COMPARATIVE INHIBITORY ACTIVITY OF BL-S640 AND TWO OTHER CEPHALOSPORINS

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(Received for publication March 20, 1975)

In vitro antibacterial activity of BL-S640 was compared to that of cephalothin and cephalexin against Gram-negative and Gram-positive bacteria isolated from clinical specimens. BL-S640 demonstrated the best activity on nearly all microbial species studied, except for *Haemophilus influenzae* and *Diplococcus pneumoniae* against which cephalothin was slightly more active.

BL-S640 (a7-(2-aryl-2-aminoacetamido)-3-(heterocyclicthiomethyl) cephalosporin) (Bristol) is a new broad-sepctrum semi-synthetic cephalosporin, which is water-soluble and well absorbed by man[1] and animals after oral administration[2].

The present study reports the comparative minimal inhibitory concentrations (MIC) for BL-S640, cephalothin (considered as the leading cephalosporin) and cephalexin (considered as the most representative of the oral cephalosporins) against strains isolated in human pathology.

Material and Methods

Microbial strains

The 99 bacteria studied were recently isolated from clinical specimens (urine, blood, pus, sputum, *etc.*) collected at the Hôpital Universitaire Brugmann, Brussels. Included in the survey were 13 strains each of *Salmonella*, *Proteus*, *Klebsiella*, *Escherichia coli*, *H. influenzae*, 8 strains of *Listeria monocytogenes*, 13 strains of *Streptococcus faecalis* and 13 strains of *D. pneumoniae*.

Antibiotic sensitivity testing

MIC were determined by an agar dilution technique, on MUELLER-HINTON Agar BBL, to which were added, in the case of *D. pneumoniae*, *Haemophilus* and *St. faecalis*, 3 μg per ml of factor V (nicotinamide-adenine dinucleotide phosphate-BDH Chemicals Ltd.) and 2% defibrinated horse blood. Serial twofold dilutions of the antibiotics (BL-S640, powder for analysis, Bristol; cephalothin and cephalexin, Lilly) were prepared, starting from initial concentrations of 100 μg per ml. Overnight MUELLER-HINTON broth cultures were diluted to contain 10^5 organisms per ml for *E. coli*, *Klebsiella*, *Listeria*, *Proteus* and *Salmonella*. *D. pneumoniae*, *Haemophilus* and *St. faecalis* were grown in the same medium supplemented with 0.5% defibrinated horse blood in a 5% CO_2-enriched atomsphere, and diluted to contain 10^6 organisms per ml. These inocula were spotted with an automatic multipoint inoculator onto the surface of the agar plates[3]. *D. pneumoniae* and *Haemophilus* were incubated in a 5% CO_2-enriched atmosphere.

Results were read after overnight incubation at 37°C. The MIC was designated as the lowest antibiotic concentration yielding no colony at the site of inoculation.

Disk diffusion tests were performed according to the method described by BAUER *et al.*[4] using the same media as in the MIC determination, and disks containing 30 μg of cephalothin, cephalexin or BL-S640.

Inhibition zones were measured with a caliper after overnight incubation at 37°C.

Table 1. Activity of BL-S640, cephalothin and cephalexin against Gram-negative bacilli

Microorganisms (No. of strains)	Antibiotic	Number of strains inhibited at each concentration (μg/ml)												
		0.048	0.097	0.195	0.39	0.78	1.56	3.12	6.25	12.5	25	50	100	>100
Salmonella (13)	Cephalothin	0	0	0	0	2	11	0	0	0	0	0	0	0
	BL-S640	0	0	0	11	2	0	0	0	0	0	0	0	0
	Cephalexin	0	0	0	0	0	0	13	0	0	0	0	0	0
Proteus (13)	Cephalothin	0	0	0	0	0	2	1	5	1	0	1	3	0
	BL-S640	0	0	0	2	0	2	4	1	0	3	0	1	0
	Cephalexin	0	0	0	0	0	0	2	3	2	2	2	2	0
Klebsiella (13)	Cephalothin	0	0	0	0	3	1	0	1	3	1	4	0	0
	BL-S640	0	0	0	3	1	0	3	3	3	0	0	0	0
	Cephalexin	0	0	0	0	0	0	8	4	1	0	0	0	0
E. coli (13)	Cephalothin	0	0	0	0	0	5	4	1	2	0	0	0	0
	BL-S640	0	0	1	5	3	1	0	2	0	0	0	1	0
	Cephalexin	0	0	0	0	0	0	9	1	2	0	0	0	0
Haemophilus (13)	Cephalothin	0	1	3	1	5	2	1	0	0	0	0	0	0
	BL-S640	0	1	0	4	0	1	7	0	0	0	0	0	0
	Cephalexin	0	0	0	0	0	4	1	2	5	1	0	0	0

Table 2. Activity of BL-S640, cephalothin and cephalexin against Gram-positive bacteria

Microorganisms (No. of strains)	Antibiotic	Number of strains inhibited at each concentration (μg/ml)											
		0.024	0.048	0.097	0.195	0.39	0.78	1.56	3.12	6.25	12.5	25	50	100
Listeria (8)	Cephalothin	0	0	0	0	0	0	0	8	0	0	0	0	0
	BL-S640	0	0	0	0	0	0	0	8	0	0	0	0	0
	Cephalexin	0	0	0	0	0	0	0	0	0	0	0	8	0
Str. faecalis (13)	Cephalothin	0	0	0	1	0	0	1	0	0	1	10	0	0
	BL-S640	0	0	0	0	0	2	0	0	2	9	0	0	0
	Cephalexin	0	0	0	0	0	0	0	1	0	1	0	1	10
D. pneumoniae (13)	Cephalothin	3	6	4	0	0	0	0	0	0	0	0	0	0
	BL-S640	1	4	5	3	0	0	0	0	0	0	0	0	0
	Cephalexin	0	0	0	0	1	2	9	1	0	0	0	0	0

Results

MIC values obtained for Gram-negative bacilli are shown in Table 1, those for Gram-positive bacteria in Table 2.

Although there sometimes exist considerable differences between susceptibility of one strain and another amongst the same species, a tentative classification of the 3 drugs, based on the median values (M) of their MIC values was done. Following results were obtained:

Salmonella: BL-S640 ($M = 0.39$ μg/ml) better than (>) cephalothin ($M = 1.56$ μg/ml)> cephalexin ($M=3.12$ μg/ml).

Proteus: BL-S640 ($M=6.25$ μg/ml)>cephalexin=cephalothin ($M=12.5$ μg/ml).

Klebsiella: BL-S640=cephalexin (M=3.12 μg/ml)>cephalothin (M=6.25 μg/ml).

E. coli: BL-S640 (M = 0.78 μg/ml) > cephalothin=cephalexin (M=3.12 μg/ml).

H. influenzae: cephalothin (M=0.78 μg/ml) > BL-S640 (M = 1.56 μg/ml) > cephalexin (M=6.25 μg/ml).

L. monocytogenes: BL-S640=cephalothin (M=3.12 μg/ml)>cephalexin (M=50 μg/ml).

St. faecalis: BL-S640 (M = 6.25 μg/ml) > cephalothin (M =12.5 μg/ml) > cephalexin (M=50 μg/ml).

Diplococcus pneumoniae: cephalothin (M= 0.048 μg/ml) > BL-S640 (M = 0.097 μg/ml) > cephalexin (M=1.56 μg/ml).

Thus, in this material, BL-S640 demonstrated the best activity on nearly all of the microbial species studied, except for *H. influenzae* and *D. pneumoniae* against which cephalothin was slightly more active. Its activity was always superior to that of cephalexin except in the case of *Klebsiella* where it was equivalent.

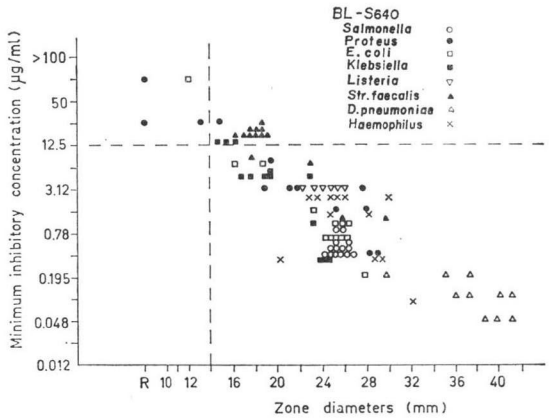
Fig. 1 illustrates the relationship between zone diameters and MIC values for BL-S640 (Fig. 1a), cephalothin (Fig. 1b) and cephalexin (Fig. 1c).

There was a good agreement between these two methods of sensitivity testing for BL-S640 and cephalothin. Only *St. faecalis* appeared slightly more sensitive by the disk method than by the agar diffusion technique; the zone diameters remain nonetheless grouped within the intermediate sensitivity zone.

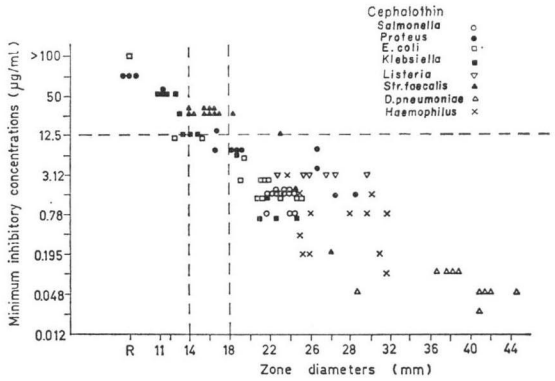
Cephalexin, on the other hand, did not demonstrate as fine a distinction between sensitive and resistant strains with the disk method, as did the other two antibiotics: several strains were found in the intermediate sensitivity zone. However, the poor activity of cephalexin on *St. faecalis*, as illustrated by a mean MIC value of 50 μg/ml, was confirmed by the disk method: 11 of the 13 strains had zone diameters inferior to the critical diameter of 14 mm.

Fig. 1. Relationship between zone diameters (30-μg disks) and MIC values for BL-S640 (Fig. 1a), cephalothin (Fig. 1b) and cephalexin (Fig. 1c)

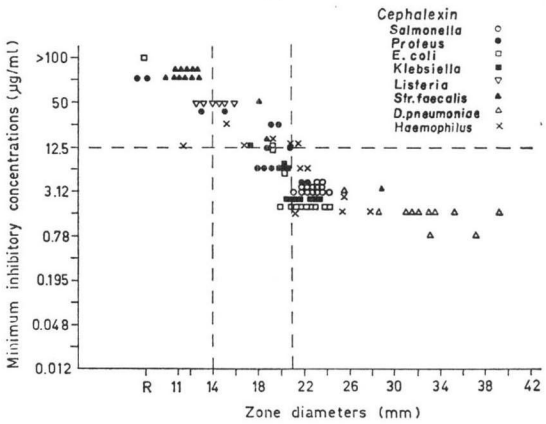
(a)



(b)



(c)



Discussion and Conclusions

BL-S640 is an oral cephalosporin presenting an *in vitro* antibacterial activity comparable to that of cephalothin (considered as the first choice among the cephalosporins) and considerably superior to that of cephalexin.

BL-S640 could be a valuable drug among the oral cephalosporins: it has been shown (in animal experiments) that peak blood concentrations of this drug are similar to those obtained with cephalexin when administered at the same oral doses[2], but blood levels are more prolonged. Moreover, volume of tissue distribution of this drug increases as dosage increases[1]. Clinical efficacy in man, as well as toxicity and side-effects of BL-S640 must be further investigated.

References

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