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Moxalactam Penetration into Normal Heart Valve, Cardiac Vegetations, and Myocardium in Relation to Protein Binding and Physiological Distribution Spaces

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Rabbits with catheters implanted in the left ventricle were given a single dose of moxalactam and sacrificed at various times thereafter for measurement of the concentration of this antimicrobial agent in serum, heart muscle, and various heart valves. Penetration into both extravascular sites was rapid; steady state was achieved within 5 min after the dose. Moxalactam showed essentially complete penetration into valve lesions, whereas concentrations in heart muscle were only 20% of those in serum. The physiological distribution of moxalactam in heart muscle was beyond the inulin space, but substantially lower than total body water. This myocardial distribution ratio was not predicted by the serum-free fraction or blood trapped in tissues alone, but was in good agreement with that of extracellular fluid plus blood trapped in tissues. The moxalactam distribution profile was most compatible with that of drugs which are excluded from cells but readily distributed throughout extracellular fluids. This explains its nearly complete penetration into heart valves as well as its incomplete penetration into heart muscle, since the two sites differ in their relative proportions of cells and extracellular fluid spaces.

On occasion, patients with abdominal infections caused by aminoglycoside-susceptible bacteria have failed to respond to aminoglycosides. Several of these have been treated with moxalactam and the infection has been cured (6, 16). The apparent reason for these responses was the penetration of moxalactam into abdominal fluids at higher multiples over the MIC than aminoglycoside penetration. If moxalactam does possess a therapeutic advantage due to higher extravascular penetration in relation to MIC, then the drug should be explored for treatment of heart valve infections. This site is devoid of host defense, and infections frequently resist treatment with antibiotics (19).

Recent studies from this laboratory have described the penetration of methicillin into heart valves (8–10) and have correlated concentrations therein with cure of experimental staphylococcal endocarditis in rabbits (F. M. Gengo, T. W. Mannion, C. H. Nightingale, and J. J. Schentag, J. Antimicrob. Chemother., in press). Serum protein binding had little influence upon the penetration of methicillin into heart valves at steady state. Methicillin, however, is only 17% bound to serum proteins, and hence a major influence was not anticipated. In contrast, moxalactam is 60% bound to rabbit serum proteins, and this could limit its extravascular penetration (4). Other studies, however, question the influence of serum protein binding upon heart valve penetration, since the heart valve vegetation contains a fluid high in protein content and similar to serum (3, 7). The purposes of the present study were to evaluate the distribution of moxalactam into heart valves and to assess the impact of its higher serum protein binding on this distribution.

MATERIALS AND METHODS

Sixteen New Zealand rabbits between 2.6 and 3.5 kg were used for this investigation. They were housed separately and fed a standard diet with free access to water. Under pentobarbital anesthesia, each had a 15-cm polyethylene catheter implanted in the heart via the carotid artery. This polyethylene catheter was passed through the aortic valve leaflets to cause fibrous scarring of the valves. Mitral and tricuspid valves are not damaged by catheter placement (9, 16).

Five days after placement of the catheter, each rabbit was given a 50-mg intravenous bolus dose of moxalactam. This dose produces a serum concentration profile similar to that produced by 1- to 2-g doses in humans. Blood samples were drawn from the marginal ear vein every 5 min for 1 h after dosage to determine the time course of the concentration of moxalactam in serum. Forty-eight hours later, a second 50-mg moxalactam dose was administered, and blood samples were drawn at 5-min intervals until sacrifice. Four rabbits each were sacrificed with 100 mg of pentobarbital at 5, 15, 45, and 60 min after the second dose. At the time of sacrifice, a final blood sample was taken from the heart. The heart was then removed, and samples of aortic and mitral valves, fibrotic heart valve lesions, and myocardium were collected and weighed (8). Tissue samples were homogenized in glass hand-held homogenizers and assayed for antibiotic concentration within 7 days of collection.

Assay. Concentrations of moxalactam in serum and tissue were measured by microbiological disk diffusion assay, with *Providencia stuartii* as the test organism. Standards were prepared by adding moxalactam to sera or tissue homogenates of untreated rabbits. The serum or tissue supernatant standard curves were employed for assays as appropriate. Serum and tissue supernatant samples were assayed in triplicate on two or three occasions, and the results were averaged. The detectable serum concentrations without dilution ranged from 2.1 to 33.3 μg/ml. Day-to-day control samples in this concentration range showed variation of less than 10%.

Pharmacokinetic analysis. The moxalactam serum concentration data were best described by a biexponential equation and were therefore fitted by nonlinear least-squares regression analysis (14). Estimates of slope and intercept values were obtained, and these values were used to calculate half-

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TABLE 1. Moxalactam concentrations and percent penetration at various time points after rabbits were given a 50-mg single intravenous bolus dose

Fluid or tissue site	Moxalactam concn (μg/ml) at:				Tissue-to-serum ratio (%) at:			
	5 min	15 min	45 min	60 min	5 min	15 min	45 min	60 min
Serum	109.1 ± 14	47.5 ± 12.3	33.1 ± 12.8	15.0 ± 1.2				
Myocardium	23.5 ± 5.8	11.1 ± 4.4	5.6 ± 3.2	ND[a]	21 ± 6	21 ± 1	15 ± 4	ND
Aortic valves		29.3 ± 6.8		10.2 ± 1.0		61 ± 20		61 ± 10
Aortic vegetations	55.9 ± 1.1	20.6 ± 7.2	22.0 ± 8.5	9.2 ± 2.07	51 ± 10	48 ± 23	64 ± 11	73 ± 6
Mitral valves	53.5 ± 8.3	27.4 ± 4.4	16.7 ± 7.8	8.8 ± 0.95	59 ± 4	60 ± 20	51 ± 11	58 ± 2

[a] ND, Not detectable.

life, clearance, and steady-state volume of distribution (11). The moxalactam tissue concentrations were best described by a monoexponential equation, and estimates of slope and intercept values were obtained. All tissue concentrations were expressed as tissue-to-serum ratios. The myocardium, vegetations, and mitral valve tissue-to-serum ratios were compared by the unpaired Student's t test, with $P < 0.05$ accepted as a statistically significant difference. All data are expressed as mean ± standard deviation. Literature-derived extracellular water and total water contents of each tissue (2, 9) and the measured moxalactam serum protein binding were used to estimate the penetration of the free fraction of moxalactam into the extracellular fluids (ECF) of each tissue.

Protein binding. The percentage of moxalactam bound to serum proteins was determined by ultrafiltration by the Amicon system. One milliliter of rabbit serum containing 25, 50, or 100 μg of moxalactam per ml was placed on the reservoir side of the apparatus and centrifuged for 5 to 10 min at 5,000 rpm. Ultrafiltrate volumes were between 0.16 and 0.27 ml. Four determinations were made at each moxalactam concentration. Both the solution remaining in the reservoir and the filtrate were assayed for moxalactam by the microbiological assay. The concentration in the filtrate was assumed to be the free fraction. Control samples ($n = 3$) containing 100 μg of moxalactam per ml in saline were handled by the same procedure to measure the extent of moxalactam bound to the ultrafiltration membrane. Degradation rates of moxalactam at the temperature, time, and medium conditions of the protein-binding experiments have been measured previously in this laboratory (21).

RESULTS

Concentrations of moxalactam in serum were between 109 ± 14 μg/ml at 5 min and 15 ± 1.2 μg/ml at 1.0 h after both 50-mg doses in all rabbits, and mean values are given in Table 1. These serum concentrations were described well by a biexponential equation ($r > 0.9$). Biexponential analysis yielded intercepts of $A = 78.8$ μg/ml and $B = 155.7$ μg/ml, and slopes of $\alpha = 23.9\ min^{-1}$ and $\beta = 1.59\ h^{-1}$. The moxalactam steady-state volume of distribution was 0.50 liters, and the total body clearance was 0.89 liters/h. The beta-phase half-life of moxalactam in serum was 26.1 min (0.44 h).

In Table 1, tissue and serum data are provided for each of the four time points. There was rapid penetration of moxalactam into both myocardium and heart valve, as maximum penetration ratios were reached at the 5-min sampling point. Myocardium penetration of moxalactam was lower than that of either normal or damaged heart valve. Concentrations in damaged aortic valves and aortic vegetations were slightly, but not significantly, higher than concentrations in normal mitral valves. Both myocardium and heart valve data fit monoexponential equations well (correlation coefficients for combined mitral valves and vegetations and myocardium were 0.97 and 0.99, respectively). The half-life of moxalactam in myocardium and heart valve was not different from the serum half-life.

Moxalactam binding to serum proteins was 56 ± 5.2%, a value independent of concentration over the range of 25 to 100 μg/ml. Moxalactam recovery from both sides of the membrane after ultrafiltration was 75 ± 1% of the amount originally present. The saline controls had a 93.4 ± 1.4% recovery rate, which eliminated degradation and membrane binding as responsible for the loss. The total fluid recovery from both sides of the membrane after the ultrafiltration procedure was never below 96% of the amount originally added to the serum side of the membrane. Apparently, some moxalactam remains on the serum side of the ultrafiltration membrane, trapped by the high amount of protein. An alternative explanation is that the accurate assay of moxalactam in the protein-rich concentrate requires standards prepared in this fluid.

In Table 2, moxalactam penetration ratios were estimated from the serum free fraction and the ECF spaces at each site. Heart valve penetration was most consistent with tissue water. Moxalactam concentration in blood space in tissues, ECF alone, serum-free fraction alone, or total body water spaces did not match the observed moxalactam partitioning in heart muscle. Consideration of total drug in ECF plus limited intracellular penetration of unbound moxalactam is one explanation. Another prediction method which describes the observed moxalactam partitioning in heart muscle is complete ECF penetration of total drug plus complete distribution throughout the blood trapped in heart muscle.

DISCUSSION

Tissue penetration is an important characteristic of an antibiotic, since infections are not only confined to blood, but also involve the extracellular spaces. There have been a large number of studies exploring the tissue penetration of antibiotics (1, 17, 18), but there does not appear to be a

TABLE 2. Measured moxalactam percent penetration for myocardium and heart valve compared with estimates of moxalactam distribution spaces based on free fraction, tissue water, and ECF

Site	Observed tissue-to-serum ratio	Estimated distribution space (%)				
		Blood in tissues[a]	ECF[b]	Tissue water[c]	ECF + (free fraction · intracellular fluid)[d]	Blood + ECF
Myocardium	19	6	11	78	40	17
Heart valve	60	0.8	45	61	52	46

[a] Expressed as a fraction of tissue weight (from reference 2).
[b] Inulin space (from reference 2).
[c] Measured by dessication.
[d] Intracellular fluid = tissue water − ECF; free fraction for moxalactam = 0.44.

consensus as to the relative importance of determinants such as protein binding, infusion rate, influence of blood trapped in tissue homogenates, extracellular water, lipophilicity, and other factors such as infection of the tissue site.

In this investigation we measured moxalactam concentrations in serum, heart valve, and heart muscle. Serum protein binding was also measured, and an attempt was made to account for the observed partitioning via the use of free fraction. The serum-free fraction was unable to predict moxalactam extravascular penetration at either site. Therefore, the known physiological distribution of blood and ECF and the size of the intracellular space were employed to predict the observed partition ratios. Total tissue water (ECF plus intracellular fluid) predicted heart valve penetration better than all other parameters. This finding is in complete agreement with our previous studies of methicillin (8).

ECF space was a logical explanation of myocardial partitioning, because the beta-lactam antibiotics appear to be largely excluded from cells (13, 15). Total tissue water overestimated penetration, and ECF alone underestimates the observed partitioning. Consideration of the serum-free fraction also did not explain the data, since the myocardium distribution space of moxalactam apparently exceeded the ECF but was not as large as intracellular penetration of free drug plus ECF distribution space.

Although there are clinical data in support of ECF penetration of total (free plus bound) moxalactam (20), the measured moxalactam partitioning in myocardium was higher than that of the ECF space alone. This site, in contrast to the valves, has intact cells capable of excluding the drug. Consistent with cell culture experiments, moxalactam is probably excluded from most body cells (5), but it could bind to cell membranes or even penetrate cells well but then be subjected to an active exclusion which lowers the intracellular concentrations below those expected by passive diffusion. There is some evidence for cell membrane binding of beta-lactam antibiotics (12), as well as evidence for partial intracellular penetration with lowered intracellular concentrations due to active exclusion from cells (5, 13). It is not necessary, however, to invoke either limited binding to cell membranes or limited intracellular penetration to explain moxalactam partitioning in myocardium. Reasonable estimates of the observed myocardium-to-serum ratio can be made by considering the ECF space plus the drug retained in the blood content of excised tissue. The data from the present study favor this hypothesis, since there is no evidence of concentration dependence in the partition ratio.

The role of tissue binding sites and blood trapped in tissues as determinants of the tissue-to-serum ratio should be further explored. Regardless of precise mechanisms, serum protein binding does not compromise moxalactam penetration into either normal heart valves or noninfected heart vegetations. Since these data were collected in tissues which were not infected, however, further studies must explore the influence of infection upon these relationships.

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