Effect of caffeine on the plasma protein binding and the disposition of ceftriaxone

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The effects of caffeine on the in-vitro protein binding and the pharmacokinetics of ceftriaxone (a highly protein bound cephalosporin) were investigated. Caffeine failed to decrease in-vitro protein binding of ceftriaxone. Rabbit plasma concentrations of ceftriaxone (30 mg kg^{-1} i.v.) were elevated significantly ($P < 0.05$ at 0·3, 0·6 and 1 h after injection) when caffeine 5 or 10 mg kg^{-1} i.v. was co-administered compared with ceftriaxone given alone. Caffeine increased the volume of distribution of the central compartment (V_1) for ceftriaxone significantly from 49 ± 38 ml kg^{-1} (mean ± s.d., n = 6) to 97 ± 33 ml kg^{-1} (caffeine 5 mg kg^{-1}, $P < 0.05$), and 94 ± 8 ml kg^{-1} (caffeine 10 mg kg^{-1}, $P < 0.05$) and decreased the volume of distribution of the peripheral compartment (V_2) from 145 ± 106 ml kg^{-1} (mean ± s.d., n = 6) to 31 ± 18 ml kg^{-1} (caffeine 5 mg kg^{-1}, $P < 0.05$) and 36 ± 31 ml kg^{-1} (caffeine 10 mg kg^{-1}, $P < 0.1$). The rate of transfer of ceftriaxone to the peripheral compartment (k_{12}) was also decreased significantly ($P < 0.05$) after caffeine. The elevated plasma concentration of ceftriaxone, increased V_1 value and the decreased V_2 and k_{12} values are probably the result of caffeine altering the distribution of ceftriaxone to the central and the peripheral compartments.

Caffeine potentiates the effect of a variety of drugs, such as acetylsalicylic acid (Dahanukar et al 1978; Collins et al 1979), paracetamol (acetaminophen) (Laska et al 1983) and benzodiazepines (Wallace et al 1981).

The purpose of the present study was to determine the effect of caffeine on the in-vitro plasma protein binding and the disposition of ceftriaxone, a cephalosporin which is extensively bound to human plasma protein (Stoeckel et al 1981; Patel et al 1981b).

Materials and methods
In-vitro plasma protein binding study
The centrifugal ultrafiltration membrane technique was used to determine the in-vitro binding of ceftriaxone and caffeine to human plasma (obtained from the City Blood Bank, Brisbane) and rabbit plasma, collected from the Australian original white male rabbits. Ceftriaxone (Roche, Basle, Switzerland) or caffeine (Ajax, Sydney, Australia) dissolved in 40 µl of distilled water was mixed with 1 ml of plasma and was shaken for 2 min by whirlimixer. After 1 h incubation at 37 °C, the sample was placed into microporous filters fitted with an ultrafiltration membrane (Type YMT, Amicon, Danvers, MA, USA) and was centrifuged at 500 rev min^{-1} for 10 min. The concentration of drug in the filtrate was determined by HPLC with uv detection. Filtrate con-

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centrations of ceftriaxone were measured in the presence of a fixed concentration of caffeine (0, 5, 10 µg ml^{-1} caffeine for human plasma; 0, 10, 20 µg ml^{-1} caffeine for rabbit plasma). Initial ceftriaxone concentration varied from 5 to 1000 µg ml^{-1} for human plasma and from 10 to 400 µg ml^{-1} for rabbit plasma.

Drug disposition study
Subjects, drug administration and sampling
Six Australian original white male rabbits, 2·6–3·2 kg, aged 8–10 months were assigned to one of three groups. Each group received ceftriaxone 30 mg kg^{-1} without caffeine, with caffeine 5 mg kg^{-1} or with caffeine 10 mg kg^{-1} and each study was conducted as a complete crossover experimental design. Both ceftriaxone and caffeine were dissolved separately in saline for injection. Caffeine dosage of 5 or 10 mg kg^{-1} was administered intravenously through a marginal ear vein as a volume of 0·2 ml kg^{-1} followed immediately by ceftriaxone 30 mg kg^{-1} as a volume of 0·24 ml kg^{-1}. The same dose of caffeine was injected again 3 h after the ceftriaxone injection. After the ceftriaxone injection, approximately 0·5 ml blood samples were collected from the articular artery of the opposite ear for 12 h. The standard blood sampling times were: 10, 20, 30 min, 1, 2, 3, 4, 6, 10 and 12 h after ceftriaxone injection. Each blood sample was transferred to a heparinized tube and centrifuged. Rabbit faeces and urine were separated via a metabolism cage and the urines were collected at each time of excretion. The plasma and urine samples obtained were stored at −20 °C until analysis.

Assay
Ceftriaxone in rabbit plasma. Measurement of ceftriaxone was based on a modification of the method of Patel et al (1981a). Plasma (50 µl) was diluted with 50 µl of distilled water and deproteinated with 100 µl of aceto-nitrile including 5 or 20 µg ml^{-1} of nitrazepam as an internal standard. The mixture was then shaken for 2 min and centrifuged for 3 min at 1500 rev min^{-1}. 20 µl of supernatant was injected into the HPLC. The mobile phase consisted of acetonitrile–cetrimide solution (10 g litre^{-1} in water)–1 M phosphate buffer (pH 7)–distilled water (42 : 30 : 1 : 27).

Ceftriaxone in rabbit urine. Rabbit urine samples were diluted to 3 times their volume with distilled water and

to 4 times their volume with acetonitrile and then treated similarly as plasma. The mobile phase consisted of acetonitrile–cetrimide solution (10 g litre^{-1} in water) -1 M phosphate buffer (pH 7) – distilled water (38:30:1:31).

Ceftriaxone in human and rabbit plasma filtrate. Filtrate samples were mixed with an equal volume of acetonitrile and 20 µl of the mixture was eluted with a mobile phase consisting of acetonitrile–cetrimide solution (10 g litre^{-1} in water)–1 M phosphate buffer (pH 7)– distilled water (40:30:1:29).

Caffeine in rabbit and human plasma filtrate. Measurement of caffeine was based on a modification of the method described by Blanchard et al (1980). Filtrate samples were mixed with an equal volume of acetonitrile and 20 µl of the mixture was eluted with a mobile phase consisting of acetonitrile–acetate buffer (pH 4·0, 10 mmol litre^{-1}) (15:85).

The HPLC system consisted of a sample injector (Model 7125, Rheodyne, Cotati, CA, USA), a solvent delivery system (Model M45, Waters Associates, Milford, MA, USA), an ultraviolet detector operated at 280 nm (Model 440, Waters), and a chart recorder (Model EB 511, Houston, Austin, TX, USA). Samples were separated on a column (µBondapak C18, Waters) with a mobile phase flow rate of 2·0 ml min^{-1} for both ceftriaxone and caffeine.

Pharmacokinetic analysis

The individual and mean data were fitted to a two compartment pharmacokinetic model using the digital computer program NONLIN (Metzler et al 1974) modified to operate on a minicomputer (Bourne & Wright 1981).

Initial estimates obtained for the pharmacokinetic parameters; volume of distribution of the central compartment (V_1), urinary elimination rate constant (k_u), miscellaneous elimination constant (k_{misc}), the rate constant of transfer to the peripheral compartment (k_{12}) and out of peripheral compartment (k_{21}) were subjected to weighted non-linear least squares analysis. Plasma data were weighted by $1/(\overline{CV})$ (Cp value)^2 and urine data were weighted by $1/(\overline{SD})^2$. Cp is the measured ceftriaxone plasma concentration for each sampling time. $\overline{CV}$ was calculated as the mean of standard deviation of the Cp value divided by the mean Cp value at each sampling time, and $\overline{SD}$ is the mean of standard deviation of the amount (% of dose) of ceftriaxone excreted in urine at each time. The area under the plasma concentration versus time curve (AUC) was calculated by the trapezoidal rule. The apparent volume of distribution at steady state was calculated by $V_{SS} = V_1(k_{12} + k_{21})/k_{21}$ and volume of distribution of the peripheral compartment (V_2) was calculated as $V_{SS}-V_1$ (Gibaldi & Perrier 1975). Total elimination rate constant (k_{el}) was calculated as $k_u +$

k_{misc}. Total clearance (CL = CL$_R$ + CL$_{misc}$) and renal clearance (CL$_R$) were calculated as ($k_u + k_{misc}$) × V_1 and k_u × V_1, respectively. Statistical evaluation of the influence of caffeine was based on the two tailed unpaired Student's t-test analysis.

Results

The extent of in-vitro protein binding of ceftriaxone to human plasma was 91·3–88·0% and to rabbit plasma was 84·6–81·1% over a range of plasma concentrations of 5 to 100 µg ml^{-1} and 10 to 100 µg ml^{-1}, respectively. Drug binding to the apparatus was slight with less than 10% of the free drug concentration bound in the absence of plasma proteins. For caffeine, the extent of protein binding was found to be 37·3–34·0% to human plasma and 35–33% to rabbit plasma over a range of plasma concentrations of 5 to 100 µg ml^{-1}. The percentage of bound ceftriaxone, in both human and rabbit plasma, decreased greatly with further increases in drug concentration from 91·3% (5 µg ml^{-1}) to 65·9% (500 µg ml^{-1}) in human plasma and from 84·6% (10 µg ml^{-1}) to 48·9% (400 µg ml^{-1}) in rabbit plasma, suggesting that the drug binding capacity of plasma protein became saturated at high drug concentration. In the presence of 5 and 10 µg ml^{-1} of caffeine in human plasma and 10 and 20 µg ml^{-1} of caffeine in rabbit plasma, the binding of ceftriaxone to plasma protein was not changed significantly. Even with a caffeine concentration of 200 µg ml^{-1} the binding of ceftriaxone (100 µg ml^{-1}) did not decrease significantly (91·2% without caffeine–88·7% with caffeine) in human plasma. These results suggest that the binding of ceftriaxone and caffeine to plasma protein is not competitive in-vitro.

The mean plasma concentration versus time data following single intravenous doses of ceftriaxone 30 mg kg^{-1} alone or with caffeine 5 and 10 mg kg^{-1} are shown in Fig. 1. The ceftriaxone plasma concentration-time curve obtained with co-administration of caffeine appeared monoexponential whereas the curve for ceftriaxone alone appeared biexponential. However all the curves were fitted by a two compartment model since a statistically improved fit with the two compartment model was obtained (f test, $P < 0·05$) (Boxenbaum et al 1974). Plasma concentrations of ceftriaxone were elevated significantly ($P < 0·05$) in the presence of caffeine at 0·3, 0·6 and 1 h after i.v. injection in both the 5 and 10 mg kg^{-1} groups.

The calculated pharmacokinetic parameters for ceftriaxone are shown in Table 1. There were no significant differences observed between the parameter values measured at the two caffeine dose levels.

The mean of the total amount of 24 h urinary excretion of ceftriaxone was not significantly different from control for either dose of caffeine (59·5, 64·3 and 64·6%, respectively). The 12 h volume of urine was increased from 40·5 ± 14 ml to 65 ± 41·8 ml ($P < 0·1$) and 80·9 ± 27·8 ml ($P < 0·02$) by caffeine 5 and
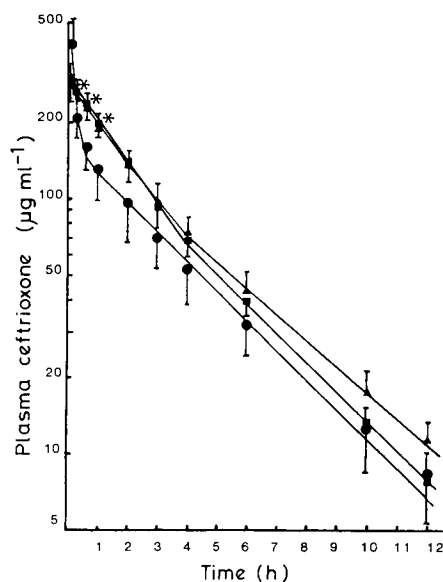
FIG. 1. Plot of mean plasma ceftriaxone concentration vs time in rabbits. Each point and vertical bar represents the mean and standard deviation of six subjects. The lines represent curves calculated by NONLIN. Key: (●), Ceftriaxone 30 mg kg^{-1} i.v.; (▲) Ceftriaxone with caffeine 5 mg kg^{-1} i.v.; (■) Ceftriaxone with caffeine 10 mg kg^{-1} i.v.; (★) $P < 0.05$.

10 mg kg^{-1}, respectively. The differences were no longer significant after 12 h post-injection. An increased consumption of water by animals after injection of caffeine was also observed.

Discussion

Caffeine has been shown to double the concentration of plasma free fatty acids (FFA) in man (Acheson et al

1980; Patwardhan et al 1980). The extent of the in-vitro plasma protein binding rate of the cephalosporins is diminished significantly in human plasma by fatty acids (Pitkin et al 1980), and an increased FFA concentration has been shown to elevate the free fraction of other drugs, including warfarin and diphenylhydantoin (Gugler et al 1974). FFA have higher affinity constants for albumin than do most drugs (Gugler et al 1974) and can displace them from binding sites. Those results suggest that caffeine may affect the pharmacokinetics of drugs, especially those drugs that are highly protein bound. Despite these findings, no effect of caffeine on the protein binding of ceftriaxone was apparent in our in-vitro study.

Generally, increasing free drug concentrations may act to increase the apparent volume of distribution in-vivo (Bowman & Rand 1980). In the present study the apparent volume of the central compartment (V_1) for ceftriaxone was approximately doubled in the presence of caffeine. However the apparent volume of distribution at steady state (V_{SS}) was not changed by co-administration of caffeine. Further, the rate of transfer of ceftriaxone from the central to peripheral compartment (k_{12}) was decreased significantly in the presence of caffeine. These results could be explained if the peripheral compartment for ceftriaxone alone is considered to consist of medium and slowly distributing components. If the caffeine was to cause improved distribution of ceftriaxone to the medium component, then all or part of this component could be included in the central compartment. This would lead to the observed results of, increased V_1, unchanged V_{SS}, and slower distribution to the remaining components of the peripheral compartment. The suggestion that caffeine causes only distribution changes in the disposition of ceftriaxone is further supported by the observation that neither the total body clearance (CL) nor the renal

Table 1. Summary of average pharmacokinetic parameters of ceftriaxone with or without caffeine administration in rabbits.

Parameter	R only[a] (n = 6)	R + C5 (n = 6)	R + C10 (n = 6)	$P <$[b] R : R + C5	R : R + C10
k_{el} (h^{-1})	1·31 (0·71)[c]	0·38 (0·17)	0·38 (0·10)	0·05	0·05
k_u (h^{-1})	0·90 (0·50)	0·23 (0·09)	0·25 (0·11)	0·05	0·05
k_{misc} (h^{-1})	0·56 (0·24)	0·15 (0·11)	0·12 (0·03)	0·02	0·02
k_{12} (h^{-1})	6·90 (6·15)	0·42 (0·69)	0·29 (0·58)	0·05	0·05
k_{21} (h^{-1})	1·54 (1·48)	0·70 (0·53)	0·43 (0·46)	NS	NS
V_1 (ml kg^{-1})	49 (38)	97 (33)	94 (8)	0·05	0·05
V_2 (ml kg^{-1})	145 (106)	31 (18)	36 (31)	0·05	NS
V_{SS} (ml kg^{-1})	194 (115)	128 (26)	130 (26)	NS	NS
$t\frac{1}{2}$ (h^{-1})[d]	4·35 (2·67)	3·38 (1·24)	3·87 (1·04)	NS	NS
CL (ml h^{-1})	112 (30)	80 (19)	94 (18)	NS	NS
CL_R (ml h^{-1})	66 (16)	55 (16)	63 (22)	NS	NS
AUC_{0-4} (μg h ml^{-1})	454 (133)	594 (71·3)	590 (72·5)	0·05	0·05
AUC_{0-6} (μg h ml^{-1})	539 (164)	711 (93·9)	694 (90)	0·05	NS
AUC_{inf} (μg h ml^{-1})	724 (190)	949 (229)	863 (112)	NS	NS

[a] R only: Ceftriaxone 30 mg kg^{-1} i.v. single dose, R + C5: Ceftriaxone 30 mg kg^{-1} i.v. single dose after caffeine 5 mg kg^{-1} i.v. injection, R + C10: Ceftriaxone 30 mg kg^{-1} i.v. single dose after caffeine 10 mg kg^{-1} i.v. injection.
[b] Unpaired t-test. [c] Mean (s.d.) of six rabbits. [d] Terminal half-life.

clearance (CL_R) are altered significantly. The terminal half-life ($t\frac{1}{2}$) of ceftriaxone was shortened slightly but not significantly by coadministration of caffeine.

In conclusion, caffeine did not affect the plasma protein binding of ceftriaxone in-vitro. Caffeine elevated rabbit plasma concentrations of ceftriaxone and increased the volume of distribution of the central compartment (V_1). Caffeine decreased the volume of distribution of ceftriaxone of the peripheral compartment (V_2) and the rate of transfer to the peripheral compartment (k_{12}) in the rabbit. The mechanism for these pharmacokinetic changes by caffeine may be by its effect on drug distribution to organs and peripheral tissue.

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Lack of effect of BW755c on glucose-induced insulin secretion in the rat in-vivo

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Glucose-induced elevations in plasma immunoreactive insulin (IRI) were examined in anaesthetized rats pretreated with BW755c, sodium meclofenamate or vehicle. Neither drug influenced the glucose-induced hyperinsulinaemia or the glucose disappearance rate. The results do not support a physiological role for arachidonic acid metabolites in the regulation of glucose-induced insulin secretion.

It has been suggested that certain metabolites of arachidonic acid produced by the lipoxygenase pathway may act as second or third messengers in coupling the glucose stimulus to the secretion of insulin (Metz et al 1984). These suggestions have been made in light of the ability of certain lipoxygenase products to stimulate

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insulin secretion and of lipoxygenase inhibitors to prevent secretion of insulin in response to glucose in-vitro (Yamamoto et al 1982; Metz et al 1983). We have examined the effect of the dual lipoxygenase-cyclooxygenase inhibitor, BW755c (Higgs et al 1979) on glucose-induced elevations in the plasma insulin concentration in the rat. This drug was compared with sodium meclofenamate, a potent cyclooxygenase inhibitor, as inhibition of cyclooxygenase has been reported to enhance glucose-induced insulin secretion (MacAdams et al 1984).

Method
Male Sprague-Dawley rats (190–350 g) were fasted for 18 h and anaesthetized with pentobarbitone sodium