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Antipyrine kinetics following partial blood exchange with Fluosol-DA in the rat

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The effects of partial blood exchange with Fluosol-DA on hepatic microsomal oxidative metabolism have been studied in the rat. Antipyrine clearance (Cl) was used as an in-vivo measure of the activity of the mixed function oxidase system. Rats were partially exchanged with Fluosol-DA and dosed with antipyrine at selected time intervals following exchange. No change in antipyrine Cl was observed at 0·5 h, but there was a statistically significant decrease at 24 h and then an increase by more than 50% relative to control at 48 and 72 h. These data indicate that the effects of Fluosol-DA on hepatic function are time-dependent and that Fluosol-DA has the potential both to inhibit and to enhance hepatic metabolism. The possibility of altered hepatic metabolism should be considered when patients transfused with Fluosol-DA are given drugs primarily metabolized by the mixed function oxidase system.

Perfluorochemical (PFC) emulsions are currently being investigated as oxygen-carrying blood substitutes. Animal studies have demonstrated the ability of PFC emulsions to sustain life (Geyer 1970, 1975; Yokoyama et al 1984). PFC emulsions have been administered in clinical trials to replace blood loss (Mitsuno et al 1982) and to increase oxygen delivery to ischaemic tissues (Mitsuno et al 1982; Handa et al 1983). The administration of a PFC emulsion has also been used as pre-operative treatment in patients suffering from severe anaemia (Tremper et al 1982).

PFCs are taken up extensively by the reticuloendothelial system and distributed primarily to the liver and spleen (Geyer 1983). Their persistence in the liver and spleen has been a source of concern. Fluosol-DA is a commercially prepared emulsion containing two PFCs, perfluorodecalin and perfluorotripropylamine. The half-life of perfluorodecalin in the rat liver, spleen, lungs, kidney, and adipose tissue is less than 10 days, while the half-life of perfluorotripropylamine in these tissues is 20 to 40 days (Yokoyama et al 1984). The half-life of perfluorodecalin has been shown to be dose-dependent (Yokoyama et al 1975). The intravascular half-life for the PFCs in Fluosol-DA is approximately 10 to 12 h in the rat, and they are undetectable in the blood 2 days after administration (Lutz & Metzenauer 1980). PFCs are eliminated primarily by expiration through the lungs. Urine and faecal excretion are insignificant and there is no evidence of metabolic degradation (Geyer 1983)

Because PFCs are sequestered in the liver, hepatic function may be affected following a Fluosol-DA transfusion or exchange. Previous studies have reported a decrease in the clearance of phenytoin (Matsumoto et al 1983) and morphine (Kemner et al 1984a) in rats partially exchanged with PFC emulsions. In contrast, no changes were observed in diazepam pharmacokinetics following administration of Fluosol-DA (Kemner et al 1984b).

The present study examined antipyrine pharmacokinetics for the first 72 h following partial blood exchange with Fluosol-DA. Antipyrine disposition in lightly anaesthetized animals immediately after Fluosol-DA exchange has been reported (Kemner et al 1984b). Antipyrine is extensively metabolized by the microsomal mixed function oxidase system and is commonly used to assess hepatic metabolism in man and animals (Kolmodin et al 1969; Sotaniemi 1973; Wilson et al 1982). It is distributed in total body water (Stevenson 1977), and its clearance is insensitive to changes in hepatic blood flow (Branch 1974) and protein binding (Soda & Levy 1975).

MATERIALS AND METHODS

Materials

Fluosol-DA was donated by Alpha Therapeutics (Los Angeles, California). Antipyrine, phenacetin, and HPLC solvents and buffers were obtained from commercial sources and used without further purification. Male Sprague-Dawley rats, 250 to 450 g, were used; they had free access to food and water.

Methods

Antipyrine kinetics were examined in unexchanged rats and rats partially exchanged with Fluosol-DA or

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normal saline (see Table 1). Partial as opposed to total blood exchange was used to avoid the need for supplementary oxygen. The saline exchange was included to differentiate between changes in antipyrine disposition due to Fluosol-DA itself and those due to loss of erythrocytes and other blood constituents. Antipyrine kinetics were examined at 0·5, 24, 48, and 72 h following the exchange as some alterations in hepatic metabolism may not be apparent until several hours or days after exchange.

A silastic cannula was implanted under light ether anaesthesia in the right jugular vein of all animals. Its patency was maintained by daily flushing with heparinized saline (50 u ml^{-1}). Animals in group I received an intravenous antipyrine dose of 20 mg kg^{-1} dissolved in 0·9% NaCl (saline) (10 mg ml^{-1}) 48 h after cannulation without undergoing any sham transfusion. Similar control groups have been used in other studies (Kemner et al 1984a, b). Preliminary data showed that antipyrine disposition was the same 48, 72, 96, or 120 h after cannulation. Animals in groups II–VIII were partially exchanged with Fluosol-DA or saline (40 ml kg^{-1}). The exchange fluid was administered in three equally divided doses at 0·5 h intervals. At each interval, a volume of blood equal to half the volume of the exchange fluid was removed just before and 1 min after infusion of the exchange fluid. The exchange fluid was infused over 1 min. The haematocrit and fluorocrit (Geyer 1983) were determined just before and after each exchange and before antipyrine dosing (20 mg ml^{-1}). The exchange procedure reduced the pre-exchange haematocrit by one-half, indicating approximately 50% blood exchange.

Serial blood samples (0·3 to 0·4 ml) were collected in polyethylene tubes containing heparin (33 u ml^{-1} of blood) and centrifuged for 5 min at approximately 12 000*g*. Plasma was harvested and stored at −20 °C until assayed. The animals were killed 24 h after antipyrine dosing, and the liver, kidneys, spleen and lungs were removed and examined for gross abnormalities. Wet liver weights were determined.

Antipyrine plasma concentrations were determined by high performance liquid chromatography. Internal standard (phenacetin 10 μg ml^{-1}) 25 μl and 1·0 M NaOH (25 μl) were added to plasma (100 μl). After vortexing, the samples were extracted with 1·0 ml of methylene chloride–n-pentane (50:50 v/v) and dried under nitrogen at 40 °C. The residue was reconstituted with 100 μl of mobile phase. Chromatographic separation was achieved with a 10 μm C18 (250 mm × 4·6 mm i.d.) column and a mobile phase of 50% methanol and 50% 0·005 M sodium acetate buffer (pH 4·3) at a flow rate of 1·6 ml min^{-1}. Ultraviolet absorbancy was monitored at 254 nm and peak area ratios were determined. Antipyrine extraction recovery was the same from water, plasma, and Fluosol-DA (approximately 80%). The standard curve was linear over the range of observed concentrations, and the assay's coefficient of variation was 3·7% at a concentration of 10 μg ml^{-1}.

The plasma concentration-time data were fitted with a one-compartment open model using the non-linear least-square regression program NONLIN (Metzler et al 1974). The significance of differences between any group and control were assessed using the Wilcoxon summed ranks test. A probability level of $P < 0·05$ was considered statistically significant.

RESULTS

21 of 23 animals survived Fluosol-DA exchange and 13 of 14 animals survived saline exchange. The average weight loss was 16 g in III, 14 g in IV, and 30 g in V. Animals exchanged with normal saline lost 7 g (VII) and gained 2·5 g (VIII). Such weight loss is only temporary, as long term studies have shown that exchanged animals grow comparably with unexchanged controls (Watanabe et al 1979).

The mean pre-exchange haematocrits for animals exchanged with Fluosol-DA and normal saline were 47 and 44, respectively. The average haematocrits after the last exchange were 21 and 24, respectively; the averages just before antipyrine dosing are in Table 1. The haematocrit remained depressed 72 h after exchange with Fluosol-DA or saline. Previous studies have shown that the haematocrit remains depressed longer after a one third exchange vs a near total exchange (Geyer 1973; Zucali et al 1979).

Table 1. Treatment groups.

Group	Exchange fluids	Time (h) between exchange and dosing of antipyrine	Dosing HCT(%)	Dosing FCT(%)
I	None	—	48·1 (2.3)[a]	—
II	Fluosol-DA	0·5	22·8 (3·2)	4·9 (1·1)
III	Fluosol-DA	24	20·6 (2·5)	—
IV	Fluosol-DA	48	25·2 (2·6)	—
V	Fluosol-DA	72	23·2 (2·8)	—
VI	Saline	0·5	23·3 (4·1)	—
VII	Saline	24	18·1 (0·9)	—
VIII	Saline	72	25·9 (2·1)	—

[a] Mean (s.d.).

Group II had an average fluorocrit of 4·9%. PFC particles were not visible at 24, 48, and 72 h after Fluosol-DA exchange.

Antipyrine plasma concentrations showed a monoexponential decline in all groups (see Fig. 1).

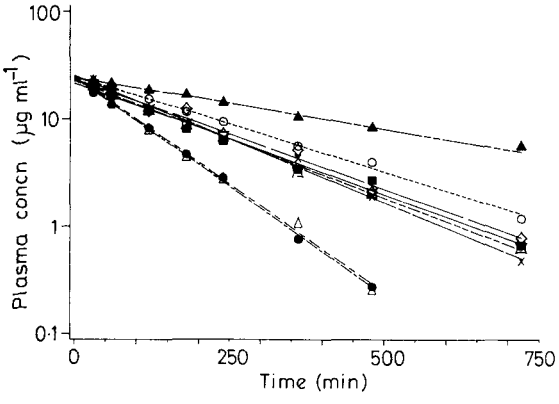

FIG. 1. Mean antipyrine plasma concentrations. Key: □ I, ■ II, ○ III, ● IV, △ V, ▲ III, ◇ VII, × VIII.

Mean disposition parameters for antipyrine are summarized in Table 2. Individual antipyrine clearances (Cl) are shown in Fig. 2. The apparent volume of distribution was not affected by Fluosol-DA or saline exchange. Antipyrine Cl was not significantly different in II, VII, and VIII compared with I. However, Cl significantly decreased with a corresponding half-life increase in III and VI compared with I. Antipryine Cl was therefore initially depressed following saline exchange, but returned to normal within 24 h. The most pronounced changes were observed in groups IV and V where Cl was increased by more than 50% relative to I. Antipyrine Cl was decreased 24 h after Fluosol-DA exchange, but dramatically increased at 48 and 72 h post exchange. There was no difference in the extent of the increased Cl at 48 and 72 h. The same overall Cl trends were observed when normalized for wet liver weight.

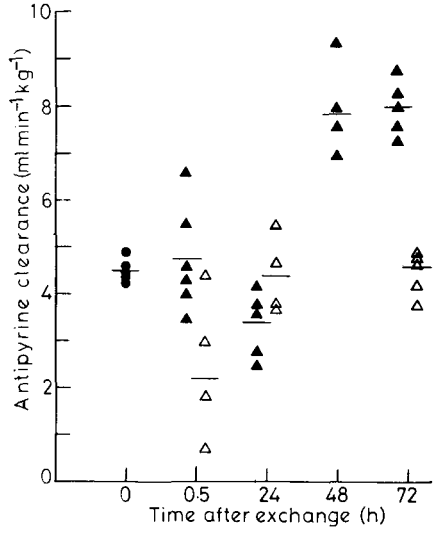

FIG. 2. Individual antipyrine clearances vs time of dosing following the exchange. Key: ● Control; ▲ Fluosol-DA; △ Saline.

Gross observations of excised organs from Fluosol-DA exchanged animals often revealed mottled and haemorrhagic lesioned lungs. Liver and spleen were normal in appearance. There was a significant increase in liver weight normalized for body weight in II versus I, but no differences were observed in any of the other groups. No abnormalities were observed in any of the other organs examined.

Table 2. Mean antipyrine disposition parameters following partial blood exchange.

Parameters	I (n = 5)	II (n = 6)	III (n = 5)	IV (n = 4)	V (n = 5)	VI (n = 4)	VII (n = 4)	VIII (n = 5)
Cl (ml min^{-1} kg^{-1})	4·51	4·74	3·38**	7·85*	8·00**	2·48*	4·43	4·47
	(0·28)[a]	(1·15)	(0·70)	(0·82)	(0·60)	(1·58)	(0·84)	(0·47)
V (ml kg^{-1})	862	839	822	872	777	781	890	847
	(43)	(102)	(57)	(86)	(61)	(63)	(281)	(71)
$t_{\frac{1}{2}}$ (min)	133	130	173**	77*	67**	329	143	133
	(9)	(38)	(27)	(5)	(6)	(256)	(25)	(18)

[a] Standard deviation.
* $P < 0·05$.
** $P < 0·01$.

DISCUSSION

Haematocrits obtained just before antipyrine dosing were reduced by approximately 50% following Fluosol-DA or saline exchange. With the saline-exchanged animals, this would indicate a 50% decrease in the oxygen carrying capacity of the circulating fluid. The Cl decrease observed 0·5 h after saline exchange may therefore be the result of inadequate hepatic oxygenation. Normal Cl values at 24 and 72 h after saline exchange indicate that adequate oxygenation is re-established within 24 h. In contrast, normal Cl values observed at 0·5 h following Fluosol-DA exchange indicate that the emulsion provides the oxygen delivery required to maintain normal antipyrine metabolism.

The short term decrease in antipyrine Cl followed by a prolonged increase in Cl after Fluosol-DA exchange is consistent with a drug-induced enhancement of microsomal enzyme activity. Potent enzyme inducers often produce inhibition before enzyme induction (Serrone & Fujimoto 1962; Kato et al 1964). Potential activity enhancing agents in Fluosol-DA could be one or both of the PFCs and/or Pluronic F-68. Since PFCs are extensively taken up and retained in the liver, one or both of the PFCs are the more likely candidates. Although Pluronic F-68 is taken up by the liver (Willcox et al 1978), the extent of the uptake and the retention time appears to be much less than that of PFCs (Lutz & Metzenauer 1980). The long retention time of the PFCs in the liver could explain their ability both to inhibit and to enhance enzyme activity after a single exposure. At least one PFC, perfluoro-n-hexane, is able to form an enzyme substrate complex with cytochrome P450 (Ullrich & Diehl 1971) which is a requirement of enzyme inducers (Greim 1981). The possibility that one constituent may act as an inhibitor and another constituent as an enhancer should also be considered.

Normal Cl values at 0·5 h after Fluosol-DA exchange followed by a significant decrease in Cl at 24 h indicates a lag time between exchange with Fluosol-DA and inhibition of enzyme activity. This lag time may be because maximal storage of total PFC in the liver is not attained until two days after administration (Lutz & Metzenauer 1980). Normal Cl values at 24 and 72 h following normal saline exchange indicate that changes in antipyrine Cl after Fluosol-DA exchange are not the result of a normal reaction to transfusion stress and/or the loss of normal blood constituents.

Recovery from pentobarbitone-induced anaesthesia was used as an index of hepatic function following intraveneous administration of a 4·4 or 10 g PFC kg^{-1} dose of Fluosol-DA to rats (Lutz & Wagner 1984). A prolongation of sleeping time was observed at 6 h and 1, 2, and 4 days after higher doses, and at 6 h and 1 day following lower doses. This appears to be in direct conflict with the present results. However, while sleeping time is well correlated with pentobarbitone Cl in normal animals, an identical relationship between the pharmacodynamics and the pharmacokinetics may not exist in transfused animals. Prolongation of sleeping time following Fluosol-DA administration may also be associated with changes in the ability of pentobarbitone to cross the blood brain barrier, and/or changes in drug interactions at the receptor site. A previous study has shown that the sleeping time is prolonged when pentobarbitone is administered with a compound which competes for serum protein binding (Pagnini 1971). The concentration of serum proteins, and hence binding capacity, would be decreased in transfused animals, suggesting that brain pentobarbitone concentrations may be increased in these animals.

Pharmacokinetic studies of phenytoin, diazepam, morphine, and antipyrine have also been conducted in animals administered PFC emulsions. Matsumoto et al (1983) examined the disposition of phenytoin in the rat 24 h after a 90% blood exchange with Fluosol-43, a 20% emulsion of perfluorotributylamine. They observed a significant decrease in phenytoin Cl with no change in the apparent volume of distribution. However, the decrease in phenytoin Cl was greater than that observed for antipyrine Cl in the present study. This may be due to differences in the PFC emulsion dose (90 vs 50% exchange); a higher dose of PFC would be expected to produce more inhibition. Alternatively, different PFC emulsions may vary with respect to the degree of metabolic inhibition produced.

Kemner et al (1984b) reported that diazepam and antipyrine pharmacokinetics in lightly anaesthetized rats were unchanged 0·5 h following exchange with 25 ml of Fluosol-DA. This observation agrees with results we have obtained. In contrast, a significant decrease in morphine Cl has been reported in rats immediately following 70% Fluosol-DA exchange (Kemner et al 1984a). The primary biotransformation pathway for morphine is glucuronidation (Yeh 1975) whereas the primary metabolic pathways of antipyrine, phenytoin, and diazepam involve the mixed function oxidase system. Therefore, it appears that several enzymatic pathways may be affected following PFC emulsion administration. The im-

mediate inhibitory response observed with morphine could be due to a greater sensitivity of this pathway to enzyme inhibition and/or the larger PFC emulsion dose administered. It should be noted that morphine Cl in the normal rat is 17 ml min^{-1} kg^{-1} (Kemner et al 1984a) which approaches hepatic blood flow (20 ml min^{-1} kg^{-1}) (Lutz & Metzenauer 1980). Therefore, changes in morphine Cl could also be the result of changes in hepatic blood flow secondary to PFC emulsion exchange. A significant decrease in hepatic blood flow has been reported 0·5 h after Fluosol-DA exchange (Bizot & Rink 1985) or 12 and 24 h following Fluosol-DA stem emulsion administration in the rat (Lutz & Metzenauer 1980).

The results of the present study, as well as previous investigations, indicate that PFC emulsions can have a significant effect on hepatic metabolism. The metabolic effects of PFC emulsions are complex in that there appears to be a time and dose dependence. These results suggest that conventional dosing regimens for agents which undergo extensive hepatic metabolism may not be applicable in patients transfused with Fluosol-DA.

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