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Antistaphylococcal Activity of Pentamidine

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Pentamidine isethionate was bacteriostatic against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus sanguis*, *Micrococcus* sp., and *Candida albicans*. *S. aureus* was inhibited by concentrations of 16 to 64 μg/ml and killed by 64 to ≥128 μg/ml. Staphylococcal killing was consistently greater in the presence of cations and was unaffected by methicillin resistance.

Pentamidine (4,4′-diamidinodiphenoxypentane) isethionate has been used for many years as an antiprotozoal agent (13) and is widely used for the parenteral treatment of infections due to *Pneumocystis carinii*. Related aromatic diamidine compounds have been known since the 1940s to possess antibacterial activity (2). However, there are no studies using current National Committee for Clinical Laboratory Standards methodology which describe this activity.

A patient with acquired immune deficiency syndrome who presented with pneumonia at our institution was treated for presumed *P. carinii* pneumonia with intravenous pentamidine (4 mg/kg of body weight per day) and showed marked clinical improvement over 24 h. *Staphylococcus aureus* was subsequently isolated from all initial blood and sputum cultures. Therapy was changed to parenteral cloxacillin, investigations for other possible pathogens (including *P. carinii*) were negative, and the patient recovered. We investigated the antibacterial activity of pentamidine.

Using disk diffusion, we screened pentamidine for its activity against a range of common pathogens. Pentamidine isethionate was obtained in pure form from Rhône-Poulenc Pharma Inc., Montreal, Canada. It was dissolved in sterile distilled water to form solutions of 100 and 1,000 μg/ml. A quantity of 0.1 ml of each solution was added to sterile 12-mm-diameter filter paper disks, resulting in disks containing 10 and 100 μg of the drug, respectively. The organisms used (see Table 1) were diluted from an overnight growth in tryptic soy broth to a density comparable to a 0.5 McFarland standard and applied as a lawn onto DST agar (Oxoid Ltd., Basingstoke, England) plates containing 5% lysed horse blood. Zone sizes of growth inhibition were measured after incubation aerobically at 35°C for 20 h.

We measured MICs according to National Committee for Clinical Laboratory Standards methods (12) and MBCs as recommended previously (14) for four strains of staphylococci: *S. aureus* ATCC 25923, the *S. aureus* strain isolated from the blood of the patient, a clinical isolate of methicillin-resistant *S. aureus*, and *Staphylococcus epidermidis* ATCC 29997. The strain of methicillin-resistant *S. aureus* was susceptible only to vancomycin when tested by both disk diffusion and the Automicrobic System (Vitek Systems, Inc., Hazelwood, Mo.). Pentamidine is known to be stable in solution (5) and at high temperatures (10). Drug concentrations were made in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) (calcium, 8 mg/liter; magnesium, 5 mg/liter), with and without additional magnesium and calcium supplements (25 and 50 mg/liter, respectively). Initial inocula of staphylococci were prepared as recommended previously (12) and were quantitated by averaging the surface colony counts for six determinations obtained by plating 0.03 ml. The final inoculum density achieved ranged from 5.4×10^5 to 8.3×10^5 CFU/ml. Each MIC was determined in duplicate in tubes containing 2 ml. The MBC was determined by subculturing 0.03 ml of broth, in triplicate, onto blood agar plates from each tube showing no visible growth after incubation. The average colony count was used to calculate the number of viable CFU per milliliter. The MBC was considered the lowest concentration of drug which killed at least 99.9% of the initial inoculum.

Zone diameters of growth inhibition are shown in Table 1. The choice of disks containing 10 and 100 μg of drug for screening activity was arbitrary, based partially on previously reported inhibitory drug concentrations (8, 11). Pentamidine showed bacteriostatic activity against all gram-positive cocci tested except *Enterococcus faecalis* and no activity against the gram-positive bacilli or gram-negative bacilli. The strain of *Candida albicans* did not display a clear zone of growth inhibition, with a few distinct colonies growing within 5 mm of the edge of the 100-μg disk. The zone size cited for this strain is an estimate of the point at which confluent growth ended. The inhibitory activity of pentamidine against *Candida tropicalis* has previously been described (1). Geometric means for the MICs and the MBCs obtained for the four organisms tested are shown in Table 2. The MBCs were lower for all the staphylococci in the cation-supplemented broth.

In 1943, Kohn reported the antibacterial effects of propamidine (4,4′-diamidinodiphenoxypropane), a diamidine related to pentamidine, under various culture conditions (11). The growth rate of *S. aureus* was inhibited by 50% in the presence of 4.3 to 15.2 μg of the drug per ml, with similar effects also noted for *Escherichia coli*. According to Kohn, complete inhibition of growth could be obtained by doubling or tripling the concentration.

Gale and Folkes reported that the growth of *S. aureus* Duncan, from an initial inoculum of 10^5 to 10^6 CFU/ml in a "fully nutrient medium," was prevented by 3 to 5 μg of pentamidine isethionate per ml (8). They suggested that interference with protein synthesis was responsible. Bichowsky-Slomnitzki noted growth inhibition at a concentration of 6 μg/ml (2). The MIC of 3 μg/ml has been cited in a recent review (13). Hicks reported that 59 μg/ml inhibited the growth of a strain of *E. coli* (9). It has been noted that antagonism of the action of the aromatic diamines may occur with amino acids (9), polyamines (3, 15), nucleic acids (3),

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TABLE 1. Results of growth inhibition by pentamidine as determined by disk diffusion

Organism[a]	Zone size (mm) for disk with:	
	10 μg	100 μg
Staphylococcus aureus ATCC 25923	0	21
Staphylococcus aureus[b]	0	22
Staphylococcus aureus (methicillin resistant)	0	26
Staphylococcus epidermidis ATCC 29997	0	26
Streptococcus pyogenes	24	32
Streptococcus sanguis ATCC 10556	0	21
Enterococcus faecalis ATCC 1943	0	0
Micrococcus sp.	16	34
Listeria monocytogenes	0	0
Bacillus cereus[c]	0	0
Escherichia coli ATCC 25922	0	0
Pseudomonas aeruginosa ATCC 27853	0	0
Candida albicans	0	26[d]

[a] All organisms are clinical isolates unless otherwise specified.
[b] Strain isolated from patient described in text.
[c] Reference strains obtained from the Laboratoire du Santé Publique du Québec, Montreal, Canada.
[d] Poorly defined zone margin (see text).

phospholipids (6), and phosphate ions (7). Therefore, we decided to look at the effect of divalent cation supplementation in the MIC and MBC tests, which seemed to be an increase in susceptibility to the drug. In addition, we found that resistance to methicillin in the strain of methicillin-resistant *S. aureus* did not affect the susceptibility to pentamidine.

Measurement of pentamidine levels in serum and tissue has not been standardized. As determined by high-pressure liquid chromatography, the mean peak level of pentamidine isethionate in plasma after an intravenous infusion (4 mg/kg of body weight) was only 0.6 μg/ml (4). An agar diffusion bioassay gave values between 0.5 and 3.2 μg/ml (1). However, the volume of distribution of the drug is high in humans (mean = 821 liters) (4), and the drug is concentrated in various tissues, including the liver, spleen, kidneys, and adrenals (1). Pentamidine levels in the human spleen reached 40 μg/ml after a single dose and more than 350 μg/ml after 2 weeks of parenteral administration (1). The MICs we report are higher than reported peak levels in serum but lower than the reported peak levels in tissue. We therefore cannot entirely discount the possibility that pentamidine has some antistaphylococcal activity in vivo.

TABLE 2. Results of pentamidine susceptibility testing by broth macrodilution

Organism	MIC[a] (μg/ml) in:		MBC[a] (μg/ml) in:	
	MH[b]	MH-S[c]	MH	MH-S
S. aureus ATCC 25923	32	32	≥91	91
S. aureus (case isolate)	64	32	≥128	128
S. aureus (methicillin resistant)	32	16	128	64
S. epidermidis ATCC 29997	16	16	128	64

[a] Geometric means of MICs and MBCs determined in duplicate for each isolate.
[b] Mueller-Hinton broth.
[c] Mueller-Hinton broth supplemented with magnesium and calcium.

We conclude that pentamidine possesses antistaphylococcal activity which may be clinically relevant at the dosages commonly used for the treatment of parasitic infections, as was observed in our patient. A clinical response to pentamidine should not a priori imply an infection with *P. carinii*.

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