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In Vitro Activity of BAY v 3522, a New Oral Cephalosporin

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The in vitro activity of BAY v 3522 was compared with the activities of cephalexin, cefaclor, cefuroxime, cefixime, and amoxicillin-clavulanate. MICs (in μg/ml) of BAY v 3522 were as follows: *Staphylococcus* spp. (except for oxacillin-resistant strains), 0.13 to 1; *Streptococcus* spp. (except for some viridans group streptococci), ≤0.015 to 0.25; *Enterococcus faecalis*, 2 to 8; other enterococci, 0.5 to >32; β-lactamase-negative *Haemophilus influenzae* and *Branhamella catarrhalis*, 0.13 to 1; β-lactamase-positive *H. influenzae* and *B. catarrhalis*, 0.5 to 4; *Pasteurella multocida*, 0.06 to 0.25; and members of the family *Enterobacteriaceae*, 0.5 to >32. Among the cephalosporins, BAY v 3522 was the most active against gram-positive cocci and cefixime was the most active against gram-negative bacilli; BAY v 3522 was similar in activity to amoxicillin-clavulanate against most species.

The various antimicrobial and pharmacokinetic properties of the oral cephalosporins have been achieved by modifying the basic cephem nucleus, primarily by substituents at C-3 or C-7 (R. N. Jones, Antimicrob. Newsl. 5:1–7, 1988). BAY v 3522 is a new oral cephalosporin with a *Z*-propenyl side chain at the C-3 position and a D-(2-aminobenzothiazol-6-yl) glycyl substituent at C-7. In this study, the in vitro activity of BAY v 3522 was compared with the activities of other oral cephalosporins and amoxicillin-clavulanate against a variety of aerobic and facultatively anaerobic pathogens.

The organisms studied included 630 bacterial strains arbitrarily selected from recent isolates (57% from blood) at the Ohio State University Hospitals. Duplicate isolates from the same patients were excluded.

BAY v 3522 was obtained from Miles, Inc., West Haven, Conn. Cephalexin, cefaclor, and cefuroxime were obtained from Eli Lilly & Co., Indianapolis, Ind. Cefixime was obtained from American Cyanamid Co., Pearl River, N.Y. Amoxicillin-clavulanate was obtained from Beecham Laboratories, Bristol, Tenn. Laboratory standards were diluted in accordance with the recommendations of the manufacturer and dispensed into microdilution plates with an MIC-2000 dispensing machine (Dynatech Laboratories, Inc., Chantilly, Va.) in $\log_2$ dilution steps from 0.015 to 32 μg/ml. For amoxicillin-clavulanate, a fixed ratio of 2:1 was tested; MICs were expressed as the concentration of amoxicillin. Plates were stored at −70°C until used.

For nonfastidious aerobic and facultative anaerobic species, MICs were determined by a standardized microdilution method (1) in 0.1 ml of cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.). For *Streptococcus pyogenes*, *Streptococcus pneumoniae*, viridans group streptococci, and *Haemophilus influenzae*, the medium was Schaedler broth (Difco) supplemented with 1% heat-inactivated horse serum and 0.5 μg of vitamin K_1 per ml. For *H. influenzae*, the medium was also supplemented with 5% Fildes enrichment (Difco). Microdilution plates were inoculated with disposable inoculators (Dynatech) so that the final inoculum was approximately 5×10^5 CFU/ml. Recommended control strains (1) were used.

The MICs of the six antimicrobial agents for the 630 study strains are shown in Table 1. BAY v 3522 was as active or more active than the other oral cephalosporins against gram-positive cocci; some viridans group streptococci and enterococci were relatively resistant to all drugs. BAY v 3522 was less active than cefixime, similar in activity to cefuroxime, and more active than cephalexin and cefaclor against *H. influenzae*, *Branhamella catarrhalis*, and *Pasteurella multocida*. β-Lactamase-positive strains of *H. influenzae* and *B. catarrhalis* were slightly less susceptible to BAY v 3522 than were β-lactamase-negative strains; this was also true for cephalexin and cefaclor but not for cefuroxime or cefixime. Against the members of the family *Enterobacteriaceae* tested, BAY v 3522 was similar in activity to cephalexin but less active than the other cephalosporins. Overall, BAY v 3522 was more similar to amoxicillin-clavulanate in its spectrum of in vitro activity (except for less activity against enterococci) than it was to any of the cephalosporins tested. The MICs of BAY v 3522 for control strains are shown in Table 2.

For five strains of each species, MICs were simultaneously determined by using inocula of 5×10^5 and 5×10^7 CFU/ml. Increasing the inoculum size had little effect (≤2 dilution steps) on BAY v 3522 MICs for most oxacillin-susceptible staphylococci, streptococci, β-lactamase-negative *H. influenzae* and *B. catarrhalis*, and *P. multocida* but resulted in increased MICs (≥3 dilution steps) for enterococci, β-lactamase-producing *H. influenzae* and *B. catarrhalis*, and the members of the *Enterobacteriaceae*. The drugs to which BAY v 3522 was compared were similarly affected but were bactericidal (except cefaclor) against β-lactamase-producing strains of *H. influenzae* and *B. catarrhalis*. The greatest inoculum effect, with all drugs, was observed with the enterococci and the members of the *Enterobacteriaceae*.

Subcultures from the MIC plates of five strains of each species were obtained for determination of MBCs. For the MBCs, 0.01-ml portions of the medium were subcultured to sterile broth by using hand inoculators (Dynatech). Colony counts confirmed that the original inoculum was 6.4×10^5 ($\pm 0.4 \times 10^5$, standard error) CFU/ml, indicating an adequate predictive value for >99.9% killing (2). Bactericidal activity for all drugs was quite variable, although some organisms (e.g., oxacillin-susceptible *Staphylococcus epidermidis*) were typically killed by all drugs, while other organisms (e.g., enterococci and *P. multocida*) were typically only inhibited by all drugs. BAY v 3522 MBCs were ≥3 dilution steps higher than MICs for oxacillin-susceptible *Staphylococcus aureus*, *Streptococcus agalactiae*, enterococci,

TABLE 1. In vitro activities of BAY v 3522 and comparative oral antimicrobial agents

Organism (type[a]) (no. of isolates)	MIC (μg/ml)[b]											
	BAY v 3522		Cephalexin		Cefaclor		Cefuroxime		Cefixime		Amoxicillin-clavulanate	
	Range	50%	Range	50%	Range	50%	Range	50%	Range	50%	Range	50%
Staphylococcus aureus (OS) (25)	0.25–1	0.5	4–8	4	1–8	4	1–2	2	8–16	16	0.5–2	1
Staphylococcus epidermidis (OS) (25)	0.13–0.25	0.13	2–4	2	0.5–1	1	0.25–1	0.5	2–8	4	0.13–0.5	0.25
Staphylococcus haemolyticus (OS) (25)	0.13–1	0.5	1–8	4	0.5–4	2	0.5–4	2	4–32	32	0.25–1	0.5
Staphylococcus hominis (OS) (25)	0.13–1	0.25	1–16	4	0.25–4	2	0.13–2	1	2–32	8	0.13–1	0.5
Staphylococcus saprophyticus (25)	0.25–1	0.5	4–8	8	1–4	2	2–8	4	32–>32	>32	0.5–1	0.5
Staphylococcus aureus (OR) (25)	8–>32	32	16–>32	>32	16–>32	>32	4–>32	>32	>32	>32	2–>32	16
Staphylococcus epidermidis (OR) (25)	0.5–16	2	8–>32	32	4–32	8	0.25–>32	4	4–>32	>32	0.5–8	2
Staphylococcus haemolyticus (OR) (25)	4–>32	>32	32–>32	>32	8–>32	>32	8–>32	>32	>32	>32	2–>32	32
Staphylococcus hominis (OR) (7)	1–16	1	16–>32	32	4–>32	8	2–>32	4	16–>32	32	1–32	2
Streptococcus pyogenes (25)	≤0.01–0.06	0.03	0.25–1	0.5	0.03–0.25	0.13	≤0.01–0.06	0.03	0.06–0.25	0.13	≤0.01–0.03	0.03
Streptococcus agalactiae (25)	0.03–0.13	0.06	1–4	2	0.25–1	0.5	0.03–0.13	0.03	0.13–0.5	0.25	0.06–0.13	0.06
Streptococcus pneumoniae (25)	0.03–0.13	0.06	0.5–4	4	0.5–1	1	≤0.01–0.06	0.03	0.13–0.5	0.25	≤0.01–0.06	≤0.01
Streptococcus bovis (18)	≤0.01–0.25	0.13	0.25–4	2	0.06–1	0.25	≤0.01–2	0.13	0.25–16	2	≤0.01–0.25	0.06
Viridans group streptococci (25)	≤0.01–16	0.25	0.13–>32	4	0.03–>32	2	≤0.01–8	0.25	0.13–>32	2	≤0.01–4	0.06
Enterococcus faecalis (25)	2–8	4	>32	>32	16–>32	>32	8–>32	>32	16–>32	>32	0.5–1	1
Enterococcus faecium (25)	0.5–>32	16	16–>32	>32	4–>32	>32	16–>32	>32	>32	>32	0.13–16	2
Enterococcus avium (16)	1–8	2	8–>32	16	2–16	4	16–>32	>32	>32	>32	0.5–2	1
Enterococcus durans (17)	2–>32	16	>32	>32	16–>32	>32	>32	>32	>32	>32	0.5–4	2
Haemophilus influenzae (−) (25)	0.13–1	0.5	2–16	8	1–4	2	0.5–1	1	0.03–0.13	0.06	0.25–1	0.5
Haemophilus influenzae (+) (25)	0.5–4	1	4–>32	8	1–8	2	0.5–1	0.5	≤0.01–0.06	0.03	1–2	1
Branhamella catarrhalis (−) (7)	0.25	0.25	1–4	2	0.25–0.5	0.25	0.5–1	1	0.06–0.5	0.25	≤0.01–0.25	0.06
Branhamella catarrhalis (+) (22)	0.5–4	2	1–4	4	0.25–1	1	0.5–2	1	0.06–0.5	0.13	0.03–0.25	0.06
Pasteurella multocida (18)	0.06–0.25	0.13	1–4	2	0.25–0.5	0.5	0.06–0.25	0.13	≤0.01–0.25	≤0.01	0.13–0.25	0.25
Escherichia coli (25)	2–>32	4	4–16	8	1–>32	2	2–8	4	0.13–1	0.25	2–16	4
Klebsiella pneumoniae (25)	0.5–>32	2	4–>32	8	0.5–>32	1	0.5–>32	4	>0.01–4	0.13	1–16	2
Klebsiella oxytoca (25)	1–>32	16	4–32	4	0.5–>32	1	2–>32	4	≤0.01–0.5	0.06	2–16	4
Citrobacter diversus (25)	0.5–>32	1	4–>32	8	0.5–>32	0.5	1–>32	4	0.03–0.5	0.06	2–16	2
Proteus mirabilis (25)	0.5–>32	2	8–>32	16	0.5–16	1	0.5–8	1	≤0.01–0.06	≤0.01	0.5–16	1

[a] OS, Oxacillin susceptible; OR, oxacillin resistant; −, β-lactamase negative; +, β-lactamase positive.
[b] 50%, MIC for 50% of the isolates.

TABLE 2. In vitro activity of BAY v 3522 against control strains

Medium and control strain (no. of tests)	MIC range (μg/ml)
Mueller-Hinton broth	
E. coli ATCC 25922 (13)	4–8
S. aureus ATCC 29213 (21)	0.25–0.5
E. faecalis ATCC 29212 (4)	2–4
Schaedler broth	
E. coli ATCC 25922 (2)	8
S. aureus ATCC 29213 (5)	0.5
E. faecalis ATCC 29212 (1)	4

β-lactamase-producing *H. influenzae*, and the members of the *Enterobacteriaceae*.

For five strains of each nonfastidious species, MICs of BAY v 3522 were also determined in Mueller-Hinton broth without cation adjustment, in cation-adjusted broth (pH 5.5), and in 50% cation-adjusted broth–50% heat-inactivated pooled human serum. Reducing the pH to 5.5 or eliminating the cation supplementation typically had no effect on MICs; when changed, they were 1 dilution step lower than in the standard medium. The addition of 50% serum frequently increased MICs 1 to 2 dilution steps, although there was a mean decrease of 2 dilution steps with *Staphylococcus epidermidis* and *Staphylococcus hominis*. Oxacillin-resistant staphylococci, fastidious streptococci, and *H. influenzae* were not studied.

In this study, the antimicrobial activity of BAY v 3522 was most similar to that of amoxicillin-clavulanate, although it was less active against enterococci. It was the most active cephalosporin against gram-positive cocci, intermediate in activity against *H. influenzae*, *B. catarrhalis*, and *P. multocida*, and only moderately active against the members of the *Enterobacteriaceae*. The spectrum of BAY v 3522 against the members of the *Enterobacteriaceae* (as for cephalexin and cefaclor) was limited to species susceptible to prototype cephalosporins such as cephalothin and cefazolin, including *Escherichia coli*, *Klebsiella* species, *Citrobacter diversus*, and *Proteus mirabilis*. Other members of the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and many anaerobes are resistant to BAY v 3522 (Miles, Inc., unpublished data). BAY v 3522 should be considered, as are all beta-lactams, inactive against oxacillin-resistant staphylococci (1).

For BAY v 3522, inoculum effects and bactericidal activities were similar to those of beta-lactams to which it was compared, and MICs were not appreciably affected by pH or cation changes in medium or by the addition of serum. The profile of in vitro activity of BAY v 3522 indicated that it has potential clinical utility.

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