Concentration of Perfluorohexyl Bromide in Dog Plasma and Selected Tissues

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ABSTRACT □ Beagle dogs received single perfluorohexyl bromide doses, either 30.2 g/kg po or 3.8 g/kg intratracheally. The apparent first-order plasma half-life during the terminal elimination phase was ~8 hr after oral treatment and >8 hr after intratracheal administration. Tissue analysis showed the highest mean concentration of the compound in abdominal fat 1 week after intratracheal administration. One dog had detectable levels in abdominal fat 4 weeks after treatment by either administration route.

Keyphrases □ Perfluorohexyl bromide—tissue and blood plasma levels in dogs after oral and intratracheal administration □ Fluorocarbons—perfluorohexyl bromide, tissue and blood plasma levels in dogs after oral and intratracheal administration □ Radiopaque contrast agents—perfluorocarbons, perfluorohexyl bromide, tissue and plasma levels in dogs after oral and intratracheal administration

Perfluorocarbon compounds were recently investigated for their potential use as radiopaque diagnostic agents. Perfluorooctyl bromide and perfluorohexyl bromide (I) are the least toxic of these compounds (1–11). Among the most frequently performed radiodiagnostic procedures with these two compounds are bronchography, alveolography (4, 5), and gastroenterography (4, 7).

BACKGROUND

One desirable property in contrast agents used in bronchography and alveolography is rapid elimination from the lungs. Radiopaque agents currently available for bronchography and alveolography are retained in the lungs for long periods; thus, they may irritate pulmonary tissues and cause varying degrees of damage (5). Vaporization of the perfluorocarbons at body temperature (4), a property not characteristic of other radiopaque agents, predisposes them to efficient elimination from the lungs. Although mucocilliary clearance is involved in elimination of the "neat" perfluorocarbons, evaporation plays the major role in elimination from the lungs (4, 5). Radiographic measurements demonstrated the elimination time from the lungs to be 30–60 min for I, as compared to 3–6 hr for the octyl derivative (4).

Good quality radiogastroenterograms have been obtained with these compounds since low viscosity and low surface tension allow them to flow freely into tiny folds and orifices (4, 6, 7). Visualization and delineation were similar for the two compounds (4). However, by vaporizing in the bowel, I provided a unique gas-contrast resolution, which allowed exceptional mucosal definition (4, 7, 8).

Elimination of the octyl derivative after oral and intratracheal administration to beagle dogs was reported (11). High concentrations of this compound were found in abdominal fat 4 weeks after medication. The present study presents elimination characteristics of I in plasma and selected tissues after oral and intratracheal administration to beagle dogs.

EXPERIMENTAL

Animal Procedure—Two groups of beagle dogs, six males and six females in each group, received either a 30.2-g/kg po dose or a 3.8-g/kg intratracheal dose of I[1]. A gastric tube was used for oral administration. The intratracheal dose was instilled under light thiopental[2] anesthesia (35 mg/kg iv); a gastric tube was inserted into the trachea until it reached a point several centimeters above the bifurcation of the right and left bronchi.

Blood samples were drawn at various intervals from the femoral vein. Potassium oxalate was used as the anticoagulant. The blood was promptly centrifuged at 2500 rpm for 10 min, and the plasma was separated and stored until analysis.

Two males and females from each group were anesthetized and killed by pentobarbital sodium[2] overdose (45 mg/kg iv) 7, 14, and 28 days after treatment. Samples of the abdominal fat, gonads, adrenals, mesenteric and thoracic lymph nodes, and lungs were stored in glass jars at −4° until assay. Previous studies showed that the highest levels of the octyl derivative were found in these tissues (5, 11). Bile samples were collected from the intratracheally tested group only.

Extraction Procedure—Plasma samples, 1 ml each, were extracted with 1 ml of isooctane containing 0.25 μg of tetrachlorodifluoroethane[3], the internal standard for the assay. The mixture was mechanically shaken for 10 min and centrifuged for 10 min at 2500 rpm. A sample of the organic phase, 2.5 μl, was analyzed by GC.

Tissue samples, up to 2 g of frozen tissue depending on the sample size, were minced with a scalpel and placed into a 5-ml polyethylene homogenization vessel[4]. A known volume of isooctane containing the internal standard, approximately two volumes per gram of tissue, was added, and the mixture was homogenized for 1 min. The homogenization vessel was submerged in an ice bath during homogenization. The homogenate was centrifuged at 2500 rpm for 10 min, and 2.5 μl of the organic phase was assayed.

Known amounts of I were added to samples of normal, control dog plasma and tissue; these samples were freshly prepared and used as standards. They were extracted and assayed as described.

GC—All samples were analyzed using a gas chromatograph equipped

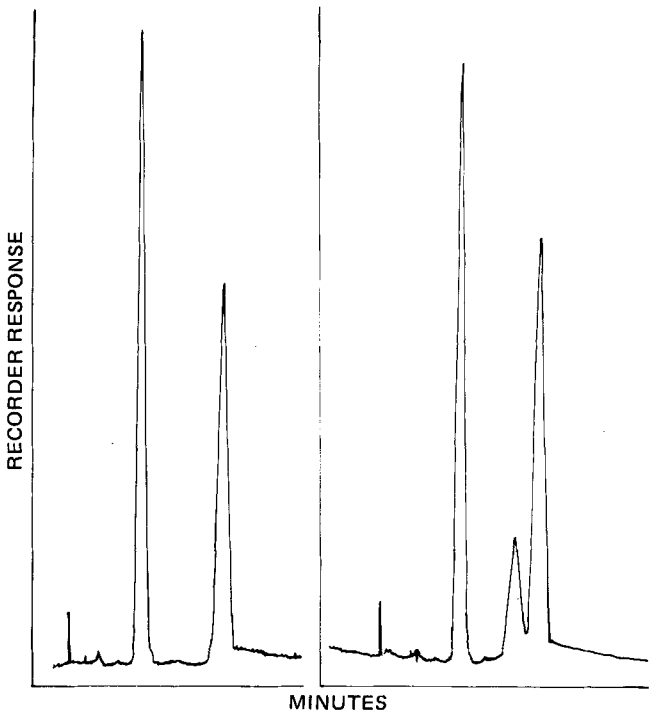

Figure 1—*Chromatogram of extracted dog plasma containing the internal standard (left) and the same sample containing 190 ng of I/ml (right).*

[1] E. I. du Pont de Nemours, Wilmington, Del.
[2] Abbott, North Chicago, Ill.
[3] Freon 112, E.I. du Pont de Nemours, Wilmington, Del.
[4] Sorvall, Norwalk, Conn.

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Table I—Tissue I Concentrations (Micrograms per Gram) in Beagle Dogs that Received 30.2 g/kg po

Dog Number, Sex, and Weight (kg)	Lung	Fat	Thoracic Lymph	Mesenteric Lymph	Adrenals	Ovaries	Testes
			1 Week				
1, M, 9.3	0.02	1.6	0.65	0.11	0.02	—	<MQL[a]
2, M, 10.1	<MQL	0.79	0.15	<MQL	<MQL	—	<MQL
3, F, 7.5	<MQL	0.08	0.03	<MQL	0.02	<MQL	—
4, F, 7.0	<MQL	1.8	0.28	0.06	0.04	<MQL	—
			2 Weeks				
5, M, 10.2	<MQL	0.07	<MQL	<MQL	<MQL	—	<MQL
6, M, 10.6	<MQL	0.06	<MQL	<MQL	<MQL	—	<MQL
7, F, 8.3	<MQL	0.08	<MQL	<MQL	<MQL	<MQL	—
8, F, 7.8	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	—
			4 Weeks				
9, M, 9.8	<MQL	0.09	<MQL	<MQL	<MQL	—	<MQL
10, M, 9.2	<MQL	<MQL	<MQL	<MQL	<MQL	—	<MQL
11, F, 7.3	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	—
12, F, 7.5	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	—

[a] Mean minimum quantifiable level (MQL) was 0.01 μg/g.

with a ^{63}Ni-electron-capture detector[5], an automatic sampler, and an integrator. The silanized glass column, 1.8 m × 3 mm i.d., was packed with 80–100-mesh, porous polymer beads[6]. The oven was operated in the isocratic mode at 175°; both the injection port and detector temperatures were 250°. The flow rate of the carrier gas, a mixture of 7% methane in argon[7], was 40 ml/min. Under these conditions, the retention times of I and the internal standard were 6.5 and 7.8 min, respectively.

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms of plasma extracted by the described procedure. The relationship between the relative peak area ratio and the concentration of I was linear up to 200 ng/ml; the computer estimated the standard line by a least-squares method. The concentration in the unknown sample was calculated from the equation for the extracted standards line. The minimum quantifiable level, defined as that value whose 80% confidence limit just encompassed zero, was 10 ng/ml or gram of tissue.

The mean plasma concentrations of I following oral and intratracheal administration are shown on Figs. 2 and 3, respectively. The apparent first-order terminal elimination half-life following oral administration of I was ~8 hr, as determined by linear regression on the logarithm of the plasma concentration and sample time data (r = 0.993). By the intratracheal route, the decline in plasma I concentration did not appear to follow first-order kinetics (Fig. 3); plasma levels were higher than observed after oral treatment, indicating greater absorption by this administration route.

In the orally dosed group, the only tissue in which I was detected beyond the 1st week was abdominal fat (Table I). No detectable level of I was found in the gonads, and only one low level was found in a 1-week lung sample. The tissue values were highly variable. In the intratracheally dosed dogs, the highest levels of I were seen in the abdominal fat (Table II); I was not detected in bile or ovaries, and only one low level was found in the testes. After 1 week, detectable levels of I were found only in abdominal fat and lymph nodes. Only one dog still had low levels of I in abdominal fat, by either treatment route, 4 weeks after medication. The octyl derivative, which was administered at similar doses in the previous study (11), also was detected in abdominal fat 4 weeks after medication.

The level of I detected in the one beagle 4 weeks after oral medication was 2% of the mean level of the octyl derivative detected in the eight animals in the previous oral study. Similarly, the level of I detected in the one dog 4 weeks after intratracheal administration was 1% of the mean level of the octyl derivative detected in the four animals that were treated by intratracheal administration of the octyl derivative in the previous study. Levels of I in abdominal fat of the remaining dogs, 4 weeks after treatment, were below the minimum quantifiable level of the assay.

Thus, detectable levels of both the octyl- and hexylperfluorocarbon compounds were seen 4 weeks after oral and intratracheal administration to beagle dogs. Levels of I observed were 1–2% those of the octyl derivative, indicating that the higher vapor pressure of I facilitated elimination from the body.

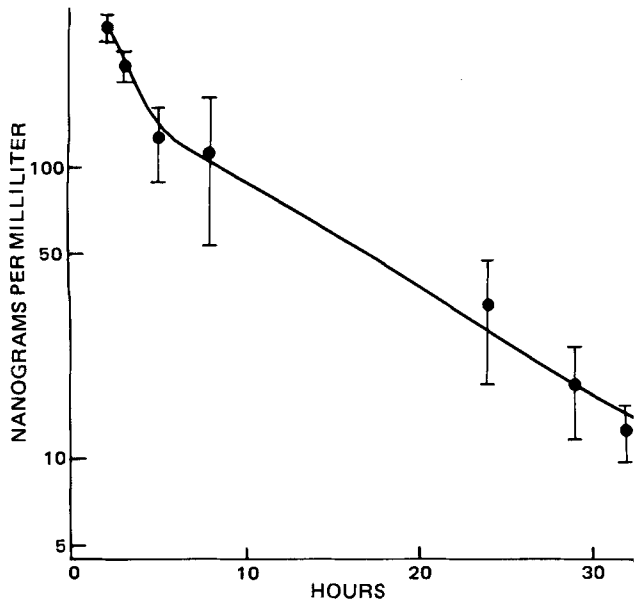

Figure 2—*Plasma concentrations in beagle dogs after oral administration of 30.2 g of I/kg;* n = *12. The vertical bar represents 2* SE.

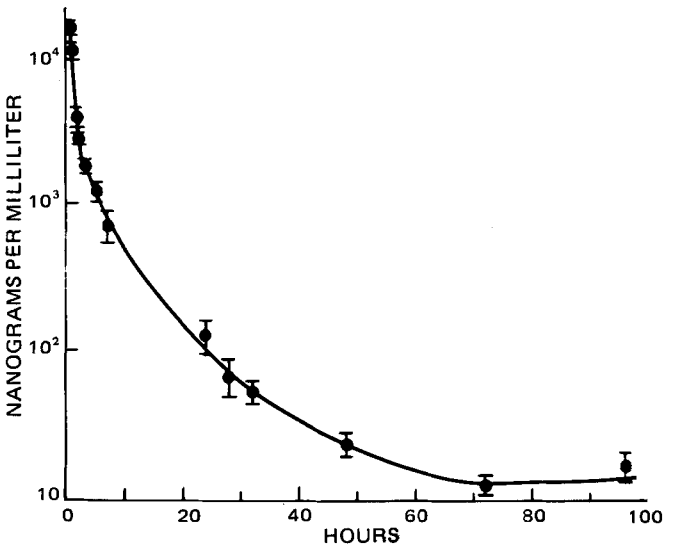

Figure 3—*Plasma concentrations in beagle hounds after intratracheal administration of 3.8 g of I/kg;* n = *12. The vertical bar represents 2* SE.

[5] Model 5710A, Hewlett-Packard, Avondale, Pa.
[6] Porapak QS, Waters Associates, Framingham, Mass.
[7] Linde, New York, N.Y.

Table II—Tissue I Concentrations (Micrograms per Gram) in Beagle Dogs that Received 3.8 g/kg intratracheally

Dog Number, Sex, and Weight (kg)	Lung	Fat	Thoracic Lymph	Mesenteric Lymph	Bile	Adrenals	Ovaries	Testes
				1 Week				
1, M, 10.4	0.01	6.5	0.30	0.70	<MQL[a]	0.11	—	<MQL
2, M, 9.2	0.01	0.96	0.26	0.09	<MQL	0.12	—	0.01
3, F, 7.3	0.01	2.5	0.31	0.11	<MQL	0.07	<MQL	—
4, F, 8.6	<MQL	0.86	<MQL	0.14	<MQL	0.05	<MQL	—
				2 Weeks				
5, M, 10.0	<MQL	0.67	<MQL	0.28	<MQL	<MQL	—	<MQL
6, M, 9.3	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	—	<MQL
7, F, 8.2	<MQL	0.16	<MQL	0.02	<MQL	<MQL	—[b]	—
8, F, 6.6	<MQL	0.67	0.03	0.05	<MQL	<MQL	<MQL	—
				4 Weeks				
9, M, 10.7	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	—	<MQL
10, M, 9.9	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	—	<MQL
11, F, 8.2	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	—
12, F, 6.9	<MQL	0.06	<MQL	<MQL	<MQL	<MQL	<MQL	—

[a] Mean minimum quantifiable level (MQL) was 0.01 μg/g. [b] No sample.

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Dose-Dependent Protein Binding and Disposition of Prednisolone in Rabbits

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Abstract □ An animal model was sought that would mimic humans with regard to the dose-dependent pharmacokinetics of prednisolone. Four rabbits were each given 0.5 and 10 mg iv of prednisolone, and timed blood samples were obtained. Plasma prednisolone and prednisone concentrations were assayed by high-performance liquid chromatography, and protein binding was assessed using equilibrium dialysis at 37°. Increases in the systemic clearance, volume of distribution at steady state, mean residence time (in three of four rabbits), and variance of residence time occurred as dose was increased. As in humans, prednisolone was partly converted to prednisone in the rabbit. Transcortin and albumin concentrations and their affinity constants for binding prednisolone were also similar to humans.

Keyphrases □ Prednisolone—dose-dependent protein binding and pharmacokinetics, rabbits □ Binding, protein—prednisolone, pharmacokinetics, rabbits □ Pharmacokinetics—prednisolone, protein binding, rabbits

Prednisolone and its pharmacologically inactive prodrug, prednisone, are synthetic glucocorticoids widely used in the treatment of various disease entities including asthma, shock, and nephrotic syndrome. Jusko and Rose (1) demonstrated nonlinear pharmacokinetics of these compounds in humans, characterized by increases in their total plasma clearance with increases in dose. Furthermore, the serum protein binding of prednisolone is nonlinear at therapeutic serum concentrations owing to the existence of two binding proteins (2). This binding appears to explain most, but not all, of the nonlinearity in prednisolone pharmacokinetics in humans (1).

To assess mechanisms contributing to this nonlinear disposition, an animal model is needed that mimics humans with regard to the serum protein binding and disposition of prednisolone. The serum protein binding of prednisolone was previously examined in rat, rabbit, canine, and human serum (3), with the rabbit being most like the human in type and degree of nonlinear binding. This study further evaluated the rabbit as an animal model for dose-dependent disposition of prednisolone.

EXPERIMENTAL

Methods—Four New Zealand White rabbits, 3.0–3.9 kg, received 0.5- and 10-mg iv equivalent doses of prednisolone as the sodium succinate.

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