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Butirosin, a New Aminoglycosidic Antibiotic Complex: Antibacterial Activity In Vitro and in Mice

C. L. HEIFETZ, M. W. FISHER, J. A. CHODUBSKI, AND M. O'. DECARLO

Biological Research and Development Department, Research and Development Division, Parke, Davis & Company, Detroit, Michigan 48232

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Butirosin is a new aminoglycosidic antibiotic complex which has broad gram-negative and gram-positive inhibitory antibacterial activity, as well as some bactericidal properties. Significantly susceptible bacteria include strains of *Staphylococcus aureus* and *Streptococcus pyogenes*, and pathogenic gram-negative species such as *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *P. vulgaris*, *Salmonella enteritidis* and *S. typhimurium*, *Shigella flexneri* and *S. sonnei*. Good activity by parenteral dosing was obtained in various acute mouse infections. Butirosin is especially interesting because of its activity against *Pseudomonas aeruginosa* in vitro, including gentamicin-resistant clinical isolates, and in experimental mouse infections at relatively low doses.

The discovery and production of butirosin, a new aminoglycosidic antibiotic complex, has been reported by Howells et al. (3) and Dion et al. (1). A description of its chemical and physical characteristics was presented by Woo et al. (7-9). In this communication, data are given on the in vitro and mouse-protective antibacterial activities of this new antibiotic.

MATERIALS AND METHODS

The antibiotics were handled, evaluated, and reported on the basis of total weight rather than base content. The most frequently used antibiotics (all sulfates) were: butirosin, 710 μg of base/mg; colistin, 630 μg of base/mg; gentamicin, 554 μg of base/mg; kanamycin, 785 μg of base/mg; nebramycin complex; neomycin, 660 μg of base/mg; paromomycin, 700 μg of base/mg; polymyxin B, 7,200 units/mg; and streptomycin, 750 μg of base/mg. Butirosin is a complex (3) containing ca. 80 to 85% component A and 15 to 20% component B.

Minimal inhibitory concentrations (MIC) were determined generally according to the methods described by Fisher et al. (2). Inocula of approximately 1,000 cells in 0.1 ml of medium were added to tubes containing twofold decremental dilutions of antibiotic in 5-ml volumes of Trypticase soy broth (TSB) or synthetic TB medium. The inoculated tubes were incubated for 18 to 24 hr (14 days for *Mycobacterium tuberculosis*) at 37 C before observation.

To determine bactericidal effects, decremental tube dilutions in 5 ml of broth were inoculated with approximately 10^6 cells per tube. After overnight incubation at 37 C, a loopful of medium from each inoculated clear tube was streaked onto a Trypticase soy agar plate. After 24 hr of incubation at 37 C, these subculture plates were observed for the presence and relative numbers of surviving bacteria. The lowest concentration of butirosin producing a distinct, marked reduction in numbers as revealed by such subculture was regarded as the minimal bactericidal concentration.

The influence of serum and inoculum size on bacteriostatic activity was determined by the method used for initial MIC determinations. However, light (10^3 cells) and heavy (10^6 cells) inocula were compared. The test media included not only TSB, but also TSB fortified with 10% bovine serum or 50% pooled human serum.

Increases in resistance for a given strain were attempted by the transfer of heavy inocula through rising concentrations of antibiotic (butirosin, gentamicin, and colistin) in TSB. Three to 10 transfers were required before the MIC values of these strains were increased to $\geq$ 100 μg/ml. By use of light inocula, the MIC values of several antibiotics were compared against the resistant variants of a *Klebsiella pneumoniae* and a *Staphylococcus aureus*, and their sensitive parent strains. In another series, butirosin, gentamicin, and colistin, as well as streptomycin, kanamycin, and paromomycin, were compared for their activity against bacteria with increased resistance to the first three of these drugs. As before, the sensitive parent cultures were included.

Clinically obtained antibiotic-resistant isolates were subjected to the same broth dilution (light inoculum) MIC determination. Tested were strains of *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *S. aureus*, *Shigella flexneri*, and *S. sonnei*.

All in vivo tests were done in 18- to 22-g female CF-1 mice (Carworth). The mice received mouse pellet diet (Rockland) and water ad libitum. In therapy studies, single subcutaneous antibiotic doses, twofold rising incremental series in 0.5-ml volumes of physiological saline, were administered concurrently with the bacterial challenge. Challenges were accomplished by the intraperitoneal injection of an estimated 100 LD_{50} suspended in 0.5 ml of either TSB (for *Klebsiella* only) or 5% hog gastric mucin. Generally, $\geq$ 90% of the untreated controls died in 48 to 72 hr. Final survival percentages, obtained after 7 days of observation among groups of 10 to 15 mice, were used to estimate median protective doses (PD_{50}) among infected and treated mice by the log-probit procedure of Miller and Tainter (4).

RESULTS

The inhibitory spectrum of butirosin is summarized in Table 1. Butirosin is most noteworthy for its activity at low levels against a wide variety of gram-negative species, especially *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *P. vulgaris*. Also susceptible are the test strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Salmonella enteritidis*, *S. typhimurium*, *Shigella flexneri*, and *S. sonnei*. *Pseudomonas pseudomallei* is less susceptible.

Activity against other species is variable. Although inhibitory at low levels against *Mycobacterium tuberculosis*, *Streptococcus pyogenes*, and strains of *Staphylococcus aureus* with limited antibiotic resistance, butirosin appears much less active against *Diplococcus pneumoniae*, *Streptococcus faecalis*, and multiantibiotic-resistant *Staphylococcus aureus* strains.

Data pertaining to the bactericidal tests are summarized in Table 2. Butirosin usually exhibited bactericidal activity at concentrations equal to or double the MIC.

Results of tests involving serum and inoculum size effects are summarized in Table 3. Subjected to a 1,000-fold increase in inoculum, butirosin MIC values were usually elevated four- to eightfold. The presence of 10% bovine serum or 50% human serum had no significant effect on activity.

In vitro antibacterial comparisons were made between butirosin and some structurally similar antibiotics. The results (Table 4) demonstrate that butirosin possesses the spectrum characteristic of gentamicin (5) and nebramycin (6) and is active at similar concentrations.

Butirosin-resistant progeny of *S. aureus* and *K. pneumoniae* strains, obtained by in vitro selection, were compared with their parent cultures for susceptibility to various other antibiotics (Table 5). The butirosin-resistant derivative strains were also resistant to all the other aminoglycosidic antibiotics tested: gentamicin, neomycin, kanamycin, paromomycin, and streptomycin. No changes in susceptibility were encountered with the unrelated compounds: novo-

TABLE 1. *Antibacterial spectrum of butirosin*

Species	No. of strains tested	Minimal inhibitory concn (μg/ml)	
		Range	Geometric mean
Enterobacter aerogenes	3	0.8–6.3	2.5
Escherichia coli	11	1.6–50	6.3
Klebsiella pneumoniae	3	0.4–6.3	2.0
Proteus mirabilis	2	12.5–25	17.7
P. vulgaris	2	6.3–25	12.5
Pseudomonas aeruginosa	35	3.1–50	11.1
P. pseudomallei	4	50	50.0
Salmonella enteritidis	3	12.5–50	25.0
S. typhimurium	2	12.5	12.5
Shigella flexneri	5	12.5	12.5
S. sonnei	4	3.1–6.3	5.3
Diplococcus pneumoniae	6	3.1–200	49.9
Staphylococcus aureus			
Antibiotic-sensitive	4	1.6–6.3	3.1
"Limited" resistance[a]	4	3.1–25	7.4
Multiresistant	7	12.5–>100	37.1
Streptococcus faecalis	1	>100	>100
Streptococcus pyogenes	3	6.3–12.5	7.9
Mycobacterium tuberculosis	1	1.6	1.6

[a] Phage type 80/81 strains resistant to penicillin and streptomycin, and susceptible to neomycin, kanamycin, and paromomycin.

biocin, erythromycin, penicillin G, tetracycline, and chloramphenicol.

An additional series of in vitro cross-resistance tests was performed. Four bacterial strains "made" resistant to either butirosin, gentamicin, or colistin, as well as their parent cultures, were tested against these three antibiotics and kanamycin, streptomycin, and paromomycin (Table 6). Complete butirosin-gentamicin cross resistance occurred in strains selected for decreased susceptibility to the related compounds: streptomycin, kanamycin, and paromomycin. Except for one instance, these strains were unchanged with regard to colistin susceptibility. Unexpectedly, colistin-resistant strains, again with one exception, were also insusceptible to the other drugs tested.

TABLE 2. *Butirosin bactericidal activity*

Test organism	MIC (μg/ml)[a]	MBC (μg/ml)[b]
Enterobacter aerogenes MGH-1	12.5	25
Escherichia coli Vogel	25	25
Klebsiella pneumoniae MGH-2	6.3	6.3
Proteus mirabilis MGH-1	100	100
P. vulgaris UC-232	100	100
Pseudomonas aeruginosa 28	50	100
P. aeruginosa 733	12.5	50
P. aeruginosa Whittington	50	100
Salmonella typhimurium V-31	200	200
Shigella sonnei C-10	25	50
Staphylococcus aureus Bail	12.5	100
S. aureus UC-76	25	50

[a] Higher MIC values in these tests resulted from heavy (10^6 cells) inocula. See Table 3.
[b] Minimal bactericidal concentration.

Clinical isolates, chosen for their insusceptibility to streptomycin and not subjected to in vitro selective manipulations, were tested for their susceptibility to butirosin and several related compounds (Table 7). Unlike the in vitro-derived cultures previously described, the clinically resistant isolates were not uniform in their cross-resistance patterns. Of the antibiotics tested, some isolates of *E. coli*, *S. flexneri*, and *S. sonnei* were resistant only to streptomycin. An *E. coli* isolate which demonstrated insusceptibility to streptomycin, paromomycin, and kanamycin was fully susceptible to butirosin and gentamicin.

Similar tests with gentamicin-resistant clinical isolates of *P. aeruginosa* are summarized in Table 8. The butirosin MIC values are identical with those obtained with gentamicin-sensitive isolates, indicating an absence of cross resistance.

Results of initial exploratory in vivo studies, not detailed here, demonstrated that butirosin was of relatively low activity in mice when given by the oral route. Single oral PD_{50} values were about seven times as high as those from subcutaneous (sc) administration in parallel treatment

TABLE 3. *Influence of serum and inoculum size on the bacteriostatic activity of butirosin in vitro*

Test organism	Inoculum size (approx no. of cells)	Culture medium	MIC (μg/ml)
Pseudomonas aeruginosa 28	10^3	TSB[a]	12.5
	10^6	TSB	100
	10^3	TSB + 10% bovine serum[b]	12.5
	10^6	TSB + 10% bovine serum	100
P. aeruginosa IM-1	10^3	TSB	6.3
	10^6	TSB	12.5
	10^3	TSB + 10% bovine serum	12.5
	10^6	TSB + 10% bovine serum	12.5
Staphylococcus aureus Bail	10^3	TSB	3.1
	10^6	TSB + 50% human serum	25
S. aureus Padgette	10^3	TSB	25
	10^6	TSB + 50% human serum	100
S. aureus S18713	10^3	TSB	50
	10^6	TSB + 50% human serum	400

[a] TSB, Trypticase soy broth.
[b] A total of 15 strains of *P. aeruginosa* were compared in TSB and TSB + 10% serum with inocula consisting of 10^3 cells. The addition of serum did not significantly influence the MIC.

TABLE 4. *Comparative inhibitory activity of aminoglycosidic antibiotics in vitro*

Test organism	MIC (μg/ml)					
	Butirosin	Gentamicin	Kanamycin	Nebramycin	Paromomycin	Streptomycin
Staphylococcus aureus UC-76	3.1	0.8	1.6	6.3	0.4	1.6
Escherichia coli Vogel	12.5	6.3	12.5	12.5	25	12.5
Klebsiella pneumoniae MGH-2	3.1	1.6	3.1	6.3	6.3	6.3
Pseudomonas aeruginosa 28	6.3	1.6	50	3.1	100	>100
P. aeruginosa 733	6.3	1.6	100	6.3	100	>100

TABLE 5. *Antibiotic resistance of butirosin-resistant derivants in vitro*

Antibiotic	MIC (μg/ml) against			
	K. pneumoniae MGH-2		*S. aureus* UC-76	
	Original	Butirosin-resistant	Original	Butirosin-resistant
Butirosin	1.6	100	0.8	>100
Gentamicin	1.6	50	ND[a]	ND
Neomycin	1.6	>100	0.2	100
Kanamycin	6.3	>100	1.6	>100
Paromomycin	3.1	>100	0.4	100
Streptomycin	3.1	100	1.6	>100
Novobiocin	50	25	0.03	0.05
Erythromycin	50	25	0.05	0.05
Penicillin G	25	50	0.01	0.01
Tetracycline	0.8	0.8	0.05	0.05
Chloramphenicol	1.6	0.8	1.6	3.1

[a] ND, Not done.

TABLE 6. *Laboratory-induced cross resistance between butirosin, gentamicin, and colistin*

Test organism		MIC (μg/ml)			
Strain	Selected for resistance to	Butirosin	Gentamicin	Colistin	Streptomycin, kanamycin, paromomycin
Escherichia coli Vogel	None	12.5	6.3	<0.2	12.5–25
	Butirosin	>100	50	<0.2	>100
	Gentamicin	100	100	<0.2	≥100
	Colistin	>100	100	>100	>100
Klebsiella pneumoniae MGH-2	None	3.1	1.6	<0.2	3.1–6.3
	Butirosin	>100	50	<0.2	≥100
	Gentamicin	>100	>100	<0.2	>100
	Colistin	>100	100	>100	>100
Pseudomonas aeruginosa 28	None	6.3	1.6	1.6	50–>100
	Butirosin	>100	25	1.6	>100
	Gentamicin	100	100	>100	>100
	Colistin	100	50	>100	>100
Pseudomonas aeruginosa 733	None	6.3	1.6	1.6	≥100
	Butirosin	>100	50	0.8	>100
	Gentamicin	>100	>100	1.6	>100
	Colistin	12.5	6.3	>100	50–>100

TABLE 7. *Cross resistance between butirosin and other aminoglycosidic antibiotics in some clinical isolates chosen for their insusceptibility to streptomycin*

Test organism[a]	MIC (μg/ml)					
	Butirosin	Gentamicin	Streptomycin	Paromomycin	Neomycin	Kanamycin
Escherichia coli (3)	3.1–6.3	1.6	>100	12.5	ND[b]	6.3–12.5
E. coli (1)	1.6	0.8	100	>100	ND	>100
Enterobacter aerogenes (2)	0.8–3.1	0.4–0.8	>100	3.1	ND	1.6–3.1
Staphylococcus aureus type UC-18 (6)	25–100	0.4–6.3	>100	>100	50–>100	ND
S. aureus type 54 (1)	>100	1.6	>100	>100	>100	ND
S. aureus type 80/81 (3)	3.1–25	ND	100–>100	0.8–1.6	0.2–0.8	ND
Shigella flexneri (3)	12.5	6.3–12.5	>100	12.5–25	12.5–25	12.5–25
S. sonnei (3)	3.1–6.3	0.8–3.1	≥100	6.3–12.5	3.1–12.5	6.3–12.5

[a] Numbers in parentheses indicate number of isolates.
[b] ND, Not done.

TABLE 8. *Butirosin susceptibility of gentamicin-resistant Pseudomonas aeruginosa clinical isolates*

Strain	MIC (μg/ml)	
	Gentamicin	Butirosin
64	>400	100
B237	>400	25
B239	>400	50
G137	>400	12.5
G138	>400	50
G236	>400	6.3
76	400	25
G30	400	50
G76	200	25
74	100	25
79	100	12.5
81	100	12.5
G20	100	25
G22	100	25
G75	100	25
71	50	12.5
72	50	12.5

groups. Consequently, all further mouse tests on butirosin used sc treatment regimens.

Results of single sc dose mouse protection studies involving butirosin, and gentamicin in some cases, are summarized in Table 9. The in vivo anti-*Pseudomonas* activities of butirosin, gentamicin, colistin, and polymyxin B were also compared (Table 10). Butirosin is effective in mice against susceptible gram-negative bacteria and staphylococci when administered parenterally. Activity against *P. aeruginosa* is obtained at doses similar to those required for the other agents.

TABLE 9. *Activity of butirosin against experimental acute bacterial infections of mice*

Infecting organism	Approx PD_{50} (mg/kg) single subcutaneous dose	
	Butirosin	Gentamicin
Staphylococcus aureus, IL-69	7	ND[a]
S. aureus, H-228	5	ND
S. aureus, UC-76	7	ND
Enterobacter aerogenes, MGH-1	21	5
Escherichia coli, MGH-1	5	ND
E. coli, 04-D	5	2
E. coli, 07-D	6	2
E. coli, 075-D	3	1
E. coli, Vogel	19	5
Klebsiella pneumoniae, AD	3	1
K. pneumoniae, MGH-2	5	ND
Proteus vulgaris, UC-232	14	ND
Pseudomonas aeruginosa, 28	64	18
P. aeruginosa, 733	25	13
P. aeruginosa, 1174C-1	150	28
P. aeruginosa, 1369-1	96	22
P. aeruginosa, Lawson	23	13
P. aeruginosa, Whittington	46	18

[a] ND, Not done.

We found the acute intravenous LD_{50} of butirosin in mice to be approximately 650 mg/kg and the sc LD_{50} to be about 4,070 mg/g.

DISCUSSION

The preceding data indicate that butirosin is an intrinsically bactericidal antibiotic which exhibits significant activity against staphylococci and a wide variety of gram-negative organisms

TABLE 10. *Comparative mouse-protective activity of four anti-Pseudomonas antibiotics*

Antibiotic	Approximate single SC[a] PD_{50} (mg/kg) against three *P. aeruginosa* strains		
	733	1359-1	Lawson
Butirosin	25	96	23
Gentamicin	13	22	13
Colistin	37	13	30
Polymyxin B	19	9	30

[a] SC, Subcutaneous.

in vitro and in experimentally infected mice. Most notable among the susceptible species are *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *P. vulgaris*.

The cross-resistance studies involving strains selected by in vitro procedures, although apparently unrelated to the cross-resistance patterns associated with clinical source strains, are yet of some interest. Strains selected for resistance to either butirosin or gentamicin are resistant to both these and the other similar agents tested, but are susceptible to colistin. In contrast, the colistin-resistant strains are generally resistant to the aminoglycosidic agents as well. Clinically derived multiple drug-resistant staphylococci showed decreased susceptibility to butirosin as well, whereas the one *E. coli* in this category was susceptible. Especially significant is the observation that butirosin is equally active against gentamicin-resistant and -sensitive *P. aeruginosa* clinical isolates in vitro. A further implication is the potential clinical effectiveness of butirosin against such types.

Butirosin given parenterally was active in mice against acute infections with staphylococci and various gram-negative bacilli. Especially interesting was its protective activity against *P. aeruginosa* infections at doses considerably below those producing acute toxicity by the same route of administration.

These aspects would recommend the further evaluation of this antibiotic.

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