Effect of Protein Binding on the Disposition of Cephalexin and Cefazolin in a Simultaneous Perfusion System of Rat Liver and Kidney

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The effect of protein binding on the disposition of cephalexin (CEX) and cofazolin (CEZ) was investigated in a simultaneous perfusion system of rat liver and kidney. In the present study, we used bovine serum albumin (BSA) or human serum albumin (HSA) as plasma protein to control the degree of perfusate protein binding of drugs. Total clearance (CL_t) of CEX perfused with BSA (0.70±0.27 ml/min) was slightly smaller than that with HSA (0.89±0.08 ml/min), corresponding to the unbound fraction of the drug in the perfusate plasma. On the other hand, CL_t of CEZ perfused with BSA (0.90±0.20 ml/min) was significantly larger than that with HSA (0.32±0.10 ml/min). The unbound fraction of CEZ to BSA (0.703±0.052) was much larger than that to HSA (0.253±0.017) and the clearance of the unbound drug did not differ significantly between two kinds of albumin perfusate (1.30±0.40 ml/min for BSA and 1.26±0.40 ml/min for HSA). These results suggest that plasma protein binding is an important factor determining the biliary clearance as well as the urinary clearance of drugs.

Keywords simultaneous perfusion system; liver; kidney; bovine serum albumin; human serum albumin; protein binding; clearance; rat

A number of theoretical considerations and *in vivo* experiments were performed with the aim of evaluating the role of plasma protein binding in drug disposition, since marked changes in the extent of binding are frequently observed in various disease states.[2,3] However, it is not easy to assess the effect quantitatively due to the many other variable factors *in vivo*. These difficulties can be partly overcome by means of a simultaneous perfusion system of rat liver and kidney that we recently developed.[4] This system is able to keep the flow rate constant, avoid the effect of the nervous system, and control the plasma protein binding of the drug by selecting the kind of perfusate protein.

Cephalexin (CEX) is not metabolized in the body and is rapidly excreted principally by the kidney, although small amounts are also excreted in the bile.[5] Another antibiotic, cefazolin (CEZ) is recovered almost completely in the intact form in urine and bile after intravenous administration to rats.[6] As to the plasma protein binding, CEX is weakly bound to human plasma proteins (less than 10%), while CEZ is stronly bound to them (more than 70%).[5] Thus, we chose CEX and CEZ as model compounds in this study and investigated the effect of plasma protein binding on the disposition of these model drugs in the simultaneous perfusion system of rat liver and kidney.

Experimental

Materials Cephalexin was obtained from Shionogi & Co. (Osaka, Japan). Sodium cefazolin was supplied by Fujisawa Pharmaceutical Co. (Osaka, Japan). Bovine serum albumin (BSA) was purchased from Poviet Producten B. V. (Amsterdam, Holland) and human serum albumin (HSA) was kindly provided by The Green Cross Corporation (Osaka, Japan). All other chemicals were of analytical grade.

Simultaneous Perfusion Studies A closed, isolated circulation of rat liver and kidney was performed as described previously.[4] The perfusate consisted of 20% (v/v) bovine red cells and 5% (w/v) BSA or HSA in Krebs–Henseleit buffer solution, equilibrated with 95% O_2+5% CO_2 to maintain a pH of 7.4 at 37 °C. The flow rate was set at 15 ml/min for liver and 5 ml/min for kidney. After equilibration for 10 to 20 min, CEX or CEZ (50 mg/kg body weight) was added to the 30 ml reservoir and samples (0.3 ml) of the reservoir solution were obtained at 3, 5, 10, 15, 20, 25 and 30 min. Bile and urine samples were collected every 10 min up to 30 min. The degree of drug binding to perfusate plasma was determined by the ultrafiltration technique for plasma samples obtained from the perfusate at the end of each experiment.[4]

Analytical Method Determination of drug concentration in plasma was performed by a slight modification of the method of Inui *et al.*[7] A plasma sample (0.1 ml) was transferred into a micro test tube and 0.2 ml of methanol was added. The sample was centrifuged, and 10—50 μl of the clear supernatant was injected onto the column. A high-performance liquid chromatograph (TRI ROTOR-II; Japan Spectroscopic Co., Ltd., Tokyo, Japan) equipped with a UV monitor and a Chemcosorb ODS column (15 cm × 4.6 mm i.d., Chemco Scientific Co., Ltd., Osaka, Japan) was used. The flow rate was 0.8 ml/min. The same method was used for bile, urine and tissue homogenates (liver and kidney were homogenized in five volumes of 0.03 M KH_2PO_4).

Data Analysis The perfusate plasma concentration–time curves were fitted to a monoexponential equation by non-linear least-squares regression analysis.[8] Total clearance of the drug (CL_t) was calculated from the equation; CL_t=dose/AUC,where area under the blood concentration curve (AUC) is obtained by integrating the monoexponential equation from time zero to infinity, and clearance of the unbound drug (CL_f) was calculated as follows; $CL_f = CL_t/f$, where f is the unbound fraction of the drug in the perfusate plasma. Biliary and urinary clearances of the drug were obtained through dividing excretion rates in the bile and urine during the collection interval by the concentration of perfusate plasma at the midpoint of the fluid collection interval. Mean values are reported with standard deviations. Statistical analysis was performed using Student's t test with $p=0.05$ as the minimal level of significance.

Results and Discussion

The disappearance of CEX and CEZ from the perfusate plasma followed monoexponential kinetics (Fig. 1). The

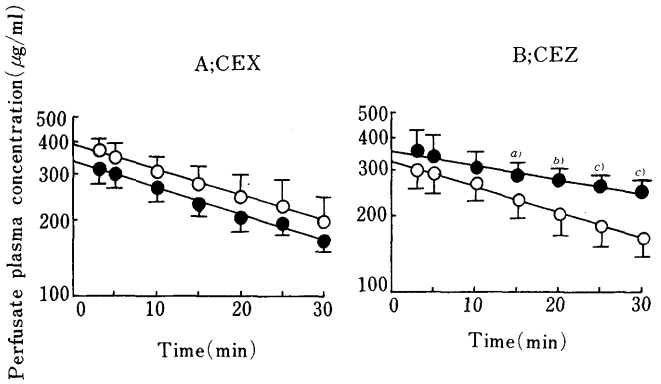

Fig. 1. Disappearance of CEX and CEZ from Perfusate Plasma in a Simultaneous Perfusion System of Rat Liver and Kidney

A; CEX (50 mg/kg body weight) was added to the reservoir perfusate at time 0. B; CEZ (50 mg/kg body weight) was added to the reservoir perfusate at time 0. Each point and vertical bar represent the mean±S.D. of 3—5 animals. ○, BSA perfusate; ●, HSA perfusate. *a*) $p<0.05$ compared to BSA perfusate; *b*) $p<0.025$; *c*) $p<0.005$.

TABLE I. Effect of Plasma Protein Binding on the Distribution and the Clearance of Cephalexin and Cefazolin in the Simultaneous Perfusion System of Rat Liver and Kidney

	Cephalexin		Cefazolin	
	BSA	HSA	BSA	HSA
No. of experiments	5	3	4	4
Unbound fraction	0.726±0.047	0.953±0.041[c]	0.703±0.052	0.253±0.017[c]
Urinary clearance (ml/min)	0.31±0.26	0.26±0.18	0.30±0.10	0.10±0.01[a]
Biliary clearance (ml/min)	0.03±0.02	0.03±0.03	0.34±0.12	0.09±0.04[a]
K/P	4.9±0.8	4.5±2.4	2.7±0.6	0.9±0.2[b]
L/P	1.1±0.5	1.2±0.5	1.6±0.4	0.7±0.1[b]

Each value represents mean±S.D. *a*) $p<0.01$. *b*) $p<0.005$. *c*) $p<0.001$ (compared to BSA perfusate).

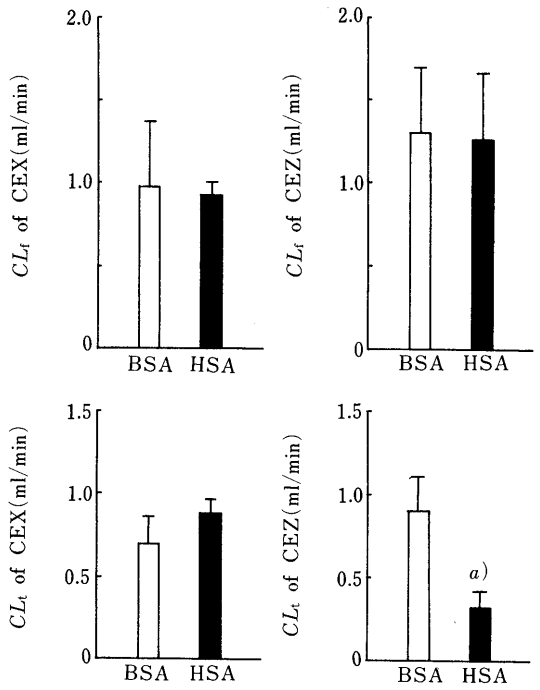

Fig. 2. CL_t and CL_f of CEX and CEZ in a Simultaneous Perfusion System of Rat Liver and Kidney

Each column and bar represent the mean±S.D. of 3—5 animals. □, BSA perfusate; ■, HSA perfusate. *a*) $p<0.005$ compared to BSA perfusate.

perfusate plasma concentrations of CEX perfused with BSA were slightly higher than those with HSA (Fig. 1A) and the unbound fractions of CEX were 0.726±0.047 ($n=5$) and 0.953±0.041 ($n=3$) for BSA and HSA, respectively ($p<0.001$). The CL_t of CEX obtained by using these perfusates showed a slight change correspoding to the plasma unbound fraction of the drug (Fig. 2). However, CL_f did not differ significantly between the two kinds of albumin. On the other hand, the perfusate plasma concentrations of CEZ perfused with BSA were significantly lower than those with HSA (Fig. 1B). The CL_t of CEZ was reduced to one-third by changing the perfusate protein from BSA to HSA (Fig. 2). The unbound fraction of CEZ to BSA (0.703±0.052, $n=4$) was much larger than that to HSA (0.253±0.017, $n=4$, $p<0.001$), and the CL_f of CEZ did not differ significantly between BSA and HSA (Fig. 2).

As is evident from Fig. 1, the distribution volume (V_d) of CEX perfused with HSA tended to be larger than that with BSA, while the V_d of CEZ perfused with BSA was slightly larger than that with HSA. These differences (statistically not significant) may reflect the different degree of plasma protein binding in each combination.

Biliary and urinary clearances of drugs are summarized in Table I. Biliary clearance of CEX was only about one-tenth of urinary clearance and neither biliary nor urinary clearance of CEX was affected by the type of albumin in the perfusate. On the other hand, CEZ was excreted equally in urine and bile, and both routes of CEZ clearance were apparently affected by the type of albumin in the perfusate. For instance, urinary clearance of CEZ decreased from 0.30±0.10 ml/min for BSA to 0.10±0.01 ml/min for HSA ($p<0.001$). Urinary clearance of the unbound drug, however, was unaltered between the two kinds of albumin perfusate (0.43±0.16 ml/min for BSA, 0.41±0.03 ml/min for HSA). In addition, there was no significant difference in biliary clearance of the unbound CEZ between BSA (0.49±0.22 ml/min) and HSA (0.36±0.16 ml/min).

In the present study, the sums of biliary and urinary clearance of drugs were at most 70% of the CL_t. These differences could not be explained by the metabolic clearance, since the metabolism of CEX and CEZ in the body is negligible.[5,6] The values of CL_t may be overestimated due to the limited perfusion time of the present experimental system (up to 30 min after the drug administration).

No significant difference was found in the tissue distribution of CEX between BSA and HSA (Table I). The concentration ratio of CEX in the kidney to the plasma (K/P) and that in the liver to the plasma (L/P) were about 5 and 1, respectively, at 30 min after the drug administration. The values of K/P for CEZ with BSA (2.7±0.6) were significantly larger than those with HSA (0.9±0.2, $p<0.005$). However, K/P_f which was obtained through dividing K/P by f, was 3.8±1.1 for BSA and 3.6±0.7 for HSA (N. S.). The values of L/P for CEZ with BSA were also larger than those with HSA, though there was no significant difference in L/P_f between BSA (2.3±0.8) and HSA (2.7±0.5). Thus, the tissue distribution of CEZ was dependent on the degree of plasma protein binding of the drug. These results are consistent with our previous results on phenolsulfonphthalein.[4]

In the present study, both CL_t and f of CEZ decreased significantly on changing the perfusate plasma protein from BSA to HSA. The unaltered values of CL_f for CEZ irrespective of the type of albumin in the perfusate suggest that the disposition of CEZ is dependent on the unbound drug concentration in the plasma. Although the binding of CEX to BSA was significantly higher than that to HSA, CL_t of the drug did not differ significantly between the two

kinds of albumin perfusate probably due to its low degree of binding to both BSA and HSA.

References and Notes

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