After intramuscular administration of the suspension in oil, the elimination half-life is about 3·5 times that after oral administration, and although the sampling scheme provided no samples after 7 h after the oral administration, extrapolation indicates that artemisinin concentrations in serum are detectable much longer after intramuscular administration than after oral administration. This apparent discrepancy in elimination half-lives is explained by the phenomenon that at low absorption rates and rapid elimination rates the absorption rate interferes with the apparent elimination rate. The relatively large variability in peak concentration and duration is seen more often with intramuscular injections. The shape (area) of the i.m. depot and/or the activity of the volunteer (blood-flow through the muscle) may affect the release rate.

The bioavailability of the aqueous intramuscular formulation seems to be much lower than the oily intramuscular formulation. A value of 49·7% is mentioned in rat studies (Niu Xinyi et al 1985). However, the observation time is not long enough to measure the release reliably. An explanation might be that the aqueous suspension behaves like an intramuscular depot with very slow release characteristics.

Factors affecting drug release from intramuscularly administered suspensions include solubility, presence of solvent and aggregation status. Artemisinin is poorly soluble in oil as well as in water. At the site of injection, the aqueous solvent is more rapidly cleared by spreading and distribution than oily solvents, which may cause aggregation of artemisinin particles and subsequent lowering of the release rate. Such a slow release is probably not clinically useful.

A similar phenomenon is probably the cause of the poor absorption of artemisinin from the rectal aqueous suspension. In this case the depot is removed daily by defaecation, therefore another approach for a rapid rectal administration is necessary.

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Influence of the method of Fluosol-DA administration on antipyrine metabolism in the rat

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Abstract—Antipyrine disposition has been determined in the rat following administration of Fluosol-DA by an intravenous infusion without blood removal or a haemodilution procedure, and compared with data from sham haemodiluted rats (blood removed and returned) and control rats which only received antipyrine. Antipyrine total body and renal clearance and the formation clearance of two of its metabolites were affected differently at 48 h by the pretreatments. The haemodilution procedure enhanced, the sham haemodilution reduced, and the intravenous infusion had no effect on the phenobarbitone inducible cytochrome P450 isoenzyme activity.

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Animal studies have shown that Fluosol administration alters the microsomal and non-microsomal mediated metabolism of drugs (Shrewsbury & White 1990a). This was not unexpected as the perfluorochemical (PFC) emulsion particles of Fluosol are taken up by the reticuloendothelial system, and reach a maximum hepatic content in two days (Lutz et al 1982; Lowe & Bollands 1985).

Two methods of administering Fluosol have been predominantly used in animal studies: infusion (administering Fluosol without removing blood); and haemodilution (administering Fluosol while removing blood). This investigation was undertaken to determine if the method used to administer Fluosol affected the microsomal metabolism of antipyrine, which is extensively metabolized by the microsomal mixed function

oxidase system and is commonly used to assess hepatic metabolism in man and animals (Rhodes & Houston 1983).

Materials and methods

Fluosol (Fluosol-DA) donated by Alpha Therapeutic Corporation (Los Angeles, California), was prepared as directed within 0·5 h of use. Antipyrine, phenacetin, 4-hydroxyantipyrine (4-OH) and HPLC grade solvents and buffers were obtained from commercial sources and used without further purification. 3-Hydroxymethylantipyrine (3-OHME) was the gift of Dr D. D. Breimer, University of Leiden. Female Sprague-Dawley rats, 173–228 g, were cannulated and received 40 mL kg^{-1} of Fluosol either by direct intravenous infusion without blood removal (Infn) or by the haemodilution procedure of Shrewsbury et al (1988). To differentiate between the effects of Fluosol haemodilution and the physical mechanics of the haemodilution procedure, sham groups (Sham) were included each time. These animals underwent the same haemodilution procedure as the groups given Fluosol, except their removed blood was returned. Control groups which were not pretreated in any manner, were included each time to show the baseline effects of cannulation on antipyrine disposition.

All animals were cannulated with Silastic tubing in the right jugular vein under light ether anaesthesia 24 h before any pretreatment. Antipyrine disposition was determined at 24, 48 and 72 h after the pretreatment by administering a single intravenous dose (20 mg kg^{-1}) through the implanted cannula. Animals were housed in metabolism cages and urine was collected over sodium metabisulphite for 24 h after antipyrine dosing.

The HPLC analysis of plasma and urinary antipyrine and its metabolites was as previously reported (Shrewsbury et al 1988). The urine extraction procedure was modified by not dividing the sample into two 0·8 mL aliquots, but extracting it first using the acidic and then the basic procedure. Calculation of the disposition parameters and statistical analysis of data were as previously reported (Shrewsbury et al 1988). The percent of metabolite excreted in urine was calculated as the moles of excreted metabolite divided by the moles of dosed antipyrine. The formation clearance (CL_F) of the 3-OHME and 4-OH metabolites was calculated as the product of the percent of metabolite excreted in urine and the antipyrine total body clearance.

Results

Table 1 shows the averaged antipyrine disposition parameters calculated from the plasma concentration-time profiles of the individual animals. The control data show that antipyrine total body clearance (CL) is significantly decreased at 72 h, but that the antipyrine renal clearance (CL_R) or the formation clearance (CL_F) of both metabolites did not contribute to the decrease (see Table 2). As expected, antipyrine $t_{\frac{1}{2}}$ tended to increase as CL decreased, but none of these increases were statistically significant.

Sham haemodiluted animals, when compared with controls, showed that the physical mechanics of removing and returning blood does influence antipyrine disposition. At 48 h, antipyrine CL and the apparent volume of distribution (V_d) were significantly decreased. The decline in CL was accompanied by significant decreases in CL_R and metabolites' CL_F. Reductions in V_d, CL, CL_R, and the metabolites' CL_F were still evident at 72 h.

After Fluosol haemodilution, antipyrine CL and V_d were significantly reduced at 24 h, but the CL was increased at both 48 and 72 h compared with the corresponding control. The dramatic increase in CL at 48 h was due in part to a two fold increase in the CL_F of the 3-OHME metabolite. These data show that the change in antipyrine disposition following sham haemodilution is opposite that following Fluosol haemodilution.

Table 1. Antipyrine disposition parameters using different methods to administer Fluosol-DA. All values are mean ± s.d. n = 5–6.

	24 h				48 h				72 h			
	Cont	Sham	Fluo	Infn	Cont	Sham	Fluo	Infn	Cont	Sham	Fluo	Infn
$t_{\frac{1}{2}}$	114·7	123·7	78·7*	194·8	163·7	141·3	87·6*	78·5*	178·7	171·3	84·3*	68·9*
(min)	±29·8	±31·3	±13·1	±118·8	±36·6	±58·5	±19·7	±23·4	±56·3	±37·4	±5·9	±15·3
V_d	1247	1051	651*	946	1585	597*	1419	643*	1195	855*	877	574*
(mL kg^{-1})	±221	±379	±225	±468	±431	±104	±296	±442	±259	±121	±222	±140
CL	7·7	5·9	5·6*	3·6*	6·7	3·2*	11·5*	5·3	4·9†	3·6	7·1*	6·1
(mL min^{-1} kg^{-1})	±1·1	±1·1	±1·1	±0·8	±0·9	±1·0	±2·6	±1·9	±1·1	±0·8	±1·3	±2·2

* $P < 0·05$ compared with corresponding control (Cont).
† $P < 0·05$ compared with 24 h control (Cont).

Table 2. Renal clearance of antipyrine and formation clearance (mL min^{-1} kg^{-1}) of the 3-OHME and 4-OH metabolites using different methods to administer Fluosol-DA.

	24 h				48 h				72 h			
	Cont	Sham	Fluo	Infn	Cont	Sham	Fluo	Infn	Cont	Sham	Fluo	Infn
Antipyrine	0·26	0·20	0·20	0·13*	0·28	0·14*	0·22	0·19	0·21	0·10*	0·19	0·19
CL_R	±0·10	±0·04	±0·11	±0·04	±0·08	±0·03	±0·05	±0·19	±0·07	±0·01	±0·10	±0·13
3-OHME	1·49	1·71	1·11	1·01	1·64	0·70*	2·95*	1·30	1·04	0·76	1·38	1·19
CL_F	±0·97	±0·31	±0·25	±0·32	±0·34	±0·24	±0·79	±0·58	±0·29	±0·35	±0·17	±0·60
4-OH	0·84	0·94	0·72	0·46*	1·16	0·50*	1·29	0·72	0·70†	0·63	0·60	0·63
CL_F	±0·24	±0·14	±0·14	±0·10	±0·15	±0·07	±0·22	±0·32	±0·16	±0·10	±0·18	±0·24

* $P < 0·05$ compared with corresponding control (Cont).
† $P < 0·05$ compared with 48 h control (Cont).

Fluosol infusion caused a significant decrease in antipyrine CL only at 24 h with significant decreases in CL_R and in the CL_F of the 4-OH metabolite. The V_d was significantly decreased at 48 and 72 h after Fluosol infusion.

Discussion

The control data show that cannulation alone causes antipyrine CL to decrease 72 to 96 h after implantation (Table 1), but the decreased CL is not due to a decrease in CL_R or metabolite CL_F. Therefore, cannulation was influencing the other metabolic pathways responsible for antipyrine disposition. The 3-methylantipyrine metabolite was also quantitated in this study, but was not found to be significant, which is consistent with our previous reports (Shrewsbury & White 1988). The cause of the CL decrease with time is not known; cannulation may cause an inflammatory response, which has been shown to decrease the intrinsic clearance of antipyrine in isolated perfused liver and rat hepatocytes (Chindavijak et al 1987, 1988).

Sham haemodilution caused the antipyrine CL to decrease compared with each corresponding control. The decrease at 48 h is partly due to the decreases in CL_R, and metabolites' CL_F. Phenobarbitone-inducible cytochrome P450 isoenzymes increase the CL_F of both metabolites (Rhodes & Houston 1983; Van der Graaff et al 1983; Teunissen et al 1983a), and 3-methylcholanthrene-inducible cytochrome P450 isoenzymes increase the 4-OH-metabolite CL_F while not changing or decreasing the 3-OHME-metabolite CL_F (Van der Graaff et al 1983; Teunissen et al 1983b). Thus the decline in the CL_F values of both metabolites suggest that sham haemodilution inhibits the phenobarbitone-inducible cytochrome P450 isoenzymes. However, a clear inhibition of the isoenzyme activity was not seen at 72 h.

Fluosol haemodilution caused an opposite disposition pattern change compared with sham haemodilution. The CL was dramatically increased at 48 h, and was still significantly increased at 72 h compared with the corresponding control. The increased CL involve an increase in the 3-OHME CL and to some extent the 4-OH metabolite CL_F, but must also involve increases in the other metabolic pathways. It appears that the presence of Fluosol overshadows the reduction in phenobarbitone-inducible cytochrome P450 isoenzyme activity which results from the physical mechanics of haemodilution.

Fluosol infusion caused a decreased CL, CL_R, and 4-OH metabolite CL_F at 24 h. However, Fluosol infusion did not enhance either metabolites' CL_F at 48 h although Fluosol haemodilution did. This unexpected observation cannot be explained from the data in this study. However, it must also be considered in the light of the Fluosol haemodilution data. At 48 h, the antipyrine CL was significantly increased after Fluosol haemodilution; at 72 h, antipyrine CL was significantly increased compared with the 72 h controls only because the cannulation effect reduced the 72 h control CL value. Thus, it is likely that antipyrine CL is only elevated at 48 h, and has returned to normal values by 72 h. The changes in the metabolites' CL_F confirm this pattern. This is not consistent with reports that a single administration of the PFCs in Fluosol increases the cytochrome P450 content for at least 72 h (Obraztsov et al 1985, 1988; Grishanova et al 1987, 1988; Huang et al 1987), and that the induction remains for several days or months (Obraztsov et al 1985; Grishanova et al 1987; Huang et al 1987); if the isoenzymes increase as suggested in these reports, antipyrine CL and the 3-OHME and possibly the 4-OH metabolite CL_F should remain elevated at 72 h instead of returning to normal levels.

Thus a generalized conclusion is that at 48 h, the three pretreatments had different effects on antipyrine CL, CL_R, and the CL_F of both metabolites and these effects are mediated through phenobarbitone-inducible cytochrome P450 isoenzymes. Sham haemodilution reduces, Fluosol haemodilution enhances, and Fluosol infusion has no influence on the isoenzyme activity. These effects occur only at 48 h, and although they involve cytochrome P450 isoenzymes, they are not due to an induction of the cytochrome P450 isoenzyme content. It is possible that a transitory substance is released as the result of the haemodilution procedure which manifests its effect on cytochrome P450 isoenzyme activity only after 48 h and that Fluosol infusion, which did not involve the haemodilution procedure, did not cause the release of such an endogenous compound. Taking this hypothesis further, our data would suggest that sham haemodilution caused the release of a substance(s) different from that released after Fluosol haemodilution.

While sham haemodilution and Fluosol infusion cause the same change in V_d at 48 and 72 h, it is most likely that each pretreatment involves different mechanisms with the same overall effect. A detailed discussion of the literature and the mechanisms which might be responsible for the V_d changes has been reported elsewhere (Shrewsbury & White 1990b).

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