《研究速報》

^{99m}Tc-(Sn)-PYRIDOXYLIDENEVALINE and ^{99m}Tc-(Sn)-PYRIDOXYLIDENEISOLEUCINE: Potential Radiopharmaceuticals for Hepatobiliary Tract Imaging

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1. Introduction

Several ^{99m}Tc-labeled radiopharmaceuticals thus far have been reported for hepatobiliary tract imaging, including ^{99m}Tc-penicillamine[1], ^{99m}Tc-tetracycline[2], ^{99m}Tc-2-mercaptoisobutylic acid (^{99m}Tc-MIBA)[3], ^{99m}dihydrothioctic acid[4], ^{99m}Tc-N-(2, 6-dimethylphenylcarbamoylmethyl) iminodiacetic acid (^{99m}Tc-HIDA)[5], ^{99m}Tc-kethoxalbis (thiosemicarbazone) (^{99m}Tc-KTS)[6] and ^{99m}Tc-pyridoxylideneglutamate (^{99m}Tc-PG)[7]. A successful investigation has been carried out to find better hepatobiliary imaging agents and this communication reports the results of preliminary studies on the two titled promising agents.

2. Materials and methods[8]

2.1 Preparation of Sn-pyridoxylidenevaline (Sn-PVal) in kit form

Pyridoxal hydrochloride (3,665 mg, 18.0 mM), L-(+)-ascorbic acid (as the stabilizer, 70 mg, 0.4 mM) and anhydrous stannous chloride (15.2 mg, 0.08 mM) were dissolved successively in 100 m*l* of sterile, apyrogenic and oxygen-free (deoxygenized by nitrogen gas bubbling) water (solution A).

In another vessel, sodium hydroxide (1,440 mg, 36.0 mM) and L-valine (2,109 mg, 18.0 mM) were dissolved in 100 m*l* of sterile, apyrogenic and oxygen-free water (solution B). Then, while stirring, solution B was poured into solution A. Finally, 2.2 m*l* of the resultant bright-yellow solution was dispensed through a 0.22 μm Millipore filter into sterile 3 m*l* ampules and each ampule was flame sealed. All of the above processes were carried out under a nitrogen atmosphere. This Sn-PVal kit reagent (pH 8.52) was stored at 4°C until use.

2.2 Preparation of Sn-pyridoxylideneisoleucine (Sn-PIle) in kit form

An analogous method to that described above was adopted with the replacement of L-valine by L-isoleueine (2,361 mg, 18.0 mM) as the only modification. (pH 8.55).

2.3 Preparation of ^{99m}Tc-(Sn)-pyridoxylidenevaline [^{99m}Tc-(Sn)-PVal] and ^{99m}Tc-(Sn)-pyridoxylideneisoleucine [^{99m}Tc-(Sn)-PIle]

These two ^{99m}Tc-labeled complexes were prepared by mixing 1.5 m*l* of each kit reagent with 1.5 m*l* of $^{99m}TcO_4^-$ solution (5-10 mCi, in isotonic saline, obtained by MEK extraction method in our laboratory) in a 3.5 m*l* sterile vial, and incubated for 1 hr at room temperature, respectively.

2.4 Thin-layer chromatography

A drop of each ^{99m}Tc-labeled complex solution

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Table 1 Organ distribution of ^{99m}Tc-(Sn)-PVal and ^{99m}Tc-(Sn)-PIle in rats*

Organ	5 min		20 min		60 min		
	(PV)	(PI)	(PV)	(PI)	(PV)	(PI)	^{99m}Tc-PGT
Liver	27.3	21.3	4.63	4.16	1.46	0.78	11.5
Small intestine	48.4	59.3	86.5	87.4	93.6	96.4	66.9
Large intestine	0.94	0.92	0.22	0.32	0.13	0.05	0.79
Stomach	0.52	0.32	0.08	0.01	0.03	0.00	0.28
Spleen	0.13	0.07	0.03	0.00	0.02	0.01	0.21
Lung	0.60	0.56	0.42	0.22	0.18	0.08	0.93
Heart	0.17	0.16	0.09	0.06	0.04	0.05	0.13
Kidneys	2.91	1.92	1.04	0.99	0.99	0.78	2.17
1 ml Blood	0.62	0.43	0.12	0.12	0.09	0.03	0.43
Carcass	14.7	12.5	6.31	5.86	2.71	1.98	18.1
Urine	7.30	8.07	13.9	11.1	14.7	11.9	47.8

(PV)....^{99m}Tc-(Sn)-PVal, (PI)....^{99m}Tc-(Sn)-PIle

* Each value represents the mean results for five rats. The values are expressed as % of activity remaining in the body except for urine in which they are expressed as % of total injected activity.

T Prepared by Baker's method[7].

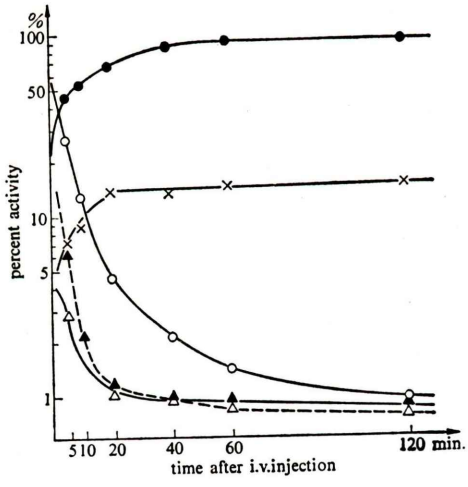

Fig. 1 Organ distribution of ^{99m}Tc-(Sn)-PVal in rats at various time intervals after i.v. injection. Each point is the mean for five rats. The data is expressed as % of activity remaining in the body except for urine in which it is expressed as % of total injected activity.
● small intestine, ○ liver, △ kidneys, × urine (cumulative), ▲ 1 m*l* blood × 10

was charged on a silica gel plate (Merck No. 60, 0.25 mm thickness) and was developed with 90% methanol-MEK (1:1) before the charged spot dried. The distribution of radioactivity on the plate was measured by a thin-layer scanner.

2.5 In vivo distribution study

Female rats (Sprague-Dawley), weighing 160–180 gm, were injected intravenously with 0.2 m*l* of the ^{99m}Tc-labeled complex solutions. At specific time intervals, the animals were killed and dissected. Blood (6–9 m*l*) was collected by aortic puncture with heparinized syringe, and isolated organs were collected in plastic cups and were counted on a scintillation counter.

3. Results

3.1 Chromatographic behavior

The analysis of ^{99m}Tc-(Sn)-PVal and ^{99m}Tc-(Sn)-PIle by thin-layer chromatography showed that the labeling efficiency to the kit reagents was practically 100%. Both of these two ^{99m}Tc-labeled complexes had an Rf value of 0.78–0.85 and showed a sharp single peak on the scanning chromatogram. With this chromatographic system, $^{99m}TcO_4^-$ had an Rf value of 0.95–0.98, both ^{99m}Tc-pyridoxal and ^{99m}Tc-Sn colloid remained at the origin.

3.2 In vivo distribution

The in vivo tissue distribution of the radio-

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activity indicated that these ^{99m}Tc-labeled complexes are very rapidly cleared from the blood by the liver, and are excreted into the small intestine (Table 1, Fig. 1). Table 1 also shows the distribution of ^{99m}Tc-PG [prepared according to the method reported by Baker et al[7]] at 1 hr after the administration. It is worthwhile to compare the in vivo behavior of our new agents with that of ^{99m}Tc-PG in animals since the clinical advantages of ^{99m}Tc-PG have been reported by several investigators[9), 10), 11)].

Chromatographic and in vivo behavior had no change for kit reagents stored for 60 days and complexes were stable for 48 hrs after ^{99m}Tc-labeling.

4. Discussion

In addition to the two titled agents, we have prepared several ^{99m}Tc-(Sn)-pyridoxylideneaminates using L-glutamic acid, L-glycine, L-alanine, L-phenylalanine, L-leucine and so forth as constituent amino acid following the analogous method to that described above, and their in vivo and chromatographic charactors were studied.

The stereochemical consideration of the most probable structure of these labeled complexes leads us to the conclusion that the in vivo behavior of these ^{99m}Tc-complexes are closely related to the molecular hydrophobicity of the complexes, because ^{99m}Tc-(Sn)-pyridoxylideneleucine also exhibits good charactors as the hepatobiliary tract imaging agent as well as the two titled compounds.

In the course of this study, it has been revealed that the charactors of ^{99m}Tc-(Sn)-pyridoxylideneglutamate are quite different from that of ^{99m}Tc-PG prepared by the autoclaving method[7)]. Snell reported that transamination proceeds up to 85% under the condition (120°C, 30 min)[12)] that adopted by Baker et al[7)], therefore, the resulting solution contains 2-ketoglutarate and pyridoxamine as well as glutamate, pyridoxal and PG. At the same time, the tautomerization of the Schiff's base occurs[13)]. These subreactions make the chemistry of the final ^{99m}Tc-PG solution quite complicated even if ^{99m}Tc was really chelated by PG. On the other hand, our method using divalent tin as the reductant enables us to label pyridoxylideneaminates with ^{99m}Tc under a mild condition.

Furthermore, it is a noteworthy result that we have succeeded in specific ^{99m}Tc-labeling of pyridoxylideneaminates in an alkaline media (pH 8–9). Many investigators indicated that "if new methods of technetium labeling could be developed in alkaline media, the number of valuable technetium radiopharmaceuticals would rapidly expand[14)]". To the best of our knowledge, this is the first report that presents the method for technetium labeling in an alkaline media using divalent tin as the reductant.

It is well known that, even under an inert atmosphere, *ionic* divalent tin, such as stannous halides, undergoes hydrolysis to form Sn-colloid when the pH of the solution is raised to the alkaline region. If large excess of strong chelating regaent, such as EDTA, however exists together with divalent tin, chelate complex formation occurs prior to hydrolysis even at pH 9–10[15)]. At the present stage of our investigation we have observed some evidence that divalent tin is first chelated by pyridoxal in solution A (pH 2–3) and the resulting Sn-pyridoxal complex is then converted into Sn-pyridoxylideneaminate complex in solution B (pH 8–9).

So, it is *not* the ionic divalent tin (stannous chloride) but it *is* the Sn-pyridoxylideneaminate complex that reduces technetium from the heptavalent to a low valency state when the kit reagent is incubated with $^{99m}TcO_4^-$. These mechanisms in the preparation of kit reagenets and technetium labeling enable us to prevent the formation of ^{99m}Tc-Sn-colloid and to succeed in specific labeling of pyridoxylideneaminates with technetium in an alkaline region.

In vivo distribution of ^{99m}Tc-(Sn)-PVal and ^{99m}Tc-(Sn)-PIle in rats indicates the possibility of these agents as hepatobiliary tract imaging radiopharmaceuticals. The initial blood clearance rate of these ^{99m}Tc-complexes is comparable to that of ^{99m}Tc-radiocolloids. Urinary excretion of the radiopharmaceuticals was relatively small and completed within a short time after the administration. The excretion of activity from liver into small intestine was very rapid.

Further work is now in progress including scintigraphic study using rabbits, studies on some other ^{99m}Tc-(Sn)-pyridoxylideneaminates, prolonged stability of kit reagents, in vitro and in vivo stability of technetium labeled complexes, chemi-

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stry of labeling mechanisms and toxicity of the pharmaceuticals.

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^{99m}Tc-(Sn)-ピリドキシリデンバリン，^{99m}Tc-(Sn)-ピリドキシリデンイソロイシン
新しい肝胆道系検査用放射性医薬品としての可能性

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要　旨

新しい肝胆道系動態検査用放射性医薬品の開発を目的として pH 8–9 においてスズ (II) を還元剤とする ^{99m}Tc-ピリドキシリデンアミネイト錯体の標識法と得られる錯体の性質について研究した．

pH 2–3 においてまず Sn^{II}-ピリドキサール錯体を生成させ，次いで pH 8–9 においてこれを Sn^{II}-ピリドキシリデンアミネイト錯体に変換し，この錯体と $^{99m}TcO_4^-$ を反応させることにより目的の ^{99m}Tc 錯体を選択的に得ることができた．

^{99m}Tc-(Sn)-ピリドキシリデンバリン，-イソロイシンについて検討した結果，標識はキット試薬と $^{99m}TcO_4^-$ 溶液を室温で混合するだけで達成され，標識率は実質的に 100% であった．

ラットを用いた経時的分布実験では静脈内に投与されたこの 2 種の ^{99m}Tc-錯体はすみやかに肝臓にとりこまれ，ついですみやかに小腸へ移行した．投与後 1 時間までに体内残存放射能の93–96% が肝臓を経て小腸へ移行した．

ラットにおける経時的体内分布をすでに臨床的有用性が指摘されている ^{99m}Tc-PG (オートクレーブ標識，Baker et al) と比較した結果，著者らの ^{99m}Tc-(Sn)-錯体は ^{99m}Tc-PG よりもはるかにすぐれた動態を示し，この 2 種の錯体の新しい肝胆道系動態検査剤としての可能性を強く示唆した．

標識機構に関する研究および考察，ウサギを実験動物とした γ-カメラによる経時的シンチフォトイメージの評価，他の種々のアミノ酸を用いて調製した同種錯体についての研究，製剤の in vitro, in vivo における安定性の評価，製剤の毒性の評価，などを続行中であり，近々詳報を発表する予定である．

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