Pancreas Accumulation of Radioiodinated HIPDM in Mice and Rats

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High pancreatic affinity for ^{131}I-labeled HIPDM was observed in mice and rats. Although the brain uptake of [^{131}I]HIPDM is very fast, the pancreatic uptake is rather slow. The pancreas to liver ratios (per gram) were 5.08 ± 0.52 in mice and 5.15 ± 0.65 in rats at 2 hr and 7.05 ± 0.53 in mice and 8.06 ± 1.14 in rats at 5 hr after administration. These ratios are higher than those obtained with routinely used pancreatic agent [^{75}Se]selenomethionine. An increase in liver uptake and decrease in pancreatic uptake was observed at higher dose of carrier HIPDM, which resulted in lower pancreas to liver ratios. HIPDM is a new type of compound which shows predilection for pancreas. Our results suggest that [^{123}I]HIPDM might be a useful agent for pancreas imaging.

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Iodine-123-labeled N,N,N′-trimethyl-N′-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine ([^{123}I]HIPDM) has been recently reported as a potential brain-perfusion imaging agent (*1*). The mechanism of brain uptake of [^{123}I]HIPDM was explained on the basis of the pH gradient that exists between blood (pH ~7.4) and brain (intracellular pH ~7.0) (*2*).

Therefore, it was presumed that [^{123}I]HIPDM might also be a useful imaging agent for other tissues with low intracellular pH, such as cardiac muscle and some types of tumors (*3*).

The extracerebral distribution of radioiodinated HIPDM was studied using whole-body autoradiographic methods. Significant accumulation of radioactivity in the pancreas was observed, which increased with time.

Our data suggest that radioiodinated HIPDM may be potentially useful as a pancreas imaging agent.

MATERIALS AND METHODS

Radiolabeling

Radioiodination of HIPDM was carried out according to the method developed by Kung et al. (*1*). Iodine-131 (^{131}I) was used in most studies for convenience (long half-life, beta-emissions suitable for autoradiography). A solution (~0.5 ml) containing 2 mg/ml HIPDM and 1.0 μg/ml sodium iodide carrier was transferred into a 10 ml multi-injection vial, and the desired amount of ^{131}I added to it.* Hydrochloric acid (0.14*N*), in a volume 1.2 times the volume of the ^{131}I used was added next and the capped vial then immersed in a boiling water bath for 30 min. Prior to boiling, the pH of the solution was checked and kept between 2 and 4. The solution was cooled and millipore filtered (0.22 μ) before use. An aliquot was analyzed for radiochemical purity by TLC-silica gel† using $CHCl_3$/EtOH/NH_4OH (8:2:0.1) as solvent (Rf:unbound iodine O; labeled HIPDM 0.5, uv positive spot). The iodination yields were consistently more than 95%.

Animal experiments

Hale-Stoner strain BNL mice (20–24 g) and Sprague-Dawley male rats (200–250 g) were used. For tissue distribution studies, each mouse or rat was injected with 0.2 ml saline solution containing 2–4 μCi [^{131}I]-HIPDM through the tail vein. Animals were killed at various times after injection. The organs of interest were excised, weighed, and counted in a NaI(Tl) well-type scintillation counter along with a diluted standard of injected dose. The effect of varying doses of carrier

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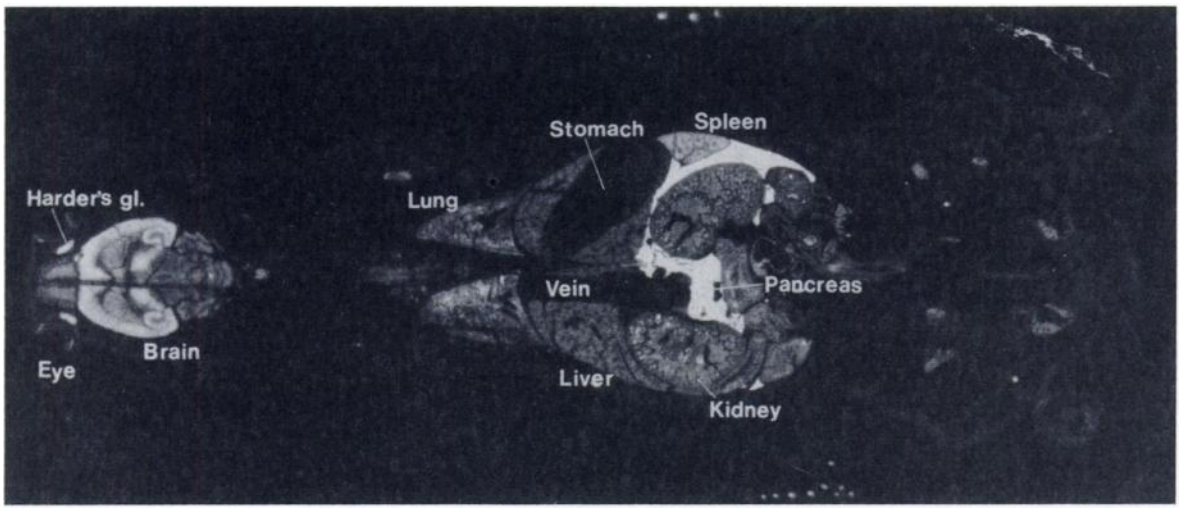

FIGURE 1
Whole-body ARG of mouse at 2 hr after [^{131}I]HIPDM administration. Intense uptake is noticed in pancreas. Almost no radioactivity is present in blood vessels, muscle, and fat tissues. Lung shows heterogeneous distribution of [^{131}I]HIPDM

(0.05–6.13 mg/kg) on biodistribution of [^{131}I]HIPDM was also studied in mice.

The tissue distribution data were expressed by percent relative concentration (%RC) (*4*) described as follows:

$$\%RC = \frac{\mu Ci\ found/g\ specimen}{\mu Ci\ administered/g\ body\ weight} \times 100$$

For whole-body autoradiography (ARG), 7 μCi and 30 μCi of [^{131}I]HIPDM was injected into mice and rats, respectively. At various time intervals after injection, the animals were killed and rapidly frozen in liquid nitrogen. After cutting into 20-μm-thick sections and freeze-drying for a few days, the specimens were placed on x-ray film‡ for exposure along with graded standards. After development, ARGs were digitized and quantitated using a videodensitometric system coupled to a minicomputer (*5,6*).

RESULTS

Figure 1 depicts the whole-body ARG of a mouse at 2 hr after [^{131}I]HIPDM administration. Intense concentration of radioactivity in the pancreas and low background activity in the surrounding organs is seen. The pancreas to liver (P/L) ratio obtained from this image by videodensitometry is 2.67 because of rather high carrier HIPDM (3.03 mg/kg).

The results obtained by tissue distribution studies in mice with carrier HIPDM dose of 0.05 mg/kg body weight are summarized in Table 1. In contrast to the very rapid brain uptake, the pancreatic uptake of [^{131}I]-HIPDM is rather slow. At 2 hr after injection, the pancreas activity is highest (4.15 ± 0.45 %dose/organ) and P/L ratio is 5.08 ± 0.52. The radioactivity in the pancreas persists even up to 5 hr after injection (3.97 ± 0.28 %dose/organ), while it is considerably diminished in the adjacent organs. Therefore, P/L ratio at 5 hr increases to 7.05 ± 0.53, which is significantly higher than that obtained with selenium-75 (^{75}Se) selenomethionine (2.70 ± 0.39 at 1 hr after injection) (*8*). The effect of carrier loading dose on biodistribution in mice is shown in Table 2. The pancreatic uptake did not show significant change up to doses of 1.23 mg/kg body weight of administered HIPDM, but at the higher loading doses it decreased. This result suggests that a threshold in mice pancreas might exist between 1.23 and 3.3 mg/kg. On the other hand, the liver uptake was increased by adding carrier HIPDM. Therefore, increasing carrier dose from 0.05 up to 6.13 mg/kg decreased the P/L ratio from 5.08 ± 0.52 to 1.43 ± 0.15.

The tissue distribution data in rats is summarized in Table 3. The radioactivity in the pancreas, was stable at 2, 3, and 5 hr after injection (2.82 ± 0.59, 2.63 ± 0.40, and 2.77 ± 0.34 %dose/organ, respectively). The P/L ratio was 5.15 ± 0.65 at 2 hr and 8.06 ± 1.14 at 5 hr after administration, which is similar to that in mice. ARG (saggital section) of a rat at 2 hr after injection is shown in Fig. 2. P/L ratio obtained from this image using the quantitative videodensitometry system was 5.10.

Thyroid uptake was 0.12 ± 0.04 %dose/organ at 1 hr and 0.09 ± 0.04 %dose/organ in rats at 3 hr after injection. The radioactivity in the stomach wall at 2 hr was very little as shown in Fig. 1. These data suggested rather low in vivo deiodination of this compound in rats.

DISCUSSION

Since its introduction by Blau and Bender (*9*), [^{75}Se]selenomethionine has been used as an agent for pancreas imaging despite its serious disadvantages.

TABLE 1
Tissue Distribution Data (%RC) of [^{131}I]HIPDM in Mice at Various Time Intervals After Injection (mean ± s.d. of Five Mice)

Item	Loading dose of HIPDM (0.05 mg/kg body weight)				
	5 min	30 min	1 hr	2 hr	5 hr
Pancreas	1.81 ± 0.20	3.02 ± 0.22	4.31 ± 0.22	5.36 ± 0.31	4.97 ± 0.66
Blood	0.38 ± 0.039	0.25 ± 0.022	0.17 ± 0.016	0.12 ± 0.032	0.053 ± 0.010
Brain	3.19 ± 0.78	2.15 ± 0.21	2.00 ± 0.49	1.61 ± 0.34	0.76 ± 0.14
Liver	1.73 ± 0.28	1.61 ± 0.15	1.48 ± 0.31	1.08 ± 0.08	0.71 ± 0.097
Spleen	1.04 ± 0.21	1.93 ± 0.28	2.63 ± 0.53	3.18 ± 0.49	1.27 ± 0.24
Stomach*	0.43 ± 0.061	0.67 ± 0.059	0.49 ± 0.12	0.38 ± 0.054	0.28 ± 0.091
Intestine*	0.82 ± 0.19	1.09 ± 0.050	1.24 ± 0.17	1.62 ± 0.23	1.25 ± 0.25
Kidneys	3.45 ± 0.34	3.22 ± 0.21	2.75 ± 0.31	2.09 ± 0.32	0.91 ± 0.21
P/L ratio†	1.08 ± 0.22	1.89 ± 0.23	2.92 ± 0.16	5.08 ± 0.52	7.05 ± 0.53

* Including contents.
† Pancreas to liver ratio.

Because of the long effective half-life (47 days), the absorbed dose is very high (total body: 8 rad/mCi, liver 25 rad/mCi); therefore, the injected dose must be limited (about 200–250 μCi in clinical use) (*8*). Moreover, the gamma ray energies of ^{75}Se (260, 410 keV) are rather high for effective imaging with a scintillation camera.

Various attempts have been made to find better pancreas imaging agents. Except for a few studies of zinc derivatives (*9,10*), most of them used ^{11}C-, ^{13}N-, or ^{123}I-labeled amino acids derivatives. The clinical ap-

TABLE 2
Effect of Loading Dose on Distribution of [^{131}I]HIPDM in Mice at 2 hr After Administration (%dose/organ, mean ± s.d. of Five Mice)

Item	Loading dose of HIPDM (mg/kg body weight)				
	0.05	0.25	1.23	3.03	6.13
Pancreas	4.15 ± 0.45	4.09 ± 0.32	4.38 ± 0.32	3.21 ± 0.36	1.95 ± 0.12
Liver	7.25 ± 0.77	7.62 ± 0.54	9.04 ± 1.34	10.40 ± 0.90	12.88 ± 1.12
Stomach*	1.27 ± 0.13	1.43 ± 0.24	1.39 ± 0.37	0.87 ± 0.08	1.11 ± 0.18
Spleen	1.58 ± 0.05	1.70 ± 0.06	1.67 ± 0.15	1.41 ± 0.15	1.34 ± 0.08
Kidneys	2.95 ± 0.41	2.70 ± 0.48	2.87 ± 0.38	2.57 ± 0.35	2.55 ± 0.25
Brain	3.45 ± 0.36	3.66 ± 0.52	3.29 ± 0.33	3.15 ± 0.38	3.01 ± 0.13
Blood†	0.58 ± 0.04	0.50 ± 0.08	0.49 ± 0.05	0.50 ± 0.05	0.53 ± 0.05
P/L ratio‡	5.08 ± 0.52	4.63 ± 0.22	4.47 ± 0.29	2.71 ± 0.29	1.43 ± 0.15

* Including contents.
† %dose/g.
‡ Pancreas to liver ratio (%dose/g).

TABLE 3
Tissue Distribution Data (%RC) of [^{131}I]HIPDM in Rats at Various Time Intervals After Injection (mean ± s.d. of Seven Rats)

Item	1 hr	2 hr	3 hr	5 hr
Pancreas	5.46 ± 0.52	6.54 ± 0.70	6.69 ± 0.94	6.61 ± 0.75
Blood	0.19 ± 0.013	0.14 ± 0.016	0.081 ± 0.0089	0.070 ± 0.011
Brain	3.65 ± 0.48	2.32 ± 0.43	1.45 ± 0.13	1.11 ± 0.18
Liver	1.66 ± 0.21	1.30 ± 0.18	0.85 ± 0.066	0.81 ± 0.17
Spleen	5.16 ± 0.48	4.60 ± 0.19	3.62 ± 0.58	2.25 ± 0.32
Stomach*	1.03 ± 0.46	0.68 ± 0.22	0.67 ± 0.19	0.51 ± 0.11
Intestine*	1.49 ± 0.076	1.61 ± 0.28	1.32 ± 0.24	1.52 ± 0.42
Kidneys	3.20 ± 0.26	2.03 ± 0.19	1.18 ± 0.22	0.78 ± 0.12
Prostate	1.53 ± 0.40	1.04 ± 0.16	1.11 ± 0.32	0.79 ± 0.21
Fat (mesenteric)	0.31 ± 0.069	0.37 ± 0.064	0.40 ± 0.11	0.23 ± 0.045
P/L ratio†	3.14 ± 0.31	5.15 ± 0.65	7.78 ± 0.95	8.06 ± 1.14

* Including contents.
† Pancreas to liver ratio.

plications of ^{11}C-, or ^{13}N-labeled amino acids (*10–13*) have been reported. However, clinical use of positron tomography is limited because of the high cost of positron cameras, need for in-house cyclotrons and extensive support for radiopharmaceutical production.

Since ^{123}I has a reasonable physical half-life (13.2 hr) and the abundant 159 keV gamma ray suitable for scintigraphy, a ^{123}I-labeled pancreas imaging agent is highly desirable. Although various kinds of radioiodinated amino acid analogs have been investigated (*14–17*), no compound has yet been developed which could replace [^{75}Se]selenomethionine for routine clinical use.

Iodine-123 HIPDM was initially developed as a brain perfusion imaging agent. The mechanism of brain accumulation was explained on the basis of pH gradient between blood (pH ~7.4) and brain (intracellular pH ~7.0). At high pH, HIPDM is neutral and lipid soluble and can diffuse into cells freely, but at the lower internal pH it becomes charged and no longer diffuses out (*1,2*). Kung et al. reported that HIPDM and N-isopropyl-*p*-[^{123}I]iodoamphetamine ([^{123}I]IAMP) displayed similar physical properties despite the differences in chemical structure because partition coefficient profiles of these compounds are similar (*1*). The pancreatic uptake of HIPDM is difficult to explain by the "pH shift theory" because intracellular pH of pancreas is not lower and blood supply to the pancreas is less than that to the liver and the kidney. Moreover, the P/L ratio of [^{131}I]IAMP in rats is much lower (0.97 ± 0.07) than that of [^{131}I]-HIPDM.

HIPDM is a diamine derivative and not an analog of

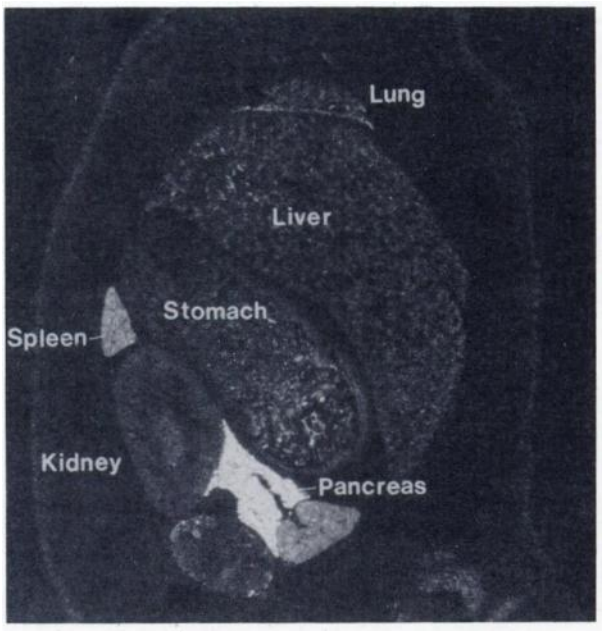

FIGURE 2
ARG (saggital section) of rat at 2 hr after [^{131}I]HIPDM injection. Intense uptake of radioactivity is seen in pancreas. Radioactivity distribution in liver is not homogenous

amino acid. Therefore, the mechanism of pancreatic accumulation of [^{131}I]HIPDM is possibly different than that of amino acid derivatives. The basic polyamines such as spermine and spermidine are present at high concentration in pancreas and prostate (*18*). The diamines are taken up by these organs as precursors of polyamines synthesis. Hydrogen-3-labeled putrescine (1,4,diamine) was reported to accumulate in the rat pancreas as well as in the ventral prostate (*19*). However, ^{14}C-labeled putrescine was concentrated in the prostate but not in the pancreas and radioiodinated analogs of diamine showed no affinity for either pancreas or prostate (*20*). Iodine-131 HIPDM was taken up by the pancreas, but the prostatic uptake was only less than one-fifth of pancreatic uptake at 2 hr after injection.

The pancreatic uptake of [^{131}I]HIPDM is rather slow and radioactivity still remains in the pancreas even at 5 hr after administration, whereas amino acids are taken up by the pancreas quickly and excreted into the intestinal tract. The P/L ratio is decreased by adding carrier HIPDM because the radioactivity shifts from pancreas to liver as the loading dose is increased. Compared to [^{75}Se]selenomethionine, high specific activity [^{131}I]-HIPDM has a significantly higher P/L ratio. Our data suggest that [^{123}I]HIPDM may be useful for pancreatic imaging, especially if imaged using a single photon emission computerized tomography system.

HIPDM is a new type of radioiodinated compound which is significantly taken up by the pancreas but the mechanism of pancreatic uptake of HIPDM is unclear at present. Further research on the mechanism of pancreas accumulation of HIPDM and the derivatives of HIPDM may result in the development of a new class of ^{123}I-labeled compounds which may turn out to be potential pancreas imaging agents. It remains to be seen whether [^{123}I]HIPDM will show similar results in other animal species and finally in man. Studies are in progress to elucidate these points.

FOOTNOTES

* NaI-131, protein iodination grade, in 0.1*N* NaOH, New England Nuclear.

† Whatman MK6F plates.

‡ Lo-Dose mammography film, DuPont.

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