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EFFECTS OF INTRAVENOUS EMULSIFIED PERFLUOROCHEMICALS ON HEPATIC CYTOCHROME P-450

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Abstract—Intravenous infusion of emulsified perfluorodecalin in rats caused a large increase in hepatic cytochrome P-450 concentration which persisted for many weeks. In contrast, hepatic cytochrome P-450 concentration was not changed significantly after infusion of perfluorotributylamine, but subsequent administration of phenobarbital caused the usual increase of cytochrome P-450. The cytochrome P-450 activity for demethylation of benzphetamine was decreased slightly after perfluorodecalin but was unchanged after perfluorotributylamine. The difference in the effects of these perfluorochemicals on hepatic cytochrome P-450 may be related to the difference in the time these compounds are retained in the liver.

Perfluorochemicals (PFC) are organic compounds in which all hydrogen atoms have been replaced by fluorine atoms. The very strong bond (11 kcal/mol) between carbon and fluorine makes these compounds very inert. Certain liquid PFC can serve as biological transporters of O_2 and CO_2 because of their high solubility for gases. The PFC are nonpolar liquids which are insoluble in water. They do not dissolve salts, glucose or other components of blood plasma and, therefore, cannot provide the functions of plasma. However, emulsions of PFC in a suitable aqueous medium can serve as adequate substitutes for the gas transport functions of the blood [1–3].

Hepatic microsomal cytochrome P-450 systems catalyze the mixed-function oxidation of many water-insoluble organic compounds and thus make them more soluble and excretable in the urine. Ullrich and Diehl [4] found that perfluoro-*n*-hexane (in contrast to *n*-hexane) is not hydroxylated when it is incubated with liver microsomes, but forms an enzyme substrate complex (spectral Type I) with the cytochrome P-450 system and stimulates oxygen consumption. They found that fluoride ion is not produced, showing that the perfluorohexane is not metabolized. They regarded the perfluoro compound as a dead end inhibitor of cytochrome P-450 which uncouples electron transport of the microsomal oxidase system. It has also been shown [5] that there is no metabolism *in vivo* of the fluorochemical after emulsified perfluorodecalin is infused intravenously. Other investigators [6, 7] found that perfluoro compounds do not affect significantly hepatic metabolic processes.

In these earlier studies, the microsomal preparations were obtained from the livers of normal, untreated rats. In the present study we measured the changes in the hepatic cytochrome P-450 system that occurred at various intervals after intravenous administration of an emulsion of either perfluorotributylamine, which is retained in the liver for many months [8], or perfluorodecalin, which leaves the liver after several days [9]. We found that both compounds altered hepatic cytochrome P-450, but that these changes were different for each compound studied. Recently, Obraztsov *et al.* [10] reported that liver microsomal cytochrome P-450 increases after intravenous injection of an emulsion which contains a mixture of perfluorodecalin and other substances, including another perfluoro compound.

MATERIALS AND METHODS

Purified perfluorotributylamine (FTBA) was obtained from the 3M Co. (St. Paul, MN), and purified perfluorodecalin (FD) was from the I.S.C. Co. (Bristol, England). Emulsions of the fluorochemicals were made by adding 4 ml of the liquid fluorochemical to 7 ml of a 10% dispersion of egg yolk lecithin (Sigma Chemical Co., St. Louis, MO) in cold Tyrode's solution (pH 7.4) and then sonicating at 100 W for 15-sec periods, eight times with intervals of 1 min between each sonication. The resulting milky white emulsion was filtered through a membrane filter (Millipore 1.2 μm porosity) to remove any large particles and any metallic dust from the sonicator tip. The emulsion contained 35–40% (v/v) emulsified fluorochemical, and the emulsified particles were about 0.1 μm in diameter. The viscosity of the emulsion was approximately that of normal blood. The chemicals and materials used in the microsomal studies have been described [11, 12].

Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 200–300 g,

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anesthesized with pentobarbital, were infused with the fluorochemical emulsion through a plastic catheter inserted into the femoral vein. About 8 ml of the emulsion was infused at a rate of 0.2 ml/min with an infusion pump, while an approximately equal volume of blood was removed through a phlebotomy in the tail vein. Since the density of the perfluoro compounds is about 1.9, a routine hematocrit measurement shows the volume percentage (F-crit) of the perfluoro compound as a white column below the red cells. Hematocrit values obtained shortly after each infusion showed that the F-crit values of the rats ranged from 19 to 22% (v/v). The F-crit decreased to 50% of the initial value during the first 2 days after infusion, and after 4 days no fluorochemical remained in the circulation.

The rats were killed by decapitation at appropriate intervals after the fluorochemicals had been administered. Immediately after decapitation, the liver was perfused *in situ*, first with normal saline to remove blood and then with 0.25 M sucrose solution. The liver was removed and weighed. A 10% homogenate of the liver was made in 0.25 M sucrose solution, and microsomes were prepared as previously described [11, 12]. Total cytochrome P-450 concentrations were measured by difference spectroscopy in whole homogenates by the method of Matsubara *et al.* [13] using the extinction coefficient of 104 $mM^{-1}\,cm^{-1}$ and in microsomes by the method of Omura and Sato [14] using 91 $mM^{-1}\,cm^{-1}$. The cytochrome P-450 catalytic activity was estimated by measuring the demethylation of benzphetamine [11]. Microsomal protein concentrations were measured by the Lowry method [15].

RESULTS

There was an increase in the weight of the liver in the rats infused with the emulsion of either fluorochemical (Fig. 1). The liver weight (expressed as a percentage of body weight) was about 10–20% higher in rats infused with FTBA emulsion than in control rats (infused with lecithin suspensions containing all the components of the emulsion except PFC). The liver weights of rats infused with FD emulsion increased much more rapidly and were markedly higher than the livers of rats infused with FTBA at all time intervals. In the control rats the liver weight remained an approximately constant fraction of the body weight, about 4 g/100 g body weight.

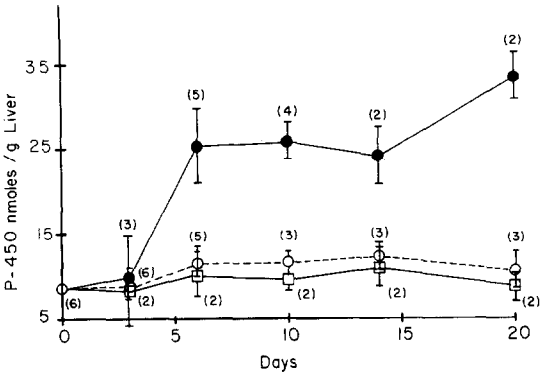

Fig. 2. Cytochrome P-450 concentration of liver (nmol/g) at times after infusion of emulsion of fluorochemicals. Values are means ± SD. All values for perfluorodecalin at times greater than 5 days were statistically significant ($P < 0.05$) compared to controls; all values for perfluorotributylamine were not significantly different. Key: (□) perfluorotributylamine; (●) perfluorodecalin; and (○) control (no perfluorochemical).

Hepatic cytochrome P-450 and protein concentration. The hepatic microsomal cytochrome P-450 concentration of control rats (infused with lecithin suspension) remained fairly constant during the 20-day period after infusion. Rats infused with FTBA emulsion showed a small (but not statistically significant) decrease in hepatic P-450 concentration as compared with controls (Fig. 2). In the rats infused with FD emulsion, after a delay of about 3 days, there was a large increase in the concentration of hepatic cytochrome P-450 which persisted beyond 20 days (Fig. 2). In these animals infused with FD, the hepatic P-450 levels reached a maximum at about 20

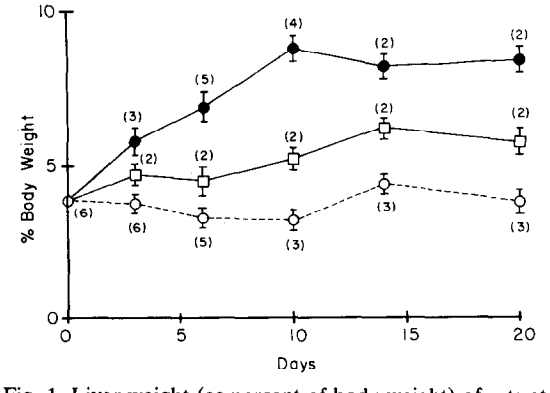

Fig. 1. Liver weight (as percent of body weight) of rats at times after infusion of emulsions of perfluorochemicals. Values are means ± SD. The differences between all experimental animals and controls were statistically significant ($P < 0.05$). Key: (□) perfluorotributylamine; (●) perfluorodecalin; and (○) control (no perfluorochemical).

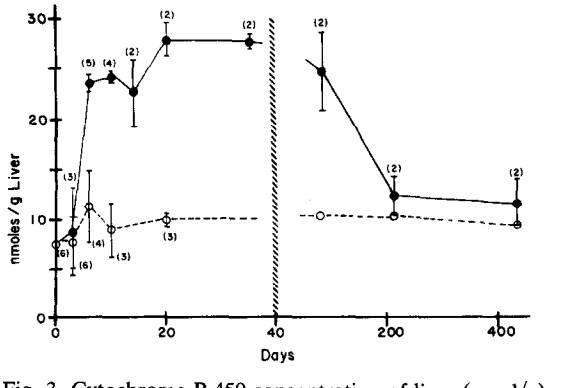

Fig. 3. Cytochrome P-450 concentration of liver (nmol/g) at times after replacing 50% of the blood of rats with an emulsion of perfluorodecalin. Values are means ± SD. All values greater than 20 nmol/g were statistically significantly ($P < 0.05$) different from controls. Key: (○) control; and (●) perfluorodecalin.

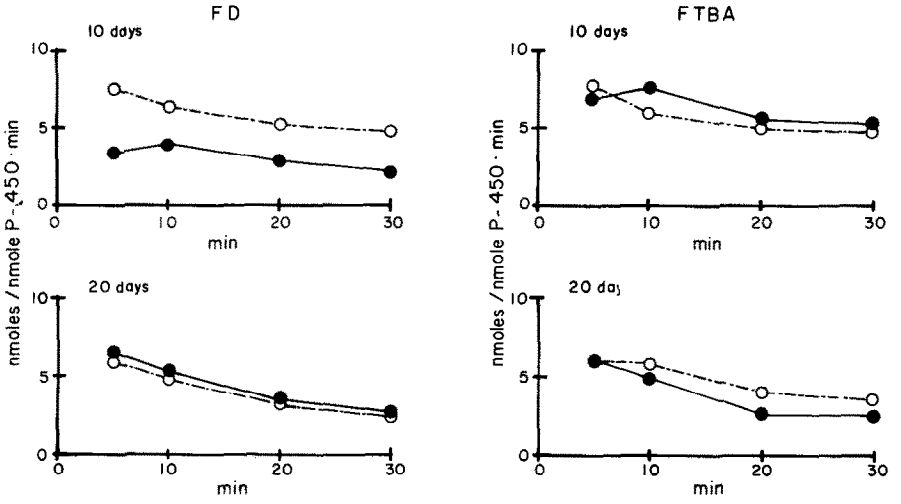

Fig. 4. Cytochrome P-450 activity of liver microsomes for the demethylation of benzphetamine (nmol formaldehyde formed/nmol P-450) 10 and 20 days after infusion of an emulsion of perfluorodecalin (FD) or perfluorotributylamine (FTBA). Values for the P-450 content (nmol/mg protein) of the microsomes were: FD: 10 days, 1.07, and 20 days, 0.90; FTBA: 10 days, 0.62, and 20 days, 0.53; controls (ave.), 0.62. Key: (○) control; and (●) perfluorochemical.

days, after which the hepatic P-450 declined and reached control levels at about 100 days (Fig. 3).

Hepatic cytochrome P-450 demethylation specific activity. In Fig. 4 are displayed the results of individual experiments in which measurements were made of the cytochrome P-450 activity for the demethylation of benzphetamine by microsomes prepared from rat livers taken 10 and 20 days after fluorochemical infusion. There was a decrease in benzphetamine demethylase activity in the rats 10 days after infusion with FD, but at 20 days after infusion this activity was not significantly different from that of control rats. In the rats infused with FTBA, both at 10 days and 20 days after infusion there was no significant difference in demethylase activity from that of the control rats. Repetition of these experiments, yielded values which differed by less than 5% from the values shown in Fig. 4.

Induction of hepatic cytochrome P-450 with phenobarbital in rats infused with FTBA. To determine whether hepatic microsomal mixed-function oxidase activity could be induced by phenobarbital in rats previously infused with FTBA, they were given four daily intraperitoneal injections of phenobarbital (80 mg/kg) 4 days after infusion, when no FTBA remained in the circulation. These rats still retained a considerable amount of FTBA in the liver. After injection of phenobarbital, these rats showed a marked increase in cytochrome P-450, from control values of 10–15 nmol/g of liver to 40–50 nmol/g of liver.

Images of the livers of these rats were made by ^{19}F magnetic resonance imaging as described previously [16] before and at intervals after administration of the phenobarbital. There was no significant decrease in the amount of FTBA in the livers of these phenobarbital-treated rats for as long as 65 days after FTBA infusion. In control rats (not treated with phenobarbital) there was also no change in the amount of FTBA after a similar period of time.

DISCUSSION

Ullrich and Diehl [4] had found that a fluorocarbon (perfluoro-*n*-hexane) stimulates the oxygen consumption of rat liver microsomes *in vitro* and causes a Type I spectral change in the difference spectrum. They interpreted these findings to indicate that the fluorocarbon uncoupled the electron transport systems of the microsomes from hydroxylation and resulted in peroxide formation in the microsomes. Our results show that the intravenous administration of an emulsion of perfluorodecalin to rats caused a large increase in the concentration of cytochrome P-450, and this increase persisted for weeks after the fluorocarbon had been cleared from the liver. In contrast, we found that infusion of a different fluorocarbon (perfluorotributylamine) into rats did not alter significantly hepatic cytochrome P-450 concentration. Subsequent administration of phenobarbital to these rats, however, caused the usual increase in cytochrome P-450, indicating that the FTBA had not blocked synthesis. In a previous study [6], incubation of rat liver microsomes with perfluorotributylamine did not stimulate oxygen consumption or cause a definite increase in hepatic cytochrome P-450 concentration. There is apparently a considerable difference between these two perfluoro compounds in their effects on hepatic cytochrome P-450, even though both are chemically inert and both are not metabolized either *in vitro* or *in vivo* (the nitrogen atom in perfluorotributylamine is not amine in nature and does not form salts with acids). In rats infused with perfluorodecalin or perfluorotributylamine, we found that fluoride was not present in blood and urine, and we recovered the infused com-

pound unchanged (boiling point and refractive index) from the liver post-mortem.

There is a large difference between these two perfluoro compounds in the time they are retained in the liver after intravenous infusion. It was tempting to speculate that perfluorodecalin leaves the liver faster because it induces P-450, whereas perfluorotributylamine does not. However, we found that inducing P-450 by administration of phenobarbital did not alter significantly the rate of removal of perfluorotributylamine from the liver. The failure to alter the rate of removal of the perfluoro compound by using another agent does not exclude the possibility that removal of the perfluoro compound from the liver is in some way related to an effect of the perfluoro compound itself on cytochrome P-450.

We found that infusion of emulsified perfluorodecalin caused only a small transient decrease in the ability of the liver microsomal preparations to carry out the *N*-demethylation of benzphetamine. Infusion of emulsified perfluorotributylamine had no significant effect on the *N*-demethylation activity. Similar results were obtained by Guaitani *et al.* [7] who found that the rate of *N*-demethylation of aminopyrine by microsomal preparations from perfused rat livers is not affected by the addition of emulsified perfluorodecalin to the perfusion fluid. It may be significant that the perfluoro compound, which has greater effects on cytochrome P-450 concentration and on demethylase activity, has a shorter retention time in the liver. An explanation for this is a subject of our continuing investigation.

Our study showed that the infusion of emulsified perfluoro compounds in rats caused changes in liver size, in benzphetamine demethylation rate, and in the metabolism of phenobarbital. In our experiments, the rats survived indefinitely; many of them remained in seemingly normal health for two years after infusion.

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