ANTIBACTERIAL ACTIVITY OF SOME CEPHALOSPORINS AND THEIR 7α-METHOXY DERIVATIVES

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In this paper certain microbiological data are reported for four antibiotics belonging to the class of cephalosporins together with their corresponding derivatives having a methoxy group in the 7α-position. The compounds examined are: Cephalothin sodium (CETNa) and the related 7α-methoxycephalothin sodium (α-CETNa); cefoperazone sodium (CEPNa) and 7α-methoxycefoperazone sodium (α-CEPNa); cefoxitin sodium (CXTNa) and demethoxycefoxitin sodium (CXTDNa); cefamandole nafate sodium (CFMDNa) and 7α-methoxycefamandole sodium (α-CFMDNa).

Cephalothin sodium is a commercialized product (Eli Lilly), its derivative, methoxylated in position 7α (α-CETNa), was synthesized by means of a reaction between cephalothin benzhydryl ester, *tert*-butyl hypochloride and lithium methoxide in methanol at −60°C[1]. The following removal of the benzhydrylic group by a treatment with CF_3COOH in the presence of anisole gives 7α-methoxycephalothin, which is then transformed into its sodium salt.

Cefoperazone sodium is a commercialized product (Pfizer Lab.); its 7α-methoxylated derivative (α-CEPNa) has been synthesized from the 3,5-di-*tert*-butyl-4-hydroxybenzylic ester of the 7β-amino-7α-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, prepared in a way similar to that followed for diphenylmethyl ester, according to the method of NAKAO *et al.*[2], which is condensed with the chloride of the 4-ethyl-2,3-dioxopiperazinecarbonylamino)-*p*-hydroxyphenylacetic acid. After a further treatment with 2-ethylhexanoate sodium, 7α-methoxycefoperazone sodium salt (α-CEPNa) is obtained.

Cefoxitin sodium is a commercialized product (Merck & Co.); its demethoxylated derivative has been synthesized by a reaction between 7-amino-3-deacetylcephalosporanic acid and α-thiophenacetylchloride. The 3-deacetylcephalothin obtained is carbamoylated in position 3 with chlorosulphonylisocianate and with a subsequent hydrolysis the product desired (CXTDNa) is obtained.

Cefamandole nafate sodium is a commercialized product (Eli Lilly); its 7α-methoxylated derivative (α-CFMDNa) has been obtained from the 3,5-di-*tert*-butyl-4-hydroxybenzylic ester of the 7β-amino-7α-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid, prepared as described for 7α-methoxycefoperazone, which undergoes a condensation with D-formylmandeloyl chloride. A further addition of 2-ethyl hexanoate sodium leads directly to 7α-methoxycefamandole nafate sodium (α-CFMDNa).

MIC was determined by the broth dilution method using Mueller-Hinton broth (Difco, U.S.A.). An overnight culture of bacteria was diluted in the same broth to about 10^6 cells/ml and this microbial suspension was sub-divided in 2 ml tubes containing serial two-fold dilutions of each antibiotic. Organisms were incubated at 37°C for 18~20 hours. The MIC of an antibiotic was defined as the lowest concentration that inhibited visible growth.

The methoxylation of CETNa results in an antibiotic (α-CETNa) which, while maintaining its activity against Gram-positive bacteria, shows markedly enhanced activity against Gram-negative bacteria, particularly *Alcaligenes faecalis*, *Escherichia coli*, *Proteus* spp. and *Shigella* spp. The methoxylation of CXTDNa, an antibiotic endowed with poor activity, gives rise to a well known antibiotic (CXTNa), with marked activity against Gram-positive as well as Gram-negative bacteria.

On the other hand, the methoxylation in position 7α of CFMDNa, an antibiotic with good antibacterial activity, leads to α-CFMDNa, a compound endowed with generally lower activity in comparison with the initial compound, particularly against Gram-negative bacteria. Finally the methoxylation in position 7α of a highly active compound like cefoperazone sodium (CEPNa) again leads to a less active compound (α-CEPNa) in comparison with the initial compound, particularly against Gram-negative bacteria.

The results reported do not allow definitive

Table 1. Comparative activity (μg/ml) of some cephalosporins and their 7α-methoxy derivatives.

Organism	CET	α-CETNa	CXTDNa	CXTNa	CFMDNa	α-CFMDNa	CEPNa	α-CEPNa
Staphylococcus aureus PT 65*	0.78	1.56	200	3.12	1.56	3.12	1.56	3.12
S. aureus PT 29*	1.56	3.12	200	3.12	6.25	6.25	3.12	3.12
S. aureus PT 32*	0.78	1.56	100	3.12	12.5	12.5	1.56	3.12
S. aureus ATCC 9144	0.78	0.78	100	6.25	6.25	6.25	1.56	3.12
S. aureus ATCC 14154	1.56	1.56	400	6.25	6.25	3.12	3.12	6.25
Streptococcus pyogenes PT 38*	12.5	12.5	200	25	200	200	25	50
S. pyogenes PT 37*	1.56	1.56	100	3.12	0.78	6.25	1.56	3.12
Alcaligenes faecalis PT 54*	>400	12.5	400	25	3.12	6.25	6.25	50
A. faecalis PT 55*	200	6.25	200	3.12	3.12	6.25	6.25	50
A. faecalis PT 60*	100	6.25	200	3.12	1.56	6.25	25	200
Bordetella bronchiseptica ATCC 4617	100	25	200	100	50	100	200	>400
Escherichia coli ATCC 10536	25	1.56	100	1.56	12.5	12.5	1.56	100
E. coli PT 11*	6.25	1.56	200	1.56	3.12	6.25	0.39	25
E. coli PT 18*	25	6.25	400	6.25	6.25	6.25	3.12	25
E. coli PT 40*	200	6.25	100	1.56	6.25	6.25	1.56	6.25
Klebsiella pneumoniae ATCC 10031	12.5	12.5	100	6.25	50	25	0.78	100
Proteus rettgeri ATCC 9250	50	1.56	400	3.12	100	25	3.12	50
P. vulgaris ATCC 6897	100	3.12	100	6.25	200	50	25	200
Pseudomonas aeruginosa ISM 66	>400	>400	>400	400	>400	400	100	>400
Salmonella typhi PT 7*	1.56	1.56	200	3.12	100	100	12.5	3.12
S. paratyphi A PT 8*	50	50	200	25	100	100	50	200
Shigella sonnei ATCC 11060	25	6.25	200	3.12	50	6.25	6.25	200
S. dysenteriae ATCC 9583	25	1.56	200	1.56	12.5	50	3.12	12.5

* Culture collection of Proter S.p.A.; β-lactamase-producing strains.

conclusions to be drawn, but the following hypotheses can be presented. Methoxylation in position 7α is useful in those compounds which do not show resistance against the action of β-lactamases. The substituent in position 7 of CETNa and CXTDNa is not sufficient to resent in resistance to the enzymatic degradation but the introduction of a $-OCH_3$ group in position 7α leads to highly active compounds of great interest, which is in accordance with the findings of other research teams[3].

In contrast, the 7α-methoxylation of compounds such as CFMDNa and CEPNa, which have side-chains which result in partial or total stability against the action of β-lactamases, does not lead to compounds with increased resistance to the action of β-lactamases, but to compounds with significantly less antibacterial activity.

It is evident that the hypotheses mentioned need further experimental data in order to become final conclusions.

References

1) KOPPEL, G. A. & R. E. KOEHLER: Functionalization of $C_{6(7)}$ of penicillins and cephalosporins. A one-step stereoselective synthesis of 7-α-methoxycephalosporin C. J. Am. Chem. Soc. 95: 2403~2404, 1973

2) NAKAO, H.; H. YANAGISAWA, B. SHIMIZU, M. KANEKO, M. NAGANO & S. SUGAWARA: A new semisynthetic 7α-methoxycephalosporin CS-1170: 7β-[[(cyanomethyl)thio]acetamido]-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid. J. Antibiotics 29: 554~558, 1976

3) GORDON, E. M. & R. B. SYKES: 3. Cephamycin antibiotics. *In* Chemistry and Biology of β-Lactam Antibiotics. Vol. 1. Penicillins and Cephalosporins. *Eds.*, R. B. MORIN & M. GORMAN, pp. 199~370, Academic Press, New York, 1982