
Technetium-99m Labeling of Red Blood Cells: In Vitro Evaluation of a New Approach

Henning Kelbaek

Department of Clinical Physiology and Nuclear Medicine, Herlev Hospital, University of Copenhagen, Denmark

By titration of two different stannous kits for ^{99m}Tc labeling of red blood cells (RBC) we found concentrations of 1–2 μg tin per ml of blood to give the highest labeling yield. Using a new kit containing 2 μg of tin and 0.1% hypochlorite (NaOCl) as an oxidizing agent we labeled RBC with ^{99m}Tc avoiding centrifugation of cells. To evaluate this new procedure we assessed the dependency of tin incubation time, and addition of 4.4% EDTA as a chelating agent on labeling efficiency. We also measured the dependency of EDTA on the stability of the label. Optimal conditions for labeling of 1 ml of whole blood using the new stannous kit were: 5–10 min of tin incubation, 0.2 ml of 0.1% hypochlorite, and 15 min of ^{99m}Tc incubation. This procedure resulted in a labeling efficiency of at least 96%. The overall effect of EDTA was not an increased labeling efficiency, and EDTA increased the stability of the label with only a few percent. The promising results of this new labeling approach encourage to further laboratory investigations and eventual clinical evaluation of the procedure.

J Nucl Med 27:1770–1773, 1986

Since the first report of technetium-99m (^{99m}Tc) labeling of red blood cells (RBC) in 1967 (1), the labeling methods have been improved constantly to reduce the cell damage, to increase the labeling efficiency and yield, and to simplify the previous time-consuming procedures (2–9).

In the present study we combined the use of very small tin amounts with the new approach described by Srivastava and Chervu (10) avoiding centrifugation of cells. The importance of each step was evaluated to optimize this new labeling procedure.

METHODS

The labeling procedure of the initial experiments was as follows. Heparinized whole blood was transferred to a vial, and the stannous kit (see "Titration of Tin Concentration") dissolved in saline was added anaerobically. Incubation was allowed for 2 min. The vial was then centrifugated upside down and 0.5 ml of packed RBC was transferred to the [^{99m}Tc] saline. After 15 min incubation the labeling efficiency was determined. The procedure of the following experiments is described here. One milliliter of heparinized whole blood was added to a kit containing 2.0 μg tin (total), 3.7 mg sodium

citrate, 5.5 mg dextrose, and 0.11 mg sodium chloride.* Centrifugation of cells was avoided—instead, sodium hypochlorite (NaOCl) was added to oxidize the extracellular tin before ^{99m}Tc incubation.

Titration of Tin Concentration

To determine the most appropriate amount of tin for preparation of RBC prior to labeling, two different stannous kits were investigated. Medronate II[†] containing stannous fluoride, and Tecephos[‡] containing stannous pyrophosphate. Initially, the stannous agents were added to 4–7 ml of heparinized whole blood in both healthy volunteers ($n = 6$) and patients with heart diseases of various origin and medication ($n = 6$) to give tin concentrations of 0, 0.5, 1, 2, 5, and 10 $\mu\text{g}/\text{ml}$ in the cell preparation. In addition, we performed tin titrations with the two kits in the interval between 0 and 1 $\mu\text{g}/\text{ml}$ to detect a possible lower border of the tin amount necessary to obtain high labeling yields. The following experiments were carried out with blood from healthy volunteers (laboratory staff).

Titration of NaOCl

In a series of six experiments (each experiment comprising four samples) 1 ml of blood was added to the 2- μg stannous kit. After 5 min of tin incubation, 0.1% NaOCl was added to the blood in volumes of 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, or 5 ml. Then, 0.3–0.5 ml 4.4% EDTA was added, and finally [^{99m}Tc]saline was added in doses of 5–10 mCi. Labeling efficiencies were determined as cell bound activity/total activity after 15 min incubation at 20°C.

Received Feb. 25, 1986; revision accepted May 27, 1986.

For reprints contact: Henning Kelbaek, MD, Dept. Clin Physiol/Nucl Med, Herlev Hospital, University of Copenhagen, DK-2730 Herlev, Denmark.

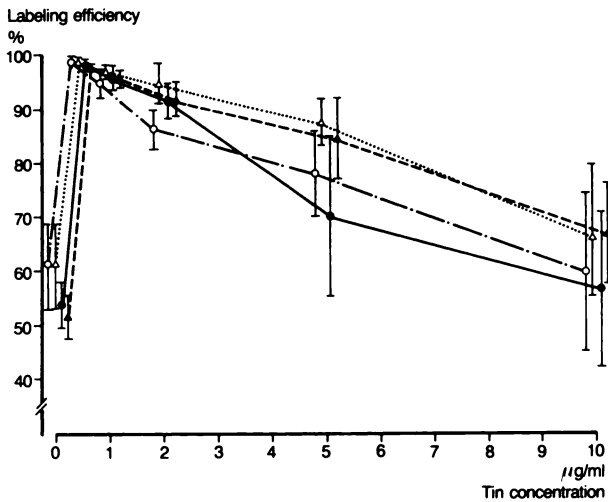


FIGURE 1
Labeling efficiency (mean \pm s.d.) as function of tin concentration in blood using stannous fluoride (triangles) and stannous pyrophosphate (circles) kit. (O, Δ): Values of six cardiac patients; (\bullet , \blacktriangle): Values of six healthy subjects

Duration of Tin Incubation

In four series of experiments (four samples) blood was incubated at 20°C with the 2- μ g stannous kit for 1, 2, 5 or 10 min. Ethylenediaminetetraacetic acid (EDTA) was added and 99m Tc incubation was allowed for 15 min before measurement of labeling efficiencies.

Titration of EDTA

The importance of EDTA for the labeling yield was evaluated in 15 series of experiments (four samples). Five minutes of tin incubation of blood was allowed before addition of 0.2, 0.4, or 0.6 ml NaOCl. To each of the NaOCl concentrations, 4.4% EDTA was added in amounts of 0, 0.5, 1, 2, or 4 ml. After 99m Tc incubation for 15 min, the labeling efficiencies were measured.

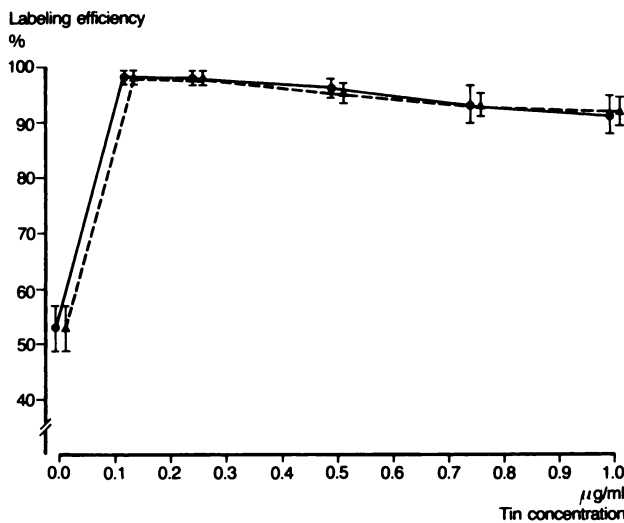


FIGURE 2
Labeling efficiency (mean \pm s.d.) as function of very low tin concentrations in blood. For explanation of symbols see Fig. 1

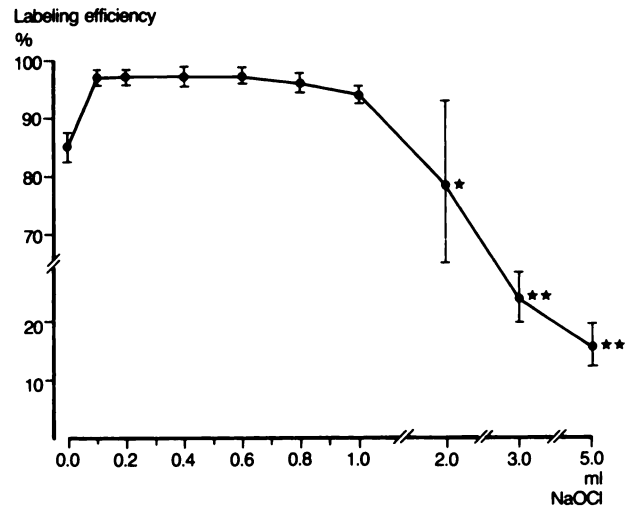


FIGURE 3
Labeling efficiency (mean \pm s.d.) as function of NaOCl addition (n = 4). * = Partial hemolysis of RBC, ** = Complete hemolysis of RBC

Duration of Technetium-99m Incubation

In six series of experiments (four samples) blood was incubated for 5 min with the stannous kit. After subsequent addition of 0.2 ml of NaOCl and 0.5 ml EDTA, 99m Tc incubation was allowed for 1, 5, 10, 15, 20, or 30 min, whereupon the labeling efficiencies were measured.

Stability of the Label

The in vivo stability of the label with and without the chelating agent EDTA was evaluated in four series of experi-

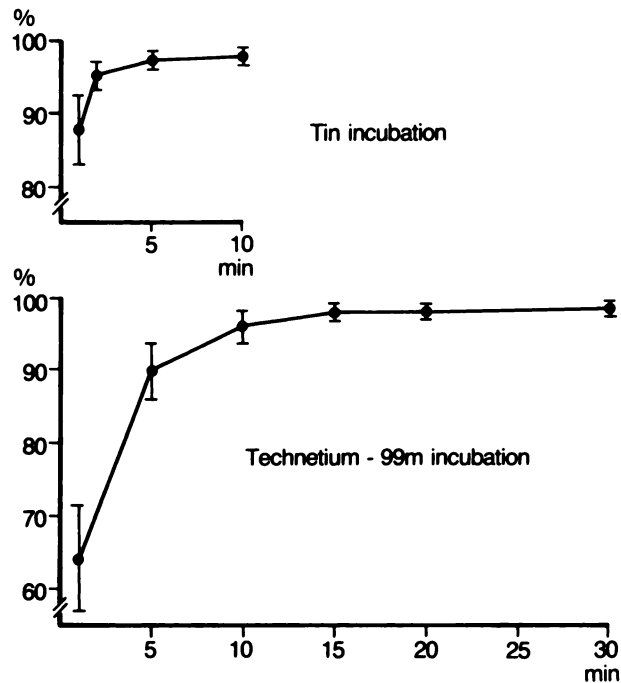


FIGURE 4
Labeling efficiency (mean \pm s.d.) as function of tin and 99m Tc incubation time (n = 4)

TABLE 1
Influence on Labeling Efficiency of 4.4% EDTA Added in Various Amounts After Preparation with 0.2 – 0.6 ml NaOCl (Mean Values \pm s.d.)

EDTA (ml)	0	0.5	1.0	2.0	3.0
NaOCl (ml)					
0.2	96.2 \pm 2.4	98.4 \pm 0.3	97.5 \pm 0.4	96.9 \pm 0.5	94.2 \pm 0.2
0.4	97.8 \pm 0.7	98.3 \pm 0.8	96.9 \pm 0.7	96.4 \pm 0.9	96.9 \pm 0.1
0.6	97.1 \pm 1.0	97.8 \pm 0.8	97.2 \pm 1.2	96.0 \pm 0.8	87.8 \pm 0.3

ments (four samples). After 5 min of tin incubation and addition of 0.2 ml NaOCl, 15 min of ^{99m}Tc incubation were allowed. Either 0 or 0.5 ml EDTA was added to the cell preparations. Each sample was transferred to a polycarbonate tube containing 6 ml of autologous plasma and gently mixed at 37°C. Labeling efficiencies were measured immediately, and then after 60 min and after 120 min.

RESULTS

The influence on labeling efficiency of the tin concentration in blood using labeling kits containing stannous fluoride and stannous pyrophosphate is shown in Fig. 1. There was a tendency towards higher yields in the stannous fluoride series, but the differences were not significant. There was no difference between healthy subjects and cardiac patients. The highest yields were found with tin concentrations $\leq 2 \mu\text{g/ml}$. In Fig. 2 it is shown that between 0.1 $\mu\text{g/ml}$ and 1.0 $\mu\text{g/ml}$ the tin concentration is not critical for the labeling yield.

The importance of the amount of added NaOCl to oxidize extracellular tin is shown in Fig. 3. Mean labeling efficiencies were $\geq 97.8\%$ in the interval from 0.1 to 0.6 ml NaOCl. When 2 ml or more NaOCl were added, partial or complete hemolysis of RBC was seen.

Tin incubation for 1, 2, 5, and 10 min gave labeling efficiencies of $88.8 \pm 4.7\%$, $96.3 \pm 1.5\%$, $98.3 \pm 0.5\%$, and $98.8 \pm 0.5\%$, respectively (Fig. 4).

Labeling yield as a function of added amount of EDTA after tin oxidation with 0.2–0.6 ml NaOCl are given in Table 1. The mean overall effect of adding 0.5, 1, 2, and 3 ml EDTA compared with no EDTA was 1.2%, 0.2%, -0.6% , and -4.0% , respectively. The greatest positive difference (2.2%) was found when 0.5 ml EDTA was added after 0.2 ml NaOCl.

The results of varying the ^{99m}Tc incubation time are given in Fig. 4. The labeling efficiency rose from $64.8 \pm 7.3\%$ at 1 min to $98.3 \pm 1.3\%$ at 15 min and $99.0 \pm 0.6\%$ at 30 min.

The labeling stability with and without the addition of EDTA is shown in Table 2; the labeling efficiency was reduced 1.4% and 1.5%, respectively, from 0 to 120 min.

DISCUSSION

Labeling of RBC with ^{99m}Tc for blood-pool imaging can be performed by in vivo (2,3), in vitro (1,3,5), and

combined in vivo/vitro methods (6,7). Neumann et al. (11) reported a higher image quality with the in vivo/vitro method compared with the in vivo technique. The background activity in studies of cardiac imaging have shown to be smallest with the in vitro labeling method (8,9). Thus, Srivastava and Chervu (10) have made efforts to reduce the time consumed by the in vitro technique, and recently they have described a new approach first introduced by Narra and Kuczynski (12)—after pretinning of RBC the extracellular tin is oxidized by hypochlorite, whereby the former loses its capacity of reducing technetium.

In the present study we have demonstrated that the highest labeling yield of RBC with ^{99m}Tc is obtained at an extremely small tin concentration in the blood using conventional in vitro labeling methods. The tin concentration in blood in our subsequent investigations using the new labeling technique was $\sim 2 \mu\text{g/ml}$. By the use of these small tin amounts we could also reduce the NaOCl addition compared with that previously reported (10). In addition, we obtained mean labeling efficiencies as high as 96.2–97.8% without the use of the chelating agent EDTA. Addition of the latter resulted in labeling efficiencies that were only 1.2% higher on the average.

A previously reported higher stability of [^{99m}Tc]RBC when preparation with 4.4% EDTA was performed prior to addition of ^{99m}Tc (10) could not be confirmed in the present evaluation.

The findings of the present study suggest that a labeling efficiency of ^{99m}Tc -labeled RBC can be obtained by preparation of 1 ml heparinized blood with a stannous kit containing 2 μg tin, and that 5 min of tin incubation seems to be sufficient. Addition of 0.2 ml 0.1% NaOCl and subsequent incubation for 15 min with ^{99m}Tc yield a labeling efficiency $>96\%$. The addition of 4.4% EDTA increases both the yield and the stability of the label by only 2%.

The promising results of this study encourage further investigations of the influence of the new reagents on

TABLE 2
Stability of Label in Autologous Plasma without (0) and with (+) Addition of 0.5 ml 4.4% EDTA (Mean Values \pm s.d.)

Plasma incubation time (min)	0		60		120	
	0	+	0	+	0	+
Labeling efficiency (%)	97.2 \pm 2.4	98.8 \pm 2.1	96.8 \pm 1.7	98.0 \pm 1.8	95.8 \pm 1.5	97.3 \pm 2.1

pH and Cl^- concentration, and analyses of radiopharmaceutical-drug interactions with drugs known to reduce the labeling yield in other methods (13). Subsequent steps in the evaluation of this new method would include the determination of the in vivo stability of the label in healthy subjects and cardiac patients.

FOOTNOTES

* Cadema, Middletown, NY.

† Amersham, Buckinghamshire, UK.

‡ Behringwerke, Frankfurt, FRG.

ACKNOWLEDGMENTS

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

The technical assistance of Kirsten Bülow is acknowledged.

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