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Effect of a Perfluorochemical Emulsion on the Rat Hepatic Mixed Function Oxidase System

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Abstract—The perfluorochemical components of synthetic oxygen transporting emulsions may persist in hepatic tissue. After a single 30% blood exchange with the perfluorochemical emulsion, Fluosol-DA 20%, the effects on the microsomal metabolism of 7-methoxycoumarin and 7-ethoxycoumarin were studied over a 9 week period. Fluosol-DA treated animals were compared with controls (sham) and hetastarch-treated controls. Changes in dealkylase activities were compared with induction by phenobarbitone and 3-methylcholanthrene. The liver to body weight ratio increased by 49% in Fluosol-DA-treated rats over the controls at 1 week and the microsomal protein was increased in the Fluosol-DA-treated rats after 4 and 9 weeks. Fluosol-DA treatment induced 7-methoxycoumarin demethylase with peak differences occurring at 1 week and a V_{max} 75% greater than controls. Fluosol-DA was a more potent inducer of demethylase than phenobarbitone. In addition, 7-ethoxycoumarin de-ethylase was induced by Fluosol-DA with a peak induction at 4 weeks. The V_{max} at 4 weeks in Fluosol-DA-treated rats was 122% greater than control. In this case, Fluosol-DA produced less induction in de-ethylase than 3-methylcholanthrene. These studies show that Fluosol-DA induces more than one form of cytochrome P450 and the effects resemble those of phenobarbitone more than those of 3-methylcholanthrene. Hetastarch, a plasma expander, did not affect liver weights, microsomal protein content, or the cytochrome P450 system.

Perfluorochemicals (PFCs) are carbohydrates in which all hydrogen atoms have been replaced by fluorine atoms. PFCs are immiscible with other fluids and are poor solvents (Geyer 1983). The solubility of physiological gases such as oxygen and carbon dioxide in PFC emulsions is higher than in whole blood. Artificial oxygen-carrying plasma expanders, such as Fluosol-DA 20%, contain the PFCs, perfluorodecalin (FDC) and perflurotripropylamine (FTPA). The potential advantages of these plasma expanders include emergency treatment of trauma victims, use in mass casualty situations in disaster or warfare, shortage of blood, availability to underdeveloped countries, transfusion for those who refuse natural blood for religious reasons, and no danger of disease transmittance.

PFC emulsions have the potential to alter the pharmacokinetics of drugs that may be administered simultaneously (Ravis et al 1991). Short-term pharmacokinetic studies in rodents have demonstrated alterations in metabolic clearance and volume of distribution (Shrewsbury 1986; Shrewsbury et al 1986, 1987a, 1989). For antipyrine (Shrewsbury et al 1986), indocyanine green (Shrewsbury et al 1987a), and phenytoin (Shrewsbury et al 1989), clearance decreased at 24 h whereas ampicillin (Shrewsbury 1986) and propranolol (Shrewsbury et al 1987a) showed no alterations in clearance over the same periods. An increased antipyrine clearance was noted (Shrewsbury et al 1986, 1987b) at periods of 48 and 72 h post-exchange in Fluosol-DA-treated rats. After a 30% blood exchange, dogs have been used as models for studying the long term pharmacokinetic effects of Fluosol-DA administration (Hoke et al 1988; Hoke & Ravis 1989; Ravis et al 1989). With test doses of antipyrine (Hoke et al 1988), a 40% lower antipyrine total body clearance was found at 48 h but an increase in total body clearance was noted from 1 week to 5 months. A similar study with lignocaine (Ravis et al 1989) showed no change in lignocaine total body clearance from 0·5 h to 5 months post-exchange.

Since several PFCs are retained in the liver for extended periods of time (Lutz & Metzenauer 1980; Lutz et al 1981), investigation of their influence on mixed function oxidase is of interest. When a non-emulsified PFC, perfluoro-n-hexane, was incubated with liver microsomes, an enzyme-substrate complex of cytochrome P450 and perfluoro-n-hexane accompanied by a type-I spectral change was noted (Ullrich & Diehl 1971). This study showed that it is a dead-end inhibitor of cytochrome P450 by uncoupling the electron transport of the microsomal oxidase system. Emulsions of PFC added to microsomes in-vitro have not been found to produce any spectral changes characteristic of binding to microsomes (Obraztsov et al 1988). Other studies (Khlopushina et al 1986; Obraztsov et al 1986, 1988; Grishanova et al 1987, 1988; Huang et al 1987) have confirmed the inducing abilities of PFCs administered separately. FDC and FTPA, both present in Fluosol-DA, appear to be inducers of the cytochrome P450 system. At three days following a blood exchange, FDC induced cytochrome $P450_b$ which is consistent with a phenobarbitone-type of induction (Grishanova et al 1987, 1988; Mishin et al 1989).

Whilst there is good evidence that FDC, when administered in an emulsified form, is a microsomal enzyme inducer, the duration of induction changes have not been well defined. In addition, the microsomal enzyme effects of Fluosol-DA administration has not been examined. In the present study, the effects of a single 30% blood exchange with Fluosol-DA on rat mixed function oxidase activity over 9 weeks was examined with the substrates 7-methoxycoumarin and 7-ethoxycoumarin. Recognizing that the blood exchange process (removal of blood and replacement) may also alter drug metabolism, microsomal activity of Fluosol-

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DA-exchanged rats were compared with rats which received blood exchange with a plasma expander.

Materials and Methods

Materials

Chemicals and reagents including 7-hydroxycoumarin, 7-ethoxycoumarin, Tris base, Tris hydrochloride, D-glucose-6-phosphate, glucose-6-phosphate dehydrogenase, 3-methylcholanthrene, phenobarbitone, and nicotinamide adenine dinucleotide phosphate (NADP) were purchased from Sigma Chemical Co. (St Louis, MO). 7-Methoxycoumarin was obtained from Aldrich Chemical Co. (Milwaukee, WI). Hetastarch (6%) in 0·9% Sodium Chloride Injection was purchased from Du Pont Pharmaceuticals (Wilmington, DE). Fluosol-DA, 20% was a gift from Alpha Therapeutics (Los Angeles, CA). All other chemicals were of analytical grade and were used as received.

Animals and treatments

Male Sprague-Dawley rats (Pratville, AL), initially weighing 180 to 200 g, were administered a 30% blood exchange with either Fluosol-DA or hetastarch. On the day of the exchange, a silastic cannula was placed in the jugular vein under ether anaesthesia. The exchanges were performed in three steps with each step involving removal of 10% of the blood volume followed by infusion of an equal volume of the exchanging material. Fluorocrits have confirmed a 28 to 30% blood replacement. Control rats underwent the cannulation procedure with no exchange. Six rats from the three treatment groups were decapitated at 24 and 72 h, and 1, 4 and 9 weeks after the exchange procedures.

Separate groups of rats were given intraperitoneal injections of either phenobarbitone (80 mg kg^{-1}) for 4 days, 3-methylcholanthrene (20 mg kg^{-1}) for 2 days, or corn oil vehicle (0·25 mL) for 3 days (control). Microsomes were obtained from these groups on the day following the last dose. These three groups were compared with another group of rats which was treated 72 h and 1 week earlier with Fluosol-DA. All treatment groups were identical in age.

Microsomal preparation

Following decapitation, livers were removed, washed with Tris-HCl buffer, pH 7·4, and weighed. Livers were then homogenized in 20 mL of chilled 1·15% KCl in 0·05 M Tris-HCl buffer (pH 7·4). The homogenate was centrifuged at 9000 *g* for 20 min at 4°C. The resulting supernatant was then centrifuged at 78 000 *g* for 60 min at 4°C. The microsomal pellets were reconstituted with chilled pH 7·4 buffer (0·05 M Tris-HCl) containing 1·15% KCl using a hand homogenizer and stored at −70°C. The microsomal protein was measured by the standard assay (Lowry et al 1951). The microsomal protein concentration was calculated from a standard curve obtained with bovine serum albumin.

Alkoxycoumarin dealkylase assay

Two alkyl analogues of 7-hydroxycoumarin, 7-methoxycoumarin and 7-ethoxycoumarin, were used to examine dealkylase activity in prepared microsomes. An NADPH generating system containing 0·4 mg of NADP, 4 mg of glucose-6-phosphate, and 0·3 units of glucose-6-phosphate dehydrogenase in 400 μL of 0·05 M Tris-HCl buffer (pH 7·4) was freshly prepared for each sample. The incubation medium contained NADPH generating system, 0·05 M Tris-HCl buffer, 5 mM $MgCl_2$, 250 μg microsomal protein, and distilled water to make 1 mL for each sample. The substrates were added at a final concentration of 1·5, 5·0 and 15·0 μM, to initiate the incubation reaction. These substrate concentrations were also used in the blood exchange studies. For studies with inducing agents, the substrate concentration was 30 μM. The incubation was performed in a metabolic shaker bath for 10 min at 37°C. The incubation was stopped by adding 150 μL of trichloroacetic acid followed by 3 mL of chloroform. The tubes were vortexed for 20 min and centrifuged at 2000 *g* for 2 min at room temperature (21°C). One mL of the lower chloroform phase was added to 3 mL of 0·01 M NaOH in 0·1 M NaCl. The mixture was vortexed for 45 s and then centrifuged for 2 min at 2000 *g* at room temperature. The upper aqueous phase was read fluorometrically with an excitation wavelength of 368 nm and an emission wavelength of 456 nm to determine generated 7-hydroxycoumarin. Formation of 7-hydroxycoumarin was expressed as μmol (mg protein)$^{-1}$ min^{-1}.

Data evaluation

Values of V_{max} and K_m were obtained by nonlinear regression methods (PC-Nonlin, Statistical Consultants, Lexington, KY) and the equation:

$$\text{rate} = (V_{max}\, C)/(K_m + C) \quad (1)$$

where C is the substrate concentration.

Parameters were determined from rates observed at 1·5, 5 and 15 μM. Correlation coefficients of the plot of rate vs C ranged from 0·97 to 1·00. Comparisons of liver weights, protein content, V_{max}, and K_m among treatment groups were performed by analysis of variance procedures (SAS, SAS Institute, Cary, NC) with a significance level of $P < 0·05$.

Results

Liver weights and microsomal protein

The liver weights of hetastarch-exchanged rats did not differ ($P > 0·05$) from control or untreated animals throughout the 9 week period (Fig. 1). The progressive decrease for control

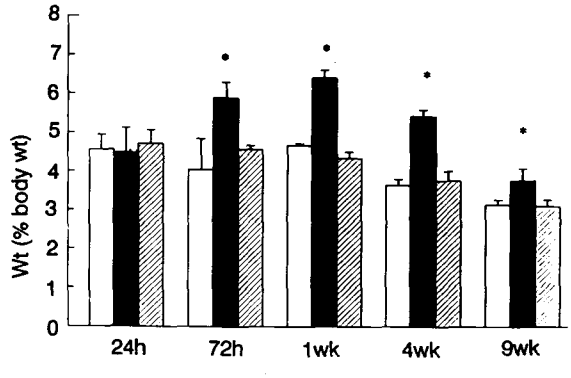

FIG. 1. Percentage weight of total body weight of livers from control (□), Fluosol-DA (■), and hetastarch (▨) rats as a function of time after exchange. Mean ± s.d. of five animals per group. *$P < 0·05$ compared with control and Hetastarch groups.

and hetastarch-treated rats in the liver weight as a percentage of body weight represents ageing and growth effects. While no body weight differences were observed amongst the three groups, animals treated with Fluosol-DA had significantly ($P<0.05$) greater percentage liver weight to body weight values at 72 h to 9 weeks compared with control and hetastarch groups. Compared with the control group, percentage liver weights were from 25 to 49% greater in Fluosol-DA animals over the 72 h to 9 week period.

Microsomal protein content did not display the same comparative changes across the groups as percentage liver weight (Fig. 2). The microsomal protein in Fluosol-DA rats was the same as control and hetastarch-treated rats at 24 h and 1 week. At 72 h, there was a slight but significant decrease in the Fluosol-DA animals compared with the other two groups at the same time period. However, at the later periods of 4 and 9 weeks, the microsomal protein appeared greater in the Fluosol-DA group. At 4 and 9 weeks, the percentage increase above controls was 24 and 30%, and above hetastarch rats was 23 and 31%, respectively. The exchange with hetastarch produced no change in microsomal protein content when compared with controls at any time period studied.

7-Methoxycoumarin demethylase activity

As shown in Table 1, differences in V_{max} and K_m values for 7-methoxycoumarin demethylase activity varied among treatment groups and time periods. Values of K_m in control rats appeared to decrease from 24 h to 1 week and then remained constant. Over 9 weeks, V_{max} for demethylase activity was constant in the control groups. In comparing the activity of microsomes between control and hetastarch groups, K_m and V_{max} values were similar except for slightly higher ($P<0.05$) K_m values in hetastarch groups at 4 and 9 weeks. A 24 h pretreatment period with Fluosol-DA had no effects on measured parameters. However, at periods of 72 h to 9 weeks, both K_m and V_{max} were greater for Fluosol-DA animals compared with either control or hetastarch groups. When compared with control rats, the increases in V_{max} were 31, 76, 63 and 49% for periods of 72 h and 1, 4, and 9 weeks, respectively. At the peak time of 1 week, the rate of demethylation in the Fluosol-DA group with a substrate concentration of 15 μM was 0.532 ± 0.031 μmol (mg protein)$^{-1}$ min^{-1} compared with rates of 0.354 ± 0.007 and 0.353 ± 0.008 μmol (mg protein)$^{-1}$min^{-1} for control and hetastarch-treated groups, respectively.

Demethylase activity of microsomes from the Fluosol-DA treated group was also compared with that of microsomes obtained from 4 day phenobarbitone- and 3 day 3-methylcholanthrene-treated rats of the same age (Table 2). Rates were evaluated with a 7-methoxycoumarin substrate concentration of 30 μM. All activities in treated groups were significantly ($P<0.05$) greater than the untreated control group. Percentage increases in demethylase rate were 36, 21, 58, and 106% for phenobarbitone, 3-methylcholanthrene Fluosol-DA (72 h), and Fluosol-DA (1 week) groups, respectively. Although the 72 h Fluosol-DA treated group was only slightly greater than the phenobarbitone group, the 1 week Fluosol-DA rats had rates 51% greater than the 3 day phenobarbitone-treated rats.

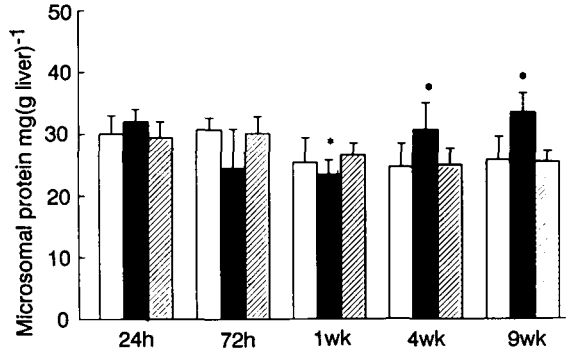

FIG. 2. Liver microsomal protein content for control (□), Fluosol-DA (■), and hetastarch (▨) rats as a function of time after exchange. Mean ± s.d. of five animals per group. *$P<0.05$ compared with control and hetastarch groups.

7-Ethoxycoumarin de-ethylase activity

The parameters of V_{max} and K_m for the de-ethylation of 7-ethoxycoumarin are presented in Table 3. In the control rats, K_m did not change over the 9 weeks but the V_{max} values increased up to 1 to 4 weeks then decreased by week 9. The same pattern in V_{max} was observed for hetastarch-treated animals. Microsomes prepared from hetastarch rats had similar K_m and V_{max} to the control groups at all periods studied. Over all the time periods, both K_m and V_{max} were different ($P<0.05$) for Fluosol-DA rats when compared with either control or hetastarch-exchanged rats. Values of K_m were 2- to 4-fold greater ($P<0.05$) for Fluosol-DA exchanged animals compared with the other groups. The smallest increase (30%) in V_{max} above controls was at 24 h and the greatest (123%) was at 4 weeks.

Table 1. 7-Methoxycoumarin demethylase activity (mean ± s.d.; n = 5).

	Treatment					
	Control		Fluosol-DA		Hetastarch	
Period	K_m (μM)	V_{max} (μmol mg^{-1} min^{-1})	K_m (μM)	V_{max} (μmol mg^{-1} min^{-1})	K_m (μM)	V_{max} (μmol mg^{-1} min^{-1})
24 h	1·46 ± 0·69	0·32 ± 0·04	1·62 ± 0·73	0·36 ± 0·05	1·27 ± 0·41	0·31 ± 0·04
72 h	0·85 ± 0·30	0·32 ± 0·03	1·83 ± 0·74*	0·42 ± 0·08*	0·76 ± 0·28	0·31 ± 0·02
1 Week	0·57 ± 0·10	0·37 ± 0·01	2·88 ± 0·88*	0·65 ± 0·08*	0·61 ± 0·10	0·36 ± 0·01
4 Weeks	0·65 ± 0·15	0·30 ± 0·04	2·05 ± 0·43*	0·49 ± 0·09*	0·90 ± 0·08*	0·29 ± 0·03
9 Weeks	0·68 ± 0·26	0·35 ± 0·04	1·86 ± 0·81*	0·52 ± 0·08*	1·02 ± 0·10*	0·32 ± 0·03

* Significantly different ($P<0.05$) from control of the same time period.

Table 2. Comparison of dealkylase activity following phenobarbitone, 3-methylcholanthrene, and Fluosol-DA 20% treatment (mean ± s.d. n = 5).

Treatment	7- Methoxycoumarin (30 μM) demethylase activity (μmol (mg protein)$^{-1}$ min^{-1})	7-Ethoxycoumarin (30 μM) de-ethylase activity (μmol (mg protein)$^{-1}$ min^{-1})
Control	0·33 ± 0·03	0·33 ± 0·02
Phenobarbitone	0·45 ± 0·02[a]	0·46 ± 0·02[a]
3-Methylcholanthrene	0·40 ± 0·06[a]	3·99 ± 0·27[ab]
Fluosol-DA (72 h)	0·52 ± 0·06[ac]	0·56 ± 0·09[bc]
Fluosol-DA (1 week)	0·68 ± 0·04[abc]	0·75 ± 0·01[abc]

[a] $P < 0·05$ compared with control. [b] $P < 0·05$ compared with phenobarbitone. [c] $P < 0·05$ compared with 3-methylcholanthrene.

Table 3. 7-Ethoxycoumarin de-ethylase activity (mean ± s.d.; n = 5).

	Treatment					
	Control		Fluosol-DA		Hetastarch	
Period	K_m (μM)	V_{max} (μmol mg^{-1} min^{-1})	K_m (μM)	V_{max} (μmol mg^{-1} min^{-1})	K_m (μM)	V_{max} (μmol mg^{-1} min^{-1})
24 h	0·55 ± 0·24	0·26 ± 0·02	1·13 ± 0·45*	0·34 ± 0·06*	0·41 ± 0·07	0·26 ± 0·03
72 h	0·66 ± 0·31	0·30 ± 0·03	1·89 ± 0·59*	0·51 ± 0·09*	0·57 ± 0·17	0·30 ± 0·02
1 Week	0·54 ± 0·03	0·34 ± 0·01	1·57 ± 0·22*	0·63 ± 0·05*	0·47 ± 0·18	0·34 ± 0·01
4 Weeks	0·53 ± 0·10	0·34 ± 0·02	2·95 ± 0·70*	0·76 ± 0·10*	0·62 ± 0·05	0·34 ± 0·01
9 Weeks	0·60 ± 0·11	0·31 ± 0·02	2·24 ± 0·52*	0·54 ± 0·11*	0·47 ± 0·14	0·29 ± 0·02

*$P < 0·05$ compared with control of the same time period.

As in the case with demethylase activity, de-ethylation following administration of phenobarbitone or 3-methylcholanthrene was compared with Fluosol-DA exchange groups at 72 h and 1 week (Table 2). With a 7-ethoxycoumarin substrate concentration of 30 μM, all treatments produced a significant change in de-ethylase activity when evaluated against controls. Treatment with phenobarbitone and Fluosol-DA, 72 h and 1 week previously, caused small but significant ($P < 0·05$) increases in de-ethylation rate of 39, 70, and 127%, respectively. A 12-fold increase in rate was observed for treatment with 3-methylcholanthrene.

Discussion

Several investigations have demonstrated that a single infusion of an emulsion of perfluorochemicals (PFCs) may alter drug disposition and biotransformation. Numerous pharmacokinetic investigations have utilized Fluosol-DA and noted both inhibition and induction of drug metabolic clearance and biotransformation. Emulsions of FDC have been shown to induce microsomal drug metabolizing enzymes (Grishanova et al 1987, 1988; Mishin et al 1989). The present study has examined the time-dependent effects of Fluosol-DA on microsomal demethylation and de-ethylation. While the circulation half-life of PFC emulsions is only a few days, the emulsion and its components remain in the liver for extended times. Complete elimination of FDC and FTPA in the rat requires 2 and 6 months, respectively (Iwai et al 1984). Of the two PFCs in Fluosol-DA, FDC is speculated to be metabolized by and be an inducer of the cytochrome P450 system.

The present studies demonstrate that a single intravenous 30% blood exchange of Fluosol-DA to rats induces demethylase and de-ethylase activity of microsomes for up to 9 weeks. In other studies, when an emulsion of FDC-FTBA was administered to rats (Obraztsov et al 1986), an increase in cytochrome P450 content, oxidation of NADPH, hydroxylation of benz(a)pyrene, and lipid peroxidation were noted at 72 h after infusion. However, major effects disappeared after 10 days. Huang et al (1987) noted that cytochrome P450 concentration in rats remained above controls for up to 200 days following an intravenous dose of emulsified FDC but not FTBA. In this same study, demethylation of benzphetamine was decreased 10 days after the FDC emulsion and unchanged at 20 days. One long-term study with emulsions of FDC by Khlopushina et al (1986) demonstrated that increases in mouse liver cytochrome P450 content as well as *N*-demethylase and *p*-hydroxylase persisted even at 12 weeks after the treatment. Our studies with 7-methoxycoumarin and 7-ethoxycoumarin substrates and Fluosol-DA dosing show that peak induction effects occurred at 1 week for demethylase activity and 4 weeks for de-ethylase activity.

The effects of Fluosol-DA blood exchange on demethylase and de-ethylase activities were demonstrated by changes in both V_{max} and K_m values. FDC and FTPA are initially engulfed by phagocytic cells present in the liver (Pfannkuch et al 1981) and this may delay the onset of microsomal effects. Phagocytized PFC particles could then coalesce intracellularly but fragment into smaller particles with time (Geyer 1983; Ohnishi & Kitazawa 1980). These PFC particles, especially FDC, interact with lipid membrane. Such interactions could cause a decrease in the affinity of the enzyme for substrate and, at the same time, could cause induction of the enzyme.

Increases in liver weight expressed as percentage of body weight have been reported previously with single administrations of PFC emulsions (Lutz & Metzenauer 1980; Huang et al 1987). Huang et al observed a near doubling of percentage

of liver to body weight over a 20 day period following an FDC emulsion. The liver weight changes in the present study are not as large; however, in those investigations, a 50% blood replacement was performed. Microsomal protein content was decreased at 72 h and increased at 4 and 9 weeks compared with the other two groups. This is less than reported by other investigators with other emulsified PFCs (Khlopushina et al 1986; Obraztsov et al 1986; Huang et al 1987) and may represent differences in the vehicle, dose, and type of PFC administered as well as protein assay methods.

Inductive effects of Fluosol-DA were also compared with activity changes produced by pretreating rats with either 3-methylcholanthrene or phenobarbitone. These studies display the selectivity of Fluosol-DA treatment on the two forms of cytochrome P450; phenobarbitone induces both demethylase and de-ethylase activity and 3-methylcholanthrene increases demethylase to a small degree but greatly increases de-ethylation. Blood exchange with Fluosol-DA seems to show the same pattern of effects on demethylation and de-ethylation as phenobarbitone but to a greater degree. Although Fluosol-DA enchanced de-ethylase activity, this was not near the 12-fold increase noted with 3-methylcholanthrene. These observations agree with previous studies (Grishanova et al 1987, 1988; Mishin et al 1989) which showed FDC, a component of Fluosol-DA to be a phenobarbitone-type inducer of the cytochrome P450 system.

These investigations with rat microsomes support microsomal studies with mice (Khlopushina et al 1986) and pharmacokinetic studies with dogs (Hoke et al 1988; Ravis et al 1989) demonstrating changes in drug metabolism several weeks after Fluosol-DA exchange.

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References

Geyer, R. P. (1983) PFC as blood substitutes—an overview. Prog. Clin. Biol. Res. 122: 157–168

Grishanova, A. Y., Gutkina, N. I., Mishin, V. M. (1988) Comparative characterization of isolated forms of cytochrome P-450 inducible by phenobarbital and perfluorodecalin. Biokhimiya 53: 368–376

Grishanova, A. Y., Obraztsov, V. V., Shekhtman, D. G., Lyakhobich, V. V. (1987) Phenobarbital type of induction of cytochrome P-450 of liver microsomes by perfluorodecalin. Ibid. 52: 1138–1143

Hoke, J. F., Ravis, W. R. (1989) Effect of a moderate blood exchange with Fluosol-DA 20% on the disposition of digoxin in the dog. Mat. Res. Symp. Proc. 110: 141–146

Hoke, J. F., Ravis, W. R., Hankes, G. H. (1988) The pharmacokinetics of antipyrine in the dog followed a blood exchange with Fluosol-DA 20%. Pharm. Res. 5: S190

Huang, R., Cooper, R. Y., Sloviter, H. A. (1987) Effects of intravenous emulsified perfluorochemicals on hepatic cytochrome P-450. Biochem. Pharmacol. 36: 4331–4334

Iwai, M., Yokoyama, K., Murashima, R., Yamada, N., Tsuda, Y., Kondo, N. (1984) Fate of FDC and FTPA in rats following intravenous injection of Fluosol-DA 20%: studies of absorption, distribution, metabolism, and excretion in rats. Yakuri. to Chirgo. 10: 3807–3848

Khlopushina, T. G., Kovalev, I. E., Lysenkova, E. M. (1986) Effect of perfluorodecalin and perfluorotributylamine on the cytochrome P-450 system of the liver. Biokhimiya 51: 664–667

Lowry, O. H., Rosenborough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Lutz, J., Metzenauer, R. (1980) Effects of potential blood substitutes (perfluorochemicals) on rat liver and spleen. Pflugers Arch. 287: 175–181

Lutz, J., Metzenauer, P., Kunz, E., Heine, W. D. (1981) Cellular response. In: Frey, R., Beisbarth, H., Stosseck, K. (eds) Oxygen Carrying Colloidal Blood Substitutes: Proceedings of the Fifth International Symposium on Perfluorochemical Blood Substitutes. W. Zuckschwerdt Verlag, Munchen, pp 73–81

Mishin, V. M., Obraztsov, V. V., Grishanova, A. Y., Gutkina, N. I., Shekhtman, D. G., Khatsenko, O. G., Lyakhovich, V. V. (1989) The phenobarbital-type induction of rat liver microsomal monooxygenases by perfluorodecalin. Chem.-Biol. Interactions 72: 143–155

Obraztsov, V. V., Shekhtman, D. G., Sklifas, A. N., Makarov, K. N. (1988) Analysis of physiological properties of fluorocarbon inducers of cytochrome P-450 in membranes of endoplasmic reticulum of liver. Biokhimiya 53: 613–619

Obraztsov, V. V., Shekhtman, D. G., Sologub, G. R., Beloyartsev, F. F. (1986) Induction of microsomal cytochromes in the rat liver after intravenous injection of an emulsion of perfluoroorganic compounds. Ibid. 51: 1220–1227

Ohnishi, Y., Kitazawa, M. (1980) Application of perfluorochemicals in human beings—a morphological report of a human case with some experimental studies using rabbit. Acta. Pathol. Jpn. 30: 489–504.

Pfannkuch, F., Schnoy, N., Ohlschlegal, C., Wilson, C. (1981) Comparative morphological studies in animal experiments after parenteral use of Fluosol-43, -DA 20%, and -DA 35%. In: Frey, R., Beisbarth, H., Stosseck, K. (eds) Oxygen Carrying Colloidal Blood Substitutes: Proceedings of the Fifth International Symposium on Perfluorochemical Blood Substitutes. W. Zuckschwerdt Verlag, Munchen, pp 61–67

Ravis, W. R., Hoke, J. F., Parsons, D. L., Hankes, G. H. (1989) Changes in lidocaine pharmacokinetics in dogs following blood replacement with perfluorochemical blood substitute. Pharm. Res. 6: S214

Ravis, W. R., Hoke, J. F., Parsons, D. L. (1991) Perfluorochemical erythrocyte substitutes: disposition and effects on drug distribution and elimination. Drug Metab. Rev. 22: 375–411

Shrewsbury, R. P. (1986) Effect of moderate haemodilution with Fluosol-DA or normal saline on ampicillin kinetics in the rat. J. Pharm. Pharmacol. 38: 647–652

Shrewsbury, R. P., White, S. G., Pollack, G. M., Wargin, W. A. (1986) Antipyrine kinetics following partial blood exchange with Fluosol-DA in the rat. Ibid. 38: 883–887

Shrewsbury, R. P., Lewis, L. M., Oliver, S. R., Womble, C. L. (1987a) Effect of moderate haemodilution with Fluosol-DA or normal saline on indocyanine green and (+)-propranolol. Ibid. 39: 592–598

Shrewsbury, R. P., Lewis, L. M., Oliver, S. R. (1987b) Effect of moderate haemodilution with Fluosol-DA or normal saline on low-dose phenytoin and (±)-5(4-hydroxyphenyl)-5-phenylhydantoin kinetics. Ibid. 39: 349–356

Shrewsbury, R. P., Oliver, S. R., Lewis, L. M., Anderson, W. T., White, L. G. (1989) The effect of varying extents of Fluosol-DA or normal saline haemodilution on the dose dependent kinetics of phenytoin in the rat. Ibid. 41: 582–584

Ullrich, V., Diehl, H. (1971) Uncoupling of monooxygenation and electron transport by fluorocarbons in liver microsomes. Eur. J. Biochem. 20: 509–512