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Accumulation and metabolism of [^{125}I] HIPDM in the rat pancreas

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Our previous studies have shown a high accumulation of [^{125}I]N,N,N′-trimethyl-N′-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine (HIPDM) in the human pancreas. In this study, the pancreatic accumulation and metabolism of [^{125}I]HIPDM were studied in rats to determine the factors influencing its uptake by this organ. In biodistribution studies, [^{125}I]HIPDM showed a high uptake by the pancreas similar to that by the brain and lungs, both organs with a low tissue pH. TLC analysis of pancreatic homogenate after the injection of [^{125}I]HIPDM showed that it was metabolically stable in this organ. Moreover, in the pancreatic homogenate, the bulk of the radioactivity was recovered from the microsomal fraction, and the radioactivity bound to microsomal particles showed release that was dependent on the Ca^{2+} or Mg^{2+} concentration in the incubation medium. These results suggest that the initial pancreatic uptake of [^{125}I]HIPDM may be a function of blood flow and governed by the pH gradient hypothesis, while subsequent retention may occur secondary to ionic binding within the pancreas.

Key words: [^{125}I]HIPDM, pancreas, pH gradient hypothesis, metabolism, cellular polyanions

INTRODUCTION

AT PRESENT, one of the most important unsolved problems in nuclear medicine is the lack of a suitable radiopharmaceutical for pancreatic diagnostic imaging.[1–3]

Recently, ^{123}I-labeled N,N,N′-trimethyl-N′-(2-hydroxy-3-methyl-5-iodobenzul)-1,3-propanediamine (HIPDM) has been proposed as a pancreatic imaging agent.[4,5] Human studies with single-photon emission computed tomography (SPECT) showed that this agent had a high affinity for the pancreas and provided an excellent pancreatic image without overlap of the radioactivity due to low accumulation in adjacent organs such as the liver and spleen. As a next step, investigation into the mechanism of the pancreatic accumulation of radioiodinated HIPDM is required, not only to verify its potential as a diagnostic agent for the pancreas, but also to allow the development of a more desirable radiopharmaceutical for this organ.

Kubota et al. compared [^{131}I]HIPDM with [^{11}C] tryptophan in murine models of various types of pancreatic pathology and demonstrated that there were some differences in the uptake of these two compounds by the pancreas.[6] However, the mechanism of the pancreatic uptake of HIPDM is still unclear.

In the present study, the accumulation and metabolism of [^{125}I]HIPDM in the pancreas were studied in rats to determine the mechanism underlying the pancreatic uptake of this radiopharmaceutical.

MATERIALS AND METHODS

Sodium [^{125}I]iodide was purchased from Amersham International Plc. and diluted with 0.1 N NaOH aqueous solution. HIPDM was kindly provided by Dr. H.F. Kung (University of Pennsylvania, U.S.A.). All other chemicals used were of reagent grade.

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Male Wistar rats were supplied by Japan SLC Co. Ltd., and were fasted overnight before the experiment.

Preparation of [^{125}I]HIPDM

Radioiodination of HIPDM was carried out according to the method reported by Tramposch et al.[7] A 1 m*l* aliquot of a HIPDM solution (1 mg/m*l*, 0.07 N HCl) was added to 100 μl of an aqueous solution of [^{125}I]NaI (18.5 MBq), sealed inside a serum vial, and heated in a boiling water bath. After 30 min, the solution was allowed to cool at room temperature and the pH was adjusted to 5–6 by the dropwise addition of 0.1 N NaOH. The radiochemical purity of the labeled [^{125}I]HIPDM was more than 96% as determined by TLC ($CHCl_3$/CH_3CH_2OH/NH_4OH=8/2/0.1, Rf=0.65—0.80 for HIPDM).

Biodistribution in rats

7.4 kBq of [^{12}I]HIPDM (0.42 μg) was injected intravenously into rats weighing 150–180 g. At designated times thereafter, the animals were killed by decapitation and their organs were removed. A blood sample was obtained by cardiac puncture immediately before decapitation. The excised organs and blood samples were weighed and their radioactivity was determined with a well-type scintillation counter. Results were expressed as the % injected dose per gram of tissue weight.

In vivo metabolism

Male rats (150–180 g) were given an intravenous injection of 1.11–1.48 MBq of [^{125}I]HIPDM in 0.5 m*l* of saline and killed at specified time intervals afterwards. The pancreas, liver, and brain were quickly dissected out and homogenized with ice-cold saline. After the addition of 100 μl of blood or tissue homogenate to 100 μl of nonlabeled HIPDM (9.4 mg/100 m*l* water), 200 μl of 1 M sodium bicarbonate (pH 9.9), and 800 μl of ethyl acetate, the mixture was vortexed and then centrifuged. The organic (upper) phase was removed and the aqueous phase was reextracted with 800 μl of ethyl acetate. The two organic phases were then combined and partially evaporated under a stream of N_2 gas. The resulting solution was analyzed by TLC on Merck silica gel ($CHCl_3$/CH_3CH_2OH/NH_4OH=8/2/0.1).

Intracellular distribution

Male rats (150–180 g) were injected intravenously with 0.5 m*l* of [^{125}I]HIPDM (1.11–1.48 MBq) solution. At specified times afterwards, the animals were killed and the pancreas was quickly removed from each rat. After the pancreatic tissue was homogenized in 9 volumes of ice-cold 0.25 M sucrose/0.01 M Tris-HCl buffer (pH 7.4), subcellular fractions were prepared by differential centrifugation as described by Kagawa.[8] The entire procedure was carried out at 0 to 4°C. The homogenate was filtered through one layer of single-nap flannelette, the filtered homogenate was centrifuged for 10 min at 600×g, and the sediment was isolated as the nuclear fraction. The supernatant was then centrifuged for a further 10 min at 8,500×g and the pellet was isolated as the mitochondrial fraction. Finally, the opalescent supernatant obtained from the second centrifugation procedure was centrifuged for 30 min at 100,000×g, and the sediment was isolated as the microsomal fraction. The radioactivity in the nuclear, mitochondrial, microsomal, and supernatant fractions, was measured with a well-type scintillation counter, and the percentage of radioactivity in each fraction was calculated by assuming the total radioactivity recovered from the filtered homogenate to be 100%.

In vitro *binding to the rat microsomal fraction and effect of Ca^{2+} and Mg^{2+} ions*

The pancreas of a rat was homogenized in 5 volumes of 0.25 M sucrose/0.01 M Tris-HCl buffer (pH 7.4) and the homogenate was centrifuged for 10 min at 8,500×g and 4°C. The supernatant thus obtained was then centrifuged for 30 min at 100,000×g and 4°C. To the resulting sediment, 0.25 M sucrose/0.01 M Tris-HCl buffer (pH 7.4) was added at a 19: 1 weight ratio to the weight of the pancreatic tissue used, and then the mixture was resuspended in a Polytron homogenizer at 4°C. Then 0.1 m*l* of [^{125}I] HIPDM (7 kBq) was added to 1.9 m*l* of this suspension and the mixture was incubated at 23°C. After 10 min, various concentrations of $CaCl_2$ or $MgCl_2$ were added and incubation was continued for another 30 min at 23°C. The mixture was then centrifuged for 30 min at 100,000×g and the radioactivity in the sediment and supernatant was measured with a well-type scintillation counter. The percentage radioactivity in each fraction was calculated by assuming the total radioactivity in the test tube to be 100%.

RESULTS

Biodistribution of [^{125}I]HIPDM

Table 1 shows the results obtained in the biodistribution study. [^{125}I]HIPDM exhibited rapid clearance from the blood after injection into the rats. The radioactivity in the pancreas increased rapidly during the first hour of the study and thereafter remained constant. The liver uptake was rather low and remained nearly constant throughout the study. Therefore, the pancreas-to-liver ratio increased with time and reached a maximum of 5.72±0.83 (mean±

Table 1 Biodistribution of [^{125}I]HIPDM in rats

Organ	Time (min)				
	2	5	30	60	120
Blood	0.25 (0.05)*	0.19 (0.01)	0.14 (0.02)	0.13 (0.01)	0.10 (0.01)
Pancreas	1.96 (0.51)	2.56 (0.72)	4.50 (0.94)	5.99 (0.74)	5.19 (1.15)
Liver	1.00 (0.22)	0.98 (0.13)	1.13 (0.14)	1.05 (0.09)	1.08 (0.14)
Kidney	4.29 (0.30)	3.87 (0.60)	2.93 (0.17)	2.27 (0.26)	1.54 (0.10)
Stomach	0.53 (0.24)	0.46 (0.07)	0.86 (0.14)	0.95 (0.32)	1.41 (0.34)
Lung	21.34 (2.77)	19.45 (1.77)	15.45 (1.66)	9.74 (1.03)	6.47 (0.97)
Brain	1.77 (0.23)	1.63 (0.14)	1.91 (0.21)	1.87 (0.20)	1.43 (0.07)
P/B**	8.14 (2.80)	13.16 (3.97)	31.73 (6.51)	46.29 (7.20)	51.71 (10.3)
P/L***	2.02 (0.70)	2.62 (0.65)	4.00 (0.83)	5.72 (0.83)	4.88 (1.35)

* Each value is the mean (S.D.) for 4 animals (% dose/g organ).
** Pancreas/blood ratio.
*** Pancreas/liver ratio.

Table 2 Relative distribution of radioactivity in various fractions of the pancreas after the intravenous injection of [^{125}I]HIPDM into rats

Tissue	Time (min)	Ethyl acetate extractable		Ethyl acetate nonextractable
		[^{125}I]HIPDM	[^{125}I]HIPDM metabolites	[^{125}I]HIPDM metabolites
		(%)	(%)	(%)
Blood	5	68.5 (7.7)	3.2 (1.1)	28.3 (6.7)
	15	45.9 (6.5)	5.2 (0.8)	48.9 (5.9)
	30	44.8 (1.2)	5.2 (0.5)	50.0 (1.2)
	60	32.2 (2.1)	7.9 (0.4)	59.9 (2.5)
Liver	5	40.1 (6.0)	19.6 (0.6)	40.3 (5.6)
	15	23.3 (4.4)	17.2 (1.5)	59.5 (2.9)
	30	17.1 (2.6)	16.4 (0.4)	66.6 (0.4)
	60	15.9 (0.3)	16.6 (0.2)	67.5 (0.4)
Pancreas	5	95.8 (1.4)	1.1 (0.3)	2.1 (1.3)
	15	94.1 (2.0)	2.0 (0.6)	3.9 (2.5)
	30	94.7 (1.7)	2.2 (0.8)	3.1 (1.0)
	60	94.0 (0.9)	2.4 (0.1)	3.7 (0.9)
Brain	5	98.3 (0.5)	1.5 (0.4)	0.2 (0.2)
	15	98.5 (0.7)	1.2 (0.5)	0.3 (0.2)
	30	98.3 (0.3)	1.4 (0.4)	0.3 (0.1)
	60	98.4 (0.3)	1.4 (0.2)	0.2 (0.1)

Each value represents the percentage of the total counts incorporated into tissue as the mean (S.D.) for four animals.

s.d.) at 60 min. A high initial uptake was also observed in the lungs and kidneys, but these organs cleared rapidly. The brain showed a relatively high uptake that persisted throughout the experiment. Our data were in good agreement with those obtained by other workers for radioiodinated HIPDM.[4,6,9]

Metabolism of [^{125}I]*HIPDM*

Analysis of fluid and tissue homogenates was carried out at various times after the injection of [^{125}I]HIPDM and the results are shown in Table 2. Control studies showed that [^{125}I]HIPDM was more than 97% extractable into ethyl acetate from either blood or tissues. Analysis of blood samples taken at 5 min after injection showed that 69% of the original compound remained at that time. Metabolic products increased in the blood with time and accounted for 68% of the blood radioactivity by 60 min after injection. In the liver, the formation of metabolic products was also rather extensive and only 16% of the total radioactivity remained as the original compound by 60 min after injection. In both the blood and the liver, ethyl acetate-nonextractable metabolites were predominant, indicating that [^{125}I]HIPDM was metabolized into hydrophilic products. In contrast, a very high percentage (94–98%) of the total radioactivity in the pancreas and brain remained as HIPDM, indicating that intact [^{125}I]HIPDM was retained by both these organs.

Intracellular distribution of [^{125}I]*HIPDM in the pancreas*

The intracellular distribution of [^{125}I]HIPDM in the rat pancreas was studied by utilizing differential centrifugation, and the results are shown in Fig. 1. The percent distribution of [^{125}I]HIPDM in each subcellular particulate fraction remained nearly constant throughout the 60-min course of the study and about 50–60% of the total radioactivity accumulated was recovered from the microsomal fraction. About 20% of the accumulated radioactivity was found in the nuclear and mitochondrial fractions, and the supernatant accounted for less than 4%.

Effect of Ca^{2+} *and* Mg^{2+} *on the binding of* [^{125}I] *HIPDM to the microsomal fraction*

The effect of Ca^{2+} and Mg^{2+} on the binding of [^{125}I]HIPDM to the microsomal fraction was ex-

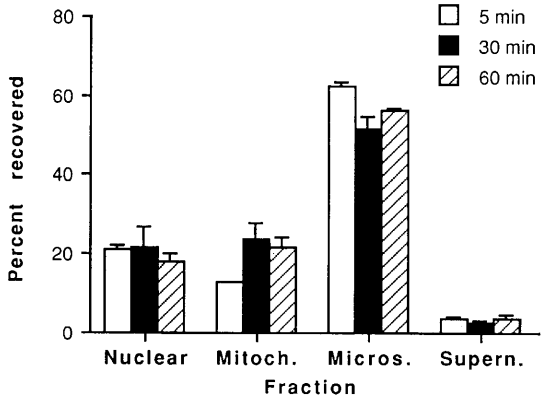

Fig. 1 Percent distribution of radioactivity in the subcellular particulate fractions obtained from the pancreas after the intravenous injection of [^{125}I]HIPDM into rats. Nuclear; nuclear fraction, Mitoch.; mitochondrial fraction, Micros.; microsomal fraction, Supern.; supernatant fraction.

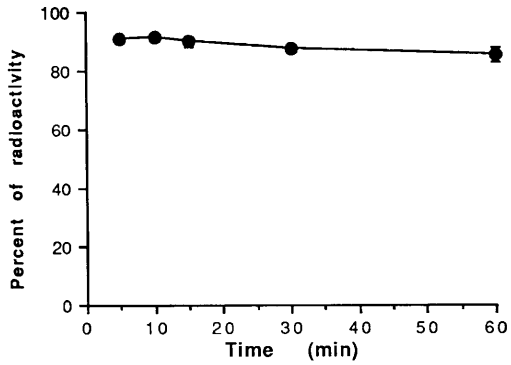

Fig. 2 Time course of binding of [^{125}I]HIPDM to the pancreatic microsomal fraction. Each point represents the mean for five experiments.

amined. In the absence of Ca^{2+} or Mg^{2+}, [^{125}I] HIPDM bound rapidly to microsomal pellets and the percentage of binding remained nearly constant over 60 min (Fig. 2). The microsomal fraction was then preincubated with [^{125}I]HIPDM for 10 min, followed by incubation with different concentrations of each cation for 30 min. As shown in Fig. 3, [^{125}I]HIPDM was released dose-dependently from the microsomal fraction, and the radioactivity detected in the supernatant reached 62% and 31% in the presence of 100 mM Ca^{2+} and Mg^{2+}, respectively.

DISCUSSION

We have obtained good quality pancreatic images with [^{123}I]HIPDM in healthy human volunteers as a result of its high pancreatic uptake and low accumulation in adjacent organs.[5] In order to determine whether this agent can be used clinically for the assessment of pancreatic function, the mechanism of

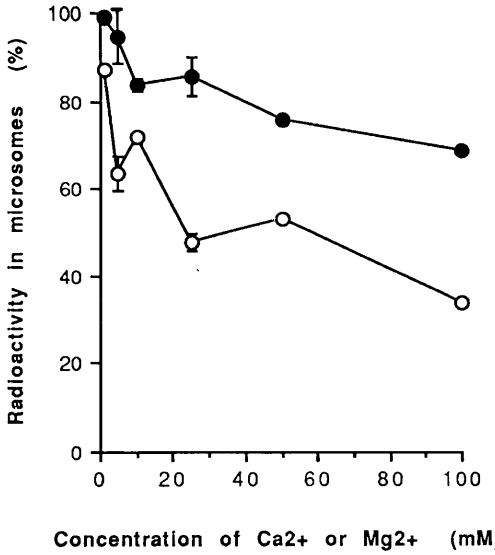

Fig. 3 Effect of Ca^{2+} (○) and Mg^{2+} (●) on the binding of [^{125}I]HIPDM to the pancreatic microsomal fraction. Each point represents the mean for five experiments.

its pancreatic accumulation was investigated in the current study.

HIPDM was originally developed as a brain perfusion agent on the basis of the pH gradient hypothesis: i.e., the compound is neutral and lipid soluble and can diffuse into the brain at a high pH, while at the lower pH existing within the brain tissue, it becomes charged and can not diffuse out again.[9,10] Although there are no reports available on the tissue pH of the pancreas, it has been estimated to be almost the same as that of the brain by calculations performed with data on the biodistribution of radiolabeled dimethyloxazolidinedione (DMO)[11] and the Waddell-Buther equation[12] (sumitted for publication). Therefore, it seems that the pH gradient hypothesis may at least partly explain the uptake of [^{125}I]HIPDM by the pancreas.

However, according to the pH gradient hypothesis, the pancreas-to-blood concentration ratio for HIPDM should always remain constant; i.e., the radioactivity should clear from the pancreas at the same rate as that in the blood.[10] Since this did not occur and the pancreas-to-blood ratio increased with time (Table 1), it appears that only the initial uptake of [^{125}I]HIPDM is explainable by the pH gradient hypothesis, and that the subsequent retention phase is due secondarily to either metabolism or binding within the pancreas.

To investigate the pancreatic metabolism of [^{125}I]HIPDM, TLC analysis of rat pancreatic homogenate was carried out. As shown in Table 2, more than 98% of the radioactivity accumulated in the pancreas was found to be in the chemical form of the original compound throughout the experiment.

This indicates that HIPDM was incorporated into the pancreas in its intact form and was metabolically stable in this organ.

On the other hand, Raina et al. have reported that ionic binding to cellular polyanions, mainly RNA, plays an important role in the hepatic retention of polyamines since the bulk of the polyamines in rat liver are recovered from the microsomal fraction and they are released from microsomal pellets as a function of the concentration of the cations (Ca^{2+} and Mg^{2+}) in the incubation medium.[13] HIPDM is a diamine derivative, so the retention of [^{125}I]HIPDM in the pancreas may be due to binding to cellular polyanions such as nucleic acids, as is the case of polyamines in the liver. To assess the accuracy of this hypothesis, we examined the intracellular distribution of [^{125}I]HIPDM in the pancreas and the effect of Ca^{2+} and Mg^{2+} on its binding to the microsomal fraction. As shown in Fig. 1, the relative distribution of [^{125}I]HIPDM radioactivity in the subcellular fractions of the rat pancreas closely resembled that of polyamines in rat liver reported by Raina et al.[13] Furthermore, microsome-bound [^{125}I]HIPDM was released dose-dependently following the addition of Ca^{2+} or Mg^{2+} to microsomal pellets (Fig. 3). Thus, our results suggest that pancreatic [^{125}I]HIPDM is retained intracellulary due to its high affinity for cellular polyanions.

In conclusion, our data demonstrate that both the pH gradient between the plasma and pancreatic tissue and the high affinity for cellular polyanions may be responsible for the high accumulation of [^{125}I]HIPDM in the pancreas. These results should provide a good basis for the clinical application of this agent to pancreatic diagnostic imaging.

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