Technetium-99m Labeling of Polymorphonuclear Leukocytes: Preparation with Two Different Stannous Agents

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A technique for in vitro labeling of human polymorphonuclear leukocytes with Tc-99m is described. Titration of stannous fluoride and stannous pyrophosphate concentrations for pretinning was performed, and optimal amounts of the stannous agents were added to polymorphonuclear leukocytes efficiently isolated from 100 ml of blood. Labeling with 10–15 mCi Tc-99m resulted, after three washings of cell suspensions, in yields of 1.6–4.8 mCi, corresponding to 20.5–33.5% of added tracer. Cell-bound activity in the final cell suspensions was 92.3% ± 1.9 of the added dose. Cell function was not impaired by the labeling technique. Sterility and exclusion of bacterial endotoxins in the final cell suspensions were demonstrated. The method may prove of diagnostic value in the isolation, labeling, and reinjection of autologous leukocytes for scintigraphic imaging of acute inflammatory lesions.

J Nucl Med 26:68–71, 1985

Indium-111 labeling and reinjection of autologous leukocytes has become a widely used procedure for the scintigraphic detection of inflammatory lesions (*1–11*). However, experiments by Chisholm et al. (*12*) have shown that In-111-oxine labeling may result in cell damage that is primarily radiation-induced. This has raised a need for the labeling of leukocytes with alternative tracers.

Experimental investigations of leukocyte labeling with Tc-99m have been encouraging (*13–19*), but though a few studies on reinjecting Tc-99m-labeled human leukocytes have been performed (*20,21*), a method suitable for clinical application has not yet become available. Preliminary results of in vitro labeling of polymorphonuclear (PMN) leukocytes with Tc-99m, using small amounts of a stannous fluoride compound for pretinning, have been reported recently (*22*). The present study was undertaken to refine this labeling technique.

Received Mar. 5, 1984; revision accepted Aug. 31, 1984.

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MATERIAL AND METHODS

To provide sterility, isolation and labeling of PMN leukocytes took place in a laminar-flow hood.

Cell separation

Human whole blood was obtained from healthy volunteers by two different procedures.

1. Samples of 500 ml of blood were collected in triple blood-bag systems and anticoagulated with citrate phosphate dextrose. The bags were centrifugated with the inlet and outlet tubes upwards for 15 min at 300 *g* and 4°C. Plasma was expelled into one of the empty bags, and the upper fifth of the visible cell layer was expelled into the other. Enough plasma was transferred to the cell bag to obtain a hematocrit of approximately 50.

2. Samples of 100 ml of blood were collected in blood bags and anticoagulated with ethylenediaminetetraacetate.

Sedimentation of red blood cells in 60-ml disposable syringes containing 2% methyl cellulose was followed by hemolysis of residual RBCs using 0.2% saline. Leukocyte separation was performed by centrifugation of the cell

TABLE 1
Separation of PMN Leukocytes from 500 ml and 100 ml Blood Drawn from Healthy Volunteers (Mean ± s.d.)

Amount of blood drawn	Cell count (in $10^6/\mu l$)	Polymorphonuclears (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
500 ml						
n = 8						
Before separation	1249 ± 395	47 ± 19	44 ± 18	6 ± 2	2 ± 2	1 ± 1
After separation	400 ± 133	92 ± 4	5 ± 3	0	3 ± 2	0
100 ml						
n = 11						
Before separation	526 ± 131	59 ± 6	33 ± 6	6 ± 3	3 ± 1	0
After separation	184 ± 77	93 ± 6	3 ± 3	0	4 ± 4	0

suspension through a sodium metrizoate/Ficoll* gradient. Buffy-coat leukocytes were washed in Gey's solution prepared according to Wilkinson (*23*), and PMN leukocytes were washed and resuspended in isotonic saline.

Cell counting was done in a Neubauer chamber after staining with methyl violet acetic acid. Differential counts were performed after staining with May-Grünwald/Giemsa.

PMN leukocyte labeling

Pretinning of the PMN leukocyte suspensions was performed in nitrogen-saturated polycarbonate tubes using one of two commercial preparations: a stannous fluoride bone-seeking agent containing 5 mg medronic acid, 0.34 mg stannous fluoride and 2 mg sodium p-aminobenzoate; or a stannous pyrophosphate solution containing 7.2 mg sodium pyrophosphate and 1.030 mg stannous chloride. The cells were incubated for 10 min with intermittent agitation, then centrifugated at 200 *g* and 4°C. After resuspension of the cells in 2.5 ml isotonic saline, 10–15 mCi sodium pertechnetate was added and allowed to incubate for 10 min. The PMN leukocyte suspension was washed three times in isotonic saline, then measured for labeling yield.

PMN leukocytes obtained from 500-ml blood samples were divided into aliquots with equal cell counts. Different amounts of the stannous agents were added in order to delimit the optimal concentrations in the cell suspensions before labeling with Tc-99m.

PMN leukocytes obtained from 100-ml blood samples were labeled with Tc-99m after pretinning the cells with the optimal amounts of stannous fluoride and stannous pyrophosphate compounds.

To elucidate an influence of cell number on labeling yield, the same labeling procedure was applied to pairs of PMN leukocyte suspensions from the same subjects in which the cell count ratio was 4 to 1. The cells of the two tubes were suspended in equal volumes of saline (2.5 ml) before labeling.

In another series, various amounts (6.9–40.6 mCi) of Tc-99m were added to equal cell numbers to evaluate a possible influence of added radiation dose on labeling yield.

Viability assays

The chemotactic function of PMN leukocytes before and after labeling was studied in modified Boyden chambers as previously described (*22*). The viability of the cells was further investigated by the trypan blue exclusion test.

Pyrogen and sterility test

In order to detect any bacterial endotoxins added to the cell suspensions during the isolation or labeling procedures a *Limulus* amebocyte lysate gelation test was applied to the final cell suspensions, with appropriate positive control samples containing *E. coli* endotoxin. Sterility of the final cell suspensions was proven by testing for growth on culturing plates. The plates were incubated for 7 days before being read.

RESULTS

Cell separation

The results of PMN leukocyte isolation from 500- and

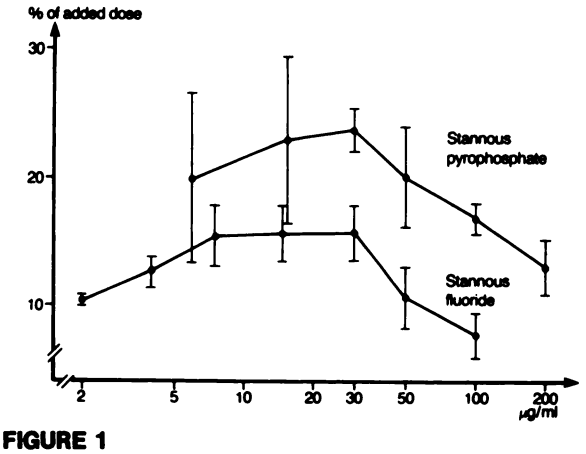

FIGURE 1
Labeling yields, in percent of added Tc-99m dose, in relation to concentrations of stannous fluoride and stannous pyrophosphate in polymorphonuclear leukocyte suspensions. Bars indicate mean ± s.d. Abscissa is logarithmic

TABLE 2
Yield of Labeled PMN Leukocytes* at Optimal Levels of Stannous Agents (n = 18)

Stannous fluoride (μg/ml)	Yield (%) (mean ± s.d.)
8	20.5 ± 3.8
15	21.0 ± 2.2
30	20.9 ± 4.0
Stannous pyrophosphate (μg/ml)	
10	29.9 ± 3.7
30	33.5 ± 3.3

* In percent of added dose.

100-ml blood are listed in Table 1. Recovery of PMN leukocytes was 55.1%. Red-cell contamination of the final cell suspensions was negligible (<1%). In PMN leukocyte suspensions from 100 ml of blood, eosinophils comprised the most common non-PMN cell type. There was no platelet contamination.

Leukocyte labeling

The relations between yields of labeled PMN leukocytes and stannous agent concentrations in the titration study are shown in Fig. 1. The optimal concentration of stannous fluoride was 30 μg/ml (22.7 μg Sn^{++}/ml) and of stannous pyrophosphate also 30 μg/ml (18.8 μg Sn^{++}/ml), with corresponding mean labeling yields of 15.7% and 23.7%. Labeling results achieved at optimal levels of stannous agents added to PMN leukocytes isolated from 100 ml of blood are given in Table 2. Cell-bound activity was 92.3% ± 1.9 (mean ± s.d.) after three washings, with radioactivity range 1.6–4.8 mCi. Labeling yields with Tc-99m were 40–45% higher by means of stannous pyrophosphate compared with the stannous fluoride labeling compound.

A certain influence of cell number on labeling efficiency was indicated by the labeling yields of 18.9% ± 3.5 (mean ± s.d.) of added dose in cell suspensions containing high cell counts, compared with 8.3% ± 3.6 in suspensions with one fourth of the cell counts of the above mentioned.

Labeling yields in percent of added doses were of the same magnitude (maximal difference of pairs 3.2%) when various amounts of Tc-99m were added.

Cell function and purity of cell suspensions

Chemotaxis and trypan blue exclusion of PMN leukocytes were not affected by the labeling procedure (Table 3). All endotoxin detection tests gave negative gelation results with final cell suspensions. Culture plates were never positive for bacterial growth.

DISCUSSION

The compounds formed by reduced pertechnetate and the stannous agents used in this study have a great affinity for human red blood cells. Thus, efficient removal of RBCs from the cell preparation is essential to the labeling procedure of PMN leukocytes with Tc-99m. Our efficient isolation of PMN leukocytes was developed in order to obtain specific scintigraphic findings in acute inflammation. Methyl cellulose sedimentation and hemolysis of red cells, followed by gradient centrifugation as described here, do not influence cell viability (*22*).

The efficiency of labeling PMN leukocytes with Tc-99m was slightly improved, compared with our preliminary results (*22*), by optimizing the amount of pretinning agent. The concentration of stannous fluoride in the cell suspension was, however, still held at a minimum, unlike previous studies on stannous reducing agents (*13,15,18,19*). Indeed, the concept of the stannous agent concentration as a crucial point in the efficiency of Tc-99m leukocyte labeling was confirmed (*16,20,24*). Incubation of PMN leukocytes with optimal amounts of stannous pyrophosphate showed a greater labeling yield compared with that of the stannous fluoride compound, making the former agent preferable. The step involving the reduction of pertechnetate seems essential in Tc-99m labeling of leukocytes, though the mechanism by which Tc-99m is incorporated into the cells is unknown (*16*). An autoradiographic study by Glenn et al. (*17*) demonstrated a probable surface attachment of the Sn-Tc compound, but Tc-99m possesses poor radiation characteristics for autoradiography, and the efficiency of the leukocyte labeling in the study was relatively low. The yield of labeled PMN leukocytes in the present study was proportional to the cell number in the final suspension, and the cell-bound activity was high after three washings. These results suggest, in accordance with electrophoretic investigations by others (*15,18*), an efficient binding of the label to the leukocytes. The ra-

TABLE 3
Chemotactic Function and Capability of Trypan Blue Exclusion of PMN Leukocytes Before and After Tc-99m Labeling in 10 Experiments

Function	Before	After	P
Chemotaxis			
Spontaneous	62 ± 19	59 ± 16	N.S.
Casein-stimulated	117 ± 19	121 ± 19	N.S.
Trypan blue exclusion	1.1 ± 0.7	1.0 ± 0.7	N.S.

dioactivity yield of the cells seems to correlate positively with the concentration of pertechnetate of the cell suspension, suggesting a rather unlimited capacity of the cell-bound stannous agent to reduce and combine with the pertechnetate. The cell-bound activity will be sufficient for scintigraphic imaging provided the leukocytes accumulate in the areas of interest.

Cell function was preserved and bacterial contamination and presence of pyrogenic material in the final suspensions of PMN leukocytes were excluded. Thus, isolation and labeling of human PMN leukocytes with Tc-99m by the technique described here may provide a valuable method for location of occult inflammatory lesions by leukocyte scintigraphy.

FOOTNOTE

*Ficoll is a sucrose polymer (MW 400,000), produced by Pharmacia, Uppsala, Sweden.

ACKNOWLEDGMENT

The skillful assistance of laboratory technician Kirsten Bülow is acknowledged.

This work was supported by The Foundation of Carl J. Becker and by The Danish Medical Research Council.

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