Effect of Sn(II) Ion Concentration and Heparin on Technetium-99m Red Blood Cell Labeling

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While convenience and economy favor the use of in vivo methods for labeling red blood cells (RBCs) with [^{99m}Tc]pertechnetate, previous reports suggested that patient medication such as heparin might interfere and thus result in inferior quality images. In this study, using a canine model, the role of stannous Sn(II) ion in in vivo and in vitro labeling of RBCs both in the presence and absence of a therapeutic dose of heparin was investigated. Our results showed that Sn(II) ion concentration of 20 μg/kg body weight levels provided better than 80% in vivo labeling efficiency enabling high quality blood-pool images even in the presence of therapeutic doses of heparin.

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The labeling of autologous red blood cells (RBCs) with technetium-99m (^{99m}Tc) pertechnetate is a daily routine procedure in most nuclear medicine laboratories. While in vitro techniques provide the highest labeling yields (*1–3*), greater patient convenience and lower equipment costs favor the use of an in vivo labeling procedure. Numerous in vivo techniques have been proposed, demonstrating adequate labeling yields for most patients (*4–9*). It has been reported, however, that several medications may interfere with radiolabeling of RBCs and consequently the performance of blood-pool studies (*10,11*). Significant reduction in labeling efficency of RBCs with ^{99m}Tc in patients where Sn-pyrophosphate and pertechnetate were administered through heparinized catheters and in patients receiving therapeutic doses of heparin has been reported (*12–14*). Furthermore, the role of Sn(II) ion in the form of stannous chloride, stannous fluoride, or stannous citrate has previously been investigated for both in vivo and in vitro methods (*1–5,8,9,12,15–17*).

Many patients referred to our laboratory for blood-pool scintigraphy and gated ventriculography are receiving therapeutic doses of heparin. It is generally administered as an initial bolus injection of 5,000–10,000 USP units followed by a constant infusion of 1,000–2,000 USP units/hr depending on laboratory and clinical parameters. Most of the clinical studies were performed using an in vitro method of labeling [^{99m}Tc] RBCs* when patients are receiving therapeutic doses of heparin. At least 20 blood-pool imaging studies resulted in poor quality images during the past 2 yr when the in vivo method was performed because of oversight in recognizing that patients are receiving therapeutic doses of heparin. To date, how therapeutic doses of heparin interfere with in vivo method of labeling RBCs with Sn(II) ion and [^{99m}Tc]pertechnetate has remained obscure. To evaluate this problem, the role of Sn(II) ion in labeling RBCs both in the presence and absence of a therapeutic dose of heparin using a canine model was investigated.

MATERIALS AND METHODS

For all aspects of the study, the source of Sn(II) ion was stannous pyrophosphate kits prepared by using the standard method described by Huberty et al. (*18*).

The kits contained 4.72 mg of stannous chloride [2.95 mg as Sn(II)] and 12.0 mg of sodium pyrophosphate decahydrate.† The kits were reconstituted with isotonic saline to a 3.0 ml volume just before use. Heparin sodium injection, USP‡, was diluted with isotonic saline to a concentration of 100 USP units per ml for in vitro experiments. For in vivo experiments, 10,000 USP units per ml were added.

For in vitro studies, 2 ml aliquots of whole blood from mongrel dogs collected with heparin (8 USP units per ml) as anticoagulant was incubated at room temperature for 10 min with varying concentrations of stannous pyrophosphate. The concentration of Sn(II) ion varied between 0.62 and 31.30 μg/ml of whole blood (Table 1).

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TABLE 1
In Vitro Labeling of RBCs with Na-$^{99m}TcO_4$ in Heparinized Dog Blood Using Different Stannous Ion Concentrations

Sn(II) concentration (μg/ml)	Whole blood (ml)	Labeling efficiency (%) Mean ± 1 s.d. Heparin in whole blood (8 USP units/ml) (n = 10)	Labeling efficiency (%) Mean ± 1 s.d. 10 USP units of additional heparin added before incubating with Na-$^{99m}TcO_4$ (n = 10)
0.62	2	90.05 ± 1.95	91.14 ± 3.32
1.56	2	90.70 ± 2.21	86.74 ± 5.47
3.13	2	66.63 ± 2.70	74.94 ± 2.75
15.65	2	45.22 ± 3.19	51.29 ± 1.02
31.30	2	34.92 ± 6.59	41.79 ± 3.18

Three to four milliliters of isotonic saline were subsequently added and the samples centrifuged at 2,000 *g* for 5 min. Two washings were performed to remove plasma and excess amounts of stannous ion. The supernatent was pipetted out, and the RBCs were incubated for 10 min with 3 to 5 mCi (111–185 MBq) of [^{99m}Tc]pertechnetate in a volume not exceeding 1 ml. All [^{99m}Tc]pertechnetate used in this study was obtained freshly eluted from generators. The labeled RBCs were then separated by centrifugation, and radioactivity associated with RBCs and supernatant was measured using a gamma ionization chamber.[§]

To investigate the interplay between heparin and stannous ion during in vitro labeling of RBCs, the above-described procedure was repeated with the addition of 10 USP units of heparin in a volume of 0.1 ml to each of the whole blood samples (Table 1). In addition, as is shown in Table 2, determinations were made with Sn(II) ion concentration held constant at 0.62 μg/ml of whole blood while heparin concentration varied between 8 and 28 units per ml of whole blood.

In vivo canine RBC labeling both with and without therapeutic doses of heparin involved i.v. injection of systematically varied doses of Sn(II) ion in the form of stannous pyrophosphate (5, 10, 20 μg/kg of body weight) followed 30 min later by an i.v. injection of 10 mCi (370 MBq) of [^{99m}Tc]pertechnetate. All mongrel dogs weighed close to 25 kg, and labeling procedures were performed either 15 to 30 min or 1 to 2 hr following therapeutic heparinization with an i.v. injection of 4,000 USP units of heparin. To determine labeling yield (Table 3), sequential venous blood samples were drawn between 5 and 60 min post [^{99m}Tc]pertechnetate injection. For the calculation of labeling yield, canine blood volume was assumed to be 9.0% of total body weight (*19*).

TABLE 2
In Vitro Labeling of RBCs with Na-$^{99m}TcO_4$ in Dog Blood with Varying Amounts of Heparin

Sn(II) concentration (μg/ml)	Whole blood (ml)	Heparin in whole blood (USP units/ml)	Labeling efficiency (%) Mean ± 1 s.d. (n = 10)
0.62	2	8	93.63 ± 4.07
0.62	2	13	92.18 ± 4.59
0.62	2	18	91.51 ± 2.23
0.62	2	28	91.33 ± 3.59

To determine the activity associated with the RBCs, blood samples were weighed, separated by centrifugation into plasma and cells, and counted using a gamma well counter.[¶] To determine the formation of Sn-heparin complex in dog blood samples, high performance liquid chromatography (HPLC) analysis was performed. Results for both in vitro (ten determinations) and in vivo (six determinations) RBCs labeling are presented in Tables 1, 2, and 3.

RESULTS

As shown in Table 1, Sn(II) concentrations of 0.62 and 1.56 μg/ml whole blood provided satisfactory labeling yields on the order of 90% with rapid falloff in labeling yield to 66% or less as Sn(II) concentration was increased above 3 μg/ml of whole blood. These results are in agreement with previous reports (*1,3,16*). An additional 10 units of heparin before in vitro incubation with [^{99m}Tc]pertechnetate did not significantly alter the relationship of Sn(II) concentration to labeling yields. Furthermore, for in vitro canine RBCs labeling with a Sn(II) ion concentration of 0.62 μg/ml as shown in Table 2, increasing the amounts of heparin up to 28 USP units per ml of blood did not have a significant effect on labeling yields.

In vivo labeling of canine RBCs with [^{99m}Tc]pertechnetate 30 min following i.v. injection of between 5 and 20 μg Sn(II) ion per kilogram of body weight is shown in Table 3.

In the presence of a therapeutic dose of heparin, the mean labeling efficiency was in the order of 80% at a concentration of 20 μg of Sn(II) per kilogram body weight. Significantly lower yields were obtained at 5 and 10 μg/kg concentrations ($p < 0.05$). The labeling yields were slightly lower although not significantly different for the 20 μg/kg Sn(II) concentration when this study was performed 15–30 min rather than 1–2 hr after an i.v. injection of 4,000 units of heparin. The blood samples collected over a period of 1 hr typically showed that close to 80% of the injected dose of radioactivity was found in whole blood. Further analysis showed that ~96% of the activity was associated with RBCs and only 4% associated with the plasma fraction. HPLC** analysis was performed to determine the formation of Sn-heparin complex in blood samples using TSK-gel G3000 PW column (7.5 mm × 30 cm) and 75 m*M* NaCl (isocratic) as solvent with a flow rate of 0.75 ml/min. No clear conclusion could be reached because of the small difference in molecular weights of heparin and Sn-heparin complex and also due to the presence of plasma proteins in the samples.

The cardiac blood-pool images, obtained in a canine model in the presence of a therapeutic dose of heparin (dogs were pretreated with 4,000 USP units of heparin 30 min prior to the study) using 10 and 20 μg of Sn(II) per kilogram of body weight, are shown in Fig. 1.

To validate the results of the canine model, gated blood-pool scintigraphy was performed in 8 patients receiving therapeutic doses of heparin using the same Sn(II) ion dose scheme. Gated cardiac blood-pool images of one patient is shown in Fig. 2.

TABLE 3
In Vivo Labeling of RBCs with Na-$^{99m}TcO_4$ in Presence of Therapeutic Dose of Heparin in Dogs

Sn(II) concentration (μg/kg)	Time interval between heparin and Sn(II) injections	Labeling efficiency (%) Mean ± 1 s.d. as function of time (min) after $^{99m}TcO_4$ injection (n = 6)					
		5	10	20	30	40	60
5	1–2 hr	63.67 ± 13.73	67.66 ± 14.60	67.34 ± 14.97	69.29 ± 13.87	69.22 ± 14.0	70.22 ± 14.28
10	1–2 hr	67.06 ± 9.55	73.96 ± 9.64	75.61 ± 10.93	76.21 ± 10.85	75.88 ± 12.59	74.88 ± 12.19
20	1–2 hr	79.85 ± 9.53	85.99 ± 10.79	82.37 ± 11.82	81.06 ± 10.54	80.48 ± 13.15	78.76 ± 11.44
20	15–30 min	78.19 ± 14.15	82.87 ± 12.25	79.04 ± 11.07	78.39 ± 14.54	76.34 ± 11.63	75.34 ± 11.47
10	Controls (no heparin)	80.02 ± 6.34	89.36 ± 6.75	91.14 ± 5.37	88.22 ± 4.82	90.73 ± 10.24	84.56 ± 4.95

DISCUSSION

In this study, in vivo [^{99m}Tc]pertechnetate labeling of RBCs in the presence of a therapeutic dose of heparin was successfully achieved by increasing the injected dose from 10 to 20 μg of Sn(II) ion per kilogram of body weight. McRae et al. (*20*) reported alterations in tissue distribution of [^{99m}Tc]pertechnetate and also toxicity of stannous ion in rats at higher concentrations than generally used in clinical studies. A dose of 2 mg Sn(II)/kg or smaller amounts showed no identifiable ill effects, whereas 8 mg Sn(II)/kg showed considerable kidney damage and significant mortality rate in rats. It should be noted that most of the kits commercially available or prepared by individual laboratories contain less than 2 mg of Sn(II) per kit. As a result, the dose in

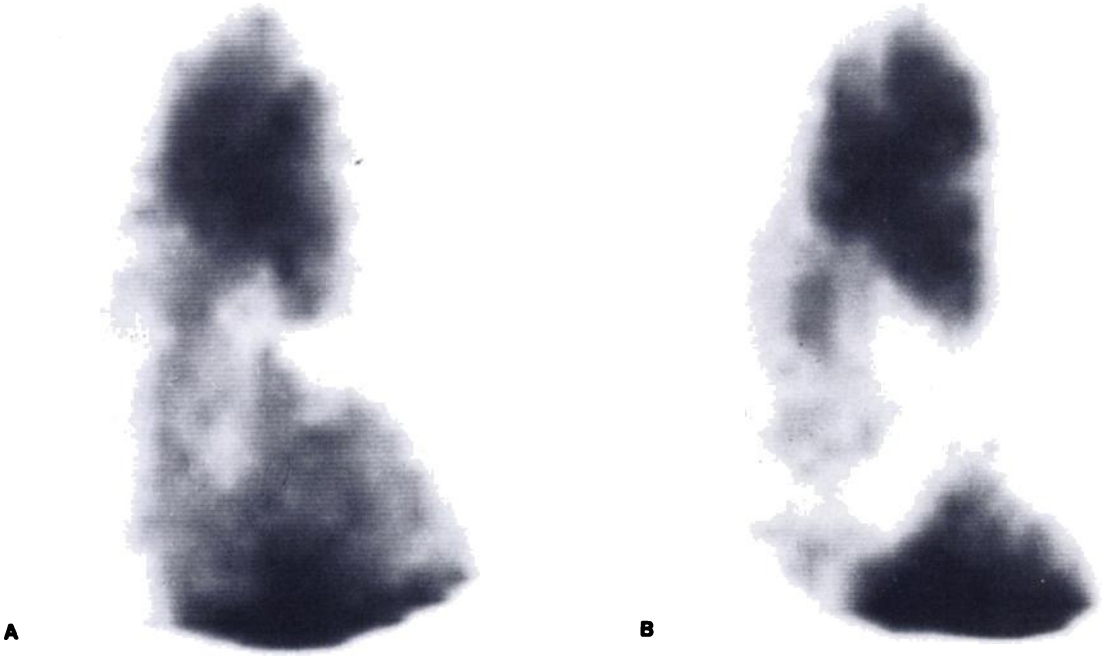

FIGURE 1
Comparision of in vivo [^{99m}Tc]RBC label with 10 μg (A) and 20 μg (B) Sn(II)/kg concentrations. Anterior view of ungated cardiac blood-pool images of dogs pretreated with 4,000 USP units of heparin intravenously 30 min prior to study. Note inferior quality of image obtained with lower Sn dose. Images were obtained following i.v. injection of 12 mCi (444 MBq) of [^{99m}Tc]pertechnetate

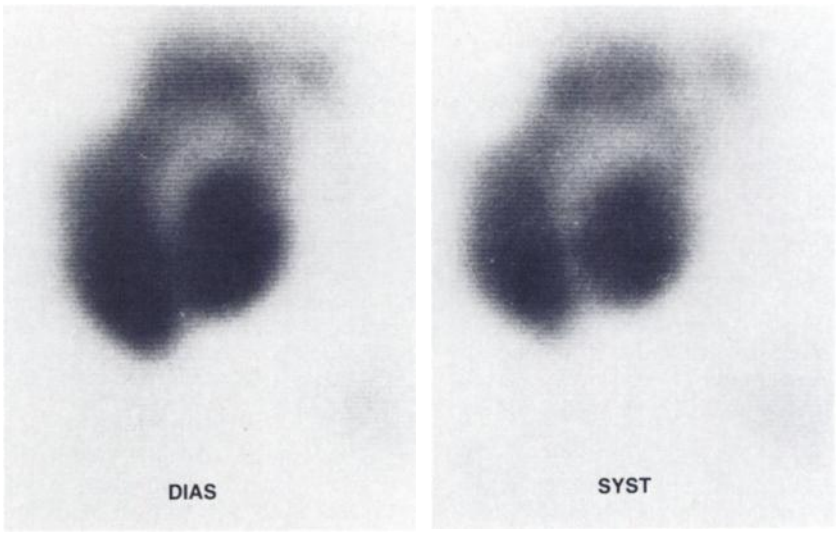

FIGURE 2
Gated end-diastolic and end-systolic cardiac blood-pool images in 45° LAO projections of one patient pretreated with therapeutic doses of heparin. Intravenously administered stannous ion concentration was 20 μg/kg which was followed by subsequent i.v. injection of 20 mCi [^{99m}Tc]pertechnetate. Despite presence of therapeutic doses of heparin visualization of cardiac blood pools appear to be of diagnostic quality

humans is on the order of 0.028 mg Sn(II)/kg, which is 70 times less than the reported toxic amounts. They also reported that even at concentrations on the order of 0.02 mg Sn(II)/kg showed considerable alterations in tissue distribution with time of [^{99m}Tc]pertechnetate administration. This is attributed to the continued presence of Sn(II) ion in some tissues longer than in the others. We elected not to consider further increases above 0.02 mg Sn(II)/kg body weight for the above-mentioned reasons.

Heparin, being a natural mucopolysaccharide possessing anionic centers of different pK values, has the ability to bind to metabolic inorganic cations such as Na^+, K^+, Ca(+2), Mg(+2), and to some organic substrates, including some drugs and natural bases (*21*). Preparation of ^{99m}Tc-labeled heparin by using stannous chloride and [^{99m}Tc]pertechnetate has been reported in the literature (*22*). Based on these established facts it may be hypothesized that heparin competes with RBCs for binding to Sn(II) ion during in vivo labeling, thus creating a situation that insufficient Sn(II) ion is available for tinning the RBCs when concentrations lower than 20 μg/kg are used. Consequently, there may be a strong possibility of formation of [^{99m}Tc]heparin compound during in vivo labeling procedure. The poor quality blood-pool images reported in the literature (*11–14*) as a result of drug induced effects on in vivo labeling could be attributed to these factors. Further investigations are warranted for better understanding of these mechanisms.

It is clearly shown in Fig. 1 that the blood-pool image of the heart using stannous ion concentration 20 μg/kg is of higher quality with clearer delineation. We suggest that a little longer wait, 30 to 40 min rather than 20 min, between stannous pyrophosphate and [^{99m}Tc]pertechnetate administration will result in much higher quality images. Figure 2 delineates diagnostic quality cardiac blood pool images of a heparinized patient. Such images could be obtained in heparinized patients by a conventional in vivo labeling method with the above-stated modifications.

In conclusion, it appears from this study that a small increase in Sn(II) ion concentration above the suggested optimum values by Hamilton and Alderson, and Billinghurst (*6,7*) would be helpful for achieving better red cell binding yields and diagnostically satisfactory cardiac blood pool images where the presence of therapeutic doses of heparin are involved.

FOOTNOTES

* Brookhaven National Laboratory Kits, Upton, NY.

† Aldrich Chemical Company, Milwaukee, WI ($SnCl_2$ and $Na_4P_2O_7 \cdot 10\ H_2O$).

‡ Heparin sodium injection, USP, The Upjohn Company, Kalamazoo, MI.

§ Radioisotope calibrator, CRC-10, Capintec, Inc., Ramsey, NJ.

¶ Auto-Gamma Scintillation Spectrometer-5220, Packard Instrument Company, Downers Grove, IL.

** Beckman Model 340 Liquid Chromatograph with Model 165 detectors, Beckman Instruments Inc., Brea, CA.

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