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In Vitro Antistreptococcal Activity of the Potassium-Sparing Diuretics Amiloride and Triamterene

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The ionophore antimicrobial agents provide evidence that perturbations of the electrolyte balance of bacterial cells exert a growth-inhibitory activity. Several drugs acting on animal cell membranes have also been shown to be active on bacterial cells. In this paper, we report preliminary susceptibility studies showing that the class of potassium-sparing diuretics acting directly on monovalent cation fluxes on animal cells possesses a selective growth-inhibitory activity on hemolytic streptococci.

The diuretic amiloride has been shown to inhibit the proliferation of several animal cells (3, 5, 6). In these cells, amiloride acts as a sodium influx blocker. However, the exact mechanism of growth inhibition is not yet fully clarified because the drug has been demonstrated to directly inhibit protein synthesis in the reticulocyte lysate (4).

We previously reported that amiloride possesses antibacterial activity (1). Moreover, we recently demonstrated that the drug causes changes in sodium and potassium concentrations in intact cells of *Streptococcus faecalis* (submitted for publication). In view of the fact that in microbial physiology intracellular potassium concentration is generally considered a fundamental parameter for metabolism and proliferation of bacterial cells, our results raised the question of whether amiloride may be able to act as a growth-inhibitory drug by interfering directly or indirectly with electrolyte balance, at least on bacterial cells. However, an as yet unknown effect of amiloride could not be excluded.

We decided that a further insight into the mechanism of action of this antibacterial activity may come from investigations of another drug, triamterene, which, although structurally different from amiloride, belongs to the same class of diuretics because it has an identical mechanism of action on animal cell membranes.

In this paper, we demonstrate that triamterene also has antibacterial activity and, like amiloride, possesses selectivity for hemolytic streptococci. Furthermore, both drugs inhibit the growth of some of the streptococci tested at concentrations well below those reported to be active in amiloride inhibition of protein synthesis in the reticulocyte lysate system.

MATERIALS AND METHODS

Microorganisms. Ten strains of hemolytic streptococci belonging to different serological groups were tested. They were isolated from specimens submitted to our clinical laboratory by methods previously described (2).

Drugs. Amiloride (*N*-amidino-3,5-diamino-6-chloropyrazinecarboxamide monohydrochloride dihydrate) was obtained from Merck Sharp & Dohme, Rahway, N.J. The product was dissolved and utilized as previously described in detail (1). Triamterene (2, 4,7-triamino-6-phenylpteridine) was obtained from Farmitalia-Carlo Erba, Montedison, Milan, Italy. The product was dissolved in HCl (0.01 N at 70°C), and microamounts of a mother solution of 1 mg/ml were used in the experiments. These did not change the pH of the medium utilized for growth inhibition experiments.

Susceptibility testing. For MIC and MBC determinations, a tube macrobroth dilution technique was used. Each drug was tested in twofold serial dilutions, and sometimes infrapoint drug concentrations were also determined. The microorganisms were transferred to Todd-Hewitt broth (Sclavo, Siena, Italy) or Mueller-Hinton medium (BBL Microbiology Systems, Cockeysville, Md.) and cultivated at 37°C to the logarithmic phase of growth. Cultures were adjusted to a turbidity of a 0.5 MacFarland standard and appropriately added to each tube to achieve a final concentration of approximately 10^5 CFU/ml. For each isolate tested, one tube contained only the inoculum in drug-free broth as a growth control. MIC was defined as the lowest drug concentration that prevented visible turbidity after 18 to 24 h of incubation at 37°C. Susceptibility testing for *S. pneumoniae* was performed by a standard method (7). MBCs were performed with an inoculum of 10^6 CFU/ml and determined by subculturing and spreading 0.01 ml of broth from each tube onto the surfaces of sheep blood agar plates.

RESULTS

The results obtained are shown in Table 1. They indicate activity against the various strains of streptococci tested, which differed according to the serological grouping of the microorganisms. However, in each strain both drugs were effective at very similar concentrations. Clearly, group A streptococci emerged as the most susceptible organisms. MBCs (not reported) were double the MICs in every case tested. We failed to test triamterene concentrations >120 μg/ml because of a slight turbidity caused by precipitation in the medium.

DISCUSSION

Amiloride and triamterene, widely used clinically for their diuretic effects, have been recognized, from work performed in our laboratory, as drugs possessing growth-inhibitory activity in bacterial cells. The general pattern which emerged from the studies performed showed that gram-positive orga-

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TABLE 1. MICs of amiloride and triamterene in 10 selected strains of streptococci from clinical isolates

Streptococcal serogroup	MIC (μg/ml) of:	
	Amiloride	Triamterene
A	7	7
A	7	15
A	15	15
A	15	30
S. pneumoniae	60	15
B	120	120
B	120	>120
C	120	120
C	120	>120
D	>120	>120

nisms were much more susceptible to these agents than were any other microorganisms. The effects of amiloride on sodium and especially potassium content in bacterial cells is a feature which might be relevant with regard to the mechanism of antibacterial activity; however, other possible actions cannot be excluded.

The preliminary investigations reported are clearly concerned with in vitro experimental pharmacology. From a clinical point of view, it should be noted that the amiloride and triamterene concentration active on some group A streptococci (7 μg/ml) is two- to threefold higher than levels obtainable in the sera of patients.

The effects of triamterene on bacterial cell membranes still remain to be elucidated, and at the present time we can only say that this diuretic also possesses antistreptococcal activity at concentrations similar to those of amiloride. The overall data, however, seem to be compatible with the conclusions that the class of potassium-sparing diuretics directly acting on monovalent cation fluxes on animal cell membranes have the interesting property of inhibiting bacterial growth and that at least one level of their action may take place at the periphery of bacterial cells.

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