In Vivo Distribution of Tc-99m(Sn)Pyridoxylideneisoleucine: Effects of Storage and Labeling Conditions. Concise Communication

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Various conditions for the storage of the stannous pyridoxylideneisoleucine (Sn-P.isoL) kit reagent, and also for the preparation of Tc-99m(Sn)P. isoL, have been evaluated experimentally using rats.

Both the frozen (−30°C) and the freeze-dried state were found to be suitable for storage of the kit reagent. For the labeling procedure, at least 40 min incubation time (Sn-P.isoL + $^{99m}TcO_4^-$) was necessary at room temperature, but the same superior results could also be obtained quickly by heating the mixed solution for 2 min in a boiling-water bath. The pertechnetate could be added in a volume up to four times that of the kit reagent. Mo(VI) and/or Al(III) in the pertechnetate solution did not interfere with the formation of Tc-99m(Sn)P.isoL up to the highest tested concentration of 40 μg/ml each.

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For every "instant kit" preparation of Tc-99m-labeled radiopharmaceuticals, it is very important to know the best storage conditions for the kit reagent and the proper procedure for the labeling with technetium. Standardized storage and labeling procedures would enable us to get highly reproducible results and hence to improve the reliability of the diagnosis.

In the course of our investigation of stannous preparations of Tc-99m-labeled pyridoxylideneaminates, we have introduced Tc-99m(Sn)pyridoxylideneisoleucine [Tc-99m(Sn)P.isoL] as one of the promising hepatobiliary radiopharmaceuticals (*1–4*). Several investigators have already reported the clinical utility of Tc-99m(Sn)P.isoL in the diagnosis of hepatobiliary disorders (*5–16*), and clinical trials of this agent are still being performed.

In response to the rapidly growing number of clinical trials, this communication explores the effects of storage and labeling conditions on the in vivo distribution of Tc-99m(Sn)P.isoL, using rats as a test animal.

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MATERIALS AND METHODS

Biodistribution testing using experimental animals is one of the suitable methods of evaluating the character of labeled materials, since there are often "analytical pitfalls" in the chromatographic methods (*17–20*). As we have reported previously, the labeling efficiency for the preparation of Tc-99m(Sn)P.isoL can be examined using a chromatographic system: a silica-gel thin-layer plate developed with methanol:water:methylethylketone (45:5:50 v/v) (*2*). The results of the chromatographic analysis, however, failed to reveal subtle differences between the preparations, and these can be examined by the biodistribution test described below.

Preparation of a Sn-P.isoL kit reagent. The procedure has been described previously (*2*). The reagent is a bright-yellow solution (pH 8.3–8.7) that consists of pyridoxal hydrochloride (90 m*M*), L-(+)-ascorbic acid (2 m*M*), anhydrous stannous chloride (1 m*M*), L-isoleucine (90 m*M*), and sodium hydroxide (180 m*M*).

Testing of storage conditions. The vials of the Sn-P.isoL kits were stored under controlled conditions: ordinary refrigeration (3 ± 1°C), in a thermostatically controlled incubator (22 ± 1°C and 31 ± 1°C), in a freezer (−30 ± 1°C); or after freeze-drying, with storage

at 3 ± 1°C. In each case the vial was purged with nitrogen. After the storage for a specified time interval, the reagent was mixed with an equivalent volume of $^{99m}TcO_4^-$ in normal saline and incubated at room temperature (22°C) for 1 hr. With the frozen kits, an equal volume of pertechnetate-saline was added immediately after the thawing; with the freeze-dried kits (original volume 1.5 ml), the added volume was 3 ml. Sprague–Dawley female rats (160–180 g), anesthetized with intraperitoneal sodium pentobarbital (30 mg/kg), were injected through the tail vein with 0.2 ml of the Tc-99m-labeled complex solution, and were autopsied 1 hr after the administration. The blood (6–9 ml) was collected by aortic puncture with a heparinized syringe; isolated organs were collected in plastic cups and counted in a scintillation counter.

Effect of incubation time at room temperature. The frozen-kit reagent (−30°C, 4 mo) was mixed with an equivalent volume of $^{99m}TcO_4^-$ in saline immediately after the thawing. After the specific incubation period at room temperature (22°C), the Tc-99m-labeled agent (0.2 ml) was injected i.v. into rats 1 hr before autopsy.

Effect of heat during the incubation. The frozen (−30°C for 4 mo) and thawed kit reagent was mixed with the same volume of pertechnetate and immersed in a boiling-water bath for 2 min. The labeled solution was then kept at room temperature (22°C) for 10 min and 24 hr, and was injected i.v. into rats (0.2 ml each), 1 hr before autopsy.

Effect of mixing volume ratio. The frozen kit reagents were thawed and mixed with various volumes of $^{99m}TcO_4^-$ in saline. The mixed solutions were incubated at room temperature (22°C) for 1 hr and then injected as above.

Effect of Mo(VI) and/or Al(III). Various amounts of $Na_2MoO_4 \cdot 2H_2O$ and/or $AlCl_3 \cdot 6H_2O$ (1, 5, 10, 20, 30,

TABLE 1. EFFECT OF STORAGE CONDITIONS OF Sn-P.IsoL KIT REAGENT ON IN VIVO DISTRIBUTION OF Tc-99m(Sn)P.IsoL IN RATS*

	% injected dose per organ†					
	Aqueous solution				6 mo	Freeze-dried;
Organ	3°C, 1 wk	3°C, 4 mo	22°C, 3 days	31°C, 3 days	at −30°C	then 6 mo at 3°C
Liver	0.91 ± 0.21	1.21 ± 0.09	1.69 ± 0.36	1.97 ± 0.33	0.70 ± 0.20	0.69 ± 0.18
Intestines	80.27 ± 2.18	75.41 ± 1.96	70.52 ± 2.18	65.09 ± 3.38	82.56 ± 1.21	83.23 ± 0.87
Stomach	0.00 ± 0.00	0.01 ± 0.01	0.15 ± 0.03	0.17 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
Lung and heart	0.11 ± 0.01	0.13 ± 0.04	1.25 ± 0.18	1.24 ± 0.17	0.11 ± 0.02	0.11 ± 0.02
Kidneys	0.89 ± 0.04	0.96 ± 0.07	1.81 ± 0.07	1.87 ± 0.08	0.69 ± 0.15	0.67 ± 0.09
1 ml blood	0.04 ± 0.00	0.06 ± 0.01	0.21 ± 0.03	0.32 ± 0.04	0.03 ± 0.01	0.03 ± 0.01
Spleen	0.01 ± 0.00	0.02 ± 0.00	0.13 ± 0.02	0.13 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Carcass	4.05 ± 1.01	6.57 ± 0.93	5.22 ± 1.00	6.22 ± 1.03	3.52 ± 0.76	2.96 ± 0.78
Urine	13.37 ± 1.96	15.04 ± 2.03	18.23 ± 1.25	20.31 ± 2.17	12.10 ± 1.73	12.00 ± 0.93

* Kit reagents, stored under stated conditions, were mixed with an equal volume of $^{99m}TcO_4^-$ in saline and incubated for 1 hr at 22°C.

† Mean results, ± 1 s.d., for five rats at 1 hr after i.v. administration.

TABLE 2. EFFECT OF INCUBATION TIME AT 22°C ON IN VIVO DISTRIBUTION OF Tc-99m(Sn)P.IsoL IN RATS*

	% injected dose per organ†						
Organ	15 sec	5 min	10 min	20 min	40 min	60 min	120 min
Liver	5.22 ± 1.26	3.22 ± 0.68	2.60 ± 0.32	1.56 ± 0.28	0.75 ± 0.14	0.69 ± 0.18	0.68 ± 0.16
Intestines	42.41 ± 3.64	58.81 ± 3.76	64.76 ± 3.72	71.50 ± 2.23	81.00 ± 1.96	82.53 ± 1.03	83.36 ± 0.62
Stomach	0.15 ± 0.03	0.13 ± 0.04	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Lung and heart	0.73 ± 0.10	0.41 ± 0.10	0.36 ± 0.06	0.23 ± 0.04	0.13 ± 0.04	0.11 ± 0.02	0.10 ± 0.02
Kidneys	6.47 ± 1.21	3.04 ± 0.49	2.44 ± 0.36	1.86 ± 0.25	0.73 ± 0.10	0.70 ± 0.11	0.69 ± 0.09
1 ml blood	0.53 ± 0.04	0.38 ± 0.03	0.25 ± 0.04	0.19 ± 0.02	0.03 ± 0.00	0.03 ± 0.01	0.02 ± 0.00
Spleen	0.18 ± 0.08	0.12 ± 0.04	0.10 ± 0.03	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Carcass	10.78 ± 1.99	8.56 ± 1.29	6.98 ± 1.21	5.22 ± 1.00	3.92 ± 0.71	3.53 ± 0.69	3.01 ± 0.52
Urine	30.39 ± 3.27	23.07 ± 2.99	20.88 ± 2.75	17.69 ± 2.81	13.16 ± 1.15	12.12 ± 1.03	12.01 ± 0.92

* Frozen kit reagent (−30°C, 4 mo) was thawed and mixed with an equal volume of $^{99m}TcO_4^-$ saline solution.

† Mean results, ±1 s.d., for five rats at 1 hr after i.v. administration.

and 40 μg metal ion/ml) were added deliberately to the pertechnetate solution. The thus contaminated $^{99m}TcO_4^-$ solution was mixed with an equivalent volume of the freshly thawed Sn-P.isoL kit reagent and incubated for 1 hr at 22°C. It was then injected into the rats.

RESULTS AND DISCUSSION

Significant instability was observed in the Sn-P.isoL kit reagent after storage as an aqueous solution for 3 days at 22°C or 31°C (Table 1), a result that urged us to try the frozen and freeze-dried storage conditions. The results shown in Table 1 indicate that the reagent kept frozen at −30°C, or freeze-dried and kept at 3°C, retains its superior characteristics as an hepatobiliary imaging agent for even 6 mo after preparation.

We have already reported that 1 hr of incubation time (Sn-P.isoL + $^{99m}TcO_4^-$) is necessary to obtain good results when the labeling is carried out at room temperature (*1–4, 14*), and the detailed results can be seen in Table 2. Neither unreacted pertechnetate nor Tc-Sn colloid was detected chromatographically (silica-gel TLC with MeOH:H_2O:MEK) (*2*) at any incubation time. Some broadening of the activity peak was observed, however, when the incubation time was less than 20 min. These biologic and chromatographic results indicate that several unstable technetium species would be formed at the early incubation stage, and they would be converted gradually into the stable species with good bile-seeking properties. These findings prompted us to carry out the labeling process at a higher temperature to accelerate the completion of the reaction. When the mixed solution (Sn-P.isoL + $^{99m}TcO_4^-$) is warmed in a boiling-water bath, the desired result can be obtained very quickly (Table 3). The table also indicates the stability of the labeled species: Tc-99m(Sn)P.isoL, once formed, is stable at room temperature for more than 2 days.

Regarding the volume ratios when pertechnetate-saline is mixed with the Sn-P.isoL kit reagent, the ratio can be as high as 4 to 1 without degrading the product's bile-seeking properties (Table 4). Therefore, with the freeze-dried kit (original volume 1.5 ml), one can safely add up to 7.5 ml of pertechnetate solution. Further increases, however, enhance hepatic and renal retention.

Hexavalent molybdenum [Mo(VI)] is one of the possible contaminants in the eluate from (n, γ) generators, and another is Al(III) ion, since almost all of the Mo-99→Tc-99m generators use alumina columns as the adsorbent for Mo-99 (*21*). We therefore studied the effects of Mo(VI) and/or Al(III) in the $^{99m}TcO_4^-$ eluate on the in vivo distribution of Tc-99m(Sn)P.isoL. The two metal ions showed no significant effect, either individually or together on the formation of Tc-99m(Sn)P.isoL—even at the maximum tested concentration of 40 μg/ml each. It has been reported that pyridoxylideneaminate Schiff bases form a stable chelate complex with Al(III) (*22–24*). The Sn-P.isoL kit reagent contains a large excess of metal-free pyridoxylideneisoleucine (*2*), and this Schiff base can scavenge Al(III) by chelate complex formation. The behavior of Mo(VI) in the Sn-P.isoL reagent remains obscure, but we have not explored the matter further.

Taking these results into consideration, we have fixed our current quality-control criteria for the in vivo distribution of Tc-99m(Sn)P.isoL as follows. Three female rats (Sprague–Dawley, 160–180 g) per each lot of the pharmaceutical are autopsied at 1 hr after the i.v. administration of the Tc-99m-labeled complex solution (0.2 ml each, with in vitro incubation time 1 hr or 12 hr at 25°C). The distribution of the radioactivity in each animal must be: less than 4% of the injected dose in the

TABLE 3. EFFECT OF INCUBATION CONDITIONS ON IN VIVO DISTRIBUTION OF Tc-99m(Sn)P.isoL IN RATS*

	% injected dose per organ†			
Organ	2 min in boiling-water bath, then 10 min at 22°C	2 min in boiling-water bath, then 48 hr at 22°C	22°C;1 hr	22°C 48 hr
Liver	0.70 ± 0.13	0.71 ± 0.17	0.69 ± 0.18	0.65 ± 0.16
Intestines	83.76 ± 0.92	84.01 ± 0.89	82.53 ± 1.03	83.40 ± 0.70
Stomach	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lung and heart	0.09 ± 0.02	0.09 ± 0.02	0.11 ± 0.02	0.09 ± 0.01
Kidneys	0.67 ± 0.13	0.69 ± 0.11	0.70 ± 0.11	0.69 ± 0.09
1 ml blood	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00
Spleen	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Carcass	2.42 ± 0.41	2.39 ± 0.08	3.53 ± 0.69	3.07 ± 0.40
Urine	12.03 ± 1.00	11.51 ± 0.96	12.12 ± 1.03	11.98 ± 0.87

* Frozen kit reagents (−30°C, 4 mo) were thawed and mixed with an equal volume of $^{99m}TcO_4^-$ saline solution.

† Mean results, ±1 s.d., for five rats at 1 hr after i.v. administration.

TABLE 4. EFFECT OF VOLUME RATIO OF $^{99m}TcO_4^-$ SALINE SOLUTION VERSUS Sn-P.isoL KIT REAGENT ON IN VIVO DISTRIBUTION OF Tc-99m(Sn)P.isoL IN RATS*

Organ	% injected dose per organ†				
	1:1‡	2:1	4:1	8:1	16:1
Liver	0.69 ± 0.18	0.88 ± 0.12	1.04 ± 0.15	2.18 ± 0.52	3.54 ± 0.62
Intestines	82.53 ± 1.03	81.62 ± 0.99	80.02 ± 0.93	72.36 ± 1.98	60.79 ± 3.16
Stomach	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.02 ± 0.01
Lung and heart	0.11 ± 0.02	0.12 ± 0.02	0.11 ± 0.01	0.16 ± 0.03	0.21 ± 0.03
Kidneys	0.70 ± 0.11	0.80 ± 0.09	0.92 ± 0.17	1.47 ± 0.30	2.31 ± 0.47
1 ml blood	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.05 ± 0.01	0.07 ± 0.02
Spleen	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.03 ± 0.01
Carcass	3.53 ± 0.69	3.21 ± 0.57	3.51 ± 0.50	6.26 ± 0.93	10.51 ± 1.37
Urine	12.12 ± 1.03	12.98 ± 1.24	14.00 ± 1.18	17.84 ± 1.72	21.72 ± 2.28

* Frozen kit reagents (−30°C, 4 mo) were thawed and mixed with various volumes of $^{99m}TcO_4^-$ saline solution.

† Mean results, ±1 s.d., for five rats at 1 hr after i.v. administration.

‡ Mixing volume ratios, $^{99m}TcO_4^-$ saline solution: Sn-P.isoL kit reagent.

liver, more than 73% in the intestines, less than 2.5% in the kidneys, and less than 15% in the urine. The pharmaceutical is now distributed both in the frozen kit form (expiration date 6 mo after production), or in the injectable Tc-99m-labeled form. The in vivo distribution test described above is performed once a week during the effective period for each lot of the kit reagent. Our current products fulfull the criteria with sufficient margin of safety throughout the effective period.

In summary, our current work has indicated practical storage conditions for the Sn-P.isoL kit reagent and the proper procedure for the preparation of Tc-99m(Sn)-P.isoL. In addition, Mo(VI) and/or Al(III) in the $^{99m}TcO_4^-$ solution did not interfere with the formation of Tc-99m(Sn)P.isoL even at 40 μg/ml each.

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