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Effect of moderate haemodilution with Fluosol-DA or normal saline on ampicillin kinetics in the rat

ROBERT P. SHREWSBURY

Division of Pharmaceutics, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, USA

The effects of haemodilution with either Fluosol-DA or normal saline on renal excretion and glomerular filtration have been studied in the rat. Ampicillin clearance and renal creatinine clearance were used as in-vivo measures of renal excretion and glomerular filtration, respectively. Rats were moderately exchanged with either fluid and evaluated after 0·5, 24, 48, or 72 h. After exchange, ampicillin clearance was higher, but not significantly different, and the apparent volume of distribution was increased significantly in some groups. These changes are consistent with the effects expected when plasma protein binding is reduced by haemodilution. The percent of ampicillin recovered in urine and the renal creatinine clearance were not statistically different in any group. Thus, moderate haemodilution with either fluid did not change renal function compared with unexchanged animals.

Perfluorochemical (PFC) emulsions are currently being investigated as blood substitutes because of their oxygen carrying capabilities. Animal studies have demonstrated that PFC emulsions can sustain life (Geyer 1975; Yokoyama et al 1984). PFC emulsions have been used clinically in man for blood loss replacement, oxygen delivery to ischaemic tissues, and severe anaemia (Mitsuno et al 1982; Tremper et al 1982). Clinical studies for safety and efficacy have been reported (Ohyanagi et al 1979a; Mitsuno et al 1982).

PFC particles are captured by the reticuloendothelial system (RES) and distributed primarily to liver and spleen, and secondarily to 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 is insignificant and there is no metabolic degradation of these compounds (Geyer 1983).

Although the kidney appears to act as a reversible store of PFC particles, there is evidence that renal function is altered. In severely Fluosol-DA (7 parts perfluorodecalin, 3 parts perfluorotripropylamine) haemodiluted monkeys, renal creatinine clearance was depressed during the 6 h exchange (Ohyanagi et al 1979b). Renal blood flow, glomerular filtration rate (GFR), *p*-aminohippuric acid (PAH) secretion, sodium reabsorption, and the production of oxygen-dependent acetylated metabolites of PAH were increased in a rat kidney perfused with the perfluorochemical FC 43 (3M Company) (Franke et al 1978).

Several investigations have reported that partial PFC emulsion-blood exchange alters drug disposition (Matsumoto et al 1983; Kemner et al 1984a). Of these studies, penicillin is the only drug which is eliminated primarily by renal processes and not metabolism. Thirty minutes after a 25 ml Fluosol-DA exchange, penicillin half-life was significantly increased from a mean of 11·7 to 18·9 min, but the total body clearance was unchanged (Hodges et al 1983). However, penicillin half-life was unaltered when determined 48 h after exchange (Hodges et al 1984). In lightly anaesthetized animals dosed immediately after a 60% exchange with Fluosol-DA, penicillin kinetics were not altered compared with the sham control (Kemner et al 1984b).

The present study examines ampicillin pharmacokinetics following partial blood exchange with Fluosol-DA or normal saline. Ampicillin is excreted from the rat by glomerular filtration and proximal tubule secretion as is penicillin (Thonus et al 1982). However, ampicillin is metabolized at a slower rate than penicillin, and the ampicillin renal clearance accounts for a larger percentage of the drug's total disposition compared with penicillin (Kind et al 1968; Dittert et al 1970). These characteristics would make ampicillin a better predictor of renal function than penicillin. Additionally, renal creatinine clearances were determined to assess the influence of haemodilution on the glomerular filtration rate.

MATERIALS AND METHODS

Materials

Fluosol-DA was donated by Alpha Therapeutics (Los Angeles, California) and prepared as directed

within a half hour of use. Commercially available lyophilized powders of ampicillin were reconstituted and used as directed for dosing solutions and HPLC standard curves. HPLC solvents and buffers were obtained from commercial vendors and filtered before use. Male Sprague-Dawley rats, 280 to 450 g, with free access to food and water, were used.

Methods

Ampicillin kinetics were examined in unexchanged rats and rats moderately exchanged with either Fluosol-DA or 0·9% NaCl (saline) in a parallel designed study. Group number assignments are given in Table 1. Moderate blood exchange was used

Table 1. Treatment groups.

Group	Exchange fluid	(h) Time between exchange and dosing of ampicillin
I	None	—
II	Fluosol-DA	0·5
III	Fluosol-DA	24
IV	Fluosol-DA	48
V	Fluosol-DA	72
VI	Saline	0·5
VII	Saline	24
VIII	Saline	48
IX	Saline	72

to avoid the need for supplemental oxygen. Saline exchanged groups were included to differentiate between changes in ampicillin disposition due to the Fluosol-DA itself and changes due to haemodilution alone.

A silastic cannula was implanted in the right jugular vein under light ether anaesthesia 48 h before any treatment or dosing. Patency of the cannula was maintained daily with heparinized saline (20 units ml^{-1}). Group I animals were not exchanged but received an intravenous ampicillin dose of 100 mg. Animals in groups II–IX were partially exchanged with Fluosol-DA or normal saline (40 ml kg^{-1}) and received the 100 mg dose 0·5, 24, 48, or 72 h later. Ampicillin kinetics were determined at these times because time-dependent alterations in renal function have been reported (Hodges et al 1983, 1984). 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 to be infused was removed just before and 1 min after infusion of the exchange fluid. The exchange fluid volume was infused over 1 min. The haematocrit (HCT) and fluorocrit (FCT) (Geyer 1983) were determined just before the exchange procedure and ampicillin dosing.

Blood samples (0·3 ml) were collected at 10, 20, 30, and 45 min after dosing in polyethylene tubes containing heparin (33 units ml^{-1} of blood) for ampicillin plasma concentration analyses. 0·7 ml blood samples were collected just before and 2 h after dosing, for creatinine plasma concentration analyses. The samples were centrifuged at 12 000*g* for 5 min and the plasma harvested. For ampicillin samples, 100 µl of plasma was taken, 200 µl of 0·33 M $HClO_4$ was added, and the sample recentrifuged for 5 mins, and refrigerated until 20–100 µl of the supernatant was analysed. Plasma for creatinine analysis was frozen at −20 °C for subsequent assay. One urine collection was made 4 h after dosing and animals were made to inhale ether for 15 to 20 s to ensure complete urination. Urine was collected in an ice bath. To 10 µl of urine collected, 90 µl of water and 200 µl of 0·33 M $HClO_4$ were added, and the mixture centrifuged for 5 min, and refrigerated until 20 µl of the supernatant was analysed. A 5 to 6 ml aliquot of the collected urine was frozen for subsequent creatinine analysis.

Ampicillin samples were assayed the same day as collected since its stability in solution is concentration-, temperature-, and pH-dependent (Stratton & Sandmann 1975; Bundgaard 1976). Ampicillin plasma and urine concentrations were determined with a modified HPLC procedure (Vree et al 1978). Chromatographic separation was achieved with a 5 µm C8 (150 mm × 4·6 i.d.) Lichrosorb column and a mobile phase of 0·067 M KH_2PO_4 buffer(pH = 4·6)–methanol(80/20) at a flow rate of 1·1 ml min^{-1}. Absorbancy was monitored at 225 nm and the peak area of ampicillin was determined. Standard curves were prepared each day and were linear over the concentration range of 10 to 1200 µg ml^{-1} with a correlation coefficient of 0·998 or better. Standard curves prepared from water, plasma from whole blood, plasma from the mixture of whole blood and saline, and plasma from the mixture of whole blood and Fluosol-DA, were identical.

Plasma and urine creatinine analyses were carried out using a reagent kit based on the kinetic degradation of a picrate acid-creatinine chromogen monitored with UV spectrophotometry (Sigma Chemical Co, St Louis). Standard curves prepared from plasma obtained from whole blood, the mixture of whole blood and saline, and the mixture of whole blood and Fluosol-DA were identical. 0·3 ml of

plasma was suitable for all plasma analysis but a 10–15 fold dilution in urine volume was needed to stay within the assay's linearity. Renal creatinine clearance was determined by dividing the creatinine excretion rate over 4 h by the plasma creatinine concentration obtained at 2 h.

Plasma concentration-time data were fitted to a non-linear least-squares regression program to obtain estimates of the intercept (C_0) and the slope (K). $t_{\frac{1}{2}}$ was calculated as (ln 2)/K and the apparent volume of distribution (Vd) was (dose/C_0 × BW) where BW was the animal body weight at dosing in kilograms. Clearance (Cl) was Vd × K, and area-under-curve (AUC) was calculated as C_0/K. The significance of difference between any group and control was assessed with the Wilcoxon two-sample test. A probability level of $P < 0.05$ was considered statistically significant.

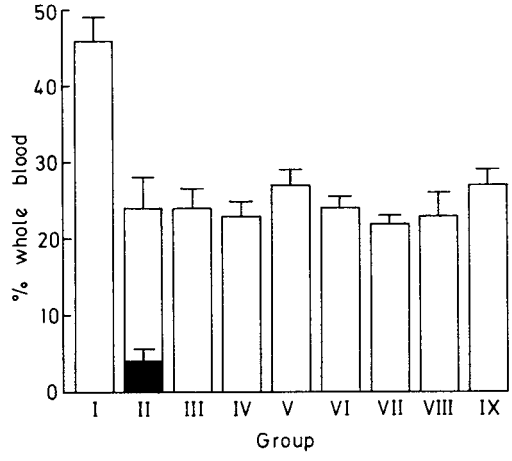

FIG. 1. Mean haematocrit and fluorocrit (black) before ampicillin dosing per treatment group. Bar represents ± s.d.

RESULTS

Animals in all groups, except group II, underwent the exchange procedure and data collection protocol as outlined with a 100% survival rate. Group II animals had a 57% survival rate when all blood samples were collected as outlined, but a 100% survival rate when the blood sample for the pre-dosing creatinine determination was not taken and replacement blood from a donor rat was given after the 2 h creatinine blood sample was collected.

Animals lost weight as the result of either Fluosol-DA or normal saline exchange, with only 8% of the animals in groups III–V and VII–IX showing a weight gain. There was no immediate weight loss in groups II and VI. Average weight losses were 11·5 g in III, 11·6 g in IV, 12·5 g in V, 10·2 g in VII, 5·2 g in VIII, and 9·3 g in IX.

The plasma creatinine concentrations taken before dosing were not significantly different between groups (group II was not collected), and ranged from an average of 0·46 to 0·57 mg dl^{-1}. There was no difference in plasma creatinine concentrations determined 2 h post dosing with values ranging from 0·56 to 0·80 mg dl^{-1}. The 2 h creatinine concentrations were higher than the pre-dosing concentration for every group, but the difference was not significant.

The exchange procedure reduced the pre-exchange haematocrit by one-half, indicating approximately a 50% blood exchange (see Fig. 1). The haematocrit remained depressed at 72 h after exchange with either Fluosol-DA or saline. Group II had an average fluorocrit of 3·8% 0·5 h post exchange. No fluorocrit was present at 24, 48, or 72 h after Fluosol-DA exchange (groups III, IV, and V).

Plasma ampicillin concentrations displayed a monoexponential decline in all groups with correlation coefficients typically greater than 0·98. The mean ampicillin disposition parameters and 4 h renal creatinine clearances are summarized in Table 2. Individual ampicillin Cl values are shown in Fig. 2. In groups IV and VI, both the $t_{\frac{1}{2}}$ and Vd were statistically higher than I, but the Cl was not different. The Vd was also higher in group IX compared with I, but the $t_{\frac{1}{2}}$ and Cl were not. VII was

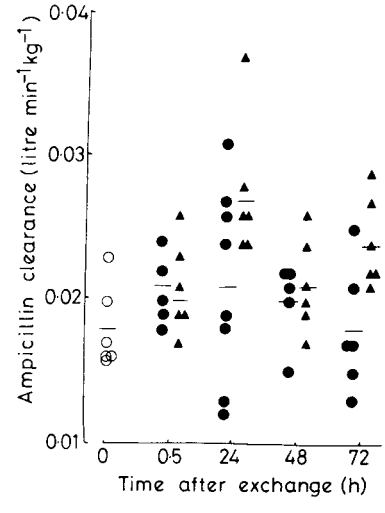

FIG. 2. Individual ampicillin clearance values grouped by dosing time after haemodilution. Bar represents mean value; ○, control; ●, Fluosol-DA; ▲, saline.

Table 2. Averaged parameters of ampicillin disposition.

Parameters	Treatment groups								
	I	II	III	IV	V	VI	VII	VIII	IX
N	6	5	8	5	6	6	6	6	6
$t_{\frac{1}{2}}$ (min)	10·9[a] (3·0)	13·0 (1·5)	12·9 (3·8)	15·3* (2·2)	10·9 (1·9)	15·5* (2·0)	9·2 (1·1)	10·2 (1·2)	12·4 (1·6)
Vd (ml kg^{-1})	274·3 (48·9)	388·2 (74·0)	376·9 (115·6)	442·2* (106·5)	277·2 (46·2)	463·0* (53·6)	363·7 (81·1)	306·7 (23·5)	434·3* (91·4)
Cl (ml min^{-1} kg^{-1})	17·8 (3·1)	20·6 (2·4)	21·3 (6·8)	20·0 (2·9)	18·0 (4·3)	20·8 (3·2)	27·5* (4·9)	21·2 (3·3)	24·2 (3·2)
% Excreted in 4 h	66·3 (16·1)	71·3 (22·4)	47·4 (17·2)	43·9 (27·2)	80·2 (23·3)	49·9 (14·3)	56·9 (9·7)	56·8 (6·5)	77·4 (6·4)
Renal creatinine clearance (ml min^{-1} kg^{-1})	6·5 (2·1)	6·6 (2·1)	5·1 (1·0)	5·5 (3·2)	6·0 (0·9)	3·8 (1·4)	6·1 (1·9)	5·0 (2·7)	5·7 (2·4)
AUC (μg min ml^{-1})	14784 (1719)	13640 (2591)	15578 (4688)	15329 (2467)	17296 (3304)	14826 (3988)	10433* (2164)	13575 (2586)	12120 (1955)

[a] mean (s.d.).
* $P < 0·05$ Wilcoxon two-sample test.

the only group showing a difference in the ampicillin Cl compared to I, although all groups had mean Cl values greater than control. This group contained the one animal with the highest Cl, but omitting the value did not change the statistical analysis. There was no significant difference in percent of dose excreted over 4 h or renal creatinine clearance compared with control. Only group VII showed a significant decline in AUC compared with the control.

DISCUSSION

It has been demonstrated that animals can survive partial blood exchange with Fluosol-DA without supplemental oxygen (Hodges et al 1983, 1984; Kemner et al 1984a). Supportive oxygen is required in severely haemodiluted animals (Hardy et al 1983; Mitsuno et al 1984); however, the exact percentage of exchange above which oxygen is required has not been established. Experience in this laboratory indicates that a 60% exchange with either Fluosol-DA or saline is the maximal exchange which can be performed without additional oxygen. Kemner et al (1984a) reported a 71% Fluosol-DA exchange without supplemental oxygen. Animals exchanged in this study did not require supplemental oxygen, but the amount of blood collected influenced the survival rate of group II animals. Group VI animals survived without the modified study protocol, indicating that the Fluosol-DA exchanged animals were compromised in some fashion. The blood volume in Fluosol-DA exchanged animals decreases to about 66% of the normal volume 3 h after severe transfusion (Watanabe et al 1979). An additional observation was that most group II animals voided a white material of plastic-like consistency which might be the Fluosol-DA emulsion without the PFCs. It is not clear from this study if one or both of these factors is sufficient to account for the decreased survival rate.

Weight loss and plasma creatinine were comparable regardless of the exchange fluid used. Weight loss must be a natural consequence of a haemodilution since all groups dosed at least 24 h after exchange with either fluid lost weight. The effect occurred for 72 h after exchange. But in long term studies (Watanabe et al 1979), exchanged animals grew comparable to unexchanged controls. Plasma creatinine concentrations increased from the predosing level probably due to depletion of the blood volume as samples were collected. None of the plasma creatinine values indicated a decreased renal creatinine clearance.

Haematocrits were reduced approximately 50% as a result of the exchange protocol. Haematocrit levels remained depressed for 72 h. A similar observation has been reported where a one-third exchange caused haematocrit levels to remain depressed longer than rats severely exchanged whose haematocrit levels returned to normal within several days (Geyer 1973; Zucali et al 1979). Fluorocrit levels were seen only in group II, with no fluorocrit detected at 24 h. The intravascular half-life of total PFC in Fluosol-DA is two days (Lutz & Metzenauer 1980). Between the PFCs being taken up by the RES and undergoing exhalation and the emulsion formulation presumably being excreted in the urine, the administered Fluosol-DA formulation must be dispersed and/or eliminated within 24 h.

Ampicillin Vd in all groups were higher than in group I, some values being statistically different. Haemodilution with Fluosol-DA or saline decreases the plasma protein concentration which requires two or more days to return to pre-exchange levels (Geyer 1970; Watanabe et al 1979). Ampicillin is 73% serum protein bound in rats (Trottier & Bergeron 1981); therefore, a decreased plasma protein content would decrease the percent of bound ampicillin leading to an increased Vd (Gibaldi & McNamara 1978).

Data in Table 2 indicate that ampicillin Cl is not statistically altered by exchange with either Fluosol-DA or saline. But each mean Cl is greater than I, a consequence of the decreased plasma protein binding (Cook & Smith 1985). It is not obvious why only group VII Cl values were significantly greater than control, but the decreased AUC and increased Cl are consistent with changes expected as plasma protein binding is decreased (Gibaldi & Koup 1981). Since $t_{\frac{1}{2}}$ equals (ln 2)Vd/Cl, the observed $t_{\frac{1}{2}}$ depended upon the relative magnitudes of change in both Cl and Vd.

Ampicillin Cl was greater than renal creatinine clearance in all groups, suggesting that ampicillin is renally excreted by both filtration and secretion. Inhibitors of the organic acid secretory system in the kidney (i.e. probenecid) significantly increase the half-life of concommitantly administered penicillins, including ampicillin (Gibaldi & Schwartz 1968). Exchange with either Fluosol-DA or saline had little influence on the secretory system since the ampicillin $t_{\frac{1}{2}}$ generally remained unchanged.

The percentages of ampicillin dose recovered in urine within 4 h was not statistically different in any group compared with the control. Ampicillin undergoes hepatic biotransformation to penicilloic acid (Kind et al 1968; Bird et al 1983), but the present data do not suggest that metabolism has been altered. Data in this laboratory showed that Fluosol-DA exchange markedly induced antipyrine metabolism after 48 and 72 h, and that saline exchange dramatically inhibited antipyrine metabolism 0·5 h after exchange (Shrewsbury et al 1986). Such a pattern of induction/inhibition is not seen with ampicillin.

Renal creatinine clearance was not significantly altered by haemodilution with either exchange fluid, and is in agreement with other estimates of GFR determined with inulin (Chayoth et al 1984; Jobin & Bonjour 1985). The data indirectly suggest that renal blood flow is not altered after exchange, although renal blood flow can vary 20% before a change in GFR is detected (Duchin & Schrier 1978). 5 to 18 percent of an ampicillin dose undergoes enterohepatic cycling (Kind et al 1968; Murakami et al 1984). These data suggest that enterohepatic cycling is not significantly altered after exchange with either fluid since the AUC are not different from control; the AUC parameter will change with variations in the percent of drug entering the enterohepatic cycle (Chen & Gross 1979; Colburn 1982).

It has been reported that the penicillin half-life was significantly longer than 0·5 h, but unchanged 48 h after a 25 ml Fluosol-DA exchange (Hodges et al 1983, 1984) and unaltered immediately after a 60% exchange in lightly anaesthetized animals (Kemner et al 1984b). The extent of exchange is similar to the present study, but the ampicillin $t_{\frac{1}{2}}$ was not altered 0·5 h after the exchange. Penicillin is eliminated almost exclusively by tubule secretion (Gibaldi & Schwartz 1968), and the inhibited elimination effect apparently disappeared within 48 h. A similar inhibitory effect was not seen with ampicillin since its elimination is not totally by secretion, but a combination of urinary filtration and secretion (Thonus et al 1982; Gibaldi & Schwartz 1968).

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