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Susceptibility of ceftazidime and other cephalosporins to a range of β-lactamases and their potential as inducing agents

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The susceptibility of ceftazidime, five other cephalosporins, moxalactam and cefoxitin to 13 different β-lactamases was determined by means of the spectrophotometric method. None of the bacterial sonicates used as crude β-lactamase solutions and which were prepared with cephalothin as an inducer, hydrolysed ceftazidime, cefoxitin or moxalactam. In comparative induction experiments one cephalosporinase (class I) was induced to some extent by cephalothin, cefuroxime, cefamandole and ceftazidime, but not by cefotaxime, cefoperazone and moxalactam. Both cephalosporinase-producing strains tested were strongly induced by cefoxitin. With the most potent inducer, cefoxitin, β-lactamase activity against ceftazidime could not be detected with either of the two cephalosporinase preparations, or with any one of 11 other β-lactamases. It is concluded that β-lactamase induction as a result of therapy does not appear to be a factor which should be taken into account in therapy with ceftazidime.

Introduction

Recent experimental data (Goering, Sanders & Sanders, 1978; Sanders & Sanders, 1979; Marre, Schulz & Freiesleben, 1980) indicated that when there is a discrepancy between β-lactamase susceptibility and *in-vitro* MIC values of a new cephalosporin, such as cefamandole, the former may be more important for the clinical outcome than the *in-vitro* values. Recently some clinical evidence indicated (Beckwith & Jahre, 1980) that the induction of β-lactamase production by cephalosporins may have some clinical relevance. Therefore it seemed worthwhile to determine and compare the β-lactamase susceptibility as well as the inducing capacities of new and old cephalosporins.

The recently developed new cephalosporin, ceftazidime, was investigated for its susceptibility to variety of β-lactamases and for its potential as a β-lactamase inducer. The results were compared with those of other cephalosporins, moxalactam and cefoxitin.

Materials and methods

Antibiotics

Ceftazidime and cefuroxime were obtained from Glaxo, Hoofddorp, the Netherlands; cephalothin, cefamandole and moxalactam from Eli Lilly, Brussels; cefoperazone from Pfizer Inc., Rotterdam; cefoxitin from Merck, Sharp and Dohme, Haarlem, The Netherlands; cefotaxime from Roussel Laboratories, Hoevelaken, The Netherlands.

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Bacterial strains

Five strains of enterobacteria (two class I β-lactamase producing strains and three TEM β-lactamase producing strains) were selected from our own collection of strains. Some characteristics regarding their activity, spectrum and β-lactamases have been described elsewhere (Mouton, Bongaerts & van Gestel, 1979; Mouton *et al.*, 1981). Eight strains, each producing a different type of β-lactamase (Table I) were supplied by Dr Iain Simpson, Glaxo Group Research Ltd, Middlesex.

Preparation of cell free extracts

The same procedure was used as previously described (Mouton *et al.*, 1979) with some minor modifications. Bacterial cells, in the presence of either cephalothin or one of the other cephalosporins (final concentration: 25% of the MIC) as an inducing agent (see Table I), were harvested in the logarithmic phase of growth, washed and disrupted ultrasonically. After centrifugation for 20 min at 100,000 g the supernatants of the disrupted cells were used for β-lactamase activity tests. For the induction of β-lactamase production a concentration of 25% of the MIC of each cephalosporin was chosen in view of a possible clinical relevance. Higher concentrations may have a stronger inducing effect but will kill most organisms.

β-Lactamase assay

β-Lactamase activity against the cephalosporins was assayed by means of the spectrophotometric method of O'Callaghan *et al.* (1969). Spectrophotometric β-lactamase assay always included a control cuvette containing the same amount of

Table I. MIC-values (mg/l) of six cephalosporins, cefoxitin and moxalactam for 13 β-lactamase producing strains

Strain	β-lact. class	CET	CXM	CMD	CTX	CPZ	CAZ	CFX	MOX
Enterobacter AZL 1	I	>128	8	4	0·5	0·12	0·5	128	0·06
Proteus indole positive 104225	I	>128	128	4	0·03	2	0·03	2	0·06
E. coli 104050	TEM	>128	32	8	1	2	8	32	0·5
E. coli 3320	TEM	32	4	16	0·06	4	0·5	4	0·12
E. coli L12	TEM	>128	32	8	1	4	2	32	0·25
Klebsiella K1$^+$	IV	>128	>128	>128	1	>128	0·5	2	0·06
Klebsiella K14	IV	>128	>128	>128	2	>128	0·5	4	0·06
E. coli J53–R455	OXA-1	8	8	1	0·12	0·25	0·25	4	0·25
E. coli J53-R46-T'	OXA-2	4	4	0·25	0·03	0·03	0·12	4	0·06
E. coli J53-R57	OXA-3	8	4	4	0·06	0·5	0·25	4	0·12
E. coli E5.2 pUZ8-RPL11	PSE-1	4	4	1	0·06	8	0·25	4	0·12
E. coli E5.2 pUZ8-R151	PSE-2	2	2	0·5	0·03	0·12	0·12	2	0·06
E. coli J53-R1010	SHV-1	8	4	2	0·03	1	0·5	4	0·12

Abbreviations used: CET: cephalothin; CXM: cefuroxime; CMD: cefamandole; CTX: cefotaxime; CPZ: cefoperazone; CAZ: ceftazidime; CFX: cefoxitin; MOX: moxalactam.

cell-free extract, but no substrate. The optimal wavelength for cephalothin, cefuroxime, cefotaxime and ceftazidime was 262 nm; the same wavelength was chosen for moxalactam and cefoxitin. Hydrolysis of cefamandole and cefoperazone was measured at 272 nm and cephaloridine at 258 nm. In those cases where only the readings for β-lactamase activity of an undiluted cell-free extract were positive whereas no activity could be found with diluted extracts, a correction for "background" activity was made. Corrections in the extinction values were necessary because of small fluctuations in the spectrophotometer. The size of these corrections was derived from a number of dummy assays and was fixed at $\Delta E_{10\ \mathrm{min}} = 0{\cdot}01$. This implied that readings for undiluted crude extracts were interpreted as follows:

$\Delta E_{10'} \leq 0{\cdot}01 \rightarrow$ no activity; $0{\cdot}01 < \Delta E_{10'} < 0{\cdot}02 \rightarrow$ trace;

$\Delta E_{10'} \geq 0{\cdot}02 \rightarrow$ calculation after deduction of 0·01.

This correction resulted in lower limits of activity assay (calculated as μmol·min/mg protein) of 0·003 for cephalothin, cefuroxime, cefamandole and cefoperazone, 0·004 for cefotaxime, 0·002 for ceftazidime; the lower limits of activity assay of moxalactam and cefoxitin could not be determined accurately since the $\Delta E_{10'}$ was never higher than 0·01.

Results

In the first series of experiments a comparison was made of activities of 13 different β-lactamase preparations against six to nine cephalosporins, including ceftazidime, and against moxalactam and cefoxitin (Table II). In these tests cephalothin was used as an inducer. Ceftazidime, moxalactam and cefoxitin were completely resistant to all β-lactamases tested.

As with cephalothin, cefamandole and cefoperazone were hydrolysed by all TEM β-lactamases tested. One class I β-lactamase was active against cefamandole, cefuroxime

Table II. Activity of 13 β-lactamase preparations against eight β-lactams

Strain	β-lact. class	CET*	CXM	CMD	CTX	CPZ	CAZ	CFX	MOX
			Percentage activity† against						
Enterobacter AZL1	I	0·055	0	0	0	0	0	0	0
Pr. vulgaris 104225	I	0·022	59	122	tr	tr	0	0	0
E. coli 3320	TEM	0·14	tr	221	0	193	0	0	0
E. coli L123	TEM	0·6	tr	72	0	68	0	0	0
E. coli 104050	TEM	0·81	0	35	0	27	0	0	0
Klebsiella K14	IV	4·7	15	109	3	2	0	0	0
Klebsiella K1⁺	IV	5·02	14	127	3	2	0	0	0
E. coli J53-R455	OXA-1	0·005	0	tr	tr	tr	0	0	0
E. coli J53-R46-T'	OXA-2	0·005	0	0	0	0	0	0	0
E. coli J53-R57	OXA-3	0·007	0	tr	0	tr	0	0	0
E. coli E5·2 pUZ8-RPL11	PSE-1	tr	0	≥1400	0	0	0	0	0
E. coli E5·2 pUZ8-R151	PSE-2	0·006	0	tr	tr	0	0	0	0
E. coli J53-R1010	SHV-1	0·02	0	253	0	800	0	0	0

For abbreviations see Table I.
* Specific β-lactamase activity against cephalothin (μmol/min/mg protein).
† Activity against cephalothin: 100%.
tr: trace.

and cephalothin, although at a low level (0·027, 0·013 and 0·022 μmol/min/mg protein, respectively).

Cephalothin and cefamandole in particular and, to a much lesser extent cefuroxime, showed susceptibility to the class IV broad spectrum β-lactamases. With cephalothin as an inducing agent, the OXA enzymes did not degrade any antibiotics except for cephalothin and this only to a small extent. The SHV-1 enzyme hydrolysed, in order of activity, cefoperazone, cefamandole, and cephalothin. The very low activity of the extract from enterobacter AZLI, against cephalothin and the lack of activity against the other antibiotics suggested non-specific hydrolysis and a lack of β-lactamases, but the clear-cut induction by cefoxitin (Table III) indicated clearly that they were in fact present.

The results for β-lactamase activity after induction with different cephalosporins (Table III) showed only slight differences from those obtained after induction by cephalothin. Comparison of β-lactamase activity in the absence of a potential inducing agent, showed clearly that cefoxitin had a strong inducing effect on enterobacter strain AZL1. The other class 1 strain showed only very weak β-lactamase activity without induction. Cephalothin and cefuroxime induced marginally more activity (0·022 μmol/min/mg of protein) but only cefoxitin appeared to be a strong inducer. No clearcut inducing effect with ceftazidime was found, perhaps with the exception of proteus 104225 (class I β-lactamase) when the inducing effect of ceftazidime was only 0·4 times the inducing effect of cephalothin.

Table III. β-Lactamase activity of 13 β-lactamase preparations against cephalothin, with and without induction by eight different β-lactams

β-lactamase	CET*	No Ind.‡	Activity against cephalothin when induced by† CXM	CMD	CTX	CPZ	CAZ	CFX	MOX
Ia (enterobacter AZL1)	0·055	0·9	1·1	1·1	1·1	1·3	1·1	32·4	1·1
I (*Pr. vulgaris* 104225)	0·022	(tr)	1·7	0·4	0·2	(tr)	0·4	18	(tr)
TEM (*E. coli* 3320)	0·14	0·9	0·6	1·1	0·5	1·1	1	1·1	1·1
TEM (*E. coli* L123)	0·6	1·2	0·8	1	0·4	0·9	0·7	3	1·1
TEM (*E. coli* 104050)	0·81	0·9	1·2	1·1	1	0·9	0·5	1·1	0·9
IV (klebsiella K14)	4·7	0·9	1	1	0·8	1·1	0·7	0·5	1
IV (klebsiella K1$^+$)	5·02	1·6	1·2	1·4	1·1	1·3	0·9	0·6	1·1
OXA-1 (*E. coli* J53-R455)	0·005	0·8	(tr)	(tr)	1·4	2·4	1·2	1·4	1·8
OXA-2 (*E. coli* J53-R46-T*)	0·005	0·8	(tr)	0	(tr)	0·8	(tr)	(tr)	0
OXA-3 (*E. coli* J53-R57)	0·007	0·9	(tr)	0·6	(tr)	1·3	0·9	1·4	0·6
PSE-1 (*E. coli* E5·2 pUZ8-RPL11)	0·011	1	0·8	1	0·5	0·9	0·4	0·7	0·8
PSE-2 (*E. coli* E5·2 pUZ8-R151)	0·006	0·7	≤0·5	2·5	2·5	1·5	(tr)	(tr)	(tr)
SHV-1 (*E. coli* J53-R1010)	0·02	2·6	1·9	2·1	1·9	3	1·4	2·8	0·9

For abbreviations see Table I.
tr: trace; relative activity could not be calculated.
* Induction by cephalothin; specific activity in μmol/min/mg of protein.
† Activity expressed as a fraction of the activity after induction by cephalothin.
‡ No induction

Results for the activity against all β-lactams, after induction by different cephalosporins, are not presented separately since all these results corresponded with those for the activity against cephalothin. This is an indication that none of the β-lactamase preparations consisted of a mixture of inducible and non-inducible β-lactamases. Such a mixture would probably have led to differences in relative activity after induction.

Discussion

The lack of activity of any of the β-lactamases tested against moxalactam agrees with the findings of Richmond (1980) and of Yoshida *et al.* (1980). However, these authors found that two of their class I cephalosporinases had some activity against cefoxitin. In our study there were no strains which produced that type of β-lactamase. As far as we could establish only O'Callaghan *et al.* (1980) have published results of β-lactamase activity tests with ceftazidime. They reported slight activity of the OXA-1 enzyme against ceftazidime. Our results do not indicate any activity of this enzyme against ceftazidime. Evidently there may be other types of β-lactamase which, in contrast to the ones tested here, may hydrolyse ceftazidime. In particular, the enzyme of *Bacteroides fragilis* should be tested in this respect.

It became clear in the induction experiments that cephalothin can only induce the cephalosporinase (class I) of proteus 104225. No clearcut induction in enterobacter strain AZL1 could be found. When compared with the non-induced β-lactamase preparations, the other cephalosporins, including ceftazidime and moxalactam, showed approximately the same inducing properties. Only cefoxitin was a strong inducing agent for the cephalosporinases. This agrees with the findings of Minami *et al.* (1980) who concluded from their induction experiments that the cephamycins such as cefoxitin, have a high inducing activity at low drug concentrations. At higher concentrations (100 mg/l) most cephalosporins also showed some inducing capacity for cephalosporinase. In our experiments the inducing effect of these higher drug concentrations was not tested and a comparison could not be made. The weak hydrolysing capacity of the OXA-1 enzyme against cephalothin is not influenced by any of the other cephalosporins when used as inducing agents. This also appears to be true for the other OXA, PSE and SHV-1 enzymes. The greatest activity against cephalothin (PSE-2, induction by cefotaxime) was only about three times higher than when no inducing agent was used. This does not seem to be true induction.

We conclude that induction of β-lactamase production by cephalosporins may be of little importance when sub-MIC concentrations are used for induction. Cefoxitin is an exception as fas as inducible cephalosporinases (class I) are concerned. It is possible that higher concentrations of inducing agents may yield different results, but in clinical situations this may be unimportant since the bacteria will be killed at concentrations at MIC (MBC) level or higher.

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