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TECHNETIUM-99m-PYRIDOXYLIDENEGLUTAMATE, A NEW AGENT FOR GALLBLADDER IMAGING: COMPARISON WITH ^{131}I-ROSE BENGAL

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Two agents used for hepatobiliary studies, ^{131}I-rose bengal and ^{99m}Tc-pyridoxylideneglutamate, have been compared in rabbits. The ^{99m}Tc radiopharmaceutical is rapidly cleared from the blood by the liver and rapidly excreted through the common bile duct into the duodenum. Because of its rapid removal from the liver, visualization of the gallbladder and biliary passages was obtained within 15 min after injection in experimental animals.

Baker, Bellen, and Ronai (*1*) reported that intravenously injected pyridoxylideneglutamate labeled with technetium is extracted from the blood by the liver, passed into the biliary tract, and transported to the gallbladder, where it is excreted through the common bile duct into the intestine. We have compared the distribution of ^{99m}Tc-pyridoxylideneglutamate with that of ^{131}I-rose bengal reported by Taplin, et al (*2*) to determine if this new agent has properties that would be helpful to the differential diagnosis of patients with jaundice.

MATERIALS AND METHODS

The nonradioactive pyridoxylideneglutamate kit was prepared according to the general method proposed by Baker, Bellen, and Ronai (*1*). Pyridoxal hydrochloride (270 mg, 1.33 m*M*) and monosodium glutamate monohydrate (250 mg, 1.48 m*M*) were dissolved in 5 ml sterile water. The pH was adjusted to 9.0 with dilute sodium hydroxide and the final volume was adjusted to 10 ml with sterile water. The solution was purged with nitrogen and 2 ml was dispensed through a 0.22-micron filter into sterile 5-ml vials. The material was lyophilized and stored at 4°C until used. The ^{99m}Tc radiopharmaceutical was prepared by adding up to 2 ml of $^{99m}TcO_4^-$ to the reaction vial and autoclaving for 30 min at 121°C.

The ^{99m}Tc radiopharmaceutical was chromatographed on silica gel plates in chloroform–methanol (75:25 V/V) (*3*). Nonradioactive pyridoxal has an R_f value of 0.56 and pyridoxamine has an R_f of 0.05. Pertechnetate has an R_f of 0.57. Pyridoxylideneglutamate remained at the origin. A second system using Whatman No. 1 paper in isotonic saline gave an R_f value of 0.74 for pyridoxylideneglutamate. The R_f value of 0.69 for $^{99m}TcO_4^-$ required that both systems be used to prove radiochemical purity. In both systems the R_f of the ^{99m}Tc activity was identical with the pyridoxylideneglutamate R_f value.

Iodine-131-rose bengal (E. R. Squibb & Sons, New Brunswick, N.J.) was chromatographed on the silica gel in chloroform–methanol system and gave a single uv-absorbing spot at an R_f of 0.32. The rose bengal was also chromatographed on silica gel plates in chloroform–formic acid solution (87:13 V/V) and gave a single uv-absorbing spot at 0.99. Free iodide remained at the origin (*4*). In both systems the R_f value of the ^{131}I activity was identical with the R_f of the single uv-absorbing spot.

White New Zealand rabbits (2–3 kg) were injected with a solution containing ^{99m}Tc-pyridoxylideneglutamate and ^{131}I-rose bengal. Tables 1 and 2 contain data for an average of 6–7 rabbits at each measurement. Three rabbits in each group were fasted and 3–4 rabbits were maintained on a normal diet. Since no statistical difference in distribution could be observed between the two pretreatments,

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TABLE 1. DISTRIBUTION OF ^{99m}Tc-PYRIDOXYLIDENEGLUTAMATE IN RABBITS*

Organ	5 min	15 min	30 min	60 min	120 min
	Percent of dose per gram				
Liver	0.16 ± 0.03	0.14 ± 0.04	0.11 ± 0.04	0.12 ± 0.04	0.10 ± 0.06
Kidneys	0.31 ± 0.07	0.14 ± 0.03	0.11 ± 0.04	0.10 ± 0.03	0.09 ± 0.02
Stomach	0.02 ± 0.02	0.03 ± 0.04	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01
Gallbladder	1.31 ± 0.75	0.94 ± 0.74	1.40 ± 1.95	1.92 ± 0.63	1.08 ± 1.03
Upper duodenum†	0.74 ± 0.40	0.51 ± 0.29	0.44 ± 0.21	0.26 ± 0.11	0.05 ± 0.03
Duodenum‡	0.37 ± 0.15	0.52 ± 0.47	0.95 ± 0.63	0.58 ± 0.44	0.06 ± 0.04
Muscle	0.04 ± 0.07	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Blood	0.10 ± 0.02	0.06 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.00
	Percent of dose per organ				
Liver	13.70 ± 2.07	12.80 ± 4.18	8.84 ± 3.38	7.60 ± 2.61	7.48 ± 4.29
Kidneys	5.22 ± 1.20	2.29 ± 0.65	1.91 ± 0.61	1.40 ± 0.27	1.19 ± 0.36
Stomach	2.12 ± 1.32	2.89 ± 4.32	0.93 ± 0.78	0.94 ± 0.96	0.63 ± 0.98
Gallbladder	1.00 ± 0.81	1.24 ± 1.41	0.92 ± 0.25	1.79 ± 0.70	1.29 ± 1.20
Upper duodenum†	3.60 ± 1.50	2.66 ± 1.08	2.99 ± 2.65	2.14 ± 1.51	0.43 ± 0.15
Duodenum‡	2.08 ± 1.50	3.35 ± 1.58	4.56 ± 3.23	4.04 ± 2.68	0.43 ± 0.22
Muscle	16.30 ± 2.00	12.00 ± 5.51	9.82 ± 1.64	6.79 ± 2.56	3.62 ± 1.12
Urine	3.95 ± 2.54	11.00 ± 2.66	13.50 ± 3.33	16.90 ± 3.66	27.40 ± 6.46
Blood	17.20 ± 3.19	10.40 ± 1.82	7.47 ± 1.74	5.66 ± 1.24	3.46 ± 0.52

* Average of 6–7 rabbits were killed for each measurement.
† Upper duodenum, 15 cm of intestine below the stomach.
‡ Duodenum, additional 15 cm of intestine below the upper duodenum.

TABLE 2. DISTRIBUTION OF ^{131}I-LABELED ROSE BENGAL IN RABBITS*

Organ	5 min	15 min	30 min	60 min	120 min
	Percent of dose per gram				
Liver	0.93 ± 0.21	0.79 ± 0.24	0.59 ± 0.09	0.27 ± 0.09	0.11 ± 0.03
Kidneys	0.18 ± 0.02	0.12 ± 0.03	0.11 ± 0.03	0.12 ± 0.03	0.11 ± 0.03
Stomach	0.01 ± 0.01	0.01 ± 0.02	0.01 ± 0.01	0.01 ± 0.02	0.01 ± 0.02
Gallbladder	0.47 ± 0.29	0.66 ± 0.35	1.20 ± 2.37	3.70 ± 1.61	1.80 ± 1.31
Upper duodenum†	0.26 ± 0.23	0.65 ± 0.37	1.63 ± 1.39	0.97 ± 0.52	0.16 ± 0.14
Duodenum‡	0.10 ± 0.04	0.41 ± 0.24	1.33 ± 0.50	1.89 ± 1.24	0.14 ± 0.11
Muscle	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Blood	0.23 ± 0.04	0.11 ± 0.04	0.08 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
	Percent of dose per organ				
Liver	74.20 ± 13.90	62.00 ± 17.80	46.60 ± 6.58	17.60 ± 5.11	8.04 ± 2.29
Kidneys	2.99 ± 0.60	1.94 ± 0.57	1.82 ± 0.49	1.65 ± 0.32	1.61 ± 0.43
Stomach	1.28 ± 0.91	1.45 ± 1.81	1.04 ± 1.03	1.14 ± 0.32	1.38 ± 2.00
Gallbladder	0.36 ± 0.23	0.79 ± 0.66	1.27 ± 1.26	3.39 ± 1.37	2.47 ± 1.76
Upper duodenum†	1.27 ± 0.86	3.73 ± 1.98	6.39 ± 5.66	5.15 ± 2.08	1.10 ± 0.54
Duodenum‡	0.54 ± 0.38	3.55 ± 3.32	7.14 ± 5.58	14.60 ± 10.10	0.91 ± 0.50
Muscle	4.01 ± 1.14	3.42 ± 1.48	4.15 ± 1.24	3.67 ± 0.69	2.71 ± 0.85
Urine	0.40 ± 0.33	1.45 ± 0.81	1.57 ± 0.95	2.23 ± 1.19	2.95 ± 0.71
Blood	38.90 ± 7.40	18.90 ± 6.36	13.03 ± 0.94	9.88 ± 1.92	7.23 ± 1.67

* Average of 6–7 rabbits were killed for each measurement.
† Upper duodenum, 15 cm of intestine below the stomach.
‡ Duodenum, additional 15 cm of intestine below the upper duodenum.

the results were combined. Approximately 9 μCi in 0.086 ml of the ^{99m}Tc compound and 0.9 μCi in 0.052 ml of the ^{131}I-rose bengal were mixed and injected. The animals were killed at the noted times, organs were removed and weighed, and weighed aliquots were counted for ^{131}I and ^{99m}Tc by dual-nuclide spectral analysis. The amount of nonradioactive compounds contained in these solutions was calculated to be equal to the suggested human dose per kilogram of body weight. The percentages for total blood and total muscle were calculated on the assumptions that 7% of the body weight is blood

and 43% is muscle (*5*). The total urinary excretion was determined by collection in metabolic cages and recovery from the bladder. In addition, ^{99m}Tc-pyridoxylideneglutamate was injected in three mice to determine the total intestine content of the radiopharmaceutical.

Studies in eight normal humans were carried out by injection of 2–3 ml of ^{99m}Tc-pyridoxylideneglutamate containing 5–8 mCi of ^{99m}Tc, followed by sequential imaging with a scintillation camera.

RESULTS

On silica gel plates in chloroform–methanol solution, the ^{99m}Tc activity remains at the origin. As the age of the nonradioactive kit in solution approached 2 weeks, another small peak (<20%) appears at $R_f = 0.33$. This did not significantly affect the in vivo animal distribution. This additional peak was not found in the lyophilized kits up to 6 weeks after preparation. In the paper chromatographic system the ^{99m}Tc activity was found in a single peak at $R_f = 0.74$.

The distribution of the radioactivity with time in the rabbit indicates that the ^{99m}Tc radiopharmaceutical is rapidly cleared from the blood by the liver and a fraction is rapidly excreted through the common bile duct into the duodenum (Table 1). In both gallbladder and intestine, the activity per gram is more than ten times that for blood from 5 min to 2 hr after the injection. The equilibrium concentration of activity in the gallbladder is reached within 5 min. It appears that passage through the intestine is rapid and therefore much of the intestinal activity was not recovered in the first 30 cm of the rabbit duodenum. However, the distribution in mice indicates that as much as 57% of the injected ^{99m}Tc activity is found in the intestinal lumen at 2 hr. The distribution in rabbits results in good visualization of the gallbladder and the intestine in scintigrams taken immediately after injection and up to 2 hr. An appreciable amount of the ^{99m}Tc activity, 27.47 ± 6.5% of the dose, is excreted in the urine of the rabbits within 2 hr. The tissue ratios important in imaging the gallbladder are given in Table 3. In normal humans, the gallbladder, intestine, and urinary bladder are visualized within ½ hr after injection.

The ^{131}I activity clears the blood and the liver at a significantly slower rate than the ^{99m}Tc activity ($p < 0.05$) (Table 2). Comparing the dose percentages per gram for each organ, a liver-to-blood ratio of 10:1 is not reached within the 2-hr analysis time. Ratios greater than 10 for gallbladder-to-blood and intestine-to-blood are reached at 30 min after injection. In our experience visualization of the gallbladder at 60 min with ^{131}I-rose bengal is infrequent despite the high gallbladder-to-nontarget ratios shown in Table 3. Urinary excretion of ^{131}I activity in rabbits is very slight (<3%). The chromatography of ^{131}I-rose bengal shows less than 3% free iodide and a single radioiodinated rose bengal peak.

TABLE 3. TISSUE RATIOS FOR ^{131}I AND ^{99m}Tc UPTAKE AND EXCRETION

	Gallbladder-to-liver ratio (% dose/gm tissue)				
Time after injection	5 min	15 min	30 min	60 min	120 min
^{99m}Tc-PG	8.1	6.7	12.3	16.2	11.2
^{131}I-rose bengal	0.5	0.8	2.0	13.5	17.0
	Gallbladder-to-blood ratio (% dose/gm tissue)				
^{99m}Tc-PG	12.8	15.3	31.5	56.9	50.8
^{131}I-rose bengal	2.0	5.8	15.2	63.2	41.6

DISCUSSION

Pyridoxal, a metabolite of vitamin B_6, is the biocatalytically active form that takes part in a number of different enzymatic reactions. One of these, transamination, was elucidated by Snell (*6*), who observed that pyridoxal reacts nonenzymatically with glutamic acid to yield pyridoxamine and α-ketoglutaric acid. The mechanism involves the initial formation of a metal chelate of the Schiff base followed by transamination (*7*).

On mixing the pyridoxal and the monosodium glutamate, the solution becomes intensely yellow. This color change is associated with the formation of the pyridoxal glutamate imine and this reaction is nearly complete at pH 9.0 (*8*). In addition, reduction of pertechnetate also occurs. From the paper chromatographic analyses, it seems unlikely that particle formation occurs.

Our comparison of the biologic characteristics of the two pharmaceuticals suggests that immediate visualization of the gallbladder is possible with ^{99m}Tc-pyridoxylideneglutamate because of its early high gallbladder-to-blood and gallbladder-to-liver ratios as compared to ^{131}I-rose bengal. The ability to inject millicurie amounts of ^{99m}Tc with safety is an additional advantage. However, after 60 min a comparable tissue concentration is obtained with ^{131}I-rose bengal.

Further work on the application of this agent to patients with jaundice is in progress.

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