ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1981, p. 30-32
0066-4804/81/070030-03$02.00/0

Vol. 20, No. 1

Epimers of Moxalactam: In Vitro Comparison of Activity and Stability

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Received 13 November 1980/Accepted 22 April 1981

Moxalactam exists in two epimeric forms, R and S. The in vitro activity of these two epimers was compared with that of material available for clinical and laboratory use (R + S moxalactam). Generally, R moxalactam was twice as active as the S form. The stability of R + S moxalactam was studied at 37, 20, 4, and −20°C in buffer and serum. Only in serum at 37°C was there any appreciable loss of activity (half-life, 8 h). The stability of R and S epimers was studied separately, and the composition of the resulting equilibrium was investigated. At 37°C in serum, one-half of the excess of either R or S over the equilibrium mixture was converted into the equilibrium mixture in 1.5 h. The proportions of R to S in an equilibrium mixture in buffer were 50:50, but in serum they were 45:55. It is doubtful whether these differences in stability and activity will have any significant clinical importance.

Moxalactam (LY127935, 6059S) is a broad-spectrum oxa-β-lactam (3). During a pharmacological study on this compound (6), it was observed that high-performance liquid chromatographic analysis of samples and standards yielded a double peak. Information from Lilly Research Centre Limited, Windlesham, U. K., indicated that these peaks corresponded to the two epimers of moxalactam. These epimers are known as R and S moxalactam and correspond to the D and L of prior nomenclature. This study compares two important properties of these epimers, the antimicrobial activity and stability.

MATERIALS AND METHODS

The two epimers, R (potency, 773 μg/mg) and S (potency, ">85%"; taken as 90%) moxalactam, together with an R and S mixture (potency, 10 mg of diacid material per sealed vial) available for clinical studies and stated to be a 50:50 mixture of the two epimers, were supplied by Lilly Research Centre Limited. Only a small amount of S moxalactam was available, and this limited the studies which could be performed. Neither the R nor the S material was pure as each contained some of the other epimer (T. Yoshida, personal communication).

The strains studied were identified by the API (API Laboratory Products Ltd., Farnborough, England) method, and the production of β-lactamase by certain strains was verified by the nitrocefin method (4).

The minimum inhibitory concentrations of R, S, and R + S moxalactam were measured for the organisms listed in Table 1 by methods previously described (5). Briefly, an agar dilution procedure was used. The medium was Isosensitest (Oxoid Limited, Basingstoke, England) (pH 7.2) enriched when appropriate. The test organisms were grown overnight and then diluted in broth to yield a viable count of about 10^6 colony-forming units, 1 μl of which was transferred to the agar surface by a multi-inoculating device; the final inoculum, therefore, was 10^3 colony-forming units. The plates were incubated for 18 h at 37°C in air, with the exception of *Bacteroides fragilis*, for which we used a GasPak jar (BBL Microbiology Systems, Cockeysville, Md.), and *Haemophilus influenzae* and *Neisseria gonorrhoeae*, which were incubated in 10% CO_2. The minimum inhibitory concentration of each test substance was defined as the lowest microgram per milliliter of medium at which there was no visible growth.

The stability of R, S, and R + S moxalactam was studied by high-performance liquid chromatography with an Applied Chromatography pump (Luton, England) and an ultraviolet detector at 254 nm. A 10-cm Hypersil 5-μm octadecylsilane column was used. The solvent was 14% methanol–0.1% nitric acid in water at a flow rate of 2 ml/min. Serum samples were prepared by mixing with an equal volume of saturated ammonium sulfate, centrifuging and injecting 20 μl of the supernatant. Buffer samples were injected without the initial precipitation step. With this procedure, a 95% base-line separation was achieved, and the retention times of R and S moxalactam were 6 and 8 min, respectively (Fig. 1).

The high-performance liquid chromatographic assay was used to determine the stability of R + S moxalactam by spiking (i) pooled human serum and (ii) phosphate-buffered saline (pH 7.2) with 100 μg of R + S moxalactam per ml, incubating at 37°C, room temperature (about 20°C), 4°C, and −20°C, and measuring the total amount of material remaining at 1, 2, 4, 8, 30, 50, and 120 h. Standards were freshly prepared on each sampling occasion.

The rate of interconversion of R to S and S to R

epimers and a comparison with the R + S mixture were also studied in both serum and buffer by similar methods.

RESULTS AND DISCUSSION

Table 1 summarizes the results obtained for the 23 isolates. In the case of 15 strains, R moxalactam was twice as active as S; they were equally active for 7 strains. Only in the case of one strain was S moxalactam more active. The R + S mixture usually reflected the activity of the R epimer. In no case was there a greater than twofold difference in activity, but these studies are in agreement with the observation of Eli Lilly Research (R. Lucas, personal communication). Other β-lactams, such as ampicillin, hydroxyl benzylpenicillin, phenethicillin, carbenicillin (1), and sulfocillin (2), exist in two epimeric forms. In the case of ampicillin, hydroxyl benzylpenicillin, and sulfocillin, the D (or R) epimer is somewhat more active than the L (or S) epimer. In the case of carbenicillin, epimerization is too rapid to detect differences in activity. Both epimers of phenethicillin have similar activities. It has been postulated (1) that the reasons behind these differences in activity are that the more active epimer would mimic more closely the pentapeptide precursor of the cell wall.

TABLE 1. *Minimal inhibitory concentrations of R and S moxalactam epimers and the R + S clinical mixture*

Strain	Minimal inhibitory concn (μg/ml) of:		
	R moxalactam	S moxalactam	R + S mixture
Escherichia coli			
1193E (TEM-1)[a]	0.06	0.12	0.06
I8	0.06	0.12	0.06
1541E[a]	1	0.5	1
Proteus mirabilis			
J23	0.25	0.5	0.25
J3	0.06	0.12	0.06
Proteus morganii			
J176	0.06	0.12	0.06
Proteus rettgeri			
J51	0.06	0.12	0.06
Proteus vulgaris			
J106	0.25	0.5	0.25
Klebsiella pneumoniae			
H19	0.06	0.12	0.12
H21	0.06	0.12	0.06
Pseudomonas aeruginosa			
10662	4	4	4
G33	4	4	4
G230 (TEM-1)[a]	2	2	2
Staphylococcus aureus			
F4	4	8	4
F105 (β+)[a]	16	32	16
Neisseria gonorrhoeae			
X52	≤0.008	≤0.008	≤0.008
X4	0.015	0.03	0.03
E40 (β+)[a]	0.015	0.015	0.015
Bacteroides fragilis			
X6	0.5	0.5	0.5
X18	2	2	1
X10	8	16	8
Haemophilus influenzae			
A43	0.03	0.06	0.06
A13 (β+)[a]	0.015	0.03	0.03

[a] Known β-lactamase-producing strain.

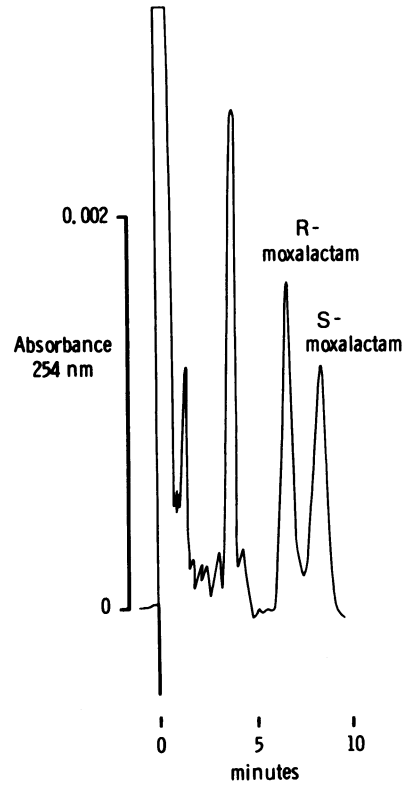

FIG. 1. *High-performance liquid chromatographic assay of R + S moxalactam.*

In Table 2 the in vitro stability of R + S moxalactam is shown and expressed in terms of the half-life. Only in serum at 37°C was there any appreciable instability of the compound. At 4 and −20°C there was less than 10% loss of material in 100 h.

The stability of R and S moxalactam is expressed as the interconversion half-life, that is, the time taken for the percentage of excess (over the equilibrium mixture) of either the R or S epimer to be reduced by half. Each epimer was stable in that form at −20°C, and each was more stable at all temperatures in buffer solution rather than serum. In serum, the S form converted to R + S more rapidly than did the R epimer.

Whereas in buffered saline the final mixture

TABLE 2. *Stability (as measured by half-life) of R + S moxalactam and the interconversion half-lives[a] of R and S moxalactam*

Medium	Stability (half-life) and interconversion half-lives of:											
	R + S at (°C):				R at (°C):				S at (°C):			
	37	RT[b]	4	−20	37	RT	4	−20	37	RT	4	−20
Serum	8	27.5	>100	>100	1.5	11	43	>100	1.5	4	13	>100
Phosphate buffer (pH 7.2)	55	>100	>100	>100	5.5	24	80	>100	8.5	22	>100	>100

[a] Time taken for the percent excess of either the R or S epimer to be reduced by one-half.
[b] RT, Room temperature (about 20°C).

was 50:50 (R to S), if one started with either R or S moxalactam in serum, the equilibrium mixture of R to S was approximately 45:55. This phenomenon was seen at 37°C, room temperature, and 4°C. The data obtained at room temperature are shown in Fig. 2. The initial sampling at time 0 was not 100% R or 100% S (i.e., 0% R) since either interconversion occurred during sample preparation and high-performance liquid chromatographic separation or the epimers were not pure as supplied to us. It can be seen from Fig. 2 that it took 7 to 10 h at room temperature for R + S moxalactam to reach this new equilibrium ratio; at 37°C the interval was about 5 h.

Although it is doubtful whether the in vitro observations have any clinical significance, we have some evidence that the apparent pharmacokinetics of the two epimers may be different in that the relative amounts of S moxalactam increase in the serum of volunteers with time (R. Luthy, R. Wise, A. Bonetti, and J. Blaster, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 239, 1980). We have also observed (N. Wright, P. J. Wills, and R. Wise, unpublished data) that patients with significant degrees of renal failure fail to show a relative increased S epimer in the serum. Possibly this is because the slow excretion allows equilibration to occur. This study does show that equilibration occurs moderately rapidly in serum. The problem is compounded by the fact that the equilibrium mixture in serum is not 50:50 R to S as it is in buffer, but 45:55. Equilibrium mixtures are usually 50:50, but it is possible that small differences in protein binding in serum may account for the different equilibrium ratio.

ACKNOWLEDGEMENT

We thank R. Lucas for his advice and support.

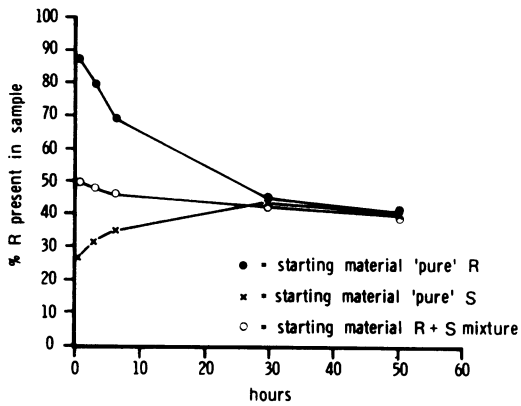

FIG. 2. *Equilibration time curve of R and S moxalactam in serum at 20°C.*

LITERATURE CITED

1. **Butler, K., A. R. English, V. A. Ray, and A. E. Timreck.** 1970. Carbenicillin chemistry and mode of action. J. Infect. Dis. **122** (Suppl):1-8.
2. **Marimoto, S., H. Nomura, T. Fugono, T. Azuma, I. Minami, M. Hori, and T. Masada.** 1972. Semisynthetic β-lactam antibiotics. II. Synthesis and properties of D and L sulfobenzyl penicillins. J. Med. Chem. **15:** 1108-1111.
3. **Neu, H. C., N. Aswapokee, K. P. Fu, and P. Aswapokee.** 1979. Antibacterial activity of a new 1-oxa cephalosporin compared with that of other β-lactam compounds. Antimicrob. Agents Chemother. **16:**141-149.
4. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler.** 1972. Novel method for detection of β-lactamase by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1:**283-288.
5. **Wise, R., J. M. Andrews, and K. A. Bedford.** 1979. LY 127935, a novel oxa β-lactam: an in vitro comparison with other β-lactam antibiotics. Antimicrob. Agents Chemother. **16:**341-345.
6. **Wise, R., S. Baker, N. Wright, and R. Livingston.** 1980. The pharmacokinetics of LY 127935, a broad spectrum oxa-β-lactam. J. Antimicrob. Chemother. **6:**319-322.