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Effects of probenecid on pharmacokinetics of cefaclor in rats

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ABSTRACT **AIM:** To investigate the effect of concurrent probenecid administration with different dosages on pharmacokinetics of cefaclor in rats. **METHODS:** Cefaclor (100 mg·kg^{-1}, ig) was given alone or in the presence of probenecid (300, 600 and 900 mg·kg^{-1}, ig) to rats. Serial blood samples were drawn according to the exact time schedule and concentrations of cefaclor were determined by HPLC method. **RESULTS:** Under the dosages of probenecid ranged from 300 to 600 mg·kg^{-1}, C_{max} and AUC of cefaclor increased, while Vd/F and Cl/F of cefaclor decreased. However, when a still higher dosage (900 mg·kg^{-1}) of probenecid was used, C_{max} of cefaclor decreased, while AUC, Vd/F and Cl/F maintained at the levels of those with probenecid 600 mg·kg^{-1}. **CONCLUSION:** Probenecid can markedly alter pharmacokinetics of cefaclor and the influential magnitude for each parameter is dependent on the dose of probenecid used in the trial. The C_{max} of cefaclor increases with proper dosage of probenecid, while decreases with over dose of probenecid, and this phenomenon may be related to the inhibition of cefaclor intestinal absorption attained with over dose of probenecid.

KEY WORDS cefaclor; probenecid; pharmacokinetics; HPLC

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The ability of uricosuric agent probenecid to inhibit renal tubular secretion and to cause increased and prolonged blood drug levels of β-lactam antibiotics is well documented[1－4]. Even though the prolongation of blood β-lactam levels in the presence of probenecid is thought to be related to the competitive inhibition of the drugs for renal active transport processes, the mechanisms underlying the increased blood drug levels are unclear. The influence of probenecid with different dosages on pharmacokinetics of cefaclor in rats was examined in the present study. It was noteworthy that overdose of probenecid might reduce C_{max} of cefaclor.

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1 MATERIALS AND METHODS

1.1 Drugs and reagents Standard cefaclor was provided by National Institute for the Control of Pharmaceutical and Biological Products. Cefaclor capsules were obtained from Eli Lilly pharmaceutical Co., Ltd, Suzhou. Probenecid tablets were obtained from Shanghai Jicheng pharmaceutical factory. Methanol was at HPLC grade, other chemicals were analytical reagent grade, and doubly distilled water was used for HPLC assay.

1.2 Animals and study design 24 Sprague-Dawley rats (either sex weighing 240－280 g) after an overnight fasting were randomly divided into 4 groups. Group Ⅰ: cefaclor 100 mg·kg^{-1}; Group Ⅱ: cefaclor 100 mg·kg^{-1} + probenecid 300 mg·kg^{-1}; Group Ⅲ: cefaclor 100 mg·kg^{-1} + probenecid 600 mg·kg^{-1}; Group Ⅳ: cefaclor 100 mg·kg^{-1} + probenecid 900 mg·kg^{-1}. Probenecid was administered intragastrically (ig) 0.5 h before cefaclor ig administration. Blood samples were collected at 0.25, 0.5, 0.75, 1, 2, 4, 6 and 8 h after cefaclor administration. Samples were promptly centrifugated to determine cefaclor levels by HPLC method.

1.3 Apparatus and chromatographic conditions Analyses were performed on a C18 column (4 mm × 125 mm) linked to a C18 pre-column (4.6 mm × 12.5 mm) with fluorescence detector. The mobile phase consisted of methanol-water (20 : 80, v/v) with acetic acid adjusted to pH = 3.1. Flow rate was 1 ml·min^{-1}. The UV detection wavelength was set at 265 nm[5].

1.4 Sample preparation Blood samples were collect-

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ed in heparinized centrifugation tubes and centrifugated at 3000× *g* for 10 min. 0.2 ml plasma in 10 ml glass centrifugation tube, 6%(*v*/*v*) $HClO_4$ 0.2 ml was added. The mixture was vortexed for 2 min and centrifugated at 3000× *g* for 10 min. An aliquot (20 μl) of supernatant was injected into the HPLC apparatus.

1.5 Quantitation Calibration curve was constructed by plotting the peak-area of cefaclor against the known concentrations of cefaclor added to drug-free plasma to cover the range of 2.5—800 mg·L^{-1}. The drug concentration was quantitated by relating the peak-area to obtain the concentration from the calibration curve.

1.6 Data analysis Experimental data were presented as $\bar{x} \pm s$. Pharmacokinetics parameters were calculated by DAS software[6]. Student *t*-test was used to determine differences between groups.

2 RESULTS

2.1 Calibration curve and assay validation Calibration curve of cefaclor was shown in Fig 1. The regression equation was: $A = 5.852C + 1.361$ ($r = 0.999$, $n = 5$). Concentrations of cefaclor were linear in a range between 2.5 and 800 mg·L^{-1}. Low limit detection of cefaclor was 0.1 mg·L^{-1} as a signal to noise ratio of 3. RSDs of within-day and between-day were all less than 7%. The analytical peaks of cefaclor were well resolved with good symmetry (Fig 2), and the retention time was 5.1 min. No endogenous interference was observed.

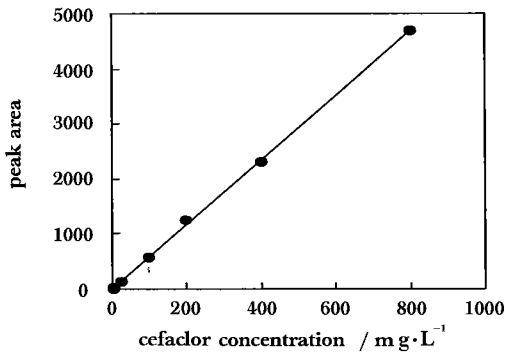

Fig 1 Calibration curve of cefaclor

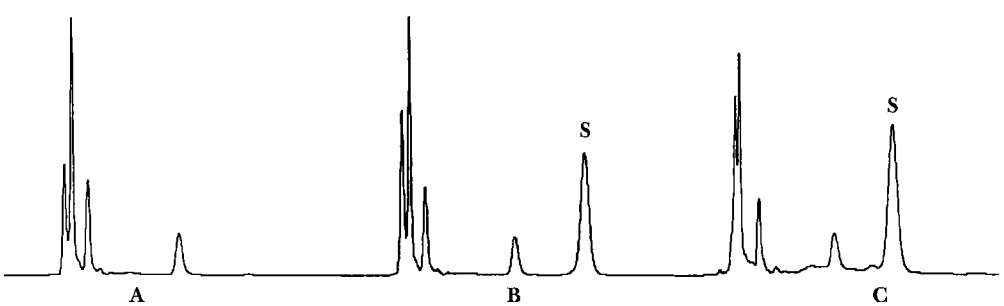

Fig 2 Chromatograms

A: blank plasma; B: standard component added to plasma; C: plasma sample after ig cefaclor; S: cefaclor

2.2 Pharmacokinetics of cefaclor The plasma concentration-time data of cefaclor after administration were fitted to a one-compartment model. Plasma concentration-time curves of cefaclor were showed in Fig 3, Mean plasma concentrations and pharmacokinetics parameters of cefaclor obtained from the 4 groups were given in Tab1 and Tab 2, respectively.

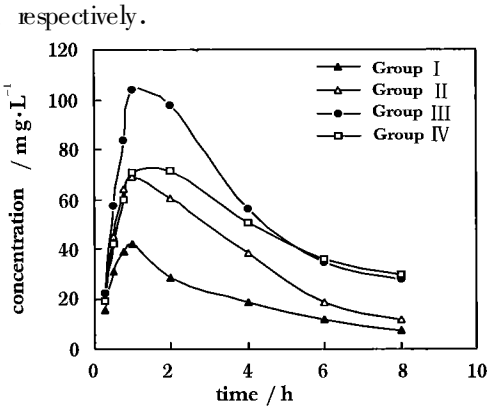

Fig 3 Mean plasma concentration-time curves of cefaclor of group Ⅰ-Ⅳ in rats($\bar{x} \pm s$, $n = 6$)

The cefaclor concentrations increased in proportion to probenecid dosage of 300 — 600 mg·kg^{-1}, maximum plasma concentration (C_{max}) and area under the plasma concentration-time curve (AUC) of cefaclor increased significantly ($P < 0.01$) compared with those in absence of probenecid, which was accompanied by a dramatic reduction ($P < 0.01$) in clearance (Cl/F) and apparent distribution volume (Vd/F). Absorption half-life ($T_{1/2ka}$) of cefaclor was prolonged ($P < 0.05$) by probenecid co-administration. Time to reach the peak plasma concentration (T_{max}), Mean residence time (MRT) and elimination half-life ($T_{1/2ke}$) also presented a tendency for prolongation, while there were no significant statistically differences for these parameters.

However, when a still higher dosage of probenecid (900 mg·kg^{-1}) was used, plasma levels of cefaclor declined as well as C_{max} strikingly decreased ($P < 0.01$), MRT and $T_{1/2ke}$ prolonged ($P < 0.05$) in opposition to those with probenecid 600 mg·kg^{-1}; no obvious changes

were observed in Cl/F and AUC. In addition, T_{max} prolonged compared with cefaclor administered alone ($P<0.05$).

Tab 1 Mean plasma concentrations of cefaclor at different times after dosing ($\bar{x}\pm s$, $n=6$, $mg\cdot L^{-1}$)

Groups	Times after dosing /h							
	0.25	0.5	0.75	1	2	4	6	8
I	16±7	31±7	38±8	42±8	28±7	19±4	12±4	7±3
II	23±14	45±21	64±24	69±22[b]	60±13[b]	38±7[c]	18±4[b]	12±4
III	22±6	57±15	84±17	104±11[c]	98±13[c]	56±25	34±16[b]	28±12[b]
IV	19±10	42±14	60±18[e]	71±18[cf]	72±12[cf]	50±7	35±8	28±9

II, III, IV *vs* I [b]$P<0.05$, [c]$P<0.01$; IV *vs* III [e]$P<0.05$, [f]$P<0.01$

Tab 2 Effects of probenecid with different dosages on pharmacokinetics parameters of cefaclor in rats($\bar{x}\pm s$, $n=6$)

Groups	T_{max} /h	C_{max} /$mg\cdot L^{-1}$	AUC /$mg\cdot h^{-1}\cdot L^{-1}$	Vd/F /L	$T_{1/2ka}$ /h	$T_{1/2ke}$ /h	MRT /h	CL/F /$L\cdot h^{-1}$
I	1.00±0.00	42±8	107±31	2.20±0.42	0.16±0.05	2.00±0.28	2.98±0.1	10.49±0.11
II	1.33±0.52	72±19[c]	177±52[c]	1.07±0.33[c]	0.33±0.17[b]	1.77±0.30	2.98±0.17	0.25±0.04[c]
III	1.17±0.55	110±9[c]	298±68[c]	1.02±0.38[c]	0.24±0.07[b]	2.35±0.34	3.13±0.34	0.13±0.07[c]
IV	1.50±0.55[b]	76±14[cf]	281±65[c]	1.30±0.19[c]	0.27±0.08[b]	4.05±1.50[cf]	3.48±0.15[ce]	0.15±0.02[c]

II, III, IV *vs* I [b]$P<0.05$, $P<0.01$; IV *vs* III [e]$P<0.05$, [f]$P<0.01$

3 DISCUSSION

The present study showed that proper dosage of probenecid increased and also prolonged circulating levels of cefaclor, which were consistent with previous studies[1-4]. Simultaneously, it was noteworthy that a reduction in cefaclor plasma concentration was also observed by co-administration over dose of probenecid, which has not been reported by other documents. Earlier studies have shown that the absorption and elimination of cefaclor and probenecid are saturable[7,8], whereas these properties can not account for a full reasonable explanation for the phenomenon.

Previous studies tend to support the traditional notion that major action of probenecid on kinetics of β-lactam antibiotics is due to its inhibition effect of renal tubular secretion of the latter. The result in this study demonstrated that cefaclor plasma concentrations elevated upon probenecid co-administration dosages of 300—600 $mg\cdot kg^{-1}$. The fact might be the result of at least three phenomena: (1) the decreased Vd/F, which could be expected to reflect as a change in the drug distribution. Some observations have shown that probenecid interferes with distribution of β-lactam antibiotics in the body. Support for this argument was found in other observations that probenecid could inhibit uptaking of various drugs by liver and decrease drug elimination from the liver[9]. Probenecid could also reduce uptaking of some β-lactam antibiotics by brain tissue[10]. Additionally, since probenecid has a high degree of plasma protein-binding (83%—95%), it has also been shown to displace other compounds from serum proteins[11], a factor which would tend to increase free drug concentration in plasma by releasing more drug. (2) When the drug was co-administered with probenecid, the decreased Cl/F of cefaclor, compared with the drug used alone, might be partly due to the competition of two drugs for the tubular excretion. (3) It has been confirmed that probenecid elimination from the kidney shows a dose-dependent manner, high dosage of probenecid might cause $T_{1/2ke}$ of the drug to prolong and yield cefaclor levels increasing positively correlated with probenecid dosage. Present study suggested that reduction in Vd/F of cefaclor produced by probenecid might be the major cause attributed to the elevation of cefaclor plasma concentration.

Of particular interest was the observation that C_{max} of cefaclor was apparently decreased by co-adminstration overdose probenecid ($900\ mg\cdot kg^{-1}$). $T_{1/2ke}$ as well as MRT markedly prolonged in group IV compared with other groups implyed that probenecid at such a higher dosage could directly inhibit cefaclor elimination from kidney, but failed to elicit the reason for cefaclor concentration decreasing contrasted with group III. Whereas there appeared to be a striking prolongation ($P<0.05$) in absorption parameters $T_{1/2ka}$ and T_{max} of cefaclor in group IV, it suggested that high dosage probenecid might prolong or inhibit cefaclor intestinal absorption. In addition, AUC of cefaclor appeared to be a progressive increase when probenecid dosage of 300—600 $mg\cdot kg^{-1}$, while maintained at the level of those with probenecid $600\ mg\cdot kg^{-1}$ in group IV, which also supported the view mentioned above.

It has shown recently that some transport systems are found both in intestine and kidney with functional and morphological similarities[12], such as peptide transport system[13], organic anion transport system[12], organic cation transport system[14] and *P*-glycoprotein-mediated transport system[15]. All these transport systems participate in the transportation process of β-lactam antibiotics in different extent. It would seem logical that inhibitors of renal transport, probenecid, for example, might also affect drug absorption in the intestine. Further studies such as experiment for cefaclor kinetics after ivadministration and experiment for cefaclor intestinal absorption are required to examine whether overdose of probenecid might have influence on cefaclor absorption in intestine.

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丙磺舒对大鼠体内头孢克罗药动学的影响

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摘要　目的: 观察不同剂量丙磺舒对大鼠体内头孢克罗药动学的影响。方法: 大鼠 24 只随机分成 4 组, Ⅰ组: 单用头孢克罗 100 mg·kg^{-1}; Ⅱ组: 头孢克罗 100mg·kg^{-1}联用丙磺舒 300 mg·kg^{-1}; Ⅲ组: 头孢克罗 100 mg·kg^{-1}联用丙磺舒 600 mg·kg^{-1}; Ⅳ组: 头孢克罗 100 mg·kg^{-1}联用丙磺舒 900 mg·kg^{-1}。各组动物灌胃给药后不同时间取血, HPLC 法测头孢克罗血药浓度, DAS 程序计算药动学参数。结果: 联用剂量在 300～600 mg·kg^{-1}范围内, 随丙磺舒联用剂量增大, 头孢克罗的 C_{max}、AUC 增高而 CL/F、V/F 减少; 当丙磺舒联用剂量达 900 mg·kg^{-1}时, 头孢克罗的 C_{max}反而降低, 而 AUC、CL/F 则稳定于联用丙磺舒 600 mg·kg^{-1}时的水平。结论: 丙磺舒可明显改变头孢克罗的药动学, 在本实验剂量范围内其影响程度与丙磺舒剂量有关, 随丙磺舒联用剂量增大, 头孢克罗的 C_{max}先升高后降低, 该现象可能与大剂量丙磺舒抑制头孢克罗的肠吸收有关。

关键词　头孢克罗; 丙磺舒; 药物动力学; HPLC