GLYCOCINNAMOYLSPERMIDINES, A NEW CLASS OF ANTIBIOTICS

V. ANTIBACTERIAL EVALUATION OF THE ISOPROPYL DERIVATIVE OF LL-BM123γ

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Isopropyl LL-BM123γ, a novel semisynthetic glycocinnamoylspermidine antibiotic, was active *in vitro* against both Gram-negative and Gram-positive bacteria with broad spectrum bactericidal activity against clinically important Gram-negative strains. In parallel tests, it was equal to or more potent than reference aminoglycoside antibiotics against *Escherichia coli, Proteus, Enterobacter-Klebsiella, Serratia, Salmonella*, and *Acinetobacter* strains. Against clinical isolates of *Pseudomonas aeruginosa*, isopropyl LL-BM123γ compared favorably with gentamicin, verdamicin and amikacin but was less potent than tobramycin. Isopropyl LL-BM123γ was active against many Gram-negative bacteria that were relatively resistant to aminoglycosides. It was rapidly absorbed following subcutaneous administration in mice and showed greater potency than gentamicin on both dosage and plasma concentration bases against several experimental infections.

LL-BM123γ is a novel antibiotic produced by a culture of *Nocardia*.[1] It has a unique structure in which a *p*-hydroxycinnamoylspermidine unit is combined with a trisaccharide.[2] It has potent antibacterial properties resembling those of aminoglycoside antibiotics.[3] Chemical modification of the γ components resulted in the preparation of an isopropyl derivative which in preliminary tests was less toxic and more potent against experimental infections than the parent antibiotic.[4] This report is concerned with the *in vitro* and *in vivo* microbiological evaluation of isopropyl LL-BM123γ.

Materials and Methods

Antibiotics

Isopropyl LL-BM123γ is a mixture of two isomers, γ_1 and γ_2.[4] Gentamicin sulfate is the commercial product of Schering Corporation. Sisomicin sulfate and verdamicin sulfate samples were kindly supplied by the Schering Corporation, tobramycin by the Eli Lilly Company and amikacin by Bristol Laboratories.

Media

Nutrient, MUELLER-HINTON and Brain-Heart Infusion broths and agars were products of Difco; trypticase-soy broth and agar were products of Baltimore Biological Laboratories.

Test strains

Clinical isolates obtained from eleven medical centers in the United States were used to assess the *in vitro* activity of isopropyl LL-BM123γ.

In vitro antibacterial activity

Minimal inhibitory concentrations (MICs) of agents were determined by means of standard two-fold serial dilution methods using agar or broth media. Agar plates were inoculated with exponentially growing cultures diluted 100-fold in broth and applied with a STEERS multiple inocula replicator.[5] The inocula in broth dilution tests were approximately 10^5 viable units per ml. The minimal inhibitory

concentration was defined as the lowest concentration of antibiotic inhibiting growth after 18~20 hours incubation at 37°C.

Bactericidal effects were determined by two methods. First, after MICs were determined in broth dilution tests after 20-hour incubation, inhibited cultures were plated for colony counts. The lowest concentration of drug that effected a 3-log reduction in the viable population of the inoculum was recorded as the minimal bactericidal concentration (MBC). Secondly, bactericidal rates were assessed by determining viable populations at various time intervals after addition of antibiotic to the culture.

In vivo antibacterial activity

Therapeutic effects of drugs were determined in experimental Gram-negative and Gram-positive infections produced in 18~20 g female mice of the CD-1 strain (Charles River Laboratories). Mice were challenged intraperitoneally with sufficient organisms suspended in 0.5 ml of trypticase-soy broth to kill 90~100% of nontreated mice within 72 hours. Acute lethal toxic effects were determined in non-infected mice. The antibiotic doses were contained in 0.5 ml of 0.2% aqueous agar and administered subcutaneously in a single dose within 1 hour after infection. In each test, 10 mice were treated at each dose level and survival ratios were determined 7 days after infection. The data from two to four separate tests were pooled for the estimation of median-effective doses (ED_{50}) and median lethal doses (LD_{50}) by the method of LITCHFIELD and WILCOXON.[6]

To determine drug concentrations in serum, single doses at various levels were administered subcutaneously and the mice were bled at intervals thereafter. Plasma samples were obtained from the pooled heart blood from 3 mice. They were assayed by a disk plate method using *Klebsiella* #602 as the test organism. Plasma concentrations associated with the median effective doses were estimated from the straight line obtained by plotting peak plasma drug concentrations logarithmically against the corresponding doses.

Results and Discussion

In vitro Potency

Isopropyl LL-BM123γ was active against both Gram-negative and Gram-positive bacteria with broad spectrum activity against Gram-negative bacteria. Its spectrum compared with five aminogly-

Table 1. *In vitro* spectrum of activity of isopropyl LL-BM123γ compared with aminoglycosides (Agar-dilution method, MUELLER-HINTON agar)

Organism	No. of strains	Range of minimal inhibitory concentrations (μg/ml)					
		Isopropyl LL-BM123γ	Gentamicin	Tobramycin	Verdamicin	Sisomicin	Amikacin
Escherichia coli	31	1~2	2~4	2~4	2~4	2	4~8
Enterobacter-Klebsiella	30	0.25 ~4	0.5 ~16	1~4	1~64	0.5~16	2~8
Serratia	24	0.25 ~2	1~8	2~16	1~4	1~8	1~16
Proteus	26	1~4	2~8	2~4	2~8	2~4	2~4
Acinetobacter	32	0.015~16	0.06~8	0.06~16			0.12~4
Pseudomonas	31	0.25 ~64	1~>128	1~16	0.5~>128	0.5~>128	1~64
Salmonella	2	<0.06~0.25	0.25~1	0.25~1			0.5 ~2
Shigella	2	<0.06~0.5	0.5 ~1				
Staphylococcus aureus	16	0.12~0.25	0.06~0.12	<0.06~0.12			0.25~1
Enterococcus	3	8~32	16				
Bacteroides	4	>128	>128				
Fusobacterium necrophorum	2	>128	>128				

Table 2. Activity of isopropyl LL-BM123γ against bacteria requiring high concentrations of some aminoglycosides for inhibition of growth (MUELLER-HINTON agar dilution tests)

Organism	Minimal inhibitory concentrations, μg/ml					
	Isopropyl LL-BM123γ	Gentamicin	Tobramycin	Verdamicin	Sisomicin	Amikacin
Klebsiella N-74-2	0.5	16	2	32	8	2
〃 N-74-4	1	16	2	64	16	4
Serratia K-101	0.5	2	8	2	4	8
〃 F-20	1	8	8	4	4	16
〃 F-21	1	8	8	4	4	16
〃 F-66	1	8	8	4	4	16
Acinetobacter Mayo-75-4	0.06	0.5	16	ND	ND	4
Pseudomonas N-74-3	4	32	16	64	32	64
〃 G236	32	>128	2	>128	>128	8

cosides is shown in Table 1. In general, isopropyl LL-BM123γ was equal to or more potent than the reference antibiotics against clinical isolates of *Escherichia coli, Proteus, Enterobacter-Klebsiella, Salmonella, Serratia,* and *Acinetobacter.* Against *Pseudomonas*, isopropyl LL-BM123γ compared favorably with most of the reference aminoglycosides but was not as potent as tobramycin. The antibiotic was active against Gram-positive bacteria but like gentamicin was inactive against anaerobic bacteria.

Several Gram-negative organisms that were relatively resistant to some aminoglycosides were susceptible to low concentrations of isopropyl LL-BM123γ (Table 2).

Factors Influencing *In Vitro* Activity

Medium ingredients affected the activity of isopropyl LL-BM123γ. The drug was more active against *E. coli* in nutrient broth than in MUELLER-HINTON, brain-heart infusion or trypticase-soy broths. Gentamicin was similarly affected. The MBCs were similar to the MICs in all media (Table 3). The addition of horse serum to MUELLER-HINTON broth had no effect on either the MIC or MBC of isopropyl LL-BM123γ, but reduced the bactericidal activity of gentamicin. Like aminoglycosides, isopropyl LL-BM123γ was more active in alkaline than in acid media.

Table 3. Effect of growth medium on the inhibitory activity of isopropyl LL-BM123γ and gentamicin against *E. coli* #311

Broth medium	Minimal concentrations, μg/ml			
	Isopropyl LL-BM123γ		Gentamicin	
	Inhibitory[a]	Bactericidal[b]	Inhibitory	Bactericidal
Nutrient	≤0.06	≤0.06	≤0.06	≤0.06
MUELLER-HINTON	0.12	0.25	0.5	1.0
MUELLER-HINTON+50% serum	0.12	0.12	0.5	4.0
Brain-Heart Infusion	0.50	1.0	4.0	4.0
Trypticase-Soy	0.50	0.50	16	16

[a] Minimal inhibitory concentration (MIC), 24 hours at 37°C

[b] Minimal bactericidal concentration (MBC)—the minimal concentration that reduced the viable population of the inoculum by at least 3 logs after 20 hours at 37°C.

Bactericidal Effects

Isopropyl LL-BM123γ was rapidly bactericidal for several bacterial strains (Fig. 1). At concentrations that were only 2- to 4-fold higher than inhibitory concentrations in MUELLER-HINTON

Fig. 1. Bactericidal effect of isopropyl LL-BM123γ against 5 bacterial strains
1 μg/ml (2 μg/ml *vs. Pseudomonas*).

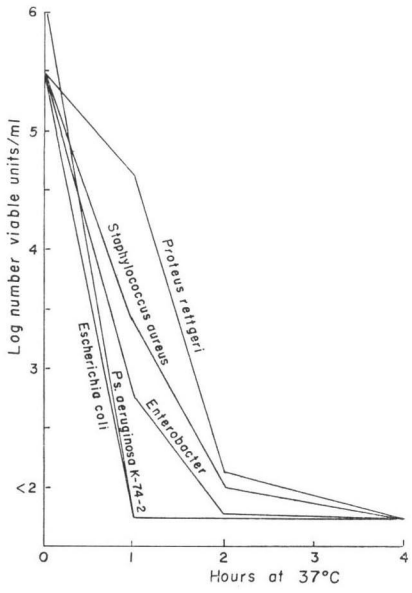

Fig. 2. Concentration of isopropyl LL-BM123γ in plasma of mice after single subcutaneous dose

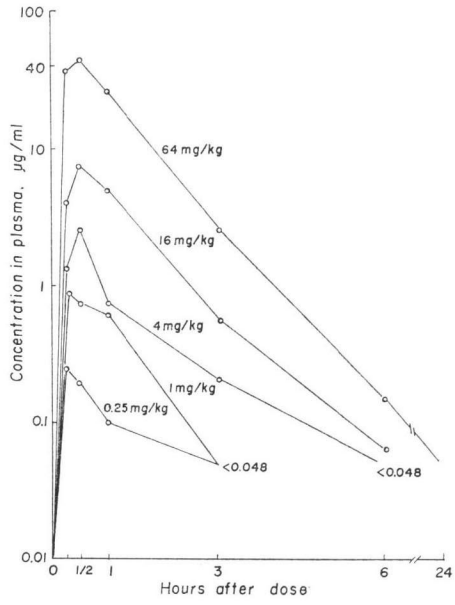

Table 4. Chemotherapeutic effects of isopropyl LL-BM123γ and gentamicin against experimental infections in mice

	Isopropyl LL-BM123γ			Gentamicin		
	ED_{50}[a] mg/kg	EC_{50}[b] mcg/ml	Safety margin[c]	ED_{50} mg/kg	EC_{50} mcg/ml	Safety margin
Klebsiella pneumoniae AD	0.02	0.027	6500	0.3	0.2	1000
Escherichia coli #311	0.08	0.09	1600	0.5	0.31	700
Proteus mirabilis #4671	0.4	0.37	330	0.4	0.22	950
Enterobacter aerogenes #75	0.9	0.76	140	0.5	0.31	700
Salmonella typhosa #6539	0.04	0.05	3300	0.1	<0.19	2700
Pseudomonas aeruginosa PA-7	8.0	5.3	16	3.7	2.9	95
Staph. aureus Smith	0.04	0.05	3300	0.2	<0.19	2300
Staph. aureus Rose	0.1	0.14	1000	0.2	<0.19	1900
Streptococcus pyogenes C-203	9.0	5.9	14	15.0	13.9	23
Streptococcus pneumoniae SV1	9.0	5.9	14	17.0	16.0	21
Median lethal subcutaneous dose, mg/kg	130			350		

[a] ED_{50}=Median effective single subcutaneous dose, mg/kg.
[b] EC_{50}=Median effective peak plasma concentration, mcg/ml.
[c] Median lethal dose/median effective dose=Safety margin.

broth, the antibiotic reduced the viable populations of *E. coli* and *Pseudomonas* cultures 99.9% in 1 hour. The killing rate was slightly slower for the *Enterobacter, Proteus*, and *Staphylococcus* cultures.

In Vivo Antibacterial Activity

Isopropyl LL-BM123γ proved to be an effective chemotherapeutic agent in mice. The drug was rapidly absorbed with peak concentrations in plasma occurring approximately 1/2 hour following subcutaneous dosage (Fig. 2). Against several infections with Gram-negative and Gram-positive bacteria in mice, isopropyl LL-BM123γ was generally more active than gentamicin on both a dosage and plasma concentration basis except against the *Pseudomonas* and *Enterobacter* infections where gentamicin was more potent (Table 4). Although isopropyl LL-BM123γ was approximately three times more lethal in acute toxicity tests in mice, its therapeutic safety margins were greater than those of gentamicin in infections produced by *Klebsiella, E. coli, Salmonella*, and *Staphylococcus aureus* Smith strains.

The antibacterial activity of isopropyl LL-BM123γ is sufficiently interesting to warrant further investigation.

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