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Comparative study of antibacterial activity of cefazolin sodium and of its crystalline modifications

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Abstract

Antibacterial activity of four crystalline modifications (ground, solvated and two desolvated) of cefazolin sodium (CZS) have been studied. Their antibacterial properties have been determined by means of minimal inhibitory concentrations (MIC_{50} and MIC_{90}) and of disk diffusion inhibition zone values. The above-mentioned crystalline modifications (Mod.) of CZS-I, II, III and IV, respectively, have been shown to have differences in their antibacterial properties in comparison with the trade CZS as well as between themselves. Mod. I has higher or the same activity as CZS. The obtained results we have explained with the formation of different intermolecular H-bonds in the modifications could be due to the rotation about $C^{\alpha}-C'$ and C–N bonds in the amide groups in their molecules.

Keywords: Antibacterial activity; Antibiotics; Cefazolin sodium; Crystalline modifications; H-bonds

1. Introduction

The existence of cefazolin sodium (CZS) in different crystalline modifications (polymorphs/pseudopolymorphs) has been reported (Pikal et al., 1978; Thoma and Serno, 1984; Borka and Haleblian, 1990; Kalinkova et al., 1990). This cephalosporin of the first generation is being successfully used in the medical practice even today (Eichenwald, 1976; Jett et al., 1995; Schulz and Schmoldt, 1997). Nine forms of CZS (two crystalline, five weak crystalline and two amorphous) were obtained by Pikal et al. (1978). Solution calorimetry and X-ray powder diffraction have been applied for their crystalline determination. Comparative studies of crystalline structures of CZS (also of other β-lactam antibiotics), as well as of their crystalline modifications, have been carried out (Kalinkova et al., 1990; Kalinkova and Dimitrova, 1995). The latter have been prepared by methods different from those described by Pikal et al. (1978).

Insufficient information for the antibacterial activity of the polymorphs/pseudopolymorphs of antimicrobial agents (especially of antibiotics) has been published in the literature. The dependence

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of antibacterial activity on drug crystallinity of azlocillin sodium has been established (Opalchenova and Kalinkova, 1997). Polymorphism has been shown in about 80% of drug substances used (Grunenberg et al., 1996). Therefore, these facts have been considered as very important for drugs and especially for such unique therapeutics as antibiotics, leading to the control of bacterial diseases in this century (Bartlett, 1997).

The aim of the present study was to investigate in vitro and to compare the antibacterial properties of the four crystalline modifications of CZS, prepared by us (amorphous 'Halo' type, solvated and two desolvated) proved using IR spectroscopy, X-ray diffraction and scanning electron microscopy (SEM).

2. Materials and methods

2.1. Antibacterial agents

Cefazolin sodium™, meeting the requirements of the USP XXI (Joint Stock Company, Razgrad, Bulgaria) was used as received to prepare the four modifications (Mod.). The suspending CZS in solvent acetonitrile (Merck, spectroscopic grade, labelled max. 0.03% water content) was applied in a concentration of 0.112 gmol/l.

Mod. I (ground) was prepared by tribomechanical grinding treatment of CZS™ for 30 min with a vibrator, type n.m.v. Ardene (Germany).

Mod. II (solvated) was prepared by suspending the CZS™ in acetonitrile. The crystalline modification was obtained via slow evaporation of the solvent from an open evaporating dish at room temperature, followed by vacuum drying for 45 min (at a constant pressure of 0.7 kPa).

Mod.III (desolvated) was prepared by suspending the CZS™ in acetonitrile. The crystalline modification was obtained via slow evaporation of the solvent from an open evaporating dish at room temperature, followed by vacuum drying for 2 h (at a constant pressure of 0.7 kPa).

Mod. IV (desolvated) was prepared by suspending the CZS™ in acetonitrile. The crystalline modification was obtained via slow evaporation of the solvent from an open evaporating dish at room temperature, followed by vacuum drying for approximately 2.5 h (at a constant pressure of 0.7 kPa).

All samples were kept over calcium chloride in a vacuum dessiccator (at a pressure of 0.7 kPa) at room temperature before use.

2.2. Analytical methods

2.2.1. Infrared spectroscopy

Infrared spectra were recorded with C. Zeiss UR20 spectrophotometer using the Nujol technique. The Nujol used was a Merck, spectroscopic grade. The very strong absorption band at 1780 cm^{-1} (stretching vibration ν C=O in the β-lactam condensed ring) was used as internal standard (at 10% T) for control of the layer thickness of all Nuyol-suspensions studied.

2.2.2. X-ray diffraction

The X-ray diffractometer used was a TUR M-62 (C. Zeiss), in the angle region 2–30° (θ°) with Cu Kα radiation, an Ni filter and a proportional counter.

2.2.3. Scanning electron microscopy

The scanning electron microscopy was an SEM Philips 515.

2.3. Antibactebial activity

The disk diffusion test and agar dilution procedures were chosen as suitable for our research purposes and performed as outlined by the National Committee for Clinical Laboratory Standards (NCCLS, 1993a,b). Antibiotic disks were prepared on the day of use by applying 30 μl of drug solution to 6-mm sterile paper disks before placing them on the agar medium.

2.4. Test-microorganisms

Two hundred and eighty recent isolates of Gram-positive and -negative microorganisms were used in the experiment. The strains were identified by conventional microbiological methods. The nutritive properties of culture media were checked according to USP 23 (1993). The number (in

brackets) of bacterial strains in the different species is assigned in Figs. 1 and 2.

3. Results

The data presented in the histograms are based on NCCLS's criteria for evaluation of the cefazolin susceptibility: MICs and diameters of the inhibition zone values. The values of MICs and inhibition zones, changing by 2-fold or more, were accepted as significant differences in comparison with the results. We chose to present the results of MIC_{90} because they were similar to those of MIC_{50}.

Fig. 1 illustrates the histograms of MIC_{90} of CZS™ and of the four CZS modifications towards 182 Gram-negative microorganisms isolated from significant urocultures. Despite the fact that these microorganisms were considered as part of the normal human body flora, they also often act as opportunistic pahtogens and are now causing more infections (Khan and Cerniglia, 1994; Hume et al., 1996). Mod. I, in comparison with CZS™ is 32-fold more active against *P. mirabilis*, 16-fold against *P. aeruginosa* and 8-fold against *A. faecalis*. Mod. II is 8-fold more active against *S. marcescens* than CZS, Mod. I and III; Mod. III is 8-fold more active against *P. aeruginosa* than CZS, Mod. II and IV; Mod. IV is 64-fold more active against *S. marcescens*. There are significant differences also in the antibacterial activity between the crystalline modifications. Mod. I is 128-fold more active against *A. faecalis* than Mod. II, 32-fold than Mod. III and 64-fold than Mod. IV; 64-fold more active for *P. mirabilis* than Mod. II and 128-fold than Mod. III and IV, 4-fold more active for *E. aerogenes* than Mod. II and III; 4-, 64- and 128-fold more active for *K. pneumoniae* than Mod. II, III and IV, respectively.

Fig. 2 presents MIC_{90} of CZS™ and of the four CZS modifications towards 98 Gram-positive microorganisms isolated by microbiological control of manufacturing of drugs (Underwood, 1987)

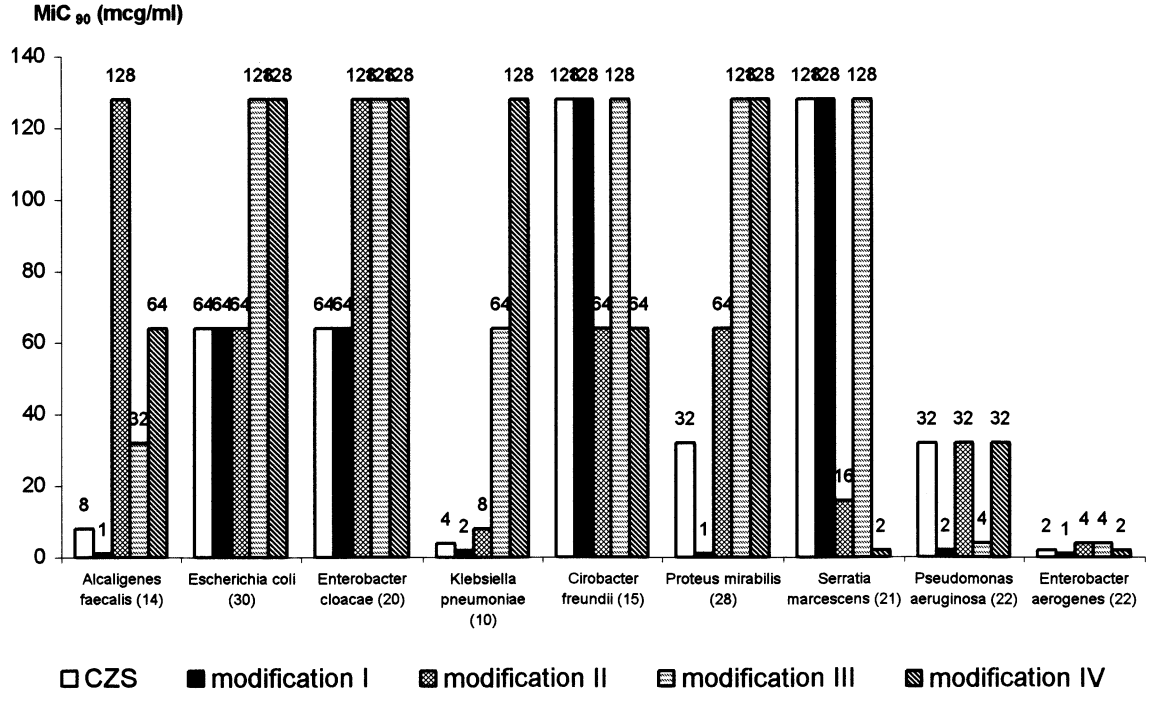

Fig. 1. Antimicrobial activity (MIC_{90} values) of cefazolin sodium and its four crystalline modifications against 182 Gram-negative microorganisms.

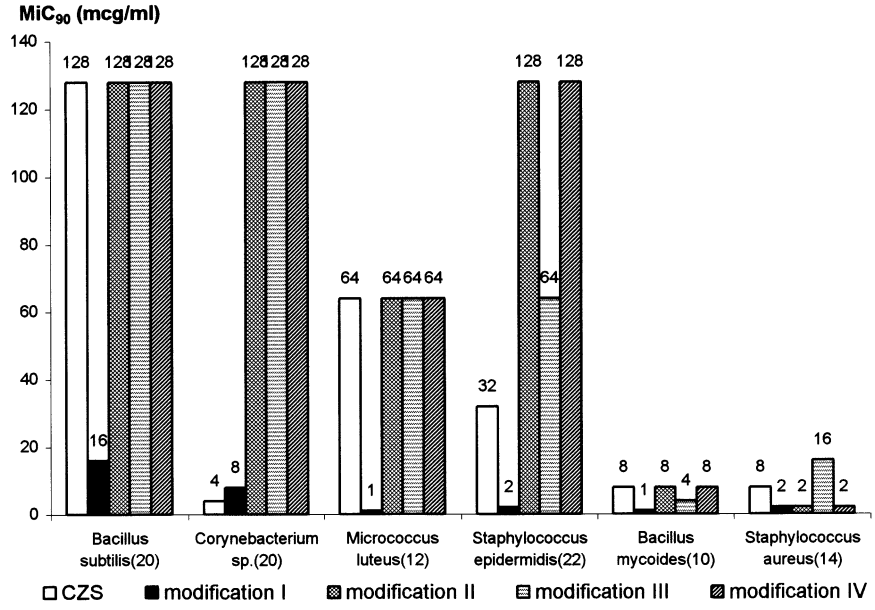

Fig. 2. Antimicrobial activity (MIC_{90} values) of cefazolin sodium and its four crystalline modifications against 98 Gram-positive microorganisms.

carried out according to the European Pharmacopoea (1997), some of wich are pathogenic for man (Cormican and Jones, 1996). The activity of Mod. I is 16-fold higher for *S. epidermidis*, 4-fold for *S. aureus*, 8-fold for *B. mucoides* and 64-fold for *M. luteus* than CZS, 8-fold higher for *S. aureus* than Mod. II and IV, as well as 8-fold higher against *B. subtilis* than Mod. II, III and IV.

The crystalline modifications I, II, III and IV of CZS show significant differences in their antibacterial properties in comparison with CZS™, as well as between themselves. Mod. I has higher or the same activity as CZS. Mod. II, III and IV have the same or lower activity than CZS. The results obtained by disk diffusion test (Fig. 3) again demonstrate greater activity of Mod. I in comparison with CZS, as well as with the other three crystalline modifications.

4. Discussion

Alterations of drug bioavailability as result of changed crystallinity have been described by many authors (Haleblian and McCrone, 1969; Bernabei et al., 1983; Cameroni et al., 1983). For example, in vitro and in vivo data for antibiotics and their polymorphs/pseudopolymorphs (novobiocin,chloramphenicol palmitate, ampicillin, etc.) have been reported (Haleblian and McCrone, 1969). The concentration and the type of the crystal polymorph presented have been evaluated as important factors influencing antibiotic availability and absorption (blood serum levels, urinary excretion rates, absorbance, etc.). The crystallinity and the availability of chloramphenicol palmitate/stearate have been investigated (Bernabei et al., 1983; Cameroni et al., 1983). Bernabei et al. (1983) made very significant conclusions connected with this phenomenon.

Our experimental data (presented in Section 3) illustrate the dependence of antibacterial activity of CZS from antibiotic crystallinity. These significant differences of the four crystal modifications of CZS may be also connected with their different physicochemical characteristics (IR spectra, X-ray diffractogrammes, SEM photographs, etc.). The crystalline modifications of CZS with their differences in amide I, II, III, IV, V and VI, and also in the region 3400–3000 cm^{-1}, have been estab-

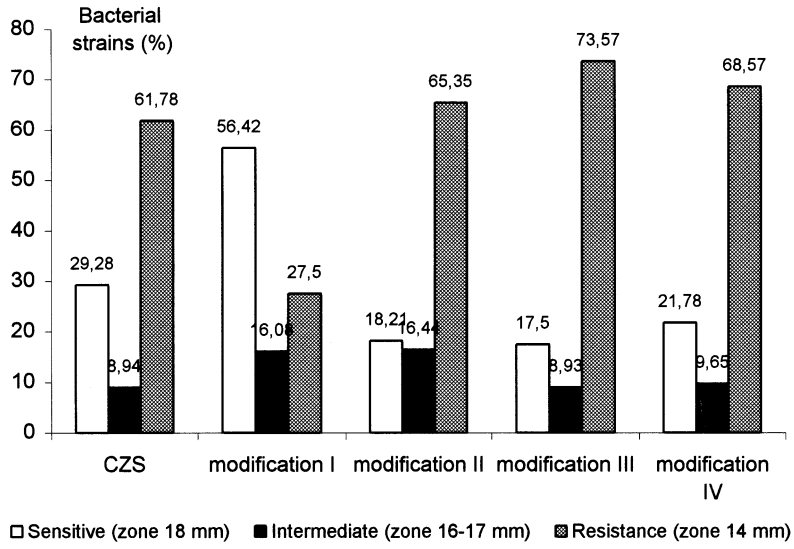

Fig. 3. Percent antibacterial activity of cefazolin sodium and its four crystalline modifications towards 280 Gram-positive and -negative bacterial strains; disk diffusion test.

lished by IR spectroscopy. The latter proved the different H-bonding formations. IR spectral data have been confirmed by Dreiding model (three-dimensional study) of CZN molecules. The possibilities of the free rotation of the amide group and its various space accesibility have been observed.

Both IR spectroscopy and X-ray diffraction of Mod. II, III and IV (solvated and two desolvated, respectively) have shown increased crystallinity.

SEM has demonstrated different morphological characteristics: Mod. I, unformed agglomerates; Mod. II, well-defined long needles with a common centre and radial disposition; Mod. III, sheafs of long crystals; Mod. IV, prismatic crystals; CZS™, inhomogeneous crystal structure (with partial amorphism). Of the four CZN modifications investigated, only Mod. I possesses the following main specific characteristics: IR spectrum, stepwise decreased intensity of the four absorption bands (range 700–900 cm^{-1}); X-ray diffractogram, 'Halo' type with a maximum at 12.5° (θ°); SEM, unformed agglomerated crystals (amorphism).

Our results are in good agreement with the Pikal's conclusion about the 'history' of sample preparation. Pikal's amorphous forms (freeze dried and vacuum dried) as well as our Mod. I have an amorphous-type 'Halo' in their X-ray diffractograms but with a different maximum in the angle region 2–30° (θ°): at approximately 22° (θ°) and at 12.5° (θ°), respectively. The two crystalline forms prepared by Pikal et al. (1978), using methods different from ours, have been characterised as pentahydrate and monohydrate, which distinguish them from ours.

The differences in the antibacterial activity can be explained in the first place with the structure of the antibiotic containing a proton donor (N–H) and two acceptors (C=O and COO^-) groups forming different intermolecular H-bonds. In the second place these differences could be due to the rotation about C^{α}–C′ and C–N bonds in the amide groups. In our opinion the type of the crystalline modifications is responsible for the changes in their antibacterial activity.

5. Conclusions

The four crystalline modifications of CZS, prepared by us, showed significant differences in their MIC values and antibacterial properties in comparison with CZS™, as well as between themselves. Mod. I exhibited the strongest activity than

CZS against the test organisms (except *E. coli*, *E. cloacae*, *C. freundii* and *S. marcescens*, where the activity was equal). Among the four CZN modifications investigated, only Mod. I possesses the following main specific characteristics: IR spectrum, stepwise decreased intensity of the absorption bands (range 700–900 cm^{-1}); X-ray diffractogram, 'Halo' type with a maximum at 12.5° (θ°); SEM, unformed agglomerated crystals (amorphism).

Mod. II, III and IV have increased crystallinity with various antibacterial activity: mainly decreased activity, but in some microbial species increased (or equal).

The results obtained proved that the antibacterial activity is influenced by antibiotic crystallinity.

Therefore, further multidisciplinary investigations in this aspect are needed to discover crystalline modifications of new drugs (more effective and stable) and to explain their mechanism of action.

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