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Effect of Probenecid on the Pharmacokinetics of Moxalactam

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The effects of probenecid on the pharmacokinetics of moxalactam were studied in normal volunteers administered a 2-min 1-g intravenous infusion. The results showed that probenecid did not alter the plasma or urinary concentrations of moxalactam, its apparent volume of distribution, plasma elimination half-life, elimination rate constant, or plasma and renal clearances. Therefore, moxalactam appears to be eliminated primarily by the kidney via glomerular filtration.

The effects of probenecid on the plasma concentrations and urinary elimination of many beta-lactam antibiotics have been studied and may vary from compound to compound (1, 5–7, 10, 20, 23–25). This study examines the effects of probenecid on the pharmacokinetics of moxalactam, a new beta-lactam antibiotic.

MATERIALS AND METHODS

Subjects. Six normal male volunteers ranging from 22 to 55 years of age and 60 to 84 kg in weight signed written statements of informed consent to participate in this study. Volunteers were judged healthy on the basis of a normal physical examination and normal clinical laboratory parameters which included complete blood count, urinalysis, prothrombin time, partial thromboplastin time, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, glucose, serum creatinine, and blood urea nitrogen. All clinical laboratory parameters were repeated at the end of the study period.

Design. Six volunteers were randomly assigned to a two-way crossover study. One volunteer did not complete the entire study because of his unwillingness to cooperate; therefore, data for only five volunteers are reported.

Two volunteers received 1 g of moxalactam intravenously in 10 ml of 5% dextrose and water over a 2-min period. Three volunteers received 1 g of moxalactam intravenously over a 2-min period, with 500 mg of probenecid orally at 6 and 12 h before the moxalactam administration, at the time of moxalactam administration, and 6 h after the start of the moxalactam administration for a total dose of 2 g. After a 7-day washout period, the two groups were crossed over so that all subjects received both treatments.

All volunteers fasted 8 h before and 2 h after each treatment and did not take other medication during the study period. Volunteers were ambulatory at all times, and no caffeine or smoking was allowed on the days of treatment.

Heparinized venous blood samples for assay of plasma concentrations of moxalactam were obtained at 0, 0.08, 0.17, 0.33, 0.5, 0.75, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, and 24.0 h after administration of moxalactam. Urine samples were collected at intervals of 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12 and 12 to 24 h after the dose. Just before initial moxalactam administration, the subjects emptied their bladders. This urine sample was used as a blank for detection of microbiologically active substances.

Heparinized venous blood samples for the assay of plasma concentrations of probenecid were obtained at 0, 2, 6, 12, and 24 h after the administration of moxalactam.

Assays. Plasma specimens were assayed microbiologically by using a standard agar well diffusion method (8) tryptic soy agar (pH 7.4) and *Escherichia coli* ATCC 4157 as the indicator organism. Antibiotic standards for assay of serum samples were prepared in a pooled serum. Concentrations greater than 0.5 μg/ml could be detected by this assay and indicator organism. All samples were run in triplicate. Samples of urine were frozen and assayed turbidometrically with an Autoturb (Elanco Products Co.), *E. coli* ATCC 4157 as the indicator organism, and Penassay broth (Difco Laboratories) (pH 6.6). The standard curve was prepared in phosphate buffer (pH 6.6). Concentrations of 10, 5, 2, and 1 μg/ml were placed in a carousel and presented to the diluter. From 1 to 100 dilutions of the curve points were made, giving the assay a sensitivity of 1 μg/ml. All samples were run in quadruplicate. The turbidometric assay of moxalactam in urine has been shown by us to have a standard error of less than 3%. This assay allows the handling of more samples and is much faster than the diffusion assay.

Plasma concentrations of probenecid were determined by a modification of the gas chromatographic procedure of Zacchei and Widner (26) which involves column methylation of probenecid with 0.2 M trimethylanilinium hydroxide in methanol. The linearity of the assay is between 10 and 80 μg/ml with a precision of ±1.5% (based on reproducibility of the slopes of the calibration curves). Based on limited data of assayed spiked plasma samples, the accuracy is about 1%. The sensitivity of the assay has been determined at 1 μg/ml. The extraction, methylation, and chromatography combined offer the specificity of the method. Based on chromatographic conditions, moxalactam does not interfere in the assay.

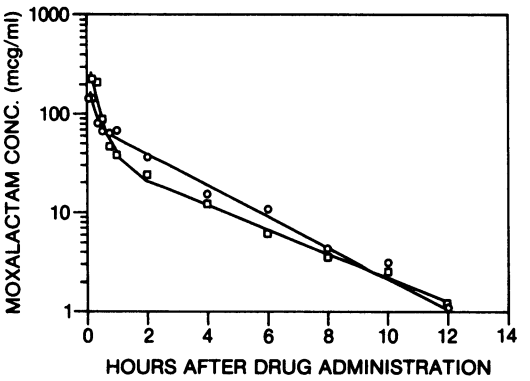

FIG. 1. Moxalactam plasma concentrations with (○) and without (□) probenecid present (subject no. 4). The line is a computer generated fit of data.

Pharmacokinetic analysis. Plasma concentrations of moxalactam were fitted to the biexponential equation: $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, as suggested by Wagner (21), using nonlinear least-squares regression analysis on the program NONLIN (12). C_p represents the plasma concentration of moxalactam in milligrams per liter. A and B are the exponential coefficients after bolus intravenous administration (A and B were corrected for infusion time as suggested by Wagner). α and β are the exponential exponents multiplying t, and t is the time elapsed after the start of the intravenous infusion. The initial estimates for A, B, α, and β were obtained with the program C-STRIP (16) (see Table 2).

RESULTS

Peak plasma concentrations with a mean of 137.4 μg/ml were achieved at 5 to 10 min after a 2-min 1-g intravenous infusion of moxalactam and 149.4 μg/ml after a 2-min 1-g intravenous infusion of moxalactam administered with probenecid (Table 1). Mean plasma concentrations of probenecid were 44.3 μg/ml at the start of the infusion, 76.1 μg/ml 2 h postinfusion (based on three subjects) and 11.5 μg/ml 24 h after the start of the infusion.

Plasma concentrations of moxalactam, when given alone or with probenecid, were well fitted to the biexponential equation for all subjects; and an example of the goodness of the fit is shown in Fig. 1 for subject 4. The pharmacokinetic parameters of moxalactam when administered alone or with probenecid are summarized in Table 2. The α (disposition rate constant) after probenecid pretreatment was 60% greater than that after moxalactam alone; however, this difference was not statistically significant ($P > 0.05$; 22). Most of this difference in α could be attributed to one subject. It should be noted, however, that this difference was not any greater than those differences we have observed in other normal volunteers. Since β is the smallest of the exponential values, it usually represents elimination of the drug from the body. Thus, the plasma half-life for moxalactam (approximately 2 h) was calculated from β. The apparent volumes of distribution indicate that the central compartment is about 50% of the total volume of distribution for moxalactam. Urinary excretion was highest in the first 4 h after the 2-min infusion. By 24 h, approximately 70% of the administered dose of moxalactam was recovered in the urine. The total 24-h urinary recovery was

TABLE 1. Plasma concentrations of moxalactam after a 2-min intravenous bolus infusion of 1 g administered with and without probenecid

Infusion	Subject no.	Plasma concn (μg/ml) of moxalactam at h[a]:											
		0.08	0.17	0.33	0.5[a]	0.75	1	2	4	6	8	10	12
Moxalactam	1	101.0	79.4	72.5	54.1	54.0	48.5	31.0	12.2	10.6	5.3	2.4	1.2
	2	113.0	110.0	105.5	84.1	73.7	70.8	62.2	45.7	23.1	5.1	3.2	2.3
	3	136.8	135.7	109.3	87.9	67.6	79.3	46.4	45.7	22.9	6.7	5.9	2.9
	4	186.5	225.6	210.1	87.4	46.7	37.9	24.1	12.0	5.9	3.5	2.5	1.1
	5	137.5	136.5	84.0	79.3	51.5	36.6	29.6	15.3	5.8	2.9	2.3	1.5
Mean		135.0	137.4	116.3	78.6	58.7	54.6	38.7	26.2	13.7	4.7	3.3	1.8
±SD		±32.8	±54.5	±54.6	±14.1	±11.4	±19.4	±15.5	±17.9	±8.7	±1.5	±1.5	±0.8
Moxalactam + probenecid (2 g)	1	118.4	74.3	76.2	41.8	49.5	37.2	37.2	10.9	3.9	3.2	2.6	1.0
	2	195.3	152.8	112.7	78.0	52.1	55.5	36.7	15.0	5.0	3.2	2.4	2.0
	3	157.2	125.6	84.2	52.8	55.6	43.7	37.9	16.4	10.6	3.9	2.7	1.3
	4	143.4	144.2	79.7	65.9	63.7	67.7	35.5	15.3	10.7	4.3	3.1	1.1
	5	132.7	116.1	78.1	63.1	69.1	71.1	34.7	16.7	11.3	8.0	5.4	3.8
Mean		149.4	122.6	86.2	60.3	58.0	55.0	36.4	14.9	8.3	4.5	3.2	1.8
±SD		±29.3	±30.7	±15.2	±13.7	±8.2	±14.7	±1.3	±2.3	±3.5	±2.0	±1.2	±1.2

[a] No values at hour 0.
[b] Significantly different from moxalactam alone ($P < 0.05$; 22).

TABLE 2. Pharmacokinetic parameters of moxalactam after a 2-min intravenous bolus infusion of 1 g administered with and without probenecid[a]

Infusion	Subject no.	Dose (mg/kg)	A (kg/liter)	B (kg/liter)	α (h^{-1})	β (h^{-1})	V_c (liter/kg)	$t_{1/2}(\beta)$ (h)	V_{DSS} (liter/kg)	V_{Darea} (liter/kg)	Cl_p (liter/kg per h)	Cl_r (liter/kg per h)	$AUC_{0-\infty}$ (kg·h/liter)
Moxalactam	1	12.8	3.97	4.94	4.87	0.34	0.11	2.0	0.18	0.19	0.065	0.058	15.3
	2	11.9	1.71	8.6	4.86	0.31	0.1	2.2	0.11	0.11	0.036	0.031	28.1
	3	16.4	4.29	5.43	3.55	0.27	0.1	2.6	0.16	0.17	0.047	0.021	21.3
	4	13.7	20.85	2.46	3.53	0.28	0.04	2.5	0.15	0.24	0.068	0.046	14.8
	5	13.9	8.5	3.48	2.89	0.32	0.08	2.1	0.18	0.23	0.073	0.057	13.7
Mean		13.74	7.86	4.98	3.94	0.30	0.09	2.28	0.16	0.19	0.056	0.043	18.6
±SD		±1.69	±7.66	±2.34	±0.89	±0.03	±0.03	±0.25	±0.03	±0.05	±0.015	±0.016	±6.1
Moxalactam + probenecid (2 g)	1	12.8	8.02	4.74	9.46	0.40	0.08	1.8	0.18	0.2	0.078	0.076	12.8
	2	11.9	14.2	5.91	4.34	0.39	0.05	1.8	0.12	0.14	0.054	0.053	18.6
	3	16.4	8.86	3.77	5.73	0.32	0.08	2.2	0.21	0.23	0.075	0.034	13.4
	4	13.7	7.99	5.71	5.68	0.36	0.07	1.9	0.15	0.16	0.058	0.048	17.3
	5	13.9	6.68	5.38	6.13	0.30	0.08	2.3	0.17	0.18	0.053	0.016	18.8
Mean		13.74	9.15	5.10	6.27	0.35	0.07	2.0	0.17	0.18	0.068	0.045	16.2
±SD		±1.69	±2.93	±0.87	±1.91	±0.04	±0.01	±0.23	±0.03	±0.03	±0.011	±0.022	±2.9

[a] Abbreviations: A and B, exponential coefficients after bolus intravenous administration; α and β, exponential exponents multiplying t; V_c, volume of the central compartment; $t_{1/2}$, plasma half-life; V_{DSS}, volume of the distribution steady state; V_{Darea}, volume of the distribution calculated from the area under the plasma concentration time curve; Cl_p, plasma clearance; Cl_r, renal clearance; and $AUC_{0-\infty}$, area under the plasma concentration time curve extrapolated to infinity.

unaltered by probenecid (Table 3). The 24-h urinary excretion was determined to be equivalent to the infinity urinary excretion by the method of Newburger et al. (13).

A statistical analysis of the plasma concentrations and pharmacokinetic parameters after intravenously administered moxalactam in the presence and absence of probenecid was performed by using an analysis of variance for a crossover design. Small differences in the plasma concentrations and pharmacokinetic parameters did not test statistically significant (22).

No adverse reaction to the drugs or significant alteration in laboratory parameters was observed during and after the study period.

DISCUSSION

In this study, plasma concentrations of moxalactam were fitted to a biexponential equation. Fitting data to a model, independent, biexponen-

TABLE 3. Urinary excretion of moxalactam after a 2-min intravenous bolus infusion of 1 g administered with and without probenecid

Infusion	Subject no.	Urinary excretion of moxalactam (mg) during collection period (h)								
		0–2	2–4	4–6	6–8	8–10	10–12	12–24	Total (mg)	% Dose
Moxalactam	1	549	176	36	48	14	14	39	876	88
	2	251	397	97	34	41	23	13	856	86
	3	298	82	15	18	15	10	13	451	45
	4	294	189	127	29	16	21	1	677	68
	5	380	149	132	55	25	19	15	775	78
Mean									727.0	73.0
±SD									±173.1	±17.5
Moxalactam + probenecid (2 g)	1	518	232	90	54	42	29	11	976	98
	2	313	360	97	68	84	35	28	985	99
	3	110	173	61	45	36	5	21	451	45
	4	85	105	66	23	8	15	7	309	31
	5	273	239	102	100	47	47	10	818	82
Mean									707.8	71.0
±SD									±310.6	±31.3

tial equation was chosen in favor of the more conventional two-compartment open model, since Wagner has reported (21) that it is extremely difficult to determine which class of models is correct when data are represented by a biexponential equation. Other investigators (2, 3, 11, 14, 15, 18) studied the pharmacokinetics of moxalactam in normal healthy volunteers. The results of these studies agree with our findings and, furthermore, indicate that the kidney is the primary route of elimination of moxalactam in humans. Probenecid does not affect the plasma concentrations or pharmacokinetic parameters of moxalactam including the volume of the distribution steady state, plasma half-life, β, and plasma and renal clearance; therefore, moxalactam appears to be eliminated primarily by the kidney via glomerular filtration.

Similar findings on the effect of probenecid on the pharmacokinetics of moxalactam have been reported (17). Unlike many other beta-lactam antibiotics (5–7, 20), tubular secretion does not appear to have a role in the renal elimination of moxalactam; however, a few beta-lactam antibiotics, such as cephaloridine, show similarities to moxalactam when probenecid is administered (4, 9, 19).

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