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ALTERATION BY PERFLUORODECALIN OF HEPATIC METABOLISM AND EXCRETION OF PHENOBARBITAL

RONG HUANG, SIDNEY S. LEVIN, HEINZ SCHLEYER, DAVID Y. COOPER, BISWANATH MUKHERJI and HENRY A. SLOVITER*

Harrison Department of Surgical Research and Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.

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Abstract—The bile duct was cannulated in rats that had been infused intravenously with an emulsion of perfluorodecalin at intervals from 2 to 34 weeks earlier. After injection of [^{14}C]phenobarbital, urine and bile were collected during the next 24 hr and were analyzed for phenobarbital and its metabolites. There was a decrease in the biliary excretion of phenobarbital and its metabolites for several weeks after infusion of perfluorodecalin, but conjugation of the metabolites was not decreased. The reduced excretion returned to normal after about 20 weeks.

Certain liquid perfluorochemicals (PFC) have high solubilities for oxygen and carbon dioxide [1] and, because of their ability to transport these gases, have been used as substitutes for red blood cells in animals [2–5] and in humans [6, 7]. Emulsified PFC, when injected intravenously, is removed gradually from the circulation and accumulates in reticuloendothelial organs, principally in liver and spleen, before ultimate elimination through the lungs in expired gas [5].

The presence of PFC in the liver for a relatively long time causes morphologic changes and alters hepatic and reticuloendothelial activity [8]. Although the PFC are foreign substances that are not metabolized [9, 10], they do have effects on the hepatic cytochrome P-450 dependent microsomal mixed-function oxidase [9, 11]. In a previous study [11], we found that intravenous administration of perfluorodecalin in rats causes a marked increase in the cytochrome P-450 concentration of microsomal preparations made from the livers of these rats. Similar results were obtained after intraperitoneal injection of liquid (not emulsified) perfluorodecalin in rats [12]. In the present study, we evaluated the change in hepatic metabolic activity *in vivo* by measuring the products of the metabolism of administered phenobarbital (PB) excreted in bile and urine of rats that had received intravenously an emulsion of perfluorodecalin at various times (2–34 weeks) prior to injection of radioactive phenobarbital. These experiments were done in rats with bile fistulas to permit easy sampling of the bile for measurement of the concentrations of phenobarbital and its metabolites [13].

MATERIALS AND METHODS

Emulsions of purified perfluorodecalin (I.S.C. Co., Bristol, England) in dispersed egg yolk lecithin were prepared and injected intravenously in male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 200–250 g, as described previously [11]. Emulsion was injected into a femoral vein by a pump while an approximately equal volume of blood was removed through a phlebotomy in a tail vein. Injection of emulsion was continued until approximately 50% of the rat's blood was replaced by the emulsion, and the F-crit value (vol. percent of PFC) of the rat's blood was in the range of 19–22% [11].

At intervals (2–34 weeks) after injection of emulsified perfluorodecalin, a polyethylene tube (PE50) was inserted and tied in the bile duct of one of the rats, under halothane anesthesia. The other end of the tube was brought through the skin of the back and inserted into a plastic vial fastened to the rat's back, as described previously [13]. The rat was kept in a metabolic cage and urine was collected; bile samples were taken from the collecting vial. About 48 hr after cannulation of the bile duct, an isotonic saline solution of phenobarbital (80 mg/kg) containing [2-^{14}C]phenobarbital (40 μCi/kg; New England Nuclear, Boston, MA) was injected intraperitoneally. Control rats received intravenous injections of dispersed lecithin suspension but no PFC, and were treated in the same way as the experimental rats including cannulation of the bile duct. Samples of bile and urine collected during the 24-hr period after the injection of radioactive phenobarbital were analyzed for total radioactivity and for the radioactivity of phenobarbital and its metabolites. The phenobarbital and the metabolites were separated and identified by reverse-phase thin-layer chromatography and scanning of the chromatograms, as described previously [13].

RESULTS

In Fig. 1 are shown representative scans of chromatograms of bile and urine from control and experimental rats (7 weeks after infusion of emulsified perfluorodecalin) and also a scan (second from top)

* Send correspondence to Dr. Henry A. Sloviter, Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6070.

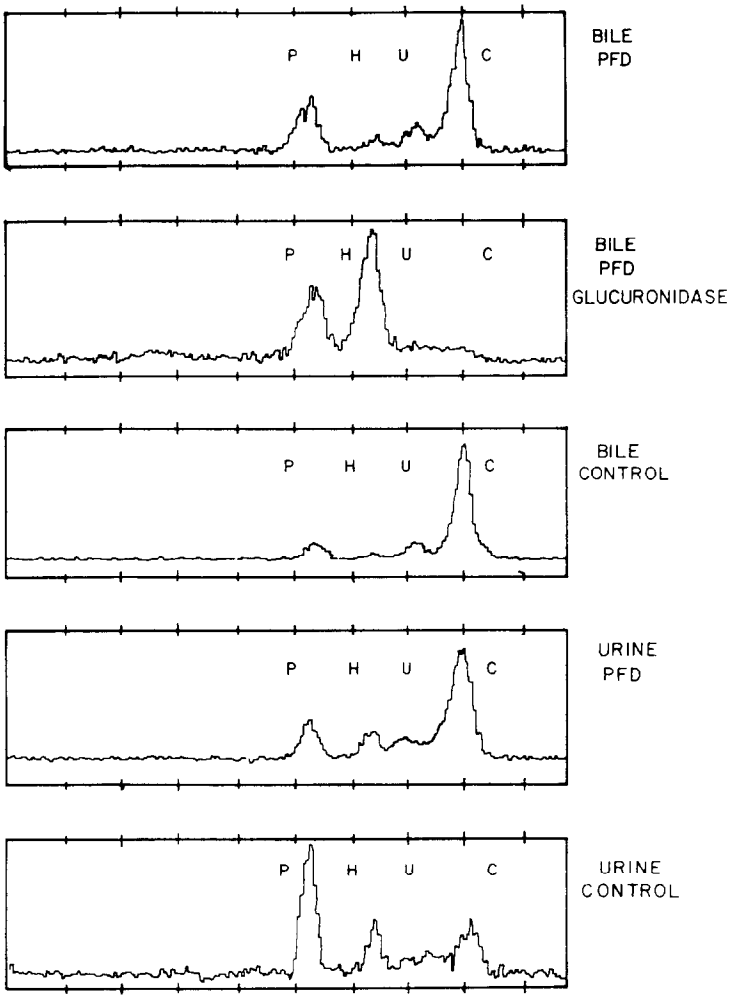

Fig. 1. Scans of thin-layer chromatograms of bile and urine samples from rats 7 weeks after intravenous infusion of perfluorodecalin emulsified in a suspension of lecithin. The samples were collected during 24 hr after intraperitoneal injection of phenobarbital (80 mg/kg) containing [2-^{14}C]phenobarbital (40 μCi/kg). Control rats received the lecithin suspension without PFD. "Glucuronidase" indicates that the sample was incubated with this enzyme before it was chromatographed. Key: P, phenobarbital; H, *p*-hydroxyphenobarbital; U, unknown, probably a conjugate; and C, conjugates.

of the chromatogram of a bile sample after treatment with glucuronidase. An imaging proportional counter (Bioscan 100) measured the radioactivity in the area under each peak, and these values were used to calculate the amounts of phenobarbital and its metabolites; the values are listed in Table 1.

In Table 1 are listed the results of the analyses for radioactive substances in the bile and urine collected during the first 24 hr after injection of [^{14}C]phenobarbital from rats that had received intravenous infusions of emulsified perfluorodecalin at intervals from 2 to 34 weeks earlier. The totals of radioactive substances (bottom of table) show that in all the control rats approximately 60% of the total excreted was in the bile and 40% in the urine. In the rats that had received emulsified perfluorodecalin, the amounts of PB and metabolites excreted in the bile were reduced markedly in the earlier periods (2–18 weeks) after perfluorodecalin infusion but by 31 weeks were equal to or exceeded the values for the controls. Thus, the ratio of biliary to urinary excretion, which was approximately 1.5 in control rats, fell to 0.18 at 2 weeks after emulsified perfluorodecalin and rose to 3.3 at 31 weeks.

The data of Table 1 also show that in control rats more than half of the radioactive substances excreted were conjugates (mainly glucuronides of hydroxyphenobarbital), and that much more was present in bile than in urine. In the perfluorodecalin rats, the amounts of conjugates in the bile were reduced markedly in the early periods and increased gradually so that, by 31 weeks after perfluorodecalin infusion, the amount was similar to that of the control.

The amount of unconjugated *p*-hydroxyphenobarbital excreted in the bile of the experimental rats was also decreased markedly in the earlier periods after perfluorodecalin, but increased gradually and by 31 weeks exceeded that of the control. The amounts of unchanged phenobarbital excreted in the bile showed less change after infusion of

Table 1. Excretion in bile and urine of phenobarbital and its metabolites during 24 hr after injection of [^{14}C]phenobarbital in rats previously infused with emulsified perfluorodecalin (PFD)*

		Percent of total ^{14}C injected									
		Weeks after PFD									
		2		7		18		31		34	
		C	PFD	C	PFD	C	PFD	C	PFD	C	PFD
PB	Bile	4.7	3.3	3.4	1.7	1.9	2.0	2.5	2.2	—	2.1
	Urine	9.0	17.7	10.3	0.9	6.3	5.5	6.0	0.8	—	0.9
	B/U	0.52	0.19	0.33	1.89	0.20	0.36	0.42	2.8	—	2.3
POH	Bile	3.0	0.2	2.5	0.2	2.4	1.1	2.2	2.9	—	2.0
	Urine	6.3	5.5	6.2	1.1	5.3	2.4	7.3	1.7	—	1.9
	B/U	0.48	0.04	0.40	0.18	0.45	0.46	0.30	1.7	—	1.5
CJ	Bile	25.6	8.8	22.5	5.9	22.8	7.8	17.7	17.0	—	12.7
	Urine	7.2	45.0	5.5	19.9	5.0	14.0	4.9	4.2	—	4.1
	B/U	3.6	0.20	4.1	0.30	4.6	0.56	3.6	4.1	—	3.1
Total	Bile	33.3	12.4	28.5	7.8	27.2	10.9	22.4	22.1	—	17.7
	Urine	22.4	68.2	22.0	21.9	16.7	21.9	18.3	6.7	—	6.9
	B/U	1.49	0.18	1.30	0.36	1.63	0.50	1.22	3.3	—	2.6

* All values for each time period (vertical column) were obtained from a single rat. Abbreviations: PB, phenobarbital; POH, *p*-hydroxyphenobarbital; CJ, conjugates; and C, control.

perfluorodecalin, and there was no definite pattern of change.

DISCUSSION

After intravenous administration of emulsified PFC, a large part of the infused PFC is retained in the liver for a relatively long time [14], but there is no inflammatory reaction to the presence of these inert foreign substances. The duration of retention varies markedly, being relatively short for perfluorodecalin and very long for perfluorotributylamine [5]. In spite of the inert nature of the PFC, their presence does affect some liver functions, e.g. cytochrome P-450 activity [11, 12] and phagocytic activity of the reticuloendothelial cells [8]. In the present study, our results provide information concerning the nature and duration of effects, measured *in vivo* on a metabolic activity, produced by perfluorodecalin, which is probably the most promising of the PFC for use in a blood substitute preparation. Our results show that in rats infused with emulsified perfluorodecalin there was a decrease in the hepatic metabolism of phenobarbital and in the biliary excretion of administered phenobarbital and its metabolites during the period from 2 to 31 weeks after the infusion of perfluorodecalin. In perfluorodecalin rats, there was no increase in the rate of hydroxylation of PB corresponding to the increase in hepatic microsomal P-450 [11]. A similar result was observed in induction of cytochrome P-450 by PB, where hydroxylation of PB also was not increased, while hydroxylation of other drugs and xenobiotics was increased [15]. It appears that the levels of cytochrome P-450 involved in hydroxylation of PB are not controlled by the same mechanism that regulates the mixed-function oxidases that metabolize other xenobiotics. A similar lack of parallelism between increases in cytochrome P-450 level and enzyme activity was reported in a study [16] of the metabolism of hexobarbital. There seems to be no impairment of the conjugation of the metabolites prior to biliary excretion. These reduced activities gradually returned to normal about 20–30 weeks after administration of perfluorodecalin. It is worthy of note that these reduced activities were apparently not due to the continuous presence of the perfluorodecalin in the liver because the effects persisted long after the perfluorodecalin had been cleared completely from the liver, which occurred at about 10–12 weeks.*

Shrewsbury *et al.* [17] used antipyrine clearance as a measure of hepatic mixed-function oxidase activity and found that this hepatic function decreases at 24 hr and then increases at 48 hr after administration of an emulsion containing PFC. Other studies [8, 14, 18] on the effects of PFC preparations on liver functions have been done with preparations that contained a mixture of perfluorodecalin and perfluorotripropylamine or perfluorotributylamine as well as a considerable amount of a synthetic surfactant. In all cases reported, the control animals were not given the surfactant. Therefore, it is not possible to conclude from these prior studies how much of the observed effects was due to each of the three components of the administered preparations. In the results reported here, our preparation contained only emulsified perfluorodecalin, and the control animals received all the components of the emulsion except the perfluorodecalin.

Our results, as well as results of prior studies, show that different perfluorochemicals, when infused intravenously in animals, all accumulated to a considerable degree in the liver and affected some hepatic functions. However, these effects varied in degree and nature among the several different

* B. Mukherji and H. A. Sloviter, unpublished results.

perfluorochemicals studied, even though none of the compounds was metabolized. It is interesting that the perfluorochemicals are very inert chemically, but that they have unsual surface activities and, as a result, may affect the functions of cells which they touch. For example, it is known that some perfluorochemicals affect the properties of blood platelets and blood coagulation factors [19, 20].

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