of blood required.[8,9)] Moreover, the almost complete inhibition of the proliferative capacity of lymphocytes to occur after labeling with ^{99m}Tc-HMPAO activities, sufficient for scintigraphy,[10)] has been described.

The criteria for a good labeling technique are: 1) high labeling efficiency; 2) high stability; 3) high cell specifity; and 4) good viability with normal maintenance of cellular function.[11)] In this paper we present an efficient method to label leukocytes with ^{99m}Tc, with high labeling uptake, stability, viability and low cost, that requires a short time for preparation.

MATERIALS AND METHODS

Mononuclear cells separation

Thirty mililiters of whole blood were obtained from rabbits by cardiac puncture using sterile heparinized (Liquemine, Roche, Brazil) tubes. Twenty mililiters of a saline solution (NaCl 0.9%) were injected into the peritoneal cavity to replace the initial physiologic conditions. For mononuclear cells isolation, the Ficoll-Hypaque technique was used.[12)] The cells were washed by centrifugation (1,000 rpm, 10 min) in saline solutions, using a clinical centrifuge. The pellet was resuspended with saline and transferred to a microcentrifuge tube for platelet removal ($500 \times g$, 1 min). This procedure was repeated three times until the

cell preparation was free of platelets. The final mononuclear cell concentration was 10^7 per ml.

Labeling of mononuclear cells with ^{99m}Tc

A freshly prepared stannous chloride solution (2400, 240, 120, 60, 12 or 6 μg/ml), as $SnCl_2 \cdot 2H_2O$ (E. Merck, Darmstadt), was added to sterile vacuum vials. Solutions were incubated for 10 min at room temperature (22℃). Following this, 185 kBq of ^{99m}Tc (sodium pertechnetate), recently milked from a ^{99} Mo/^{99m}Tc generator (Instituto de Pesquisas Energêticas e Nucleares, Brazil), was added. Incubation continued for another 10 min. After which the cells were washed once with saline.

Determination of the labeling efficiency

The second washing of the supernatant was separated and measured in a gamma counter, as well as the pellet. The percentages of radioactivity uptakes for each stannous chloride concentration were determined as previously described for red blood cells.[13]

Stability

In vitro experiments were conducted to determine the stability of the ^{99m}Tc mononuclear labeled cells preparations in two different ways: 1) the labeled cells were washed three times with saline and 2) the labeled cells were incubated for 24 h. The percentage of residual radioactivity was calculated by the ratio between the final radioactivity (after washing or incubation) and the initial one.

Viability

The labeled mononuclear cell preparations were evaluated for cell viability by a dye exclusion test.

Biodistribution study

Whole-body images of rabbits were obtained with a scintillation camera (Acticamera CGR, model 3420) for: (A) free ^{99m}Tc, (B) ^{99m}Tc-colloidal sulphur, (C) ^{99m}Tc-glucoheptonic acid (GHA), and (D) ^{99m}Tc-dietilen-triaminopentacetic acid (DTPA), in order to study their scintigraphic map. The ^{99m}Tc-mononuclear cells (obtained by the cited labeling conditions with a chosen concentration of 12 μg/ml of stannous chloride solution and a ^{99m}Tc activity of 37 MBq) were injected into the rabbits.

Table 1. Mononuclear cell labeling efficiences.

$SnCl_2 \cdot 2H_2O$ (μg/ml)	% of radioactivity in leukocytes
0	6.03 ± 0.70
6	78.19 ± 6.44
12	87.79 ± 5.76
60	81.82 ± 9.46
120	85.35 ± 8.14
240	84.37 ± 7.66
2400	83.25 ± 6.20

Table 2. Stability of labeled mononuclear cells after three centrifugations.

$SnCl_2 \cdot 2H_2O$ concentrations	Washings		
	1	2	3
0	12.20	8.51	7.84
6	81.86	72.48	66.60
12	85.44	77.89	73.00
60	85.64	77.87	71.67
120	85.50	78.55	73.01
240	78.01	71.37	66.45
2400	83.01	75.98	71.28

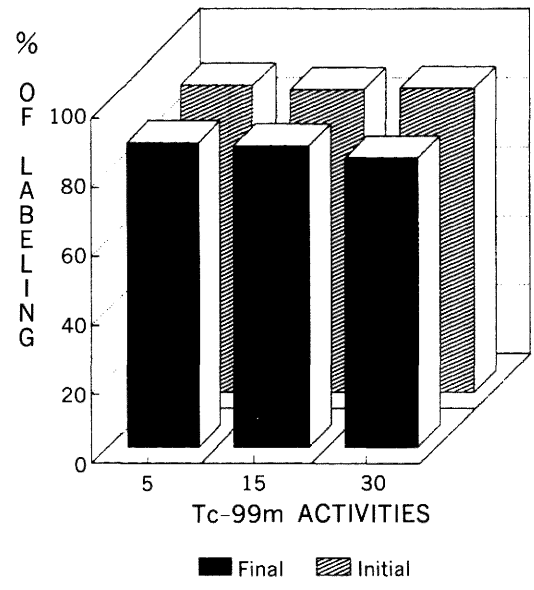

Fig. 1. Mononuclear cells were incubated with a stannous chloride solution of 12 μg/ml and then with different ^{99m}Tc activities. These labeled cells were incubated for 24 h at room temperature. Residual radioactivity percentages were calculated.

RESULTS

The mononuclear cells preparations were found to be platelets and red blood cell free. Mononuclear cell labeling efficiencies are shown in Table 1. Our results, with different stannous chloride concentrations, indicated that the radioactivity uptake reaches constant values with concentrations of 6 μg/ml and above. No statistical differences were found. The control, without stannous chloride, demonstrated the lowest labeling efficiency.

The stability of the ^{99m}Tc-labeled mononuclear cells after three washings is shown in Table 2. The radioactivity remained constant after washing for all tested stannous chloride concentrations.

The ^{99m}Tc-stability after 24 h of incubation of the labeled mononuclear cells with different activities (185, 555 and 1110 MBq) is shown in Fig. 1. Results show that there is no ^{99m}Tc spontaneous release.

Viability of the ^{99m}Tc-labeled mononuclear cells begins to decrease as the stannous chloride concentration reaches values up to 120 μg/ml (results not shown).

In order to extend the experiments, we have chosen the stannous chloride concentration of 12 μg/ml, which also presents a viability of 100%.

Figure 2 shows the scintigraphic map of healthy rabbits obtained with (A) free ^{99m}Tc, (B) ^{99m}Tc-colloidal sulphur, (C) ^{99m}Tc-GHA, and (D) ^{99m}Tc-DTPA.

Figure 3 shows the scanning obtained two hours after ^{99m}Tc-mononuclear cell administration in a healthy rabbit. ^{99m}Tc labeled mononuclear cells were distributed in the lungs, liver and spleen. Imaging studies displayed an initial uptake of labeled cells in the lungs followed by clearance. There was also progressive increase in activity in the liver with time. There were no thyroid, stomach or heart uptakes.

DISCUSSION

^{99m}Tc labeling of white blood cells has potential advantages over other radionuclides.[14] In general, a small amount of plasma is used in the incubation medium to label leukocytes,[15] resulting in a low labeling efficiency. Others use a high stannous chloride concentration although there is a consensous that this method decreases cell viability.[16]

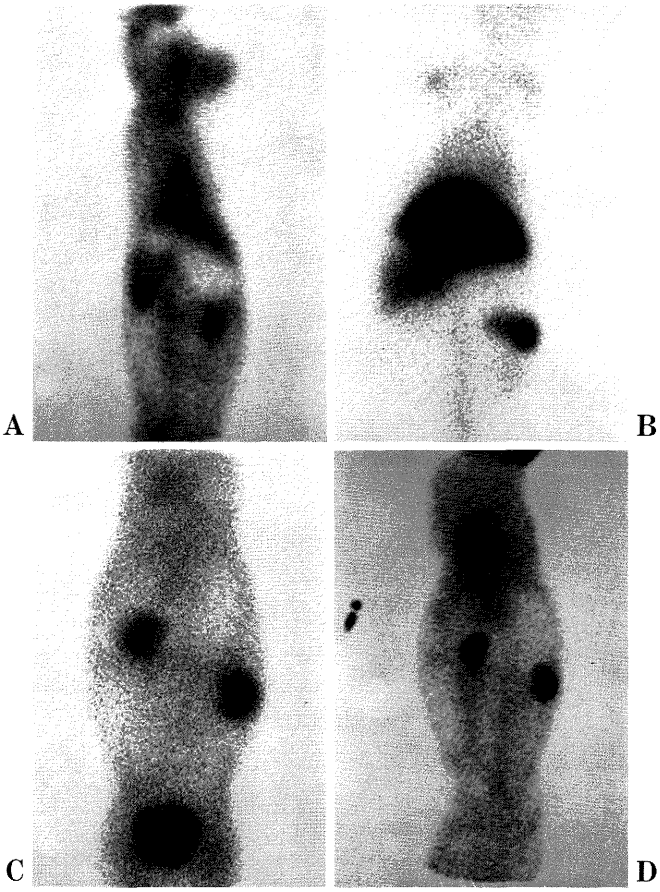

Fig. 2. (A) free ^{99m}Tc, (B) ^{99m}Tc-colloidal sulphur, (C) ^{99m}Tc-GHA and (D) ^{99m}Tc-DTPA were injected in rabbits after which scans were obtained.

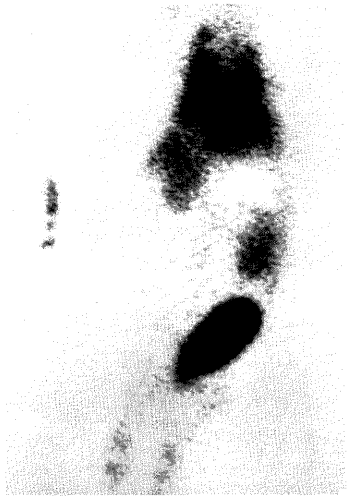

Fig. 3. ^{99m}Tc-labeled mononuclear cell (obtained with 12 μg/ml of stannous chloride and ^{99m}Tc activity of 37 MBq) scan in rabbits obtained 2 h after injection show an initial uptake in the lungs, liver and spleen. There are no thyroid, stomach or heart uptakes.

The results of our method show that the labeled preparations here presented are stable, at least after 3 washings or after 24 h incubation.

In our proposed method of ^{99m}Tc-labeling mononuclear cells, we have chosen to use a stannous chloride concentration of 12 μg/ml due to the high radioactivity uptake (87.79 $\pm$ 5.76%), high stability and viability found in these labeled cells.

Following administration of the labeled cell preparation in control animals, a rapid clearance of the blood-pool activity occurs. Organ distribution of ^{99m}Tc labeled mononuclear cells in rabbits were found in accordance with data from the literature.[17] The high specifity of labeling, the minimal red blood cell and platelet contamination, the favorable labeling properties and viability support the usefulness of this method in the clinical evalution of infection sites. Human clinical applicability for diagnostic scanning procedures are now in progress.

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