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Protein binding of cefpiramide in the plasma of various species

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Abstract—The plasma protein binding of cefpiramide in man, dog, rabbit and rat was examined. Cefpiramide was more strongly bound to human plasma than to animal plasma and was exclusively bound to albumin. The characteristics of the protein binding of cefpiramide is one of the factors influencing the extrapolation of drug disposition from animals to man.

Drug binding to plasma protein can influence drug distribution and elimination kinetics, changing the pharmacological effects (Levy & Moreland 1984; Lin et al 1987). The protein binding of drugs is altered by numerous endogenous and exogenous factors, especially by conformation changes of plasma components during disease and postoperatively (Horiuchi et al 1987; Kemp et al 1987). Cefpiramide sodium ((6*R*, 7*R*)-7-[(*R*)-2-(4-hydroxy-6-methyl-3-pyridyl-carboxamido)-2-(*p*-hydroxyphenyl)acetamido]-3-[[(1-methyl-1H-tetrazol-5-yl)-thio]methyl]-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylate) is a widely used cephem antibiotic (Fukasawa et al 1983). Cefpiramide is highly bound to protein and its biological activity parallels the concentration of the free drug in the bioassay medium (Matsui et al 1982, 1983). This study has investigated the characteristics of protein binding of cefpiramide in man, dog, rabbit, and rats and discussed the implications of extrapolation of animal pharmacokinetics to man.

Materials and methods

Materials. Cefpiramide was kindly supplied by Yamanouchi Pharmaceutical Co. Ltd (Tokyo, Japan). Human plasma samples were obtained from healthy volunteers. Animal plasma samples were obtained from male beagle dogs 15 kg, male JW-NIBS rabbits 3 kg, and male Wistar rats 300–350 g. Human serum albumin (Fraction V), and serum albumin (Fraction V) of dog, rabbit and rat were obtained from Sigma Chemical Co. (St Louis, MO). Other chemicals and reagents, purchased from commercial sources, were of analytical grade and were used without further purification. Cefpiramide, to give a final concentration of 15 μg mL^{-1}, was dissolved in each plasma sample or pH 7·4 phosphate buffer sample containing added albumin; minimal inhibitory concentrations against various clinically isolated pathogens are greater than 12·5 μg mL^{-1} (Yamasaku et al 1983). All samples were filtered through a 0·22 μm membrane filter (Millex GV, Nihon Millipore Ltd, Osaka, Japan) before analysis.

Methods. A commercially available ultrafiltration apparatus, MPS-1 (Amicon Corp., Danvers, MA) was used. The device was centrifuged at 2000 *g* (Hitachi centrifuge SCR20B, Hitachi Koki Co. Ltd, Tokyo, Japan) for 15 min at room temperature (25 ± 2°C) and the ultrafiltrate obtained was then assayed for free drug concentration. Adsorption of cefpiramide to the device was negligible. The total and free (ultrafiltrate) concentrations of cefpiramide were measured by HPLC (Ohshima et al 1988). An LC-5A system (Shimadzu Corp., Kyoto, Japan) with a SPD-2A (Shimadzu Corp.) set at 273 nm and Pinkerton column (250 × 4·6 mm i.d., Regis Chemical Co., IL) was used at room temperature. The sample was eluted with a mixture of 0·1 M KH_2PO_4 (pH 6·8) and isopropanol (98:2, v/v) at a constant flow rate of 1·0 mL min^{-1}. The protein concentration was measured by the method of Lowry et al (1951). Albumin concentration was measured by the bromcresol green method of Doumas et al (1971).

The cefpiramide in the ultrafiltrate obtained from the sample was measured (Levy & Moreland 1984). The free fraction (FF) was then calculated using the following:

$$FF(\%) = \frac{\text{free cefpiramide in sample}}{\text{total cefpiramide in sample}} \times 100.$$

Results and discussion

The FF values of cefpiramide in the plasma of the four species are shown in Table 1. The FF values varied significantly among species (dog > rat > rabbit > man). The total protein concentration, albumin concentration, and pH of the plasma specimens are also shown in Table 1.

The FF of cefpiramide in the presence of albumin (exclusively acidic drug binding, 4 g dL^{-1}) of the four species was measured (dog > rat > man > rabbit, Table 1). These results agreed well with the FF values in plasma as shown in Fig. 1. The regression equation and the coefficient of correlation were $Y = 1{\cdot}120\ X + 4{\cdot}580$ and $r = 0{\cdot}992$. This result suggests that cefpiramide was exclusively bound to albumin. In addition, the regression equation excluding human data was $Y = 1{\cdot}068\ X + 7{\cdot}267$ ($r = 0{\cdot}999$). The binding of cefpiramide to animal plasma, especially rabbit, may be related to proteins other than albumin, since the regression equation of the relationship of the FF values between plasma and albumin solutions, excluding the human data, was parallel to $Y = X$.

The protein binding of a drug is an important factor in controlling drug pharmacokinetics. Species differences in binding of many drugs to plasma protein have been reported (Lázníček et al 1987; Nakashima et al 1987). These are due to two main factors: (i) a difference in the concentration of binding protein and (ii) a difference in the characteristics of the binding protein itself (binding site, binding affinity). Changes in the conformation of albumin molecules are observed under various conditions (Aoki & Foster 1957; Bachmann et al 1980; Otagiri et al 1980; Kuwata et al 1985; Kemp et al 1987). In our study, it is suggested that the FF values of cefpiramide in human and animal plasma cover a wide range (2–60%) and the cefpiramide was bound exclusively to albumin. Therefore the difference of binding to each plasma sample was mainly due to differences of binding to albumin. In addition, there was a good correlation ($r = 0{\cdot}984$) between the FF obtained in this study and the volume of distribution (V_d) in each species of cefpiramide previously reported (Matsui et al 1982; Nakagawa et al 1983). It is suggested that the volume of distribution in man can be estimated by FF data from animal studies.

It is suggested that FF of cefpiramide may be changed by the

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Table 1. Binding of cefpiramide to plasma protein and to albumin solution, and protein content in plasma.

Species	Free fraction (%)	Total protein conc ($g\ dL^{-1}$)	Albumin concn ($g\ dL^{-1}$)	pH	Free fraction in albumin soln (%)
Man	1·61 ± 0·12	7·02 ± 0·13	3·80 ± 0·18	7·77	1·81 ± 0·02
Dog	60·21 ± 0·65	6·67 ± 0·24	2·87 ± 0·05	7·87	49·59 ± 0·25
Rabbit	7·16 ± 0·28	7·11 ± 0·07	2·17 ± 0·03	7·65	0·00 ± 0·01
Rat	12·61 ± 0·04	6·65 ± 0·31	2·91 ± 0·11	7·35	4·93 ± 0·07

In binding studies, cefpiramide was dissolved in plasma or in pH 7·4 phosphate buffer containing albumin (15 $\mu g\ mL^{-1}$). Values are the mean ± s.d. of five measurements.

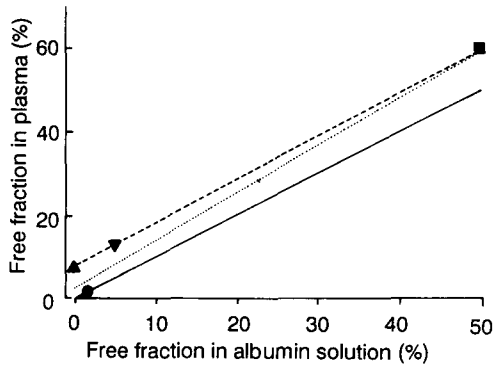

FIG. 1. The relationship between free fraction in plasma and in albumin solution. ● Human, ■ dog, ▲ rabbit, ▼ rat (values are the mean of at least five experiments). The solid line shows the regression line, Y = X, and the dotted line shows the regression line obtained using the least squares method (containing all species' data). The regression line of the data excluding human data is shown by the broken line.

conformation transition of albumin during disease (diabetes mellitus, uraemia). Therefore, extrapolation of pharmacokinetic data of highly protein-bound drugs, such as cefpiramide, should be considered carefully allowing for different species and disease states.

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