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Evaluation of a new polymorph azlocillin sodium by its antibacterial activity

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Abstract

The polymorph azlocillin sodium (obtained by us from acetonitril as recrystallized solvent), has been examined in order to evaluate the influence of the crystal form (state) on its antibacterial activity. Differences in minimal inhibitory concentrations (MIC) values have shown differences in the antibacterial properties of azlocillin sodium (AZ) and its polymorph AZ. These data illustrate the dependance of antibacterial activity on drug crystallinity. © 1997 Elsevier Science B.V.

Keywords: Azlocillin sodium; Polymorphism; Antibacterial activity; Minimal inhibitory concentration; Test microorganisms

1. Introduction

The crystallinity of a drug is of significant importance. Crystal habits influence many characteristics which are related to technopharmaceutical behaviours and therefore to drug bioavailability (Fini et al., 1995). Borka shows that the lability of the system of biologically active vs. inactive substances is usually an underestimate and may in turn produce an interior batch of a well-established drug (Borka, 1976). The evaluation of antibacterial activity is a selection criterion for antimicrobial agents in the constant search for optimizing the physical and biological properties of pharmaceutical crystals (Chow et al., 1995).

In a previous paper we reported that azlocillin sodium (AZ) can exist in a new crystal polymorph AZ, formed by recrystallization from acetonitrile. There were significant differences in the characteristics obtained between the lyophillized AZ and the polymorph AZ analyzed by IR spectroscopy, TG-DTA and SEM analysis (Kalinkova and Stoeva, 1996).

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The aim of the present study was to investigate in vitro the antimicrobial properties of the new crystal polymorph AZ prepared by us in comparison with the lyophilized AZ.

2. Materials and methods

Azlocillin – Batch N 070294 meeting the requirements of the US Pharmacopoeia XXI (Joint Stock Company, Razgrad, Bulgaria) was used as received. The evaluation of antibacterial activity was made on the base of minimal inhibitory concentration (MIC) on the test-microorganisms. The broth macrodilution procedures for testing of antimicrobial susceptibility was chosen as suitable for our research purposes and performed as prescribed by the National Committee for Clinical Laboratory Standards (NCCLS, 1993). In the experiment we used the reference strains *Escherichia coli* 25 922 and *Pseudomonas aeruginosa* 27 853; the standard strains *Staphylococcus aureus* 25 923, *S. aureus* 6538 and *Bacillus pumilus* 8241, all from ATCC and *Klebsiella pneumoniae* 52 145 (Institute Pasteur-Paris) listed in Table 1, as well as seven recent isolates from significant urocultures listed in Table 2 and 12 listed in Table 3 recent isolates by microbiological control for validation of aseptic conditions by sterility testing or manufacturing of drugs carryed out according to the European Pharmacopoea, 1983. The strains were identified by conventional microbiological methods. The interpretation of the results are based on NCCLS criteria.

Table 1
Minimal inhibitory concentrations (MIC) of azlocillin sodium and of its polymorph to reference and standard bacterial strains

Bacterial strains	MIC (μg/ml)	
	Azlocillin sodium	Polymorph of azlocillin sodium
E. coli ATCC 25 922	8	512
P. aeruginosa ATCC 27 853	8	16
S. aureus ATCC 25 923	1	128
S. aureus ATCC 6538	0.5	16
B. pumilus ATCC 8241	256	64
K. pneumoniae 52 145 (Institute Pasteur-Paris)	8	512

Table 2
Antimicrobial activity of azlocillin sodium and of its polymorph on clinical isolates

Microbial strains	MIC (μg/ml)	
	Azlocillin sodium	Polymorph of azlocillin sodium
E. coli 113	32	512
K. pneumoniae 167	1024	1024
Serratia marcescens 206	1024	1024
K. oxytoca 244	1	0.03
K. oxytoca 202	512	512
K. oxytoca 200	32	1024
Enterobacter cloacae 313	1024	1024

Table 3
Antimicrobial activity of azlocillin sodium and of its polymorph on isolates by microbiological control for validation of aseptic working conditions

Microbial strains	MIC (μg/ml)	
	Azlocillin sodium	Polymorph of azlocillin sodium
Staphylococcus epidermidis 2	8	0.5
S. epidermidis 11	1	4
S. epidermidis 12	1	4
S. epidermidis 14	2	64
S. epidermidis 17	1	2
S. epidermidis 18	0.25	16
S. epidermidis 20	0.015	4
Micrococcus luteus 3	1	2
M. luteus 19	0.065	2
M. luteus 21	1	2
Bacillus subtilis 1	1	2
B. subtilis 10	4	32

3. Results

Table 1 presents MICs for two reference and four standard strains, which are susceptible to AZ. All strains have a greater MIC for polymorph AZ than those for AZ, except *B. pumilus*. For the reference strain *P. aeruginosa* 27 853 MIC is only 2-fold higher and does not change the determination of the strain as 'susceptible'. All other strains are resistant to polymorph AZ, with 5- to 7-fold higher MIC. MIC values of seven isolates from significant urocultures are presented in Table 2. They are all resistant to AZ except *K. oxytoca* 244, which is susceptible. All these strains have greater MICs for polymorph AZ and are determined as 'resistant', except *K. oxytoca* 244, which has a lower MIC and is susceptible to polymorph AZ. Table 3 presents MICs of 12 strains isolated by environmental microbiological control after finishing the aseptic work. They are all susceptible to AZ. For nine of them the qualification 'susceptible' to polymorph AZ is valid and three are graded as 'resistant'. The results obtained for MICs of the tested strains show different values for AZ and its polymorph. For 18 strains these values are higher for polymorph AZ, in some cases manyfold. Seven of the strains determined as 'susceptible' to AZ become 'resistant' to polymorph AZ and 18 are not changed. With a few exception the polymorph has less antibacterial activity than the parent compound and in some cases proves to be resistant to the tested microorganisms. In our previous paper (Kalinkova and Stoeva, 1996) we reported significant differences in the IR spectral characteristics (amide I, II, III, IV, V and VI, as well as in the 3600–3100 cm^{-1} range) among the lyophilised AZ and the polymorph AZ. These results give us ground to consider that the amide group in the molecule of the antibiotic AZ is responsible for the formation of different intermolecular H-bonds as it contains a proton donor (N–H) and an acceptor (C=O). In our opinion, the formation of these different intermolecular H-bonds related to the change in crystalline state (polymorphism) of the antibiotic AZ as well as to change of it's antibacterial activity.

4. Conclusion

Polymorph AZ demonstrates differences in its MIC values and antibacterial properties in comparison with AZ.

There are insufficient data in the literature for antibacterial activity of polymorphs of antimicrobial agents. It has been shown that about 80% of drug substances are polymorphic (Grunenberg et al., 1996).

We are of the opinion that such an evaluation of the antimicrobial agents is very important and necessary because it is related to their therapeutic activity.

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