ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1985, p. 515–519
0066-4804/85/040515-05$02.00/0

Vol. 27, No. 4

In Vitro Activity of BMY-28142 in Comparison with Those of Other β-Lactam Antimicrobial Agents

AKIOYOSHI TSUJI,† ANTONY MANIATIS,‡ MIRIAM A. BERTRAM, AND LOWELL S. YOUNG*

Division of Infectious Diseases, University of California, Los Angeles Medical Center, Los Angeles, California 90024

Received 8 November 1984/Accepted 15 January 1985

The in vitro susceptibility of 406 clinical isolates to BMY-28142, a new semisynthetic cephem, was evaluated and compared with cefpimizole, HR 810, ceftazidime, cefotaxime, moxalactam, and cefoperazone in a broth microdilution assay. On a weight basis, the activity of BMY-28142 against *Escherichia coli*, *Proteus* species, and *Klebsiella-Enterobacter-Serratia* was superior to the other cephems. Against gentamicin-susceptible and -resistant *Pseudomonas aeruginosa*, BMY-28142 was more active than the other cephems, except ceftazidime and HR 810. A total of 74% of gram-negative and 56% of gram-positive isolates resistant to third-generation cephalosporins were susceptible to ≤8 μg of BMY-28142 per ml. Finally, for 83% of tested isolates, the bactericidal concentration of BMY-28142 was within one dilution of the inhibitory concentration.

The cephem antibiotics are used extensively in the therapy of various bacterial infections. The development of new cephems has involved changes in the chemical structure designed to increase β-lactamase stability, to broaden the spectrum of activity, and to enhance activity against gram-negative bacilli. However, these new cephems show poor activity against gram-positive cocci and are moderately active against *Pseudomonas aeruginosa* (1–3, 5, 7, 9–11).

BMY-28142 is an aminothiazolyl methoxyimino cephem such as HR 810 and cefotaxime. The complete chemical name is 7-[alpha-(2-aminothiazol-4-yl)-alpha-(Z)-methoximinoacetamido]-3-(1-methylpyrrolidinio)-methyl-3-cephem-4-carboxylate (Fig. 1).

This study compares the in vitro activity of BMY-28142 against *Enterobacteriaceae*, non-glucose-fermenting gram-negative bacilli, and gram-positive cocci with those of other recently developed β-lactam agents.

MATERIALS AND METHODS

BMY-28142 was obtained from Bristol-Myers (Syracuse, N.Y.). The other antibiotics were provided by the following firms: HR 810 and cefotaxime, Hoechst-Roussel Pharmaceuticals Inc. (Syracuse, N.Y.); cefpimizole (U-63196E), The Upjohn Co. (Kalamazoo, Mich.); ceftazidime, Glaxo Inc. (Research Triangle Park, N.C.); cefoperazone, Pfizer Inc. (New York, N.Y.); moxalactam, Eli Lilly Research Laboratories (Indianapolis, Ind.); gentamicin, Schering Corp. (Bloomfield, N.J.); and oxacillin (OX), Bristol Laboratories (Syracuse, N.Y.).

The majority of organisms (349 in total) tested were recent clinical isolates from the University of California, Los Angeles, Medical Center Bacteriology Laboratory. The gentamicin-resistant *P. aeruginosa* isolates (23 isolates) were all resistant to gentamicin at a MIC of ≥8 μg/ml. Some of these isolates were also resistant to amikacin (MICs, ≥16 μg/ml) or tobramycin (MICs, ≥8 μg/ml) or both. MICs for OX-resistant *Staphylococcus aureus* isolates (13 isolates) were ≥4 μg/ml. Fifteen *Klebsiella* spp. multiply resistant isolates were also tested and were resistant to gentamicin (MICs, ≥8 μg/ml) and cefoperazone (MICs, ≥16 μg/ml). These strains were obtained from stock clinical isolates which had been collected and stored at −70°C.

The MICs were determined by the broth microdilution method with cation-supplemented Mueller-Hinton broth (8). The organisms were inoculated into 0.5 ml of brain heart infusion broth and incubated at 35°C to stationary phase (4 to 6 h). A sample of this suspension was diluted and inoculated into antibiotic-containing wells to a final concentration of ca. 5×10^5 CFU/ml. After incubation of microtiter plates at 35°C for 18 to 24 h, the MIC was defined as the lowest concentration of antibiotic resulting in the complete inhibition of growth.

The BMY-28142 MICs for standard quality control strains were as follows (in micrograms per milliliter): *Escherichia coli* ATCC 25922, ≤0.06; *P. aeruginosa* ATCC 27853, 2 to 4; *Streptococcus faecalis* ATCC 29212, ≥32; *Staphylococcus aureus* ATCC 25923, 1 to 2; and *Staphylococcus aureus* ATCC 29213, 2 to 4.

The MBC was determined by transferring 10-μl amounts from each microdilution well without visible growth onto antibiotic-free tryptic soy agar plates. After incubation at 35°C for 20 h, the lowest antibiotic concentration at which the number of colonies on the plate was fewer than three was regarded as the MBC. This was considered a 3-log reduction (99.9%) in CFU per milliliter.

The effect of inoculum size on MIC was also determined in the broth microdilution assay with cation-supplemented Mueller-Hinton broth. The final CFUs deposited in each well were approximately 10^8, 10^7, 10^6, and 10^5/ml; the final inoculum size per milliliter was determined by viable counts.

The bactericidal activities of BMY-28142, cefpimizole, cefoperazone, and ceftazidime were compared over a defined time frame (1, 3, 5, and 24 h) against *P. aeruginosa*

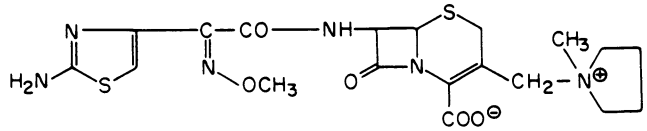

FIG. 1. Chemical structure of BMY-28142.

* Corresponding author.
† Present address: Toho University School of Medicine, Tokyo, Japan.
‡ Present address: Hellenic A. F. Hospital, Athens, Greece.

TABLE 1. Antibacterial activities of BMY-28142 and other antimicrobial agents

Organism (no. of strain)/ antimicrobial agent	MIC (μg/ml)[a]		
	Range	50%	90%
Actinetobacter spp. (14)			
BMY-28142	0.5–32	4	32
Cefpimizole	16–>32	>32	>32
HR 810	0.5–>32	4	>32
Ceftazidime	1–16	4	16
Cefotaxime	1–32	16	32
Cefoperazone	>32	>32	>32
Moxalactam	16–>32	>32	>32
Gentamicin	0.12–32	0.5	16
Citrobacter freundii (22)			
BMY-28142	≤0.06–1	≤0.06	0.5
Cefpimizole	2–>32	16	>32
HR 810	≤0.06–4	≤0.06	0.12
Ceftazidime	0.12–>32	0.5	>32
Cefotaxime	≤0.06–>32	0.12	32
Cefoperazone	0.12–>32	1	>32
Moxalactam	≤0.06–16	0.12	4
Enterobacter aerogenes (15)			
BMY-28142	≤0.06	≤0.06	≤0.06
Cefpimizole	1–>32	4	>32
HR 810	≤0.06–0.25	≤0.06	0.25
Ceftazidime	0.12–8	0.25	4
Cefotaxime	≤0.06–8	≤0.06	4
Cefoperazone	0.25–16	0.5	8
Moxalactam	0.12–4	0.25	1
Enterobacter cloacae (21)			
BMY-28142	≤0.06–2	≤0.06	0.5
Cefpimizole	2–>32	32	>32
HR 810	≤0.06–16	0.12	0.5
Ceftazidime	0.12–>32	0.5	8
Cefotaxime	≤0.06–>32	0.25	8
Cefoperazone	0.25–>32	1	32
Moxalactam	≤0.06–32	0.25	16
Escherichia coli (37)			
BMY-28142	≤0.06–1	≤0.06	≤0.06
Cefpimizole	0.5–>32	4	>32
HR 810	≤0.06–0.5	≤0.06	≤0.06
Ceftazidime	≤0.06–0.5	0.25	0.25
Cefotaxime	≤0.06–0.25	≤0.06	0.12
Cefoperazone	≤0.06–>32	0.5	16
Moxalactam	≤0.06–0.5	0.12	0.25
Klebsiella oxytoca (23)			
BMY-28142	≤0.06	≤0.06	≤0.06
Cefpimizole	1–≤32	8	≤32
HR 810	≤0.06–1	≤0.06	≤0.06
Ceftazidime	≤0.06–1	0.12	0.25
Cefotaxime	≤0.06–0.5	≤0.06	≤0.06
Cefoperazone	0.25–8	4	8
Moxalactam	≤0.06–0.5	0.12	0.25
Klebsiella pneumoniae (24)			
BMY-28142	≤0.06–0.5	≤0.06	0.12
Cefpimizole	1–8	4	4
HR 810	≤0.06–0.12	≤0.06	≤0.06
Ceftazidime	0.12–0.5	0.25	0.5
Cefotaxime	≤0.06–0.12	≤0.06	0.12
Cefoperazone	0.25–4	1	2
Moxalactam	0.12–0.5	0.25	0.25
Proteus mirabilis (26)			
BMY-28142	≤0.06–0.25	≤0.06	≤0.06
Cefpimizole	0.5–>32	1	4
HR 810	≤0.06–0.12	≤0.06	0.12
Ceftazidime	≤0.06–1	≤0.06	≤0.06
Cefotaxime	≤0.06–0.5	≤0.06	≤0.06
Cefoperazone	0.25–32	0.5	1
Moxalactam	≤0.06–0.25	0.12	0.25
Proteus vulgaris (13)			
BMY-28142	≤0.06–0.25	≤0.06	≤0.06
Cefpimizole	2–>32	>32	>32
HR 810	0.12–8	0.12	4
Ceftazidime	≤0.06–0.12	≤0.06	≤0.06
Cefotaxime	≤0.06–2	0.12	1
Cefoperazone	0.5–32	8	16
Moxalactam	≤0.06–0.25	0.25	0.25
Pseudomonas aeruginosa			
Gentamicin susceptible (26)			
BMY-28142	1–16	2	8
Cefpimizole	4–>32	16	32
HR 810	2–32	8	16
Ceftazidime	1–16	2	4
Cefotaxime	8–>32	32	>32
Cefoperazone	16–>32	16	>32
Moxalactam	8>32	16	>32
Gentamicin	0.12–4	4	4
Gemtaimcin resistant (29)			
BMY-28142	2–16	8	16
Cefpimizole	8–>32	32	>32
HR 810	4–32	16	16
Ceftazidime	1–32	2	16
Cefotaxime	16–>32	32	>32
Cefoperazone	8–>32	32	>32
Moxalactam	8–>32	32	>32
Gentamicin	8–>32	>32	>32
Pseudomonas maltophilia (16)			
BMY-28142	4–>32	32	>32
Cefpimizole	16–>32	>32	>32
HR 810	8–>32	>32	>32
Ceftazidime	2–>32	32	>32
Cefotaxime	≤32	>32	>32
Cefoperazone	16–>32	>32	>32
Moxalactam	4–>32	8	32
Gentamicin	4–>32	>32	>32
Serratia marcescens (24)			
BMY-28142	≤0.06–1	≤0.06	0.5
Cefpimizole	4–>32	32	>32
HR 810	≤0.06–1	0.12	0.5
Ceftazidime	0.12–2	0.25	0.5
Cefotaxime	≤0.06–>32	0.5	2
Cefoperazone	1–>32	8	>32
Moxalactam	≤0.06–8	0.5	8
Staphylococcus aureus			
OX susceptible (17)			
BMY-28142	2–4	4	4
Cefpimizole	16–>32	32	>32
HR 810	0.5–1	1	1
Ceftazidime	8–16	16	16
Cefotaxime	1–>32	4	32
Cefoperazone	4–8	8	8
Moxalactam	16–32	16	32
OX	0.5–2	1	2

Continued on following page

TABLE 1—*Continued*

Organism (no. of strain)/ antimicrobial agent	MIC (μg/ml)[a]		
	Range	50%	90%
OX resistant (13)			
BMY-28142	4–32	8	32
Cefpimizole	>32	>32	>32
HR 810	1–8	4	8
Ceftazidime	16–>32	>32	>32
Cefotaxime	2–>32	16	32
Cefoperazone	8–>32	32	>32
Moxalactam	16–>32	>32	>32
OX	4–>32	>32	>32
Coagulase-negative *Staphylococcus* spp. (24)			
BMY-28142	0.25–16	2	16
Cefpimizole	2–>32	32	>32
HR 810	0.12–8	1	8
Ceftazidime	2–32	16	32
Cefotaxime	0.25–16	2	16
Cefoperazone	2–16	8	16
Moxalactam	16–>32	>32	>32
OX	0.12–>32	2	>32

[a] 50% and 90%, MICs for 50 and 90% of the isolates, respectively.

ATCC 27853; the time-killing curve method was used (4). Mueller-Hinton broth containing antibiotic was inoculated with log-phase *P. aeruginosa* to a final concentration of 10^6 CFU/ml and a final antibiotic concentration of 2, 1, 1/2, or 1/4 MIC. A growth control of Mueller-Hinton broth without antibiotic was also inoculated. The tubes were incubated with shaking for 24 h at 37°C. Samples were quantitatively subcultured onto antibiotic-free Mueller-Hinton agar to determined viable counts after 1, 3, 5, and 24 h of incubation.

RESULTS

The comparative activities of BMY-28142 and other agents are shown in Table 1. BMY-28142 was very active against the *Enterobacteriaceae*, with the MIC_{90}s of *Citrobacter* spp. (8 *C. diversus*, 22 *C. freundii*), *Enterobacter* spp., *Serratia marcescens*, *Klebsiella* spp., *Proteus* spp., *Morganella morgani* (8 species tested), and *E. coli* ranging from ≤0.06 to 0.5 μg/ml.

On a weight basis, the in vitro activity of BMY-28142 against *E. coli* and *Klebsiella* species is comparable to those of HR 810 and cefotaxime; against *Proteus vulgaris* and *Enterobacter* species, it is more active than HR 810, cefpimizole, cefotaxime, cefoperazone, and moxalactam; and against *Enterobacter cloacae*, it is similar to HR 810 and better than the other cephems. *Serratia marcescens* BMY-28142 activity is comparable to those of HR 810 and ceftazidime and superior to those of moxalactam, cefpimizole, cefotaxime, and cefoperazone.

Most *Actinetobacter* spp. (79%) were susceptible at 8 μg of BMY-28142 per ml, a lower concentration than cefpimizole, cefotaxime, moxalactam, and cefoperazone; however, gentamicin inhibited 79% of these strains at 2 μg/ml. *P. maltophilia* isolates were resistant to tested antimicrobial agents, and the MIC_{90} of BMY-28142 was ≥32 μg/ml.

The MIC_{90}s of BMY-28142 against gentamicin-susceptible and -resistant *P. aeruginosa* isolates were 8 and 16 μg/ml, respectively. The activity of BMY-28142 for gentamicin-susceptible strains was comparable to those of HR 810 and ceftazidime and superior to those of the other cephems. High concentrations of all cephems tested (MIC_{90}, ≥16 μg/ml) were required to inhibit gentamicin-resistant *P. aeruginosa*.

The pattern of susceptibility to BMY-28142 followed the OX susceptibility of the gram-positive isolates. The MIC_{90} for OX-susceptible *S. aureus* isolates was 4 μg/ml; thus, BMY-28142 was more active than cefpimizole, ceftaxidime, cefotaxime, and moxalactam, but less active than HR 810. The MIC_{90}s of OX-resistant *S. aureus* isolates, coagulase-negative staphylococci, and enterococci were 32, 16, and ≥32 μg/ml, respectively.

Gram-negative and gram-positive isolates resistant to the third-generation cephalosporins, cefoperazone, cefotaxime, or moxalactam (MICs, ≥32 μg/ml) were compared for susceptibility to BMY-28142 (MICs, ≤8 μg/ml; Table 2). A total of 100% of resistant *Enterobacteriaceae*, 62% of resistant non-glucose-fermenting gram-negative isolates, and 56% of resistant gram-positive isolates were susceptible to BMY-28142. When the *Staphylococcus* spp. were separated from enterococci, 94% of resistant isolates were susceptible to BMY-28142.

The relationship between the MBC and the MIC of BMY-28142 was examined for *C. freundii*, *Enterobacter cloacae*, *E. coli*, *K. pneumoniae*, *Serratia marcescens*, *P. aeruginosa*, and *S. aureus*. For 83% of the 213 strains tested, the MBC was the same or one dilution higher than the MIC (MBC/MIC, ≤2). However, for *Serratia marcescens*, the MBC/MIC ratio for 11 of 24 strains (45.8%) was 4 or 8.

In time-killing studies of the bactericidal activity of BMY-28142, ceftazidime, cefpimizole, and cefoperazone against *P. aeruginosa*, BMY-28142 and ceftazidime at 1/2 MIC (2 μg/ml), 1× MIC (4 μg/ml), and 2× MIC (8 μg/ml) exhibited a bactericidal effect with a reduction of viable cell counts per milliliter of up to 4.5 logs when compared with the control. A substantial 3-log reduction was also observed for BMY-28142 at 1/4 MIC (1 μg/ml). In contrast, the bactericidal effect of cefoperazone and cefpimizole, although significant at 1× MIC (8 μg/ml) and 2× MIC (16 μg/ml), was substantially reduced at 1/2 MIC (4 μg/ml) and 1/4 MIC (2 μg/ml).

The effect of inoculum size on the MIC of BMY-28142 is shown in Table 3. When 10^6 CFU/ml was compared with 10^5 CFU/ml, at most a one-dilution increase in MIC was observed. For *S. aureus*, an increase to 10^7 and 10^8 CFU/ml resulted in an increase of one to two dilutions. When the MIC at 10^7 and 10^8 CFU/ml was compared with that at 10^5

TABLE 2. Susceptibility to BMY-28142 of isolates resistant to third-generation cephalosporins

Organism (total no. tested)	No. resistant to third-generation cephalosporins[a]	No. of resistant isolates susceptible to BMY-28142[b]
Enterobacteriaceae, (242)	33	33
Pseudomonas aeruginosa (55)	41	32
P. maltophilia (61)	16	1
Acinetobacter spp. (14)	14	11
Staphylococcus aureus (30)	17	15
Coagulase-negative staphylococci (24)	19	19
Enterococci (25)	25	0

[a] Resistant to third-generation cephalosporins: MIC ≥ 32 μg/ml for cefoperazone, cefotaxime, or moxalactam.

[b] Susceptible to BMY-28142: MIC ≤ 8 μg/ml.

TABLE 3. Effect of inoculum size on MICs of BMY-28142

Organism (no.)	CFU/ml[a]	No. with MIC (μg/ml) of:										
		≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
Escherichia coli (5)	1.9×10^5	5										
	1.9×10^6	5										
	1.9×10^7	1	2			1	1					
	1.9×10^8						1	4				
Pseudomonas aeruginosa (5)	1.9×10^5				1	4						
	1.9×10^6					1	4					
	1.9×10^7						3	2				
	1.9×10^8						1					4
Serratia marcescens (6)	3.7×10^5	6										
	3.7×10^6	3	3									
	3.7×10^7											6
	3.7×10^8											6
Staphylococcus aureus (5)	3.6×10^5						4	1				
	3.6×10^6						2	3				
	3.6×10^7							5				
	3.6×10^8								4	1		
Streptococcus faecalis (5)	1.9×10^5									1	3	1
	1.9×10^6										2	3
	1.9×10^7											5
	1.9×10^8											5

[a] Average of inoculum.

CFU/ml for *P. aeruginosa*, *E. coli*, and *Serratia marcescens*, a more significant increase was observed; *P. aeruginosa* and *E. coli* increased as much as five wells, and *S. marcescens* increased as much as ten wells. For *Streptococcus faecalis*, the effect of inocula of 10^7 and 10^8 CFU/ml could not be determined since the MICs were at the upper range of concentrations tested.

DISCUSSION

Our results show that the in vitro activity of BMY-28142 is highly encouraging and confirms the recent observations of Khan and associates (6). For *E. coli*, *Proteus* species, and the *Klebsiella-Enterobacter-Serratia* group, the MICs are comparable to HR 810 and other cephems. Against gentamicin-resistant and -susceptible *P. aeruginosa*, BMY-28142 activity was comparable to those of HR 810 and ceftazidime and superior to those of cefotaxime, cefpimizole, cefoperazone, and moxalactam. BMY-28142 does not appear to effect an improved coverage of the gram-positive cocci. When a group of multiply resistant *Klebsiella* species were examined (resistant to gentamicin and cefoperazone), BMY-28142 activity was comparable to ceftazidime, cefotaxime, and moxalactam activity and superior to HR 810 and cefpimizole activity. It will prove interesting to examine this new agent against other species of resistant organisms.

In the microdilution assay system utilized here, BMY-28142 proved to be bactericidal within one dilution of the MIC for a substantial percentage of isolates tested (83%). The variation between MIC and MBC for *Serratia marcescens* strains, however, was substantial, and it is intriguing that the greatest inoculum effect was also observed in this group. These findings may result from specific interactions between BMY-28142 and *Serratia marcescens* which are different from those between BMY-28142 and other genera of organisms.

When BMY-28142 bactericidal activity against *P. aeruginosa* was examined over a defined time period, the activity was directly comparable to that of ceftazidime and superior to those of cefoperazone and cefpimizole. It is encouraging that a significant 3-log reduction of viable cell count was observed even at 1/4 the MIC for BMY-28142.

Thus, BMY-28142 appears to have excellent in vitro activity against clinically important negative bacilli and anti-staphylococcal activity that is better than moxalactam and ceftazidime. Furthermore, when isolates resistant to third-generation cephalosporins were examined, 77 of 104 gram-negative isolates and 34 of 61 gram-positive isolates were susceptible to ≤8 μg of BMY-28142 per ml. The boundaries for susceptibility and resistance will depend on phase I pharmacokinetic studies in humans, after which efficacy studies may be possible. These will determine, in part, the ultimate therapeutic application of this agent in humans.

LITERATURE CITED

1. **Bertram, M. A., D. A. Bruckner, and L. S. Young.** 1984. In vitro activity of HR 810, a new cephalosporin. Antimicrob. Agents Chemother. **26**:277–279.
2. **Brooks, G. F., and S. L. Barriere.** 1983. Clinical use of the new beta-lactam antimicrobial drugs. Ann. Intern. Med. **98**:530–535.
3. **Cullman, W., W. Opferkuch, and M. Stieglitz.** 1983. Relation between beta-lactamase production and antimicrobial activity: comparison of the new compound HR 810 with cefotaxime. Eur. J. Clin. Microbiol. **2**:350–352.
4. **Goto, S., H. Sakamoto, M. Ogawa, A. Tsuji, and S. Kuwahara,** 1982. Bactericidal activity of cefazolin, cefoxitin, and cefmetazole against *Escherichia coli* and *Klebsiella pneumoniae*. Chemotherapy **28**:18–25.
5. **Jones, R. N., C. Thornsberry, and A. L. Barry.** 1984. In vitro evaluation of HR 810, a new wide-spectrum aminothiazolyl α-methoxyimino cephalosporin. Antimicrob. Agents Chemother. **25**:710–718.

6. **Khan, N. J., J. A. Bihl, R. F. Schell, J. L. leFrock, and S. J. Weber.** 1984. Antimicrobial activities of BMY-28142, cefbuperazone, and cefpiramide compared with those of other cephalosporins. Antimicrob. Agents Chemother. **26**:585–590.
7. **Kurtz, T. O., D. Winston, J. A. Hindler, L. S. Young, W. Hewitt, and W. J. Martin.** 1981. Comparative in vitro activity of moxalactam, cefotazime, cefoperazone, piperacillin, and aminoglycosides against gram-negative bacilli. Antimicrob. Agents Chemother. **18**:645–648.
8. **National Committee for Clinical Laboratory Standards.** 1983. Tentative standard M7-T. Standard method for dilution antimicrobial susceptibility tests for bacteria which grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
9. **Neu, H. C.** 1982. The new β-lactamase stable cephalosporins. Ann. Intern. Med. **97**:408–419.
10. **Neu, H. C., and P. Labthavikul.** 1983. In vitro activity and β-lactamase stability of U-63196E, a novel cephalosporin. Antimicrob. Agents Chemother. **24**:357–382.
11. **Phillips, I., C. Warren, K. Shannon, A. King, and D. Hanslo.** 1981. Ceftazidime: in vitro antibacterial activity and susceptibility to β-lactamases compared with that of cefotaxime, moxalactam and other β-lactam antibiotics. J. Antimicrob. Chemother. **8**(Suppl. B):23–31.