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Placental Binding and Transfer of Radiopharmaceuticals: Technetium-99m *d*, 1-HMPAO

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Placental binding and transfer of ^{99m}Tc *d*, 1-hexamethyl propyleneamine oxime (HMPAO) was studied in vitro using human placenta and pregnant guinea pigs. **Materials and Methods:** Five pieces of human placenta were incubated in 50 ml Earle's solution containing 1.85 MBq ^{99m}Tc *d*, 1-HMPAO. The percent binding of the tracer to the placenta per 1 ml standard solution was calculated. Pregnant guinea pigs representing first, second and third trimesters were each injected with 74 MBq ^{99m}Tc *d*, 1-HMPAO through the jugular or femoral vein after sedation was induced with pentabarbital sodium. Static images were obtained, the guinea pigs were killed, and the fetuses were removed, weighed and imaged separately. The placentas, maternal and fetal brains, lungs, livers and kidneys also were removed, and the radioactivity was assayed in a dose calibrator for each organ. The percent radioactivity in each organ was calculated. **Results:** The binding of ^{99m}Tc *d*, 1-HMPAO to human placenta ranged from 2.95% ± 1.5% to 5.82% ± 0.3% per 1 ml standard solution. Both the binding of ^{99m}Tc *d*, 1-HMPAO to guinea pig placenta and its transfer to the fetus increased with gestational age. The percent binding ranged from 0.09% ± 0.06% to 0.43% ± 0.05%, whereas that of transfer to the fetus ranged from 0.05% ± 0.03% to 2.19% ± 0.64%. Of the amount transfered to the fetus, the order of accumulation in the fetal organs was liver > blood ≫ brain > lungs > kidneys > heart. **Conclusion:** Technetium-99m *d*, 1-HMPAO binds to the placenta, and a minimal amount crosses the placental barrier and is transfered into the fetal circulation, mostly in the liver but a measurable amount is found in brain tissue.

Key Words: technetium-99m *d*, 1-hexamethyl propyleneamine oxime; placental binding; transfer; guinea pigs

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Radiopharmaceuticals are administered for diagnostic and therapeutic purposes. They could be given inadvertently during pregnancy or on rare occasions, be given deliberately to save the life of either the fetus or the mother (*1–3*) and for placental localization (*4–6*). In either situation, an accurate estimate of the radiation absorbed dose to the fetus is necessary. Such a dosimetric calculation would be based on the amount of radioactivity transferred to the fetus across the placenta and/or irradiation from adjacent organs such as the urinary bladder and the placenta. For obvious ethical reasons, there are few human studies on placental binding and transfer of radiopharmaceuticals. Even in experimental animals, few studies have been reported on the transfer of radiopharmaceuticals across the placenta to the fetus (*7*). Hence, there is a need to systematically study placental transfer, biodistribution and kinetics of radiopharmaceuticals in the fetus.

In this report the binding of ^{99m}Tc *d*, 1-hexamethyl propyleneamine oxime (HMPAO) to human and guinea pig placentas and its transfer and distribution in the guinea pig fetus are described.

MATERIALS AND METHODS

In Vitro

Placental binding was studied using human placental villous tissue within 45–50 min of delivery, according to the modified method described by Smith et al. (*8*) and Guerre-Millo et al. (*9*). Five fragments each weighing approximately 0.5 g were excised from the placentas and thoroughly washed in Earle's solution (pH 7.4). The fragments were incubated in 50 ml Earle's solution equilibrated with a gas mixture of 95% oxygen and 5% carbon dioxide for 5, 10, 15, 30 or 60 min. An aliquot of 1.85 MBq (50 μCi) of the original formulation of ^{99m}Tc *d*, 1-HMPAO was added to the incubation medium. At the appropriate time interval, the fragments were blotted with tissue paper and the radioactivity was measured. A standard solution of 1.85 MBq of ^{99m}Tc *d*, 1-HMPAO diluted in 50 ml was prepared, and the radioactivity of 1 ml of it also was measured. The percent binding per radioactivity in the 1-ml standard solution was calculated. The above procedure was repeated four times for each incubation time period. A graph of

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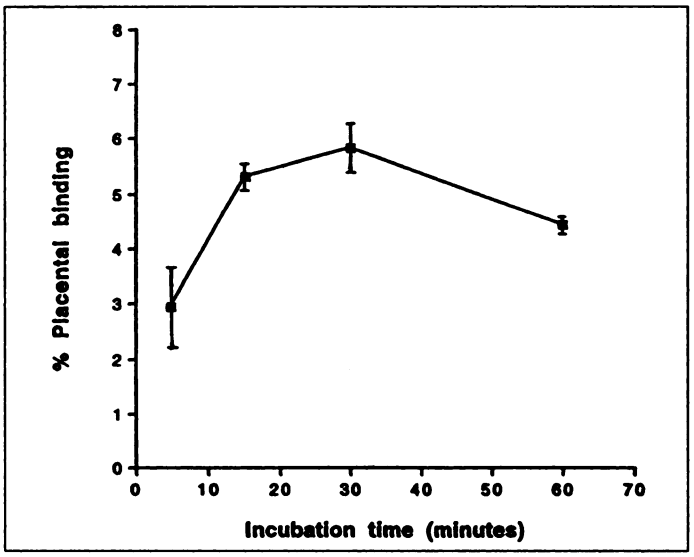

FIGURE 1. Percent binding of ^{99m}Tc *d*, 1-HMPAO to human placenta with respect to incubation period.

percent binding of ^{99m}Tc *d*, 1-HMPAO to the placental tissue compared with time was plotted.

In Vivo

Three groups of pregnant guinea pigs representing first, second and third trimesters were studied. Guinea pigs have a gestation period of 68–72 days, consequently each trimester is 20–24 days long, beginning from the last mating day. The guinea pigs were mated over a 24-hr period during estrus. Mating was determined by the presence of sperm in the vaginal smear and the day of mating was designated as Day 0 of gestation. Three pregnant guinea pigs were used for each trimester. Each pregnant guinea pig was anaesthetized by intraperitoneal injection of 30 mg/kg body weight pentabarbital sodium (saggital). Approximately 30 min later, a 22-GA-1 cannula was inserted into the jugular or femoral vein. Technetium-99m *d*, 1-HMPAO 74 MBq (2 mCi) was injected through the cannula within 15 min of its preparation. A standard solution of the ^{99m}Tc *d*, 1-HMPAO was prepared using the same amount of injected dose.

A static image of the guinea pigs was obtained 15–30 min after injection of ^{99m}Tc *d*, 1-HMPAO. Then the guinea pigs were killed and the fetuses were removed. Each fetus was weighed separately and imaged using a low-energy, all-purpose collimator and a zoom factor of 1.5. The total activity in the fetus was assayed in a dose calibrator.

The placentas, maternal and fetal brains, lungs, livers and kidneys were removed from the guinea pigs in the second and third trimester groups. The fetal organs were too small in the first trimester guinea pigs and were not removed. The radioactivity in each organ, in 1 ml blood (maternal or fetus) and in a standard solution of the tracer was assayed separately in a dose calibrator. The percent activity in each maternal organ and placenta was calculated relative to the injected dose, whereas that of the fetal organs was calculated relative to both the injected dose and the total activity in the fetus.

RESULTS

The percent binding of ^{99m}Tc *d*, 1-HMPAO to human placenta is shown in Figure 1, and it ranged from 2.95% ± 1.58% to 5.82% ± 0.3% per milliliter of the standard solution.

Images of the pregnant guinea pig showed accumulation of radioactivity in the brain, liver, kidney, fetuses and injection site (Fig. 2). The number of sites identified as fetuses on the image corresponded to the actual count of the fetuses, particularly in the third trimester. The number of fetuses per mother varied from two to a maximum of seven. The amount of radioactivity that accumulated in the placenta varied according to the gestational age from 0.09% ± 0.06% to 0.43% ± 0.05% (Table 1). The average percent injected dose transferred to the fetus also varied from 0.05 ± 0.03 (first trimester) to 2.19 ± 0.64 (third trimester) Table 1. The percent uptake in the fetal organs relative to the total radioactivity transfered to each fetus is given in Table 2 for second and third trimester fetuses. For fetuses in the first trimester, the fetal organs were too small to dissect and the radioactivity in them was not assayed. Equally important is the fact that a small amount of radioactivity (0.05% ± 0.03%) of the injected dose was transfered to each fetus during the first trimester (Table 1). For both second- and third-trimester fetuses, of the radioactivity transfered to the fetus, the order of accumulation in the fetal organs was liver > blood ≫ brain > lungs > kidneys > heart (Table 2).

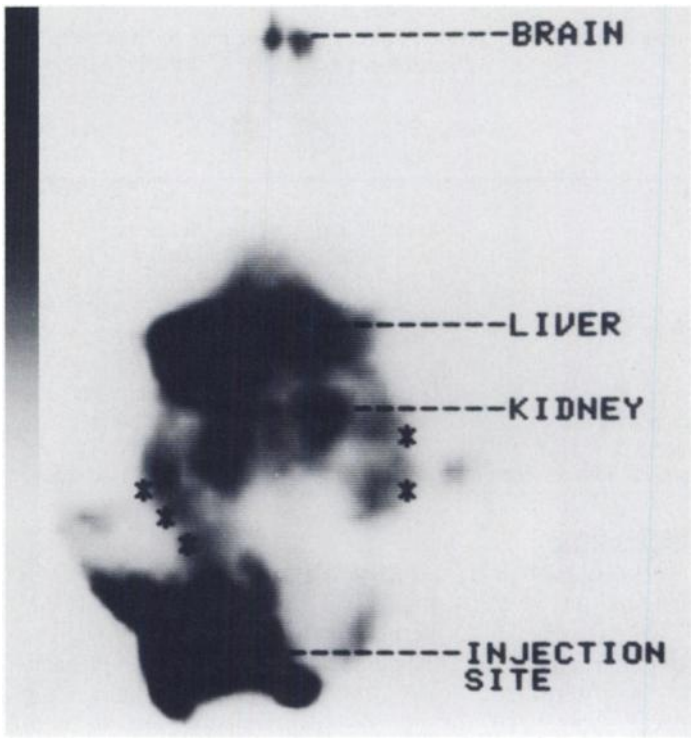

FIGURE 2. Radionuclide image of pregnant guinea pig. X denotes fetal site.

In both the second and third trimesters, the percentage uptake of ^{99m}Tc *d*, 1-HMPAO was approximately the same in the fetal organs except in brain and blood. The percentage brain uptake increased from 0.86% ± 0.22% in the second trimester to 1.56% ± 0.37% in the third trimester, whereas there was a corresponding decrease in the amount retained in the blood from 3.02% ± 1.42% to 1.46% ± 0.76%, respectively. Relative to the total activity in the fetus, a higher amount was extracted into the fetal brain than into the maternal brain (Fig. 3). Images of the third-trimester fetus clearly showed accumulation of radioactivity in different parts of the brain (Fig. 4).

TABLE 1
Percent Uptake of Injected Dose in Placentas and Fetuses and Fetal Weight According to Trimester

Trimester	Uptake (%)		Weight of fetus (g)	No. of fetuses
	Placenta	Fetus		
First	0.09 ± 0.06	0.05 ± 0.03	1.93 ± 1.00	14
Second	0.31 ± 0.02	0.95 ± 0.19	55.00 ± 10.00	11
Third	0.43 ± 0.05	2.19 ± 0.64	103.37 ± 5.71	11

TABLE 2
Percent Uptake per Gram in Fetal Organs Relative to Radioactivity Transferred to Fetus

Organ	Uptake according to trimester (%)	
	Second	Third
Brain	0.86 ± 0.22	1.56 ± 0.37
Liver	48.06 ± 1.30	47.33 ± 5.13
Lungs	0.74 ± 0.03	0.89 ± 0.10
Kidneys	0.63 ± 0.12	0.61 ± 0.17
Heart	0.38 ± 0.05	0.54 ± 0.07
Blood (1 ml)	3.03 ± 1.42	1.41 ± 0.76

First trimester fetal organs were small and not dissected, nor was radioactivity assayed. Average weight of each fetus was only 1.93 ± 1.00 g. Amount of ^{99m}Tc *d*, 1-HMPAO extracted in the brain increased during third trimester, with corresponding decrease of circulating radioactivity in blood.

DISCUSSION

Technetium-99m *d*, 1-HMPAO binds to placenta both in vitro and in vivo. A higher percentage of the ^{99m}Tc *d*, 1-HMPAO is bound to the placenta in vitro than in vivo. The difference could be due to a number of factors. Human placenta was used for the in vitro experiment, whereas guinea pig placenta was used for in vivo experiments. The in vitro technique of incubating fragments of placenta is basically crude. Therefore, there was the possibility of nonspecific binding as a result of cross-sectional cuts of the placental tissues. However, this incubation technique had been used previously to study the binding of various drugs to placenta (*9*). It is equally important to point out that the in vivo experiment involved not only the binding but also the transfer of ^{99m}Tc *d*, 1-HMPAO across the placental tissue.

The guinea pig was chosen as the appropriate animal model for the placental transfer of radiopharmaceuticals because of its small size and because its placenta closely resembles that of a human. Both are hemochorial placentas but that of the guinea pig has a labyrinthine vascular bed whereas that of humans is villous. The guinea pig also was chosen because, at full gestational period or birth, the fetal guinea pig is developmentally as mature as a human fetus.

In this study, the in vivo placental transfer of ^{99m}Tc *d*, 1-HMPAO was performed in guinea pigs during three different trimesters. Technetium-99m *d*, 1-HMPAO crossed the placental barrier and was transferred into the fetal circulation, where most of the activity accumulated in the liver followed by the brain (Table 2). The highest amount of ^{99m}Tc *d*, 1-HMPAO was transferred during the third trimester, corresponding to placental development and fetal maturity. The amount transferred during the first trimester was only 0.05% ± 0.03% (Table 1). If this calculation is extrapolated to a standard pregnant woman given 740 MBq (20 mCi) ^{99m}Tc *d*, 1-HMPAO, approximately 0.148–0.592 MBq (4–16 μCi) could be transferred to the fetus during the first trimester, when the danger of inadvertently administering a radiopharmaceutical to a pregnant woman is at its highest. This amount in and of itself might not deliver a damaging radiation dose to the fetus; however, the fetus would also receive radiation exposure from the adjacent organs.

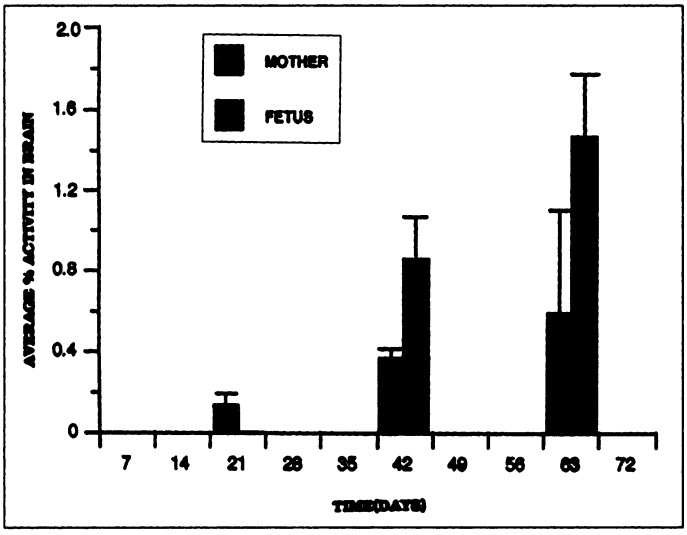

FIGURE 3. Relative percent uptake of ^{99m}Tc *d*, 1-HMPAO in maternal and fetal brains.

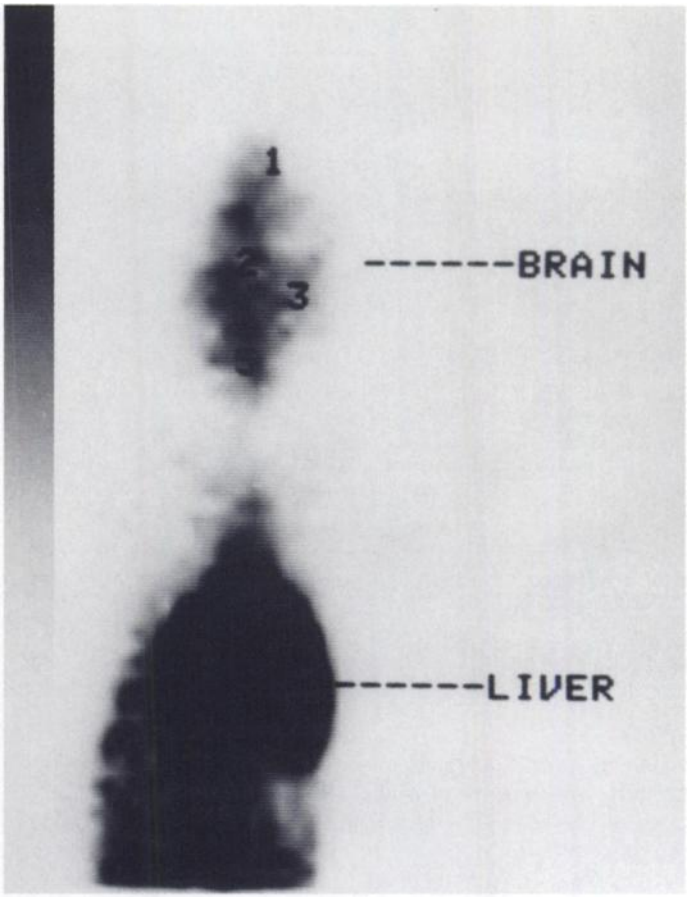

FIGURE 4. Typical radionuclide image of fetus shows different parts of the brain.

In vivo, ^{99m}Tc *d*, 1-HMPAO is converted to a secondary form that is polar and that does not cross the blood-brain barrier (*10*). A measurable amount of radioactivity was found in the fetal guinea pig brain. Hence, it can be presumed that the nonpolar parent ^{99m}Tc *d*, 1-HMPAO, crossed the placental barrier. It is suggested that the placental transfer of ^{99m}Tc *d*, 1-HMPAO is instantaneous and that the rate of placental transfer is greater than that of conversion to a secondary form.

The radioactivity in the fetal brain was detected easily with an external monitor when the fetus was imaged alone. The cerebrum, mid brain, cerebellum, pons and medulla oblongata could be identified easily (Fig. 4). Relative to the radioactivity in the fetus, there is a higher amount of ^{99m}Tc *d*, 1-HMPAO in the fetal brain compared with the amount of injected dose that penetrated the maternal brain (Fig. 3). Therefore, it appears that ^{99m}Tc *d*, 1-HMPAO can more easily cross an immature than a

mature blood-brain barrier. This phenomenon has been observed with other substances (*11,12*).

CONCLUSION

Technetium-99m *d*, l-HMPAO binds to both human and guinea pig placentas. It is transfered across the placental barrier into fetal circulation mostly in the liver with increasing gestational age.

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Biological Properties of Biotin–Chelate Conjugates for Pretargeted Diagnosis and Therapy with the Avidin/Biotin System

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Three-step pretargeting increases target-to-background ratios in radioimmunodetection and can potentially decrease harmful radiation to normal tissues in radioimmunotherapy. We studied four biotin–chelate conjugates (BCCs) for use in the avidin/biotin pretargeting system. **Methods:** Pharmacokinetics and biodistribution were studied in normal BALB/c (IA^k-negative), normal C_3H (IA^k-positive) and LS174T tumor-bearing BALB/c severe combined immunodeficient mice. Streptavidin alone and antibody–streptavidin conjugates [monoclonal antibody (MAb) 10-3.6 anti-IA^k IgG2a] were used. Indium-111- or ^{88}Y-BCCs were given alone intravenously; they were mixed with streptavidin or MAb–streptavidin conjugate and given intravenously; or streptavidin and MAb–streptavidin conjugate were pretargeted, and 2–3, 5 and 21 hr later, BCCs were injected intravenously. Samples were taken 2–3 hr after intravenous injection of labeled BCCs. **Results:** Three of the four BCCs were rapidly excreted by the kidneys, with <2.5%/g in any organ or tumor at 2–3 hr. Gut excretion eliminated biotinyl-(S)-1-*p*-aminobenzyl-ethylenediaminetetraacetic acid (EDTA) for use in pretargeting. Ninety percent of BCCs were bound to circulating pretargeted streptavidin at 1–6 hr, and ~15% were bound to pretargeted streptavidin at 24 hr. Kidney uptakes were: preformed streptavidin–BCC given intravenously, ~80%/g (24 hr); streptavidin pretargeted for 2–3 hr, ~60%/g; and streptavidin pretargeted for 5–21 hr, ~10%–20%/g. Kidney uptake was dose-dependent: 0.2, 0.67 and 1.0 nmol of streptavidin pretargeted for 21 hr showed increasing concentrations (24 hr). Uptake of monoclonal anti-IA^k–streptavidin–BCC complex into spleen (70% ± 10%/g; $p < 0.05$) and lymph nodes (10% ± 3.5%/g; $p < 0.01$) was higher in IA^k-positive C_3H mice than it was in IA^k-negative control BALB/c mice, and it was much higher than that in streptavidin controls. No significant target uptake was seen with anti-IA^k MAb–streptavidin pretargeted for 3 or 20 hr. Kidney uptake ~20%/g, which was lower than that of streptavidin alone. **Conclusion:** Three biotinyl chelates bind the diagnostic and therapeutic radiometals ^{111}In and ^{88}Y (and, by analogy, ^{90}Y) with the required in vivo stability and physiological properties for pretargeted diagnosis and therapy. Kidney uptake of streptavidin was decreased by conjugation to MAb. Failure of anti IA^k MAb–streptavidin conjugate to bind BCC after pretargeting may be due to rapid internalization of MAb–streptavidin-IA^k complex by the lymphocyte or to endogenous biotin. Either or both of these would make streptavidin unavailable to subsequent BCCs.

Key Words: radioimmunoimaging; radioimmunotherapy; yttrium-90; indium-111; pretargeting

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Radiolabeled monoclonal antibodies (MAbs) have shown promising clinical results in the diagnosis and therapy of cancer (*1–5*). Radionuclides such as ^{111}In and ^{90}Y have been of particular interest in radioimmunoscintigraphy and radioimmunotherapy (RAIT) due to their nuclear properties (*6–9*). Among the radionuclides for therapy, ^{90}Y is of particular interest due to its superior properties, including pure beta emission and the high-dose yield per nanomole (*10*). Bifunctional chelating agents (*11,12*) complex these metal ions and attach the chelated radionuclide to a protein or MAb (*13*). These conjugates act as carriers of radiometals for tumor targeting and radiotherapy. Chelates that hold radiometals with high stability under physiological conditions are essential to avoid excessive radiation damage to nontarget cells (*14*).

Renn and Meares (*15*) reported the large-scale synthesis of

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