In Vivo Labeling of Red Blood Cells with Tc-99m with Stannous Pyridoxylideneaminates

Makoto Kato

Nihon Medi-Physics Co., Ltd., Takarazuka, Japan

Several stannous pyridoxylideneaminates were evaluated as stannous ion sources for the in vivo labeling of red blood cells (RBCs) with Tc-99m. In spite of a considerable variety of stannous preparations, rapid and efficient RBC labeling was obtained with each stannous chelate. These results suggest that the role of the ligands is merely to stabilize the divalent state of the tin. The optimal time interval between Sn(II) and $^{99m}TcO_4^-$ injections, and the best stannous-ion concentration, was found using stannous pyridoxylideneisoleucine (Sn-P.isoL). Maximal in vivo labeling of the RBCs was obtained with an i.v. dose of 10–20 μg Sn(II)/kg of Sn-P.isoL followed 15–30 min later by i.v. administration of pertechnetate.

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The procedure of in vivo labeling of red blood cells (RBCs) with Tc-99m has been attracting much interest because of its clinical utility in blood-pool imaging (*1–8*). Among others, Thrall et al. (*8*) have recently reported that Tc-99m RBCs labeled in vivo provide a superior blood-pool imaging agent compared with Tc-99m HSA in terms of the simplicity of the procedure, labeling efficiency, and in vivo stability. The i.v. injection of stannous pyrophosphate (Sn-PPi) followed by sodium pertechnetate is a widely accepted procedure (*6*). Hamilton et al. have reported that several stannous-ion preparations (Sn-PPi, Sn-HEDP, Sn-MDP, or Sn tartrate) had no significant effect on labeling efficiency (*7*).

The present study was undertaken to evaluate the usefulness of various stannous pyridoxylideneaminates, a new series of stannous preparations in alkaline media (*9–11*), for the in vivo labeling of rat RBCs with Tc-99m.

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For reprints contact: Makoto Kato, Technical Dept., Nihon Medi-Physics Co., Ltd., 4, 2-1, Takatsukasa, Takarazuka, Hyogo, Japan 665.

MATERIALS AND METHODS

The preparation of stannous pyridoxylideneaminates has been described previously (*10*). Only the concentrations of Sn(II) were varied in this study to permit injection of the appropriate amount of Sn(II) into the rat in a volume of 0.1–0.2 ml. Stannous diphosphonate (Sn-HEDP)*, stannous methylenediphosphonate (Sn-MDP)†, and stannous pyrophosphate (Sn-PPi)‡ were obtained commercially.

Stannous ion [Sn(II)] was administered intravenously through the tail vein of female Sprague–Dawley rats (7 wk old, 160 ± 15 g) on a basis of micrograms of Sn(II) per kilogram. After specific time intervals, 0.2 ml of $Na^{99m}TcO_4$ saline solution ($-TcO_4$) was injected through the other tail vein. One hour later, 5–7 ml of whole blood were collected by aortic puncture with a heparinized needle and syringe, and a 1-ml aliquot was counted on a gamma counter against an appropriate standard to obtain the percentage injected dose per milliliter of whole blood ($\%ID/ml_{wb}$). This value was normalized to a body weight of 160 g by multiplying by W/160.

The remaining 3–5 ml of whole blood were centrifuged

TABLE 1. EFFICIENCY OF IN VIVO RBC LABELING USING VARIOUS STANNOUS AGENTS*

Agent	%ID/ml$_{wb}$ (norm)	$\%_p$	$\%_{RBC}$	%ID/ml$_{RBC}$ (norm)	%ID (all RBCs)
MDP	9.07 ± 0.44	1.25 ± 0.21	98.75	19.64 ± 0.92	91.11 ± 3.10
HEDP	8.64 ± 0.18	1.00 ± 0.11	99.00	18.75 ± 0.39	88.92 ± 1.85
PPi	8.38 ± 0.19	1.10 ± 0.19	98.90	18.17 ± 0.40	86.18 ± 1.91
P.isoL	9.58 ± 0.31	1.05 ± 0.06	98.95	20.77 ± 0.67	98.52 ± 2.19
P.Val	9.04 ± 0.01	0.96 ± 0.03	99.04	19.62 ± 0.02	93.05 ± 0.12
P.Leu	8.79 ± 0.14	1.02 ± 0.05	98.98	19.09 ± 0.30	90.51 ± 1.42
P.phAl	8.75 ± 0.27	1.10 ± 0.11	98.90	18.98 ± 0.59	90.01 ± 1.82
P.Al	9.51 ± 0.19	1.10 ± 0.05	98.99	20.64 ± 0.41	97.94 ± 1.95
P.Gly	9.51 ± 0.18	1.29 ± 0.20	98.71	20.60 ± 0.39	97.71 ± 1.83
P.Glu	9.34 ± 0.21	1.25 ± 0.11	98.75	20.23 ± 0.46	96.00 ± 2.23

* Mean result for five rats ±1 s.d. at 1 hr after pertechnetate injection. Rats received 20 μg/kg Sn(II) followed 30 min later by 0.5 mCi of $-TcO_4$.

(20 min at 800*g*) to provide a hematocrit (%Hct = percentage, by volume, of RBCs in whole blood), and 1 ml of the plasma was then counted in the geometry used for whole blood so that c/ml$_p$ and c/ml$_{wb}$ could be directly compared. For any given sample of whole blood, then, the percentage of radioactivity carried in the red cells ($\%_{rbc}$) and in plasma ($\%_p$) could be calculated as follows.

$$\%_p = \frac{c/ml_p}{c/ml_{wb}}\left(1 - \frac{\%Hct}{100}\right) \times 100,$$

and

$$\%_{rbc} = 100 - \%_p.$$

Finally the normalized %ID in 1 ml of RBCs was calculated:

$$\%ID/ml_{rbc}(norm) = \%ID/ml_{wb} \times \frac{W}{160} \times \frac{\%_{rbc}}{\%_{Hct}}$$

The percentage of injected dose carried in all of an animal's circulating RBCs can be estimated by assuming that circulating blood (ml) = 6.5% of body weight (g) (*12–14*). Then:

$$\%ID \text{ in all RBCs} = \%ID/ml_{wb} \times W \times 0.065 \times \%_{rbc}/100$$

Using the experimental procedure described above, the following evaluations were performed:

1. Comparison of stannous preparations. Rats were injected through the tail vein with 20 μg Sn(II)/kg as HEDP, PPi, MDP, pyridoxylideneisoleucine (P.isoL), or its analogs of valine (P.Val), leucine (P.Leu), phenylalanine (P.phAl), alanine (P.Al), glycine (P.Gly), or glutamate (P.Glu). The injection was followed 30 min later by 0.5 mCi of $-TcO_4$.

2. Optimal Sn(II) concentration as Sn-P.isoL. Rats received tail-vein injection of various concentrations (1, 5, 10, 20, or 40 μg/kg) of stannous ion as Sn-P.isoL, followed 30 or 60 min later by 0.5 mCi of $-TcO_4$.

3. Optimal time intervals between Sn-P.isoL and $-TcO_4$ injections. Rats were injected through the tail vein with 10 μg of stannous ion as Sn-P.isoL. After various time intervals (1, 15, 30, 60, or 120 min), 0.5 mCi of $-TcO_4$ was injected through the tail vein of each animal.

RESULTS AND DISCUSSION

All of the stannous pyridoxylideneaminates either equalled or surpassed the three phosphate preparations (Sn-MDP, −HEDP, and −PPi) in the in vivo labeling of RBCs with Tc-99m (Table 1). Our preliminary experiments showed that the efficiency of RBC labeling was affected by the concentration of previously administered stannous ion (as Sn-pyridoxylideneaminate), and the dose response of the labeling efficiency closely followed that reported by Hamilton et al. (*7*). We therefore adopted a stannous concentration of 20 μg/kg for the comparison of various stannous preparations, since with this concentration the labeling efficiency has already reached a plateau in each case.

In spite of a wide variety of stannous preparations, all gave efficient RBC labeling with Tc-99m. This suggests that the role of the ligand to the stannous ion is merely to stabilize the divalent state of the tin. The stannous ion would dissociate from the ligand at a moderate rate after the i.v. injection, and would be transferred smoothly to the RBCs. As reported previously, each stannous pyridoxylideneaminate was prepared as an injectable solution under a nitrogen atmosphere using deoxygenized water (*10*). We suggest that the difference in labeling efficiency between the phosphates and the pyridoxyli-

TABLE 2. EFFICIENCY OF IN VIVO RBC LABELING USING Sn-P.isoL*

Sn(II) Concentration μg/kg	%ID/ml$_{wb}$ (norm)	$\%_p$	$\%_{RBC}$	%ID/ml$_{RBC}$ (norm)	%ID (all RBCs)
1	1.12 ± 0.09	59.04 ± 2.50	40.16	0.98 ± 0.03	4.64 ± 0.15
	(0.91 ± 0.11)	(58.11 ± 1.24)	(41.89)	(0.84 ± 0.09)	(3.95 ± 0.41)
5	6.26 ± 0.33	1.62 ± 0.27	98.38	13.50 ± 0.77	64.03 ± 3.59
	(2.86 ± 0.09)	(7.86 ± 1.62)	(92.14)	(5.54 ± 0.40)	(27.38 ± 0.38)
10	9.52 ± 0.14	0.90 ± 0.11	99.10	20.68 ± 0.29	98.08 ± 1.37
	(8.11 ± 0.14)	(1.55 ± 0.26)	(98.45)	(17.53 ± 0.34)	(83.12 ± 1.63)
20	9.58 ± 0.31	1.05 ± 0.06	98.95	20.77 ± 0.67	98.52 ± 2.19
	(9.59 ± 0.43)	(1.32 ± 0.04)	(98.68)	(20.75 ± 0.94)	(98.38 ± 1.45)
40	8.56 ± 0.20	1.39 ± 0.21	98.61	18.49 ± 0.46	87.71 ± 2.16
	(9.34 ± 0.18)	(1.16 ± 0.27)	(98.84)	(20.24 ± 0.42)	(98.00 ± 1.99)

* Mean results for five rats ±1 s.d. at 1 hr after pertechnetate injection. Pertechnetate was injected at 30 min (without parentheses) or at 60 min (in parentheses) after stannous ion injection.

deneaminate preparations (Table 1) arises because the pyridoxylideneaminates form stable chelates with divalent tin, and the large excess of ligand protects the stannous ion from oxidation. The formyl group of pyridoxal can work as a scavenger of oxidants.

The results in Table 1 indicate that Sn-P.isoL (stannous pyridoxylideneisoleucine), a promising hepatobiliary agent (*10*), can be also used as a stannous ion source for the in vivo labeling of RBCs with Tc-99m. The optimum stannous ion concentration is 10–20 μg/kg (Table 2), and the best time interval between injections of tin and pertechnetate is 15–30 min (Table 3). Hamilton et al. report very similar findings for stannous phosphate preparations (*7*).

When $-TcO_4$ was administered 30 min after the 10 μg/kg stannous injection, the labeling efficiency was slightly higher than that obtained by $-TcO_4$ at 60 min, and the opposite result was obtained with a 40 μg/kg stannous injection; no significant difference in labeling efficiency was observed between each $-TcO_4$ injection time with stannous concentration of 20 μg/kg (Table 2). These results, as well as those shown in Table 3, suggest that the stannous ion, administered as Sn-P.isoL, is gradually transferred from the plasma to the RBCs, and is then moderately metabolized or oxidized.

The intravenous stannous ion dose of 15 μg/kg corresponds to a 3.0-ml i.v. injection of 2.95 m*M*/liter Sn(II) solution to a 70-kg man. We have already reported the wide margin of safety for the i.v. injection of Sn-P.isoL (*10*).

In summary, the study shows that the Sn-pyridoxylideneaminates are promising stannous ion sources for the rapid and efficient in vivo labeling of RBCs with Tc-99m. Further work with Sn-P.isoL, in higher animal species

TABLE 3. EFFICIENCY OF IN VIVO RBC LABELING USING Sn-P.isoL*

Time interval between Sn(II) and $-TcO_4$ (min)	% ID/ml$_{wb}$ (norm)	% p	$\%_{RBC}$	% ID/ml$_{RBC}$ (norm)	% ID (all RBCs)
5	8.66 ± 0.27	1.02 ± 0.14	98.98	18.78 ± 0.59	89.08 ± 2.81
15	9.27 ± 0.39	0.71 ± 0.06	99.29	20.18 ± 0.83	95.69 ± 1.97
30	9.52 ± 0.14	0.90 ± 0.11	99.10	20.68 ± 0.29	98.08 ± 1.37
60	8.11 ± 0.14	1.55 ± 0.26	98.45	17.53 ± 0.34	83.12 ± 1.63
120	3.66 ± 0.14	4.96 ± 0.02	95.04	7.62 ± 0.31	36.59 ± 1.20

* Mean results ±1 s.d. for five rats at 1 hr after pertechnetate injection. Rats received 10 μg/kg Sn(II) as Sn-P.isoL.

and including scintigraphic studies, is now in progress.

FOOTNOTES

* Diphosphonate, Dainabot Radiosotope Laboratories, Tokyo, Japan.

† MDP Bone Agent, The Radiochemical Centre, Amersham, England.

‡ Pyrophosphate, Daiichi Radioisotope Laboratories, Tokyo, Japan.

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