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DEVELOPMENT OF LIOPHYLIZED KIT OF TIN-GLUCOHEPTONATE FOR *IN VITRO* LABELING LEUCOCYTES WITH 99m-TECHNETIUM

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ABSTRACT

Many radiopharmaceuticals are applied in the scintigraphic evaluation of inflammatory and infection. The inflammatory reaction represents the reaction of the organism to any traumatic condition and this process atracts leucocytes to the inflamatory focus (chemotaxy) and substances (enzymes, antibodies and complements) that represents the functions of defense and repair, respectively. Labeled leucocytes are recognized as the best radiopharmaceutical to the detection of inflammatory and infection. This work intended the development of a tin-glucoheptonate lyophilized kit to be applied in the *in vitro* labeling of leucocytes with 99m-technetium. The leucocytes labeling procedure using the kit was optimized and the principal parameters studied were the volume of the saline reconstituted tin-glucoheptonate kit, reaction incubation time and temperature in the pre-stanization procedure. Then the leucocytes were incubated with ^{99m}Tc (185 MBq), at different times at room temperature. Labeling yield were superior than 90%. Lyophilized tin-glucoheptonate kit was stable for 90 days. Scintigraphic images obtained at 1,2 e 3 hours after the administration of labeled human leucocytes in rabit with inflammatory focus promoted by tupertine injection showed high and persistent uptake of the labeled leucocytes in the lesion .

1. INTRODUCTION

The scintigraphic images can be applied in the identification and localization of inflammatory and infection focus. The scintigraphy using labeled leucocytes was introduced in Nuclear Medicine at 1976 and is now a routine procedure applied to different pathologies related to leucocytes infiltration, including intestinal inflammatory disease, bone prosthetic infections and ulcers of diabetic feet. Nowadays, there are many radiopharmaceuticals applied in the visualization of inflammation and infection focus , being classified in non specific (colloidal radiopharmaceuticals, liposome, immunoglobulin, human albumin) and specific radiopharmaceuticals, including labeled leucocytes, specific monoclonal antibodies and receptor-specific small peptides and proteins. All these radiopharmaceuticals present advantages and disadvantages. The labeled leucocytes are considered the gold standard in visualization of infection and inflammation because of high specificity (they are attracted to the focus by chemotaxy). The aim of this study was the development of a tin-glucoheptonate lyophilized kit to be applied in the *in vitro* leucocytes labeling with 99m-technetium. The labeling method was based in the pre-stanization of the leucocytes and posterior incorporation of reduced technetium. This method represents an alternative to the labeling of leucocytes with the lypophilic ^{99m}Tc-HMPAO complex [6,2,9,10,1,8]

2. MATERIALS AND METHODS

2.1. Materials

Calcium glucoheptonate and stannous chloride ($SnCl_2.2H_2O$) were purchased from Sigma. Plasmasteril® was used in the cell sedimentation of blood samples. Pertechnetate solution (^{99m}Tc) was obtained from ^{99}Mo-^{99m}Tc generator (IPEN-TEC).®

2.2. Methods

Preparation of tin-glucoheptonate lyophilized kit

Each vial of the lyophilized tin-glucoheptonate kit contains 100mg of calcium glucoheptonate and 263μg of Sn (II). The kit was prepared by the dissolution of calcium glucoheptonate in distilled water (prepared under nitrogen atmosphere) followed by the addition of a freshly prepared solution of $SnCl_2.2H_2O$ in HCl. The pH of the preparation was adjusted to 5.0-5.5. Aliquots of 1 mL of this reaction mixture were dispensed in vials for lyophilization. After lyophilization process, the vials were stored under refrigeration (4ºC) and the stability was studied for three month. To study the labeling of leucocytes using this tin-glucoheptonate kit, 10 mL of saline (NaCl 0.9%) was introduced in the lyophilized kit and 4 mL of this preparation was incubated at 37ºC with the leucocytes preparation.

Preparation of the leucocytes sample

The procedure employed in the preparation of the leucocytes sample was developed under aseptic conditions, using sterile and apirogenic materials (ethic code # 98/ CEP-IPEN/SP).
- **Blood sampling** – the leucocytes were obtained from blood samples of human volunteers (20 normal volunteers). 50 mL of blood was obtained from each volunteer using ACD as anti-coagulant agent (10 mL). The blood was transferred to a conical tube and 6.0 mL of Plasmasteril® 6% was introduced to promote natural sedimentation of the erythrocytes at room temperature. After cell sedimentation (60-120 minutes) the plasma was separated and centrifuged at 1000 rpm. To remove residual erythrocytes, 5 mL of distilled water was introduced followed by 5 mL de NaCl 1.8%. The cell preparation was centrifuged by 5 minutes at 1000 rpm. This procedure was repeated until the elimination of the residual erythrocytes. The leucocytes were suspended in 1 mL of NaCl 0.9%. 50μL of the cell preparation was diluted with 950μL of NaCl 0.9% and an aliquot of this preparation was introduced in an Newbauer camera to determine the number of cells in the leucocytes preparation under microscopic observation. All labeling procedures in this study was developed using the same number of cells ($2.2\text{-}2.6 \times 10^7$). The cell viability was evaluated by microscopic observation using Triplan Blue [5] .

2.2.3 Leucocytes stannization

The tin-glucoheptonate lyophilized kit was reconstituted with 10 mL of NaCl 0.9% and 4 mL of this preparation was added at leucocytes preparation. The preparation was incubated at

37°C for 40 minutes. After the incubation time the cell suspension was centrifuged for 10 minutes at 1000 rpm, and the cells was washed with 10 mL of saline and centrifuged again to remove the tin excess.

2.2.4 Labeling of the leucocytes with ^{99m}Tc

After stanization, the leucocytes were suspended in 1mL of saline solution and the suspension was divided in three parts to obtain three leucocytes samples to be applied in the labeling studies. The leucocytes samples contain 2.2- 2.5 x 10^{7} cell. to the leucocytes sample was introduced the pertechnetate ($^{99m}TcO^{-}_{4}$) solution with 185 MBq and the mixture was incubated for 20 minutes at room temperature.

2.2.5 Quality Control

After reaction time, the samples were centrifuged at 1000 rpm for 10 minutes and the % efficiency labeling (EL) was determined by:

$$EL\ (\%) = \text{Leucocytes Activity/ Total Activity x } 100$$

2.2.6 Biodistribution Study

Scintigraphic images were obtained from a *New Zeland* rabit after the intravenous administration of 59.2MBq (1.6 mCi) of labeled human leucocytes. An inflammatory focus was developed at left tight of the animal, by the intramuscular administration of tupertine, 48 hours before the leucocytes administration. Planar images were obtained 1, 2 and 3 hours after the dose administration in a gamma camera using low energy collimator at Centro de Medicina Nuclear da Faculdade de Medicina da USP.

3. RESULTS

Table 1 shows the influence of the tin-glucoheptonate solution volume in the labeling efficiency of the leucocytes preparation.

Table 1. Influence of tin-glucoheptonate volume in the leucocytes labeling efficiency

Volume of Glucoheptonate/ Sn^{2+}	LABELING EFFICIENCY (%)		
	10 minutes	20 minute	40 minutes
1 mL /26.3 μg	29.10 ± 0.11	30.00± 0.10	38.20 ± 0.03
2 mL / 52.6 μg	32.20 ± 0.10	76.40 ±0.10	87.80 ± 0.03
4 mL /105.2 μg	55.60 ± 0.01	79.20 ± 0.06	90.50± 0.15

Figure 1 shows the whole body image of the rabbit after 3 hours of the administration of the ^{99m}Tc-leucocyte suspension and Figure 2 to 4 show the images of rabbit thighs 1, 2 and 3 hours after the dose administration, respectively. The left thigh presents high uptake in the inflammatory focus.

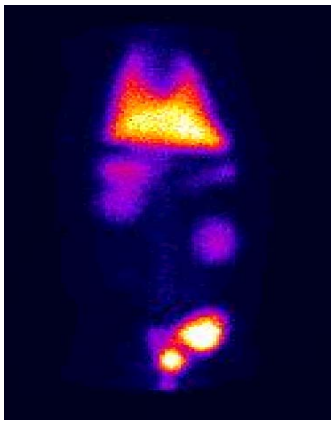

Figure 1. Whole body planar scintigraphy of rabbit after 3 hours of the ^{99m}Tc-leucocytes administration

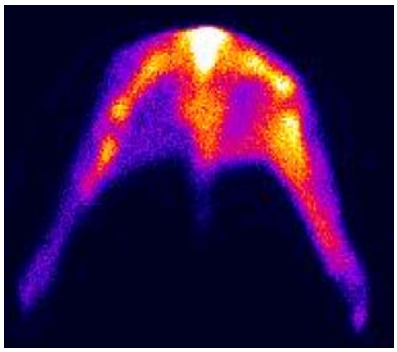

Figure 2. Scintigraphy of inflammatory focus in the left tight of rabbit 1 hour after dose administration of ^{99m}Tc-leucocytes

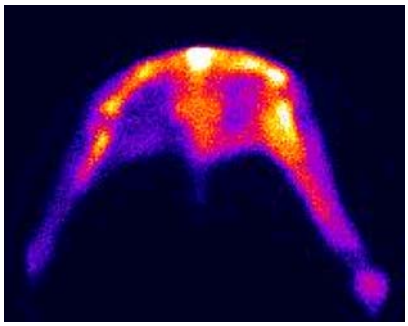

Figure 3. Scintigraphy of inflammatory focus in the left tight of rabbit 2 hour after dose administration of ^{99m}Tc-leucocytes

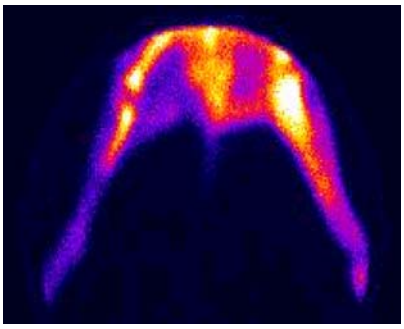

Figure 4. Scintigraphy of inflammatory focus in the left tight of rabbit 3 hour after dose administration of ^{99m}Tc-leucocytes

4. DISCUSSION

The volume of 50 mL of blood was sufficient to ensure a good number of leucocytes in the sample (more than 10^7 leucocytes) and contributed to the efficiency of labeling. The methodology employed in the leucocytes separation was recommended by ISORBE (International Society of Radiolabeled Blood Elements) [4]

Cellular viability was superior to 98% and confirmed the efficiency of the technique employed in the leucocytes preparation. This high labeling efficiency was similar to the obtained using ^{99m}Tc-HMPAO complex in the preparation of labeled leucocytes [6,5,3,4].

All the labeling study was developed using NaCl 0.9% instead residual plasma in the cell preparation, as previously described, in order to guarantee the labeling efficiency [2]. The labeling of pre-stannized leucocytes with TcO^-_4 was developed at room temperature and not at 37ºC as previously described and the efficiency labeling was very high.

Scintigraphic images showed the biodistribution of the labeled leucocytes with high uptake on lungs after 1 hour of the administration. The uptake on liver and kidneys were compatible with the metabolization and excretion of the compound, respectively. There was no uptake on thyroid and stomach that reveals the *in vivo* stability of the compound. The labeled leucocytes concentrated in the inflammatory focus promoted by tupertine injection and the uptake was high and persistent after 3 hours of the injection, suggesting the specific uptake in the focus.

5. CONCLUSIONS

The pre-stannization technique applied on labeling leucocytes with 99m-technetium using a tin-glucoheptonate lyophilized kit represents a good and economic alternative for labeling leucocytes with 99m-technetium, with high efficiency, to be applied in clinical determination of inflammatory and infection in Nuclear Medicine.

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