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Comparative Pharmacokinetics of Cefazolin in Awake and Urethane-Anesthetized Rats

TETSUYA TERASAKI,[a] AKIRA TSUJI,*,[a] EMI NAKASHIMA,[a]
KAZUNORI NISHIDE,[a] CHARLES H. NIGHTINGALE[b]
and TSUKINAKA YAMANA[c]

Faculty of Pharmaceutical Sciences[a] and Hospital Pharmacy,[c] Kanazawa University, Takara-machi, Kanazawa 920, Japan and Hartford Hospital,[b] Hartford, Connecticut 06115, U.S.A.

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The influence of urethane and pentobarbital anesthesia on the pharmacokinetics of cefazolin has been investigated in rats. No significant difference of total body clearance or distribution volume was observed between urethane-anesthetized and non-anesthetized rats. There was also no significant difference in plasma concentration of cefazolin at the terminal phase between urethane-anesthetized, pentobarbital-anesthetized and non-anesthetized rats. Good coincidence was demonstrated between the observed tissue-to-plasma partition coefficient, K_p, of non-anesthetized rats and the predicted K_p value based on the previously proposed model. Hence, it is considered that there is no significant influence of urethane or pentobarbital anesthesia on the distribution and elimination of cefazolin. The proposed physiological model could be useful to predict the distribution of cefazolin in non-anesthetized rats.

Keywords——urethane anesthesia; pentobarbital anesthesia; awake rat; cefazolin; pharmacokinetics; tissue-to-plasma partition coefficient (K_p); physiological model; β-lactam antibiotic; probenecid

Introduction

In recent years there has been an increased awareness of the role of anesthesia in modifying the pharmacokinetics of several drugs.[1a,b] Recently the renal clearance of thiamine was shown to be decreased significantly by urethane anesthesia.[1a] The mechanism of this effect, however, was not elucidated. A pharmacokinetic model for β-lactam antibiotics, including cefazolin, has been presented,[2] but the experimental values were obtained in urethane-anesthetized rats. Since cefazolin is mainly eliminated from the body by urinary excretion, it is important to investigate carefully the effect of anesthesia on the elimination and distribution of this drug in order to confirm the previously proposed physiological pharmacokinetic model. The purpose of the present study therefore, was to evaluate the influence of anesthesia on the pharmacokinetics of cefazolin in rats.

Experimental

Male Wistar rats (Sankyo Laboratory Animal Co., Ltd., Toyama, Japan), weighing 240—270 g, were used. The animals were divided into five experimental groups; no anesthesia, urethane-anesthetized, pentobarbital-anesthetized, no anesthesia plus treatment with probenecid and urethane anesthesia plus treatment with probenecid. The left femoral artery and vein were cannulated for blood collection and drug administration, respectively. For the non-anesthetized group, cannulation was performed under light ether anesthesia in the evening, and the experiments were carried out during the next day. The urethane- or pentobarbital-anesthetized rats were fasted overnight and the experiments were carried out at least 3 h after the cannulation under urethane anesthesia (1.3 g/kg *i.p.*) or under pentobarbital anesthesia (30 mg/kg *i.p.*). The body temperature of each rat was monitored with a digital thermometer,

DIGIMULTI Model D611 (Takara Thermistor Instruments Co., Ltd., Yokohama, Japan). Animals were maintained at 37±1 °C by means of a thermostatically controlled cage. Cefazolin, dissolved in saline (20 mg/ml), was immediately injected *via* the catheter in the femoral vein (20 mg/kg). Blood samples (0.2 ml) for experiments in non-anesthetized, urethane-anesthetized and awake probenecid-treated rats were obtained periodically at 2, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min after dosing in heparinized polyethylene centrifuge tubes. Blood samples (0.2 ml) for experiments in pentobarbital-anesthetized and urethane-anesthetized plus probenecid-treated rats were also obtained at 30, 60, 90 and 120 min in the same manner as described above. Plasma was separated by centrifugation for 2 min in a microfuge (Centrifuge 5412, Eppendorf). Cefazolin concentration was measured in plasma by a high-performance liquid chromatographic (HPLC) method as follows. A 0.1 ml aliquot of plasma, 0.3 ml of methanol and 0.2 ml of 0.05 M phosphate buffer (pH 7.4) were mixed vigorously. The mixture was centrifuged for 2 min. The supernatant was filtered through a membrane filter TM-2P (Toyo Roshi Co., Ltd., Tokyo, Japan) and subjected to HPLC on a constant-flow high-performance liquid chromatograph (HPLC), consisting of a solvent delivery system, BIP-I or TRI ROTOR-II (Japan Spectroscopic Co., Tokyo, Japan), a guard column, C18/CORASIL, a reversed-phase column, μBondapak C18 (30 cm × 3.9 mm i.d.; Waters Associates, Inc. Milford, Mass), packed in this laboratory, and a variable ultraviolet (UV) detector, UVIDEC 100-V or UVIDEC 100-III (Japan Spectroscopic Co., Ltd., Tokyo, Japan) set at 270 nm. Cefazolin was eluted with a mobile phase of 10% acetonitrile in 0.01 M ammonium acetate. The column and solvent were kept at ambient temperature. The flow rate was controlled at 1.5 ml/min and the injection volume was 10—80 μl. Peak areas, recorded with a CHROMATOPAC C-R1B (Shimadzu Co., Kyoto, Japan), were used for quantification.

The tissue-to-plasma partition coefficient K_p, of cefazolin in the non-anesthetized group was determined as follows. The animals were killed by exsanguination 60, 90 and 120 min after IV drug administration. Each tissue was immediately excised, rinsed with saline and stored at −70 °C. Within a week after collection, the concentration of cefazolin in the tissue was determined by HPLC analysis as described above, with the modification that the tissue homogenates were pretreated by using a disposable extraction column BOND ELUT™ C18 (Analytichem International, Inc.). The K_p value was corrected based on the apparent tissue-to-plasma concentration ratio at the terminal phase K_p, app, according to the method described by Chen and Gross.[3]

The influence of probenecid on the renal excretion of cefazolin in non-anesthetized and urethane-anesthetized rats was examined by intravenously administering a primary dose of 56 mg/kg of probenecid followed by an infusion (9.6 mg/kg/h) as a sustaining dose. One hour after the beginning of probenecid infusion, cefazolin was administered intravenously (20 mg/kg). Blood collection and sample analysis were performed as described above. The plasma concentration of probenecid was determined by the previously reported HPLC method.[2]

Results and Discussion

The time course of cefazolin in arterial plasma in non-anesthetized and urethane-anesthetized rats is shown in Fig. 1. The data were fitted to a two-compartment pharmacokinetic model by using the NONLIN program,[4] with the aid of a digital computer (FACOM M-170F, Data Processing Center, Kanazawa University). The pharmacokinetic parameters are listed in Table I. There was fairly good agreement between the concentration *versus* time profiles of cefazolin in awake rats and urethane-anesthetized rats. Statistical analysis of the pharmacokinetic parameters indicated no significant differences between the mean parameters

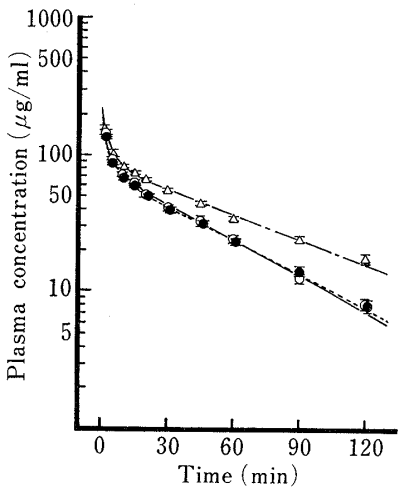

Fig. 1. Plasma Concentration *versus* Time Profile after Intravenous Bolus Administration of a 20 mg/kg Dose of Cefazolin in Awake (○), Urethane-Anesthetized (●) and Probenecid-Coadministered (△) Rats

Each point represents the mean ± S.E.M. of three to four rats. The points are experimental and the line is theoretical based upon the parameters listed in Table I.

TABLE I. Comparison of Pharmacokinetic Parameters of Cefazolin Distribution after Intravenous Administration to Non-anesthetized, Urethane-Anesthetized and Probenecid-Infused Rats[a]

Parameter	Unit	Non-anesthetized[b]	Urethane-anesthetized[c]	Probenecid-infused[b]
A	μg/ml	146±6	137±5	127±5
B	μg/ml	78.9±1.1	74.7±0.7	86.0±0.9
α	min^{-1}	0.364±0.019	0.395±0.017	0.331±0.017
β	min^{-1}	0.0199±0.0003	0.0189±0.0002	0.0141±0.0002
V_c	ml/kg	89.0±2.6	94.3±2.4[d]	93.9±2.3[d]
V_{dss}	ml/kg	210±16	227±14[d]	207±14[d]
Cl_{app}	ml/min/kg	4.59±0.09	4.65±0.07[d]	3.08±0.06[e]

a) Parameters and standard error of mean (S.E.M.) were calculated by biexponential curve fitting by using the NONLIN program.[4] *b*) Mean±S.E.M. from three rats. *c*) Mean±S.E.M. from four rats. *d*) Not significantly different from the results for non-anesthetized rats at $p=0.05$ by the t-test. *e*) Significantly different from the results for non-anesthetized rats at $p<0.01$ by the t-test.

TABLE II. Comparison of Plasma Concentrations of Cefazolin at the Terminal Phase after Intravenous Bolus Injection at a Dose of 20 mg/kg to Awake, Urethane-Anesthetized, Pentobarbital-Anesthetized and Probenecid-Coadministered Rats[a]

Condition of rats	Number of rats	Plasma concentration of cefazolin (μg/ml) Time (min)			
		30	60	90	120
Awake	3	40.7±2.9	23.9±2.1	12.5±1.0	8.18±0.82
Urethane	4	39.7±1.6 [d]	23.2±1.4 [d]	14.2±1.2	8.16±1.1
Pentobarbital	3	34.9±1.6	21.1±1.0	14.1±2.0 [d]	12.0±2.7 [d]
Awake+probenecid[b]	3	54.8±1.6	34.4±1.4	24.3±1.4 [e]	17.8±1.4 [d]
Urethane+probenecid[c]	4	49.7±12.9	34.2±8.2	27.7±7.2	19.3±3.5

a) Mean±S.E.M. from three to four rats. *b*) Probenecid was coadministered to awake rats. *c*) Probenecid was coadministered to urethane-anesthetized rats. *d*) Significantly different at $p<0.01$ by the t-test. *e*) Significantly different at $p<0.02$ by the t-test.

for anesthetized and non-anesthetized rats. The influence of pentobarbital anesthesia on the plasma time course of cefazolin was also examined. As shown in Table II, the plasma concentrations of cefazolin at 30, 60, 90 and 120 min after intravenous injection were not significantly different ($p=0.01$) among awake, urethane-anesthetized and pentobarbital-anesthetized rats. These results suggest that urethane anesthesia does not influence the distribution or elimination of cefazolin in rats.

Figure 1 and Table I also show the effect of probenecid on the disposition of cefazolin. The plasma concentration of probenecid in the steady state was determined to be 97.3±9.4 (μg/ml) ($n=4$). The concentration of probenecid was nearly equal to the previously reported value for sufficient inhibition of tubular secretion of β-lactam antibiotics.[2] The clearance rate of probenecid-infused rats was significantly less (3.08 ml/min/kg) than that of the non-probenecid-infused rats (4.59 ml/min/kg), but no difference in the volume of distribution of cefazolin was observed. The present results are consistent with the finding of the inhibition of cefazolin elimination by *p*-aminohippuric acid in rabbits[5] and suggest that cefazolin, like other β-lactam antibiotics, is eliminated not only by glomerular filtration, but also tubular secretion. Previously, we reported that probenecid inhibited the renal elimination of penicillin G, but we were unable to demonstrate any effect of probenecid on the elimination of cefazolin

in urethane-anesthetized rats.[2] Since the body temperature of the rats in the previous experiment was not controlled, this apparent contradiction might have been caused by a drop of the temperature below 37 °C. In this study, cefazolin was given to probenecid-coadministered rats under urethane anesthesia, with body temperature control at 37±1 °C. The plasma concentrations of cefazolin in awake, urethane-anesthetized, and probenecid-coadministered rats are compared in Table II. There was a significant difference in the plasma concentrations at 30, 60, 90 and 120 min between awake rats and probenecid-coadministered rats. There was also a significant difference of plasma concentration at 90 and 120 min between urethane-anesthetized rats and probenecid-coadministered rats under urethane anesthesia. From these results it seems reasonable to assume that the body temperature of the previously studied rats had dropped below 37 °C leading to a significant reduction of renal tubule secretion of cefazolin.

Thiamine pharmacokinetics was reported to be affected by urethane anesthesia.[1a] This basic drug, however, is exclusively excreted from the kidney by glomerular filtration, secreted by the tubular cells and reabsorbed in the renal tubules.[6] The difference between cefazolin and thiamine with respect to the influence of urethane anesthesia on the renal elimination may be attributed to the different mechanisms of renal tubular secretion of an organic anion and cation, as reviewed by Pitts.[7]

The tissue distribution of β-lactam antibiotics was investigated by several workers[2,8a,b] and reviewed by Bergan[9a] and Brogard *et al.*[9b] It was determined that β-lactam antibiotics are localized in the extracellular tissue space except for eliminating organs such as the liver and kidney. The tissue-to-plasma partition coefficients can be predicted from the unbound antibiotic concentrations in plasma, the interstitial fluid volume and the ratio of interstitial to plasma albumin concentration.[2] Fairly good agreement between the predicted and observed K_p values was demonstrated previously in the urethane-anesthetized rat. The tissue-to-plasma partition coefficients in non-anesthetized rats are summarized in Table III and compared with the predicted K_p values in anesthetized animals.[2] It was suggested that the proposed pharmacokinetic model[2] could also be used to predict the distribution of cefazolin in the non-anesthetized rat. Good coincidence was observed in all tissues except the gut and lung. Of the administered dose, 13.1% was recovered in the bile 6 h after bolus injection of cefazolin (10 mg/kg) to lightly ether-anesthetized rats.[10] Although the extent of the intestinal absorption

TABLE III. Comparison of Experimentally Determined and Predicted Values of Tissue-to-Plasma Partition Coefficient (K_p) of Cafazolin

Tissue	Volume[a] V_T (ml)	K_p values Observed[b]	K_p values Predicted[c]	Observed K_pV_T (ml)
Plasma	11.3	1.000	1.000	11.3
Skin	43.0	0.313±0.033	0.300—0.300	13.5
Muscle	108.0	0.052±0.005	0.074—0.079	5.6
Gut	16.0	0.248±0.047	0.085—0.086	4.0
Bone	26.0	0.073±0.008	0.045—0.049	1.9
Lung	1.6	0.182±0.013	0.103—0.106	0.3
Heart	1.0	0.073±0.014	0.055—0.056	0.1
Liver	8.0	0.131±0.016	—	1.1
Kidney	2.0	0.814±0.140	—	1.6
Total	216.9	—	—	39.4

a) Based on a 250-g rat.[2] *b*) The mean value±S.E.M. of eight to nine rats. The value was corrected according to the method described by Chen and Gross.[3] *c*) The value, corresponding to the *in vivo* plasma concentration (5—50 μg/ml) at the terminal phase after *i.v.* injection (20 mg/kg), was calculated from the previously proposed model and parameters.[2]

of cefazolin was reported to be relatively less than that of orally administered β-lactam antibiotics, about 10% of cefazolin was absorbed from the rat small intestine during a two-hour *in situ* perfusion experiment.[11] Thus, the excretion of cefazolin in bile and its incorporation into intestinal tissue might be responsible for the higher concentration of cefazolin observed in the gut than predicted. However, the reason for the difference between the predicted and observed K_p values of lung is unclear at present.

Table III also gives the values of $K_p \times V_T$. The values of skin and plasma were greater than those of other tissues. These results suggest that skin and plasma play an important role in the disposition of cefazolin. Although the total value of $K_p V_T$ (158 ml/kg) was less than the steady-state distribution volume (210 mg/kg), it represents 75% of the tissue distribution volume. Distribution to the remaining tissues, *e.g.* adipose, spleen, *etc.*, may account for the difference between the value of total $K_p V_T$ and V_{dss}.

In any experiment involving difficult or prolonged surgery as part of a pharmacokinetic experiment, continuous and stable anesthesia should be used. As urethane anesthesia is known not to influence renal blood flow rate in rats,[12] urethane is a useful anesthetic for this purpose. If the body temperature is maintained at 37 °C, the pharmacokinetics of β-lactam antibiotics in urethane-anesthetized rats should not differ significantly from that in non-anesthetized rats.

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