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The effect of Fluosol-DA and Hespan haemodilution on *S*-warfarin elimination in the rat

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Abstract—Fluosol and Hespan haemodilution in rats did not change *S*-warfarin elimination within 72 h although previous studies had indicated that Fluosol haemodilution caused an increased cytochrome $P450_b$ and $P450_e$ activity at 48 h. This study showed that the increased activity at that time was specific for those isoenzymes.

Perfluorochemical (PFC) emulsions are being investigated for a variety of clinical applications because of their ability to dissolve oxygen. Studies in the rat have shown that a single PFC emulsion administration can increase the hepatic cytochrome P450 content for several months (Huang et al 1987) and that perfluorodecalin and perfluorotripropylamine, the two PFCs in Fluosol-DA (Fluosol), induce the phenobarbitone inducible cytochrome $P450_b$ and $P450_e$ isoenzymes after 72 h (Grishanova et al 1987, 1988; Obraztsov et al 1988). Previous work in this laboratory had shown that Fluosol increased the phenobarbitone inducible cytochrome P450 isoenzyme activity at 48 h but not at 72 h after haemodilution (Shrewsbury & White 1990). This investigation was undertaken to determine if Fluosol haemodilution caused a non-specific enhancement of cytochrome P450 isoenzyme activity in the first 72 h following adminstration in the rat.

Non-specific cytochrome P450 isoenzyme activity was assessed using *S*-warfarin elimination as the marker, as its metabolism is mediated by several cytochrome P450 isoenzymes in the rat liver (Bachmann et al 1988; Kaminsky 1989). *S*-Warfarin was used instead of the racemic mixture since the two enantiomers differ in their elimination kinetics (Yacobi & Levy 1977), and the use of a single enantiomer would circumvent possible interpretive problems in data analysis. The effects of Fluosol haemodilution were compared with haemodilution with Hespan (6% hydroxyethyl starch, HES) as Fluosol contains 3% HES, and both PFCs and HES are taken up by the reticuloendothelial system (Messmer 1988; Lowe 1988).

Materials and methods

Albino, male, Sprague Dawley rats, 297–357 g, were separated into three groups. The left jugular vein was cannulated under light ether anaesthesia 24 h before any pretreatment or *S*-warfarin dosing. Two groups (FLUO, n = 6; HESP, n = 8) were haemodiluted with 40 mL kg^{-1} of Fluosol or Hespan using a published procedure (Shrewsbury et al 1988). This haemodilution procedure reduces the haematocrit by approximately one-half. The control group (n = 6) was not haemodiluted.

Thirty min after Fluosol or Hespan haemodilution, all groups received an intramuscular injection of 4 mg kg^{-1} of vitamin K_1 (Bachmann et al 1988). After 10 min, 4 mg kg^{-1} of *S*-warfarin was administered through the implanted cannula. Blood samples were collected between 16–24, 40–48, and 64–72 h after *S*-warfarin administration. Serum *S*-warfarin concentrations were determined by HPLC, and the overall elimination rate constant (K_{el}) within each 8 h period was calculated from the slope of the serum concentration-time profile. Half-life ($t_{\frac{1}{2}}$) was calculated as $0.693/K_{el}$. Differences in K_{el} and $t_{\frac{1}{2}}$ between groups were determined by the Wilcoxon two sample test (SAS), considering $P < 0.05$ as statistically significant.

To each serum sample (0·1 mL), 0·1 mL of 1 M HCl, 0·05 mL of internal standard (4-chlorowarfarin, 1 mg L^{-1} in methanol), and 0·25 mL of methanol were added. The sample was vortexed (30 s), centrifuged at 13000 *g* (4 min), and the supernatant was injected into the HPLC. Duplicate *S*-warfarin injections were made using an Econosil C_{18}, 5 μm column (4·6 i.d. × 250 mm), a mobile phase of 1·5% glacial acetic acid : acetonitrile (55 : 45 v/v) adjusted to pH 4·7 with 1 M NaOH, and a flow rate of 1·4 mL min^{-1}. Fluorescence detection with an excitation wavelength of 320 nm and emission wavelength of 390 nm was used. Standard curves were prepared (50–4000 ng mL^{-1}) using serum from donor control animals or animals haemodiluted with the appropriate haemodiluent.

Results

Table 1 shows the mean K_{el} for each 8 h period. Fluosol and Hespan haemodilution decreased *S*-warfarin K_{el} compared with control, although none of the reductions were statistically different. Table 2 shows the mean $t_{\frac{1}{2}}$ for each 8 h period. Again, both Fluosol and Hespan haemodilution slowed *S*-warfarin elimination, although none of the groups were statistically different from control.

Discussion

The study design to collect *S*-warfarin blood samples during three 3 h periods was selected so that all the blood samples would

Table 1. Averaged *S*-warfarin elimination rate constant (K_{el} h^{-1}) after Fluosol or Hespan haemodilution (mean ± s.d.).

	Collection times (h)		
	16–24	40–48	64–72
Control	0·062 ± 0·063	0·077 ± 0·089	0·065 ± 0·044
FLUO	0·048 ± 0·024	0·056 ± 0·027	0·026 ± 0·009
HESP	0·028 ± 0·007	0·045 ± 0·028	0·029 ± 0·010

Table 2. Averaged *S*-warfarin half-life ($t_{\frac{1}{2}}$ h) after Fluosol or Hespan haemodilution (mean ± s.d.).

	Collection time (h)		
	16–24	40–48	64–72
Control	20·6 ± 15·4	20·2 ± 17·9	16·6 ± 13·7
FLUO	19·3 ± 12·0	16·4 ± 10·2	29·3 ± 11·6
HESP	25·7 ± 6·0	20·8 ± 11·3	27·6 ± 14·4

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be collected during the terminal phase. Also, previous studies had also shown that Fluosol increased cytochrome $P450_b$ and $P450_e$ activity at 48 h, but not at 72 h (Shrewsbury & White 1990). Separating the blood sample collection into three periods would allow any isoenzyme enhancement to be clearly identified.

S-Warfarin $t_{\frac{1}{2}}$ reportedly ranges from 10 to 48 h with a mean of 22·4 h in the rat (Yacobi & Levy 1977; Yacobi et al 1980). The control $t_{\frac{1}{2}}$ data in Table 1 were consistent with these reports. The results further showed that neither Fluosol nor Hespan haemodilution significantly altered cytochrome P450 isoenzyme activity before 72 h. This is somewhat unexpected as Fluosol haemodilution markedly enhanced antipyrine clearance at 48 h. Antipyrine clearance is mediated by the phenobarbitone inducible cytochrome $P450_b$ and $P450_e$ isoenzymes, which are the same isoenzymes induced by the two Fluosol PFCs (Grishanova et al 1987, 1988; Obraztsov et al 1988). It is possible that Fluosol did indeed enhance the phenobarbitone inducible cytochrome P450 isoenzymes at 48 h, but that their overall contribution to *S*-warfarin metabolism is minor. Cytochrome $P450_b$ and $P450_e$ reportedly constitute 6·0 and 2·5%, respectively, of the cytochrome P450 isoenzymes (Grishanova et al 1988).

There was one other notable observation in this study. In the control group, the first *S*-warfarin serum concentration (ca 16 h) ranged from 1489–1951 ng mL^{-1}. In the FLUO group, one-half of the animals had serum concentrations from 1581–1709 ng mL^{-1}, and the remainder ranged from 778–961 ng mL^{-1}. In the HESP group, 2 animals had serum concentrations of 1283 and 1637 ng mL^{-1} but the remainder ranged from 513–724 ng mL^{-1}. These ranges suggest that the *S*-warfarin apparent volume of distribution (V_d) increased after Fluosol and Hespan haemodilution. It was not possible to measure V_d in this study because of its design, but the result was not unexpected. An increased *S*-warfarin free fraction has been shown to increase the *S*-warfarin V_d (Yacobi et al 1980). An increased free fraction could result from the decreased serum protein concentration following haemodilution, or a displacement of *S*-warfarin from serum protein binding sites by either Fluosol or Hespan or an endogenous substance released secondary to haemodilution. The displacement of racemic warfarin from albumin by Fluosol in-vitro has been reported (Parsons et al 1985; Parsons 1987; Parsons & Nadkarni 1987), and McCoy et al (1989) have reported an array of endogenous substances released after severe Fluosol haemodilution. Protein binding studies will be necessary to delineate these interactions. A second mechanism that would account for the reduced *S*-warfarin serum concentrations after Fluosol or Hespan haemodilution is an intravasation of interstitial fluid. Hespan, which is 6% HES in saline, is well known for its plasma expanding properties. The Fluosol formulation used in this investigation contained 3% HES, and showed the same volume effects as Hespan, but to a lesser degree.

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References

Bachmann, K. A., Jahn, D., Yang, C., Schwartz, J. (1988) The use of single sample clearance estimates to probe hepatic drug metabolism in rats: II. Xenobiotica 18: 161–167

Grishanova, A. Y., Obraztsov, V. V., Shekhtman, D. G., Lyakhobich, V. V. (1987) Phenobarbital type of induction of cytochrome P450 of liver microsomes by perfluorodecalin. Biokhimiya 52: 1138–1143

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

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

Kaminsky, L. S. (1989) Warfarin as a probe of cytochromes P-450 function. Drug Metab. Rev. 20: 479–487

Lowe, K. C. (1988) Biological assessment of perfluorochemical emulsions. In: Lowe, K. C. (ed.) Blood Substitutes: Preparation, Physiology and Medical Application. VCH Publishers, New York, pp 149–172

McCoy, L. E., Elliott, L. A., Lucas, C. E., Ledgerwood, A. M. (1989) Regenerative responses to exchange perfusion. In: Chang, T. M. S., Geyer, R. P. (eds) Blood Substitutes. Marcel Dekker, New York, pp 575–583

Messmer, K. (1988) Characteristics, effects and side-effects of plasma substitutes. In: Lowe, K. C. (ed) Blood Substitutes: Preparation, Physiology and Medical Application. VCH Publishers, New York, pp 51–70

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

Parsons, D. L. (1987) Perfluorochemical emulsion effect on warfarin binding by fraction V human albumin. Arch. Int. Pharmacodyn. 286: 23–30

Parsons, D. L., Nadkarni, S. R. (1987) Effects of perfluorochemical emulsion components on human albumin binding of warfarin. Ibid. 288: 165–174

Parsons, D. L., Ravis, W. R., Clark, C. R. (1985) Binding of warfarin by human albumin in the presence of a perfluorochemical blood substitute. Ibid. 277: 4–14

Shrewsbury, R. P., Oliver, S. R., Anderson, W. T., Lewis, L. M., White, L. G. (1988) The effect of varying percentages of haemodilution with Fluosol-DA or normal saline on antipyrine metabolism in the rat. J. Pharm. Pharmacol. 40: 392–398

Shrewsbury, R.P., White, L.G. (1990) Evaluation of cytochrome P-450 mediated metabolism of antipyrine in the rat at different extents of Fluosol-DA hemodilution. Res. Commun. Chem. Path. Pharmacol. 67: 411–414

Yacobi, A., Levy, G. (1977) Comparative pharmacokinetics of coumarin anticoagulants XXIX: elimination kinetics and anticoagulant activity of (S)-(-)-warfarin in rats before and after chronic administration. J. Pharm. Sci. 66: 1275–1277

Yacobi, A., Slattery, J. T., Levy, G. (1980) Comparative pharmacokinetics of coumarin anticoagulants XLVI: effect of treatment with phenobarbital on pharmacokinetics of (S)-(-)-warfarin in rats. Ibid. 69: 634–363