COUMAMIDINES, NEW BROAD SPECTRUM ANTIBIOTICS OF THE CINODINE TYPE

III. MICROBIOLOGIC ACTIVITY OF COUMAMIDINE γ1

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The coumamidines are novel antibiotics with activity against a wide spectrum of aerobic Gram-positive and Gram-negative bacteria. All microbiological studies were performed on coumamidine γ1. The MIC_{90}s (μg/ml) of coumamidine are as follows: *Staphylococcus aureus* 1.0, *Streptococcus pyogenes* 8, Enterobacteriaceae 2.0, *Pseudomonas aeruginosa* 8, *Campylobacter jejuni* and *Campylobacter coli* 1, *Legionella pneumophila* 8, *Haemophilus influenzae* 0.5, *Neisseria gonorrhoeae* 0.5. Coumamidine had MICs ranging from 8 to >64 for most anaerobes, except some *Peptostreptococcus* strains. The aminoglycoside super-sensitive strain, *P. aeruginosa* BMH 10, was also super-sensitive to coumamidine (MIC 0.2 μg/ml). Coumamidine was rapidly bactericidal for *S. aureus*. The viable bacterial count in logarithmic phase cultures was reduced to less than 10 cfu within 2 hours after exposure to 4 times the MIC (3.12 μg/ml) of coumamidine. The frequency of resistance development was $<1\times10^{-9}$ for *Escherichia coli* and *S. aureus* when selected at 4 and 8 times the MIC. The Cmax in mouse serum after a single subcutaneous dose of 25 mg/kg of coumamidine was 4.5 μg/ml and $t_{1/2}$ was 1 hour. Coumamidine is stable in serum. In mouse protection tests against *S. aureus* NCTC 10649 the ED_{50} was <0.6 mg/kg/day when it was administered subcutaneously at 1 and 5 hours after infection. Coumamidine was not absorbed after oral administration. The antibacterial spectrum, bactericidal activity, stability in serum and low frequency of resistance make this an interesting new class of antibiotics.

Broad spectrum antibiotics have been useful in treating infections prior to identification of the causative organism or in patients with mixed bacterial infections. Although effective broad spectrum antibiotics, such as aminoglycosides and imipenem, are currently available, the need for new broad spectrum agents of novel structural types exists because of the occurrence of resistance to the older agents[1,2]. Coumamidines were discovered in a screen designed to find novel broad spectrum antibiotics showing no cross-resistance with known classes of antibiotics. The fermentation, isolation, identification and structural elucidation of these compounds are described in the accompanying publications[3,4]. The microbiologic studies were performed on coumamidine γ1 and will be referred to as coumamidine in this study.

Materials and Methods

Bacterial Strains

The bacterial strains used in this study were isolated in hospitals in the United States and from the American Type Culture Collection (ATCC, Rockville, Maryland, U.S.A.).

Antibiotic

Coumamidine was isolated and purified at Abbott Laboratories.

Determination of *In Vitro* Activity

MICs were determined by the agar dilution method as recommended by the National Committee for Clinical Laboratory Standards[5]. Streptococci were tested on Mueller-Hinton agar supplemented with 5% sheep blood. *Neisseria gonorrhoeae* was tested on Proteose No. 3 agar supplemented with 1% bovine haemoglobin and 1% Kellogg supplement. *Legionella pneumophila* was tested on buffered - charcoal - yeast extract agar. *Haemophilus* were tested on *Haemophilus* test medium[6]. Anaerobic bacteria were tested on Wilkins-Chalgren agar[7]. All other non-fastidious bacteria, such as *Staphylococcus aureus*, Enterobacteriaceae, *Pseudomonas aeruginosa* and *Campylobacter*, were tested on Mueller-Hinton agar.

Effect of Serum and pH on *In Vitro* Potency

The *in vitro* activity of coumamidine was determined against *S. aureus* 553, *P. aeruginosa* 5007 and *Escherichia coli* Juhl in Mueller-Hinton broth supplemented with 50% human or mouse serum and in broth medium at pH 6.5, 7.3 and 8.0.

Determination of Bactericidal Activity

The bactericidal activity of coumamidine was determined by adding 4 and 8 times the MIC of coumamidine to logarithmic phase cultures of *S. aureus* 553. Viable bacterial counts were determined at 0.5, 1, 2, 4, 6 and 24 hours after adding the antibiotic.

Resistance Frequency Determination

Ten-fold serial dilutions of 18-hour broth cultures grown from single colonies of *S. aureus* CMX 642A and *E. coli* Juhl were spread on agar plates containing 4 and 8 times the MIC of coumamidine. The plates were incubated for 48 hours and the number of colonies growing on the antibiotic containing plates were used to determine the resistance frequency[8]. The resistant colonies were subcultured 10 times on antibiotic-free agar and their susceptibility to coumamidine, cefotaxime and gentamicin was determined after the last subculture in order to determine the stability of resistance and cross-resistance to other classes of antibiotics.

Mouse Protection Tests

Mice were infected intraperitoneally with 10 LD_{50} doses of *S. aureus* NCTC 10649 containing 6.3×10^5 cfu suspended in 5% hog gastric mucin. The mice were treated with coumamidine subcutaneously by injection or orally by gavage at 1 and 5 hours after infection. The ED_{50}s were calculated on the 6th day after infection on the basis of cumulative mortalities.

Pharmacokinetic Studies

A single dose of 25 mg/kg of coumamidine was administered subcutaneously by injection or orally by gavage to mice. Blood was collected at 0.5, 1, 2, 3, 6 and 24 hours and the serum was used for determination of serum concentrations of coumamidine. Urine was collected for 24 hours to determine the percent urine recovery. The concentration of coumamidine was determined by bioassay using *S. aureus* ATCC 6538P as the assay organism and Streptomycin assay agar (Cockeysville, Maryland, U.S.A.) as the growth medium.

Results

In Vitro Potency

The MIC_{50}s, MIC_{90}s and the range of MICs for coumamidine against a broad spectrum of aerobic and anaerobic bacteria are shown in Table 1. Coumamidine had activity against aerobic Gram-positive and Gram-negative bacteria including *P. aeruginosa*. It also had weak activity against anaerobic bacteria. Coumamidine was active against gentamicin-resistant strains, *Staphylococcus epidermidis* CMX 724g, *Serratia marcescens* 740c and *P. aeruginosa* 350f. One gentamicin-resistant strain, *P. aeruginosa* 719a, was also resistant to coumamidine. The aminoglycoside super-sensitive strain, *P. aeruginosa* BMH 10, was also super-sensitive to coumamidine.

Table 1. *In vitro* activity of coumamidine.

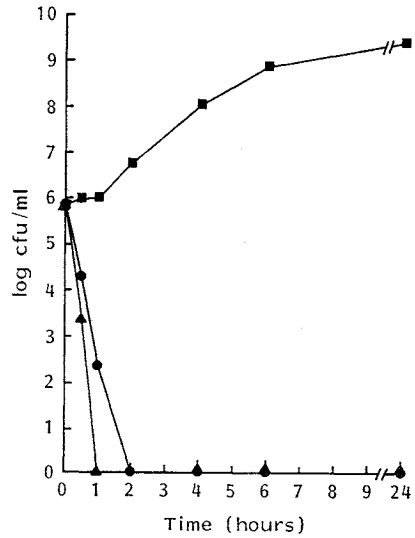
Organism (No. of strains)	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Staphylococcus aureus (20)	0.25~1	1	1
Enterococcus faecalis (11)	1~16	8	16
Streptococcus pyogenes (15)	4~8	4	8
Enterobacteriaceae (27)	0.25~4	1	2
Pseudomonas aeruginosa (11)	0.12~>32	4	8
Pseudomonas sp. and *Acinetobacter* sp. (14)	0.12~>32	8	16
Haemophilus influenzae (17)	0.03~1	0.25	0.5
Neisseria gonorrhoeae (12)	0.5~1	0.5	0.5
Legionella pneumophila (11)	4~8	4	8
Campylobacter jejuni and *C. coli* (8)	0.03~1	0.12	1
Gram-negative anaerobes (18)	8~>64	64	64
Gram-positive anaerobes (12)	2~>64	64	>64

Effect of Serum on *In Vitro* Potency

The *in vitro* activity of coumamidine against *E. coli* and *P. aeruginosa* was increased by two 2-fold dilutions after the addition of 50% human serum to the growth medium. The effect of mouse serum was different from that of human serum in that the activity of coumamidine against *E. coli* was decreased by two 2-fold dilutions, while the activity against *P. aeruginosa* was unaffected by mouse serum. The activity of coumamidine against *S. aureus* was unchanged in medium containing human serum and one 2-fold dilution lower in medium containing mouse serum. Coumamidine was two 2-fold dilutions more active at pH 8.0 than at pH 7.3 and one to three 2-fold dilutions less active at pH 6.5 than at pH 7.3.

Fig. 1. Time-kill curves of coumamidine for *Staphylococcus aureus* 553.

■ Control (no antibiotic), ● 3.12 μg/ml (4× MIC), ▲ 6.24 μg/ml (8× MIC).



Killing Kinetics *In Vitro*

Coumamidine was rapidly bactericidal for *S. aureus* 553 (Fig. 1). The viable counts were reduced to <10 cfu in 2 hours after addition of 4 times the MIC of coumamidine to a logarithmic phase culture of *S. aureus*.

Frequency of Resistance

The frequency of resistance was $<1.0\times10^{-9}$ for *S. aureus* CMX 642A and *E. coli* Juhl when selected at 4 and 8 times the MIC of coumamidine.

Mouse Protection Tests

Coumamidine was tested only against *S. aureus* NCTC 10649 in mouse protection tests because of the limited quantities of the compound available. When administered subcutaneously, coumamidine protected 100% of the mice at a dose of 0.6 mg/kg/day. It was ineffective in this test at 40

mg/kg/day when administered orally by gavage.

Pharmacokinetics in Mice

After administering 25 mg/kg subcutaneously, the peak serum concentration was 4.5 μg/ml, area under the serum curve was 7.3 μg·hour/ml and the serum half life was 0.95 hour. The percent urine recovery was 21%. Coumamidine was not detected in the serum of mice after oral dosing.

Discussion

Coumamidine is a novel antibiotic with activity against Gram-positive and Gram-negative aerobic bacteria. It was active against some gentamicin-resistant bacteria. It was similar to aminoglycosides in being more active against *P. aeruginosa* BMH 10. Unlike aminoglycosides, coumamidine had activity against anaerobic bacteria. Although the activity against anaerobes was weak, the fact that some activity was noted indicates that the transport mechanism of coumamidine into bacterial cells must be different from that of aminoglycosides which depends upon aerobic growth[9]. Chemically, coumamidine is related to the cinodines[10]. The *in vitro* activity of the coumamidines is also similar to that of the cinodines[10].

Aminoglycosides have been extensively used because of their potency and spectrum. The other useful characteristics of aminoglycosides are that they are bactericidal and that the frequency of resistance is generally less than 10^{-9}. Coumamidines have all the useful properties of the aminoglycosides and in addition, they have activity against some gentamicin-resistant strains.

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