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Effect of moderate haemodilution with Fluosol-DA or normal saline on low-dose phenytoin and (±)-5-(4-hydroxyphenyl)-5-phenylhydantoin kinetics

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Phenytoin kinetics were determined in the rat following moderate (50%) blood exchange with either Fluosol-DA or normal saline. Rats received an intravenous phenytoin dose (10 mg kg^{-1}) 0·5, 24, 48, or 72 h after exchange and were compared with non-exchanged controls. Phenytoin $t_{\frac{1}{2}}$ was not altered by exchange with either fluid. Its Cl and V_d were decreased and AUC increased 24, 48, and 72 h after saline exchange and 24 h after Fluosol-DA exchange. (±)-5-(4-Hydroxyphenyl)-5-phenylhydantoin (HPPH), a major metabolite of phenytoin, showed a decreased $t_{\frac{1}{2}}$ and V_{HPPH} 24, 48, and 72 h after exchange with either fluid; $t_{\frac{1}{2}}$ was also reduced 0·5 h after Fluosol-DA exchange. The decreased V_d and V_{HPPH} may result from changes in cardiac output secondary to haemodilution, or may represent a redistribution in the microcirculation. Fluosol-DA appears to enhance phenytoin and HPPH metabolism 48 and 72 h after exchange.

Perfluorochemical (PFC) emulsions are currently being investigated as potential blood substitutes because of their plasma-expanding property and their ability to dissolve oxygen (Gould et al 1985). Animal studies have demonstrated that they can sustain life (Hardy et al 1983; Yokoyama et al 1984) while in man they have been used clinically for blood loss replacement, oxygen delivery to ischaemic tissues, and severe anaemia (Ohyanagi & Saitoh 1982; Mitsuno et al 1982; Tremper et al 1985).

PFC particles are captured by the reticuloendothelial system (RES) and distributed primarily to the liver and spleen, and secondarily to the kidney, bone marrow, and lungs (Mitsuno et al 1984). Upon release from these organs, monocytes carry the PFCs to the lungs where exhalation is the primary route of elimination. Urinary and faecal excretion of PFCs are insignificant and there is no metabolic degradation of these compounds (Geyer 1982, 1983).

The time course of PFC uptake in the liver shows that the maximal storage occurs two days after PFC administration (Lutz & Metzenauer 1980; Lutz et al 1982). PFC particles have been found in Kupffer cells, hepatocytes, mononuclear phagocytes, and 'foamy' macrophages (Lutz et al 1982; Mitsuno et al 1984; Lowe & Bollands 1985). Their presence in the liver may alter normal hepatic function, such as hepatic blood flow, biliary excretion, or microsomal enzymatic activity. Studies with indocyanine green have suggested that hepatic blood flow or enterohepatic cycling may be compromised (Lutz & Metzenauer 1980; Bizot & Rink 1985), while studies with sulphamethazine (Kemner et al 1984b), pentobarbitone (Lutz & Wagner 1984), phenytoin (Matsumoto et al 1983; Matsumoto-Kikuchi et al 1983), and morphine (Kemner et al 1984a) suggest that hepatic metabolism may be compromised as well. A time related effect may also play a role in the total influence of PFC emulsions on drug disposition. It was reported that the penicillin half-life was significantly longer in the rat, 0·5 h, but was unchanged 48 h after a 25 mL Fluosol-DA exchange (Hodges et al 1983, 1984).

Fluosol-DA (Fluosol) is a commercially prepared PFC emulsion containing two PFCs, perfluorodecalin and perfluorotripropylamine. The intravascular half-life of the two PFCs in Fluosol is approximately 10 to 13 h in the rat, and is undetectable in the blood after 2 days (Lutz & Metzenauer 1980; Yokoyama et al 1982). The half-life of perfluorodecalin in the rat liver, spleen, lungs, kidney, and adipose tissue is 7 to 8 days, while that of perfluorotripropylamine in the tissues is greater than 60 days (Geyer 1982; Yokoyama et al 1984; Mitsuno et al 1984).

Phenytoin undergoes extensive hepatic metabolism, only 1 to 2% being recovered unchanged in human and rat urine (Maynert 1960; Borga et al 1979). 5-(4-Hydroxyphenyl)-5-phenylhydantoin (HPPH) is the principal metabolite in both species (Atkinson et al 1970; Chang et al 1972), and is subsequently glucuronidated with less than 6% of

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the free metabolite excreted unchanged in the urine (Hoppel et al 1977). Phenytoin also displays a pronounced dose-dependent elimination in both rats and man with linear kinetics observed at 10 mg kg^{-1} doses and 40 mg kg^{-1} doses resulting in non-linear kinetics (Ashley & Levy 1973; Perucca et al 1978). In rats, the dose-dependency is the result of HPPH inhibition of phenytoin hydroxylation to HPPH (Ashley & Levy 1972; Vicuna et al 1980) while in man phenytoin biotransformation is influenced by enzyme saturation (Richens 1979).

Two previous studies reported phenytoin disposition immediately after an exchange with Fluosol-43 (Matsumoto et al 1983; Matsumoto-Kikuchi et al 1983). These reports are a beginning in understanding the influence of PFC emulsions on phenytoin disposition. There has been no investigation of the time course influence of PFC emulsion exchange on phenytoin or HPPH disposition. The present study reports the effect of moderate haemodilution with Fluosol on low-dose (10 mg kg^{-1}) phenytoin and HPPH kinetics, and investigates the exchange effects for 72 h post-exchange.

MATERIALS AND METHODS

Materials

Fluosol was donated by Alpha Therapeutics, Inc. (Los Angeles, CA) and prepared as directed within 0·5 h of use. For intravenous administration, sodium phenytoin (Parke-Davis Co., Morris Plains, NJ) was diluted to 5 mg mL^{-1} with propylene glycol–ethanol–water (40 : 10 : 50). HPLC standard curves were prepared using methanolic stock solutions of 5,5-diphenylhydantoin (phenytoin), 99+% and (±)-5-(4-hydroxyphenyl)-5-phenylhydantoin (HPPH), 99+% (Aldrich Chemical Co., Milwaukee, WI), and plasma from unexchanged rats, or rats exchanged with either Fluosol or 0·9% NaCl (saline) as appropriate. HPLC solvents and buffers were obtained from commercial vendors and filtered (0·45 mm filter) before use. Male Sprague-Dawley albino rats, 265 to 487 g, were used without abstinence from food or water.

Methods

Phenytoin and HPPH kinetics were examined in unexchanged rats and rats moderately exchanged with either Fluosol or saline in a parallel designed study. Moderate blood exchange was used to avoid the need for supplemental oxygen. Saline exchanged groups were included to differentiate between changes in phenytoin and HPPH disposition due to the Fluosol itself and changes due to haemodilution alone.

A silastic cannula was implanted in the right jugular vein under light ether anaesthesia 24 h before any exchange or drug administration. Animals recovered from anaesthesia within 1 h, and were fully conscious and unrestrained during all other procedures. Patency of the cannula was maintained daily with heparinized saline (20 u mL^{-1}). One group of rats was not exchanged but received an intravenous phenytoin dose of 10 mg kg^{-1} 24 h after surgery (see Table 1). To demonstrate that cannulation alone did not alter phenytoin disposition, two additional groups received the drug 72 and 96 h after surgery (not shown in Table 1). Rats in groups II to IX were partially exchanged with Fluosol or saline (40 mL kg^{-1}) and received the phenytoin dose 0·5, 24, 48, or 72 h after the exchange. The exchange fluid was administered in three equally divided doses at 0·5 h intervals. At each interval, a volume of blood equal to ½ the volume of the exchange fluid to be infused was removed just before and 1 min after infusion of the exchange fluid. The exchange fluid volume was infused over a period of 1 min. The haematocrit (HCT) was determined just before the exchange procedure and DPH dosing.

Table 1. Treatment group designation and average haematocrit at phenytoin (DPH) dosing.

Group	Exchange fluid	Time between exchange and DPH dosing	Average haematocrit at time of DPH dosing (%)
I	None (control)	–	46·9 (1·4)†
II	Normal saline	0·5 h	18·1 (3·7)*
III	Normal saline	24 h	27·0 (8·1)*
IV	Normal saline	48 h	24·5 (3·5)*
V	Normal saline	72 h	25·9 (2·6)*
VI	Fluosol-DA	0·5 h	24·4 (6·6)*
VII	Fluosol-DA	24 h	27·0 (3·1)*
VIII	Fluosol-DA	48 h	28·6 (4·1)*
IX	Fluosol-DA	72 h	29·9 (3·8)*

† Mean (s.d.).
* Statistically different from control ($P < 0·05$).

Blood samples (0·3 mL) were collected at 30, 45, 60, 90, 120, 150, 180, 240 and 300 min after dosing, in polypropylene tubes containing heparin (33 u mL^{-1} of blood) for phenytoin and HPPH plasma concentration analysis. The samples were centrifuged at 12 000*g* for 5 min and the plasma harvested and frozen. For HPLC analysis, 100 µL of plasma was taken, 100 µL of a suspension con-

taining 20 000 u mL^{-1} of β-glucuronidase (Patella vulgata, Type L-II, Sigma Chemical Co., St. Louis, MO) in 0·2 M sodium acetate (pH) was added, and incubated in a water bath at 37 °C for 20 h. 10 μL of phenacetin (20 μg mL^{-1} in methanol, internal standard) and 1 mL of ethyl acetate was added, the mixture shaken for 15 min, centrifuged for 2 min, and the organic layer collected by freezing the aqueous layer in dry ice. The organic solvent was evaporated under nitrogen, reconstituted in 100 μL methanol, and 40 μL injected into the instrument.

Phenytoin and HPPH plasma concentrations were determined by HPLC with a 10 μm C18 (250 × 4·6 mm i.d.) Alltech column and a mobile phase of acetonitrile–methanol–water (26:2·5:71·5) (pH = 2·6 adjusted with H_3PO_4) at a flow rate of 1·7 mL min^{-1}. Absorbency was monitored at 200 nm (0·005 AUFS) and the peak area ratio of phenytoin or HPPH to internal standard was determined. The standard curves were prepared using plasma from whole blood or plasma from animals which had been exchanged with the appropriate haemodilution fluid. Standard curves were linear over the concentration range of 0·5 to 10·0 μg mL^{-1} for both phenytoin and HPPH with correlation coefficients of 0·93 or better.

Plasma concentration-time data were simultaneously fitted by SAAM27, a non-linear least-squares regression program (Berman & Weiss 1978) with the plasma concentrations weighted by their reciprocal since the coefficient of variation was dependent upon the concentration assayed. The equation to describe phenytoin disposition was:

$$C = C_o e^{-kt}$$

and for HPPH:

$$C = C_m(e^{-k_m t} - e^{-kt})$$

where C_o is the DPH dose administered divided by the DPH apparent volume of distribution (V_d), k is the phenytoin elimination rate constant, t is the time post administration, k_m is the HPPH elimination rate constant, and C_m is the HPPH formation rate constant (k_f) times the phenytoin dose divided by the quantity of the HPPH apparent volume of distribution (V_{HPPH}) times the difference ($k - k_m$) (Gibaldi & Perrier 1982). Parameter estimates of these values were used in subsequent calculations such as phenytoin half-life ($t_{\frac{1}{2}}$), clearance (Cl), and area-under-curve (AUC) and HPPH $t_{\frac{1}{2}}$, HPPH AUC and HPPH fraction (k_f/V_{HPPH}). The significance of difference between any group was assessed with the Wilcoxon Rank Sum Test (SAS Institute, Inc., Cary, NC) with a probability level of $P < 0·05$ considered statistically significant.

RESULTS

There was no significant difference in any phenytoin or HPPH parameter between the three unexchanged (control) groups, and therefore group I was taken as the control for the study. All animals underwent the exchange procedure and data collection protocol as outlined with a 100% survival rate. Animals generally lost weight as the result of either Fluosol or saline exchange; however, 27% of the animals in III to V and VII to IX showed a weight gain and 3% had no weight change. There was no immediate weight change in II and VI. The average weight loss was 6·7 g in III, 4·3 g in V, 13·4 g in VII, and 1·5 g in VIII; average weight gains were 0·8 g in IV and 1·6 g in IX.

The exchange procedure reduced the pre-exchange haematocrit by one-half, indicating a 50% blood exchange (see Table 1). The haematocrit remained depressed at 72 h after exchange with either Fluosol or saline.

Phenytoin and HPPH plasma concentrations displayed a one-compartment disposition as expected. The mean phenytoin and HPPH kinetic parameters are summarized in Tables 2 and 3, respectively. Phenytoin Cl and V_d were statistically decreased (approximately 50%) and HPPH fraction increased (approximately 2- to 3-fold) in III, IV, V, and VII compared with I. HPPH fraction was also statistically increased in VIII and IX, while phenytoin V_d tended to be reduced in those groups. Phenytoin AUC was significantly increased in III, IV, V, and VII; only group VI showed a statistically different HPPH AUC. The AUC ratio (HPPH AUC/phenytoin AUC) was not different from I, although the mean of every group was less than the control.

Phenytoin $t_{\frac{1}{2}}$ in any group was not different from I suggesting that the observed decreased Cl was due to V_d changes alone since Cl equals $(\ln 2)V_d/t_{\frac{1}{2}}$. HPPH $t_{\frac{1}{2}}$ was statistically different from I in III, VI, and VII, and tended to be decreased in other groups.

DISCUSSION

Numerous investigators have demonstrated that animals can survive partial blood exchange with Fluosol without supplemental oxygen (Hodges et al 1983, 1984). A 71% Fluosol exchange without oxygen has been reported (Kemner et al 1984a). Rats in the present study underwent approximately a 50% exchange with Fluosol or saline without casualties. Although most animals lost weight as the result of the exchange, long term studies have shown that exchanged animals grow comparably with unexchanged animals (Watanabe et al 1979).

Table 2. Averaged disposition parameters of phenytoin.

	Groups								
Parameters	I	II	III	IV	V	VI	VII	VIII	IX
N	4	5	5	6	5	5	6	4	4
Half-life	24·2†	24·7	19·9	23·5	26·8	23·0	16·6	15·5	16·1
(min)	5·7	7·1	6·2	12·1	11·3	12·5	3·8	3·3	4·8
Volume	1288·8	1407·3	662·5*	645·7*	646·1*	1560·2	469·0*	855·7	984·0
(mL kg^{-1})	332·5	725·1	219·2	187·0	176·1	422·2	86·8	182·7	188·9
Clearance	37·0	39·3	24·5*	20·8*	17·6*	51·8	20·0*	39·0	43·6
(mL min^{-1} kg^{-1})	4·5	12·7	7·8	5·9	4·0	15·6	3·7	8·2	6·6
AUC	273·3	282·6	464·6*	524·6*	592·5*	208·5	513·7*	266·0	233·6
(μg × min mL^{-1})	34·9	110·7	225·1	185·1	142·8	67·8	86·7	61·5	36·1

† Mean s.d.
* Significantly different from control ($P < 0·05$).

Table 3. Averaged disposition parameters of (±)-5-(4-hydroxyphenyl)-5-phenylhydantoin (HPPH).

	Groups								
Parameters	I	II	III	IV	V	VI	VII	VIII	IX
N	4	5	5	6	5	5	6	4	4
Half-life	128·9†	102·0	65·7*	92·2	89·1	44·3*	74·3*	105·1	63·6
(min)	49·2	47·9	6·9	50·8	54·0	25·4	28·4	67·2	37·8
HPPH fraction	2·7	4·1	7·0*	6·0*	5·3*	4·1	9·6*	5·5*	5·9*
(× 10^5)	0·8	2·0	1·8	2·1	1·5	2·3	3·8	1·5	2·4
HPPH AUC	609·7	484·1	610·0	876·7	825·7	165·5*	757·1	509·4	346·8
(μg × min mL^{-1})	356·0	136·1	170·5	238·7	260·0	55·5	352·8	210·9	158·9
AUC ratio	2·3	2·1	1·4	1·9	1·4	0·9	1·5	1·9	1·5
	1·5	0·6	0·5	1·0	0·3	0·6	0·7	0·4	0·7

† Mean s.d.
* Significantly different from control ($P < 0·05$).

Haematocrits were reduced by approximately 50% as a result of the exchange protocol and levels remained depressed through 72 h (see Table 1). Previous studies have shown that the haematocrit remains depressed longer after a one third exchange compared with a near total exchange (Zucali et al 1979; Gould et al 1983; Mitsuno et al 1984). It has been proposed that animals of the size used in this study have slower haematopoeisis than smaller animals (Geyer 1982).

Haemodilution by any substance will decrease the plasma protein content (Messmer 1975), leading to an increased free plasma drug fraction (f_B). Phenytoin is about 82% plasma protein bound to albumin in the rat (Colburn & Gibaldi 1977), and the albumin concentration is dramatically reduced after haemodilution (Lowe et al 1985). The reduced albumin content would increase phenytoin f_B which would result in an increased V_d (Gibaldi & McNamara 1978). The phenytoin V_d was found not to increase in any group (see Table 2), suggesting that even though f_B is increasing following haemodilution, such changes are not significantly influencing the V_d.

The pharmacokinetic pattern of decreased Cl and V_d, but no change in $t_{\frac{1}{2}}$, has been observed for lignocaine (lidocaine) and procainamide (Klotz 1976; Benowitz & Meister 1978) and quinidine (Ueda & Dzindzio 1978) in patients with congestive heart failure. The decreased V_d was attributed to a decreased cardiac output resulting from the compromised heart. It is well documented that haemodilution increases cardiac output due to a decreased viscosity of the circulatory fluid and decreased peripheral resistance (Kohno et al 1979; Faithfull et al 1984). There is increased flow to the brain, heart, liver, skeletal muscle, splanchnic region and kidneys (Handa et al 1983; Haneda et al 1983; Goslinga 1984). This is followed by a rapid decrease in the circulating blood volume which is reduced by one-third 3 h after the exchange and remains depressed at least 24 h post-exchange (Matsumoto et al 1977; Ohyanagi et al 1979; Watanabe et al 1979).

Phenytoin Cl will be influenced by liver blood flow (Q), f_B, and the hepatic intrinsic clearance (Cl_{INT}) in the following relationship (Nies et al 1976; Oie & Benet 1980):

$$Cl = Qf_BCl_{INT}/Q + f_BCl_{INT}$$

since phenytoin has an extraction ratio of 0·4 to 0·5 in the rat (Shand et al 1975; Rane et al 1977). $f_BCl_{INT}/Q + f_BCl_{INT}$ is defined as the extraction ratio (ER), and therefore phenytoin Cl is influenced by the product:

$$Cl = Q^* \cdot ER$$

Initially in haemodilution, both Q and f_B increase; at a constant Cl_{INT}, Q would decrease ER while f_B would increase ER. Therefore, the relative magnitudes of change in Q and f_B will determine the overall effect of the ER and partially in turn, Cl. In groups II and VI, phenytoin was dosed 0·5 h after the completion of the exchange; therefore, the data are maximally influenced by Q and f_B. V_d did increase though not significantly, probably due to the increased f_B which is approximately the same in both groups since the HCT and percent exchange are similar (see Table 1). Phenytoin Cl was only slightly higher in II but tended to be much higher in VI.

If Q was the only other parameter influencing phenytoin Cl besides f_B, the data suggest that the exchange fluids affect Q differently. Fluosol must increase Q dramatically to cause the increased phenytoin Cl and overcome the decrease in ER that results from increasing Q. Saline must not be increasing Q to the same extent as Fluosol since the phenytoin Cl is not increased as much. Cardiac output, although increased in haemodilution, is also increased based on oxygen demand (Kosugi et al 1979). Perfluorochemicals have been shown to dissolve more than 40% volume oxygen, while saline dissolves 3% volume (Nagasawa et al 1981). Therefore cardiac output should be less in group VI compared with II, not greater. Thus, the data suggest that the third variable, Cl_{INT}, must significantly influence phenytoin Cl, and that Fluosol and saline might have different effects or differing degrees of influence on Cl_{INT}.

Cl_{INT} is a function of the drug partitioning between blood and the hepatocyte (P), the inherent metabolic capability of the hepatocyte (K), and the liver volume (V_L) described by the following relationship (Branch et al 1973):

$$Cl_{INT} = PV_LK$$

It is not known whether haemodilution fluids or an approximate 50% exchange would influence the partitioning of phenytoin between blood and Kuppfer cells or hepatocytes (i.e. P). Schneeberger (1983) reported that ferritin transport was increased across non-fenestrated pulmonary endothelium in the intact animal after severe haemodilution with Fluosol-43, while McCoy et al (1984) reported that Fluosol caused increased leucocyte adhesion, fibrin disposition, and sub-endothelium exposure in pulmonary endothelial cells. Severe haemodilution with dextran 60 caused the tissue-to-blood transport of [^{51}Cr]EDTA from dog skeletal muscle to be slower than blood-to-tissue transport (Dahlberg & Lewis 1975). Whether P changes in the equation for Cl_{INT} given above cannot be determined from the present study.

Liver volume (V_L) might be expected to change in haemodiluted animals as a result of increased microcirculation secondary to the fall in peripheral resistance and increased arterial pressure resulting from increased cardiac output. PFC emulsions cause liver weights to increase, reaching a maximum gain 16 days after an exchange with minimal changes occurring within the time frame of this experiment (Lutz et al 1982). Therefore, V_L is not thought to change significantly within 72 h after Fluosol exchange, and should have little effect on Cl_{INT}.

Therefore, changes in Q, f_B, and the metabolic capacity (K) are thought to be the predominant determinants of the observed data. 0·5 h after exchange with either fluid, Q and f_B both increase; however, phenytoin Cl in VI tended to be higher than I and II, while HPPH $t_{\frac{1}{2}}$ and HPPH AUC were dramatically decreased. After haemodilution, the oxygen capacity of the blood is necessarily reduced. Oxygenation of tissues then becomes dependent upon the oxygen capacity of the remaining blood plus the fluid used in haemodilution. Fluosol has been shown to have a higher oxygen capacity than plasma, dextrans, hydroxyethyl starch, Lactated Ringer's solution, Collin's solution, saline and water (Honda 1983; Kloner et al 1983; Peerless 1983; Tabuchi et al 1983; Gould et al 1985). Thus oxygen delivery to the liver is less following saline haemodilution even with an increased cardiac output compared with Fluosol. Phenytoin is greater than 95% metabolized by the microsomal mixed function oxidases which require molecular oxygen, and HPPH is almost quantitatively glucuronidated (Asconape & Penry 1982). Therefore, the enhanced phenytoin Cl and HPPH elimination after Fluosol might result from adequate or enriched hepatic oxygenation, while saline may be providing limited or insufficient oxygenation. PFC emulsions have been shown to maintain the cerebral cytochrome c oxidase redox state and hepatic cytosolic and mitochondrial redox states better than Krebs-Henseleit-albumin or Krebs-Ringer-bicarbonate solutions, respectively (Goodman et al 1973; Sylvia et al 1982).

24 h after exchange, III and VII show the same

disposition for both phenytoin (decreased Cl and V_d, increased AUC) and HPPH (decreased $t_{\frac{1}{2}}$, increased fraction), but the disposition differed from 0·5 h post-exchange. The intra-vascular half-life of PFCs in Fluosol is 10 to 13 h; thus the PFC content might be reduced approximately 75% after 24 h and any unique influence of Fluosol seen 0·5 h after exchange may have dissipated. The oxygen capacity of the vascular fluid would be reduced in VII, and physiologically may be similar to III. The HPPH $t_{\frac{1}{2}}$ is similar in III and VII, and both are significantly less than the control. It has been suggested that rats have a reserve of glucuronyl transferase and that the functional activity of the enzyme can increase in hepatotoxic animals (Desmond et al 1981; James et al 1981). Such an increased activity could account for the enhanced HPPH elimination in III and VII, and may also be responsible for the very dramatic effect seen in VI.

The volume in which both phenytoin and HPPH distribute is reduced 24 h after haemodilution as indicated by the decreased phenytoin V_d and increased HPPH fraction. This change is possibly due to the ensuing hypovolaemia after exchange or may be a redistribution of the drug in the microcirculation. Such microcirculation redistributions following haemodilution have been reported in hepatic (Kessler & Messmer 1975) and cardiac tissue (Buckberg & Brazier 1975).

48 and 72 h after exchange, the saline exchange groups (IV, V) show the same phenytoin disposition as 24 h after exchange, while the Fluosol groups (VIII, IX) differ from VII. The depressed phenytoin V_d values and increased HPPH fraction in IV and V, since similar to III, suggest that the effects of hypovolaemia or redistribution may persist beyond 24 h post-exchange. But phenytoin Cl and V_d in VIII and IX return toward control values, and HPPH AUC suggests that less metabolite is formed in these groups. Thus it appears that Fluosol may enhance phenytoin hydroxylation to HPPH 48 to 72 h after exchange, and may have some acceleratory effect on the subsequent glucuronidation of HPPH since HPPH $t_{\frac{1}{2}}$ tended to be lower than the control. Such changes do not appear to occur after saline exchange.

Fluosol is taken into the RES while saline is not (Mitsuno et al 1983). The extended PFC retention time in the liver could explain their ability initially to inhibit and then to enhance enzymatic activity after a single exposure. Potent enzyme inducers often produce inhibition before induction (Serrone & Fujimoto 1962; Kato et al 1964). Several PFC compounds have been shown to form an enzyme substrate complex with cytochrome P450 (Geyer 1982) which is a requirement of enzyme inducers (Greim 1981). The possibility that one or more emulsion constituents may act as an inhibitor and/or enhancer should also be considered.

The present study both compares and contrasts with other reports of phenytoin disposition following extreme haemodilution with Fluosol-43 (Matsumoto et al 1983; Matsumoto-Kikuchi et al 1983). Immediately after a 90% exchange and with oxygen supplementation, phenytoin Cl was significantly reduced while $t_{\frac{1}{2}}$ and AUC were increased, with little change in the V_d. In one study (Matsumoto-Kikuchi et al 1983), the exchange percent was decreased to 70% and phenytoin Cl, $t_{\frac{1}{2}}$, AUC and V_d were not altered. But when the supplemental oxygen was decreased, the Cl and $t_{\frac{1}{2}}$ were similar to the 90% exchange with full oxygen supplementation. These studies support the role of blood oxygen capacity on phenytoin disposition as suggested in the present report which further reveals the long term effects of moderate haemodilution on phenytoin disposition.

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REFERENCES

Asconape, J. J., Penry, J. K. (1982) Epilepsia 23 (Suppl. 1): S65–S79

Ashley, J. J., Levy, G. (1972) Res. Comm. Chem. Path. Pharmacol. 4: 297–306

Ashley, J. J., Levy, G. (1973) J. Pharmacokinet. Biopharm. 1: 99–102

Atkinson, A. J., MacGee, J., Strong, J., Garteiz, D., Gaffney, T. E. (1970) Biochem. Pharmacol. 19: 2483–2491

Benowitz, N. L., Meister, W. (1978) Clin. Pharmacokinet. 3: 177–201

Berman, M., Weiss, M. F. (1978) SAAM Manual, U.S. DHEW Publication No. (NIH) 78–180, Washington D.C.

Bizot, W. B., Rink, R. D. (1985) Experientia 41: 1127–1129

Borga, O., Hoppel, C., Odar-Cederlof, I., Garle, M. (1979) Clin. Pharmacol. Ther. 26: 306–314

Branch, R. A., Nies, A. S., Shand, D. G. (1973) Drug Metab. Disp. 1: 687–690

Buckberg, G., Brazier, J. (1975) in: Messmer, K., Schmid-Schonbein, H. (eds) Intentional Hemodilution, Karger, Basel, pp 173–189

Chang, T., Okerholm, R. A., Glazko, A. J. (1972) Res. Comm. Chem. Path. Pharmacol. 4: 13–23

Colburn, W. A., Gibaldi, M. (1977) J. Pharmacol. Exp. Ther. 203: 500–506

Dahlberg, J. B., Lewis, D. H. (1975) in: Messmer, K., Schmid-Schonbein, H. (eds) Intentional Hemodilution, Karger, Basel, pp 34–43

Desmond, P. V., James, R., Schenker, S., Gerkens, J. F., Branch, R. A. (1981) Biochem. Pharmacol. 30: 993–999

Faithfull, N. S., Fennema, M., VanAlphen, W. A., Smith, A. R., Erdmann, W., Essed, C. E., Kok, A., Lapin, R. (1984) Acta Anaesthesiol. Belgica 35 (Suppl.): 69–78

Geyer, R. P. (1982) in: Frey, R., Beisbarth, H., Stosseck, H. (eds) Oxygen Carrying Colloidal Blood Substitutes: Fifth International Symposium on Perfluorochemical Blood Substitutes. W. Zuckschwerdt Verlag, Munchen, pp 214–219

Geyer, R. P. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitute Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 157–168

Gibaldi, M., McNamara, P. J. (1978) Eur. J. Clin. Pharmacol. 13: 373–378

Gibaldi, M., Perrier, D. (1982) Pharmacokinetics, 2nd edition. Marcel Dekker, New York, pp 17–20

Goodman, M. N., Parrilla, R., Toews, C. J. (1973) Am. J. Physiol. 225: 1384–1388

Goslinga, H. (1984) Blood Viscosity and Shock: The Role of Hemodilution, Hemoconcentration and Defibrination. Springer-Verlag, New York, pp 34–35

Gould, S. A., Rosen, A. L., Sehgal, L. R., Moss, G. S. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 331–342

Gould, S. A., Sehgal, L. R., Rosen, A. L., Sehgal, H. L., Moss, G. S. (1985) Ann. Emerg. Med. 14: 798–803

Greim, H. (1981) in: Jenner, P., Testa, B. (eds) Concepts in Drug Metabolism, Part B. Marcel Dekker, New York, p. 225

Handa, H., Nagasawa, S. Yonekawa, Y., Naruo, Y., Oda, Y. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 299–306

Haneda, K., Honda, K., Kanno, M. (1983) Ibid. p. 438

Hardy, R. N., Lowe, K. C., McNaughton, D. C. (1983) J. Physiol. 338: 451–461

Hodges, G. R., Reed, J. S., Hignite, C. E., Snodgrass, W. R. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitute Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 430–431

Hodges, G. R., Worley, S. E., Kemner, J. M., Reed, J. S. (1984) Antimicrob. Agents Chemother. 26: 903–908

Honda, K. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 327–330

Hoppel, C., Garle, M., Rane, A. (1977) Clin. Pharmacol. Ther. 21: 294–300

James, R., Desmond, P., Kupfer, A., Schenker, S., Branch, R. A. (1981) J. Pharmacol. Exp. Ther. 217: 127–132

Kato, R., Chiesara, E., Vassanelli, P. (1964) Biochem. Pharmacol. 13: 69–83

Kemner, J. M., Snodgrass, W. R., Worley, S. E., Hodges, G. R., Clark, G. M., Hignite, C. E. (1984a) J. Lab. Clin. Med. 104: 433–444

Kemner, J. M., Snodgrass, W. R., Worley, S. E., Hodges, G. R., Melethil, S., Hignite, C. E., Tschanz, C. (1984b) Res. Comm. Chem. Path. Pharmacol. 46: 381–500

Kessler, M., Messmer, K. (1975) in: Messmer, K., Schmid-Schonbein, H. (eds) Intentional Hemodilution. Karger, Basel, pp 16–33

Kloner, R. A., Glogar, D., Rude, R. E., Khuri, S. F., Muller, J. E., Clark, L. C., Braunwald, E. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 381–390

Klotz, U. (1976) Clin. Pharmacokinet. 1: 204–218

Kohno, S., Baba, T., Miyamoto, A., Niiya, K. (1979) in: Naito, R. (ed.) Proceedings of the Fourth International Symposium on Perfluorochemical Blood Substitutes. Excerpta Medica, Amsterdam, pp 361–371

Kosugi, I., Takagi, Y., Kitagaki, T., Okada, K. (1979) Ibid. pp 401–408

Lowe, K. C., Bollands, A. D. (1985) Med. Lab. Sci. 42: 367–375

Lowe, K. C., McNaughton, D. C., Hardy, R. N. (1985) Klin. Wochenschr. 63: 1028–1034

Lutz, J., Metzenauer, P. (1980) Pflugers Archiv. 387:175–181

Lutz, J., Wagner, M. (1984) Artif. Organs 8: 41–43

Lutz, J., Metzenauer, P., Kunz, E., Heine, W. D. (1982) in: Frey, R., Beisbarth, H., Stosseck, K. (eds) Oxygen Carrying Colloidal Blood Substitutes: Fifth International Symposium on Perfluorochemical Blood Substitutes. W. Zuckschwerdt Verlag, Munchen, pp 73–81

Matsumoto, T., Watanabe, M., Hamano, T., Hanada, S., Suyama, T., Naito, R. (1977) Chem. Pharm. Bull. 25: 2163–2171

Matsumoto, J., Bianchine, J., Thompson, R., Sharp, C., Andresen, B., Ng, K., Gerber, N. (1983) Proc. West. Pharmacol. Soc. 26: 403–407

Matsumoto-Kikuchi, J., Bianchine, J. R., Gerber, N. (1983) Pharmacologist 25: 151

Maynert, E. W. (1960) J. Pharmacol. Exp. Ther. 130: 275–284

McCoy, L. E., Becker, C. A., Goodin, T. H., Barnhart, M. J. (1984) Scan. Electron Microsc. Part 1: 311–319

Messmer, K. (1975) Surg. Clin. N. America 55: 659–678

Mitsuno, T., Ohyanagi, H., Naito, R. (1982) Ann. Surg. 195: 60–69

Mitsuno, T., Tabuchi, Y., Ohyanagi, H., Sugiyama, T. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 257–263

Mitsuno, T., Ohyanagi, H., Yokoyama, K. (1984) Artif. Organs 8: 25–33

Nagasawa, S., Handa, H., Naruo, Y., Oda, Y., Yonekawa, Y., Takebe, Y. (1981) Neurolog. Res. 3: 237–250

Nies, A. S., Shand, D. G., Wilkinson, G. R. (1976) Clin. Pharmacokinet. 1: 135–155

Ohyanagi, H., Saitoh, Y. (1982) in: Frey, R., Beisbarth, H., Stosseck, K. (eds) Oxygen Carrying Colloidal Blood Substitutes: Fifth International Symposium on Perfluorochemical Blood Substitutes. W. Zuckscherdt Verlag, Munchen, pp 178–186

Ohyanagi, H., Sekita, M., Yokoyama, K., Itoh, T., Toshima, K., Ohamoto, M., Kawa, Y., Mitsuno, T. (1979) in: Naito, R. (ed.) Proceedings of the Fourth International Symposium on Perfluorochemical Blood Substitutes. Excerpta Medica, Amsterdam, pp 373–389

Oie, S., Benet, L. Z. (1980) Ann. Report Med. Chem. 15: 277–287

Peerless, S. J. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 353–362

Perucca, E., Makki, K., Richens, A. (1978) Clin. Pharmacol. Ther. 24: 46–51

Rane, A., Wilkinson, G. R., Shand, D. G. (1977) J. Pharmacol. Exp. Ther. 200: 421–424

Richens, A. (1979) Clin. Pharmacokinet. 4: 153–169

Schneeberger, E. E. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 249–256

Serrone, D. M., Fujimoto, J. M. (1962) Biochem. Pharmacol. 11: 609–615

Shand, D. G., Kornhauser, D. M., Wilkinson, G. R. (1975) J. Pharmacol. Exp. Ther. 195: 424–432

Sylvia, A. L., Proctor, H. J., Goldsmith, M. M., Jobsis, F. F. (1982) J. Trauma 22: 815–819

Tabuchi, Y., Ohyanagi, H., Nakaya, S., Usami, M., Hattori, M., Suenaga, S., Saitoh, Y. (1983) in: Bolin, R. B., Geyer, R. P., Nemo, G. J. (eds) Advances in Blood Substitutes Research: Progress in Clinical and Biological Research. Vol. 122, Alan R. Liss, New York, pp 452–453

Tremper, K. K., Levine, E. M., Waxman, K. (1985) Int. Anesthesiol. Clin. 23: 185–197

Ueda, C. T., Dzindzio, B. S. (1978) Clin. Pharmacol. Ther. 23: 158–164

Vicuna, A., Lalka, D., DuSouich, P., Vicuna, N., Ludden, T. M., McLean, A. J., (1980) Res. Comm. Chem. Path. Pharmacol. 28: 3–11

Watanabe, M., Hanada, S., Yano, K., Yokoyama, K., Suyama, R., Naito, R. (1979) in: Naito, R. (ed) Proceedings of the Fourth International Symposium on Perfluorochemical Blood Substitutes. Excerpta Medica, Amsterdam, pp 347–358

Yokoyama, K., Watanabe, M., Naito, R. (1982) in: Frey, R., Beisbarth, H., Stosseck, H. (eds) Oxygen Carrying Colloidal Blood Substitutes: Fifth International Symposium on Perfluorochemical Blood Substitutes. W. Zuckschwerdt Verlag, Munchen, pp 214–219

Yokoyama, K., Suyama, T., Okamoto, H., Watanabe, M., Ohyangi, H., Yoichi, S. (1984) Artif. Organs 8: 34–40

Zucali, J. R., Mirand, E. A., Gordon, A. S. (1979) J. Lab. Clin. Med. 94: 742–746