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The effect of mafenide on dihydropteroate synthase

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Using intact bacterial cells, it was found that *Pseudomonas aeruginosa* was more susceptible to mafenide than *Escherichia coli*, that *p*-aminobenzoic acid (pABA) did not reverse or prevent inhibition by mafenide and that pABA itself was inhibitory. Under the experimental conditions used in these studies, pABA was more inhibitory to *E. coli* than to *P. aeruginosa*. It is proposed that pABA could be of use in the topical treatment of burn wounds. At the enzyme level, it was shown that mafenide did not inhibit dihydropteroate synthase. Thus, mafenide appeared not to exert its inhibitory effects in the same manner as the structurally related sulphonamides.

Introduction

Mafenide is a synthetic antimicrobial agent structurally related to the sulphonamides as well as to *p*-aminobenzoic acid (pABA). It has low bacteriostatic activity against a wide spectrum of both Gram-positive and Gram-negative bacteria, especially *Pseudomonas aeruginosa*, a troublesome organism in burn wound tissues, (Jelenko *et al.*, 1966; Reynolds, 1982). The first extensive use of mafenide was in the treatment of burn wound infections (Lindberg *et al.* 1965a, b) and this remains the primary use of mafenide today.

Chemically mafenide is *p*-aminomethylbenzene sulphonamide. Its structure differs from that of the sulphonamides in that it has an aminomethyl group in the *para* position on the benzene ring instead of an unsubstituted amino group. It would be expected, nevertheless, that the mechanism of action of mafenide would be the same as that of the sulphonamides, which being structural analogues of pABA, compete with pABA for the same binding site on dihydropteroate synthase (EC 2.5.1.15). In so doing they interfere directly with the synthesis of dihydropteroic acid, a precursor of tetrahydrofolic acid, the coenzymatic form of folic acid. Dihydropteroic acid is synthesized from the condensation of *p*-aminobenzoate by dihydropteroate synthase with 2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine-pyrophosphate to form dihydropteroate + pyrophosphate.

The mechanism of action of mafenide is considered to differ from that of the sulphonamides (Garrod *et al.*, 1981; Reynolds, 1982) on the basis of certain indirect observations. These are that the inhibitory action of mafenide is not antagonized by pABA, serum, pus or tissue exudates and that there is no correlation between bacterial sensitivities to mafenide and to the sulphonamides (Brown, 1962; Rickey & Brown, 1971). A search of the literature revealed no published evidence at the enzyme level that

the mechanism of action of mafenide is different. The purpose of the experiments described in this article was to measure directly the effect of mafenide on dihydropteroate synthase.

Materials and methods

Organisms and media

The two experimental bacterial strains used in these studies were *Escherichia coli*, a clinical isolate from a urine specimen, and *P. aeruginosa* ATCC 27317. Unless otherwise indicated, the organisms were grown in a chemically defined basal salts medium supplemented, in final concentration, with 20mM glucose (BSG) (Eagon & Phibbs, 1971). BSG was used in order to avoid the presence of intermediaries and end products of folic acid metabolism which might mask weak inhibitors of dihydropteroate synthesis.

Minimal inhibitory concentration (MIC) determinations

The tube serial dilution technique was used to determine MIC using BSG as the test medium. The MIC was defined as the lowest concentration of drug giving no visible growth.

Experimental methods

Preparation of cell-free extracts, protein determinations and assay procedures for the effect of various agents on dihydropteroate synthase were carried out as previously described (Eagon & McManus, 1989).

Reagents

Mafenide-HCl, [ring-UL-¹⁴C]pABA, (specific activity, 6.8 mCi/mmol), DNase and RNase were purchased from the Sigma Chemical Co., St Louis. All other reagents were purchased from commercial sources in their highest state of purity.

Results

MIC of mafenide, sulphanilamide and pABA for P. aeruginosa and E. coli in BSG medium

The results in Table I show that *P. aeruginosa* was more susceptible to inhibition by mafenide than *E. coli*, an observation paralleled by clinical evidence (unpublished observations). Sulphanilamide was used as a control since it is a weak inhibitor of dihydropteroate synthesis. A comparison of the inhibition of *P. aeruginosa* by mafenide with that by sulphanilamide showed that half as much again of mafenide as sulphanilamide was required to inhibit this organism (Table I). Upon continued inhibition, sulphanilamide remained the better inhibitor. Mafenide was less effective against *E. coli* than against *P. aeruginosa* whereas sulphanilamide was about equally effective against *E. coli* and *P. aeruginosa*.

pABA was not effective in overcoming the inhibitory effects of mafenide against *P. aeruginosa* irrespective of the concentration of pABA used (Table I). Surprisingly, pABA itself was inhibitory to both *P. aeruginosa* and *E. coli* under these experimental

Table I. Susceptibilities of *P. aeruginosa* and *E. coli* to mafenide and sulphanilamide, in the absence or presence of pABA, and to pABA alone

Agent	MIC (mg/l)	
	<i>P. aeruginosa</i> ^a	<i>E. coli</i> ^b
Sulphanilamide	200 (600)	200
Sulphanilamide + 200 mg/l pABA	>1000	400
Mafenide	400 (>1000)	>1000
Mafenide + 5 mg/l pABA	600 (>1000)	ND
Mafenide + 10 mg/l pABA	500 (>1000)	ND
Mafenide + 100 mg/l pABA	400 (>1000)	ND
Mafenide + 200 mg/l pABA	400 (>1000)	>1000
pABA	500 (500)	200

^aThe non-parenthetical numbers were results after 24 h incubation while the parenthetical numbers were results after 48 h incubation.

^b*E. coli* grew slowly on BSG and turbidity did not occur until in excess of 24 h incubation. Thus, the results shown here represent 48–72 h incubation.

ND, Not done.

conditions. A concentration of 500 mg/l of pABA inhibited *P. aeruginosa* in a 24 h incubation period compared with 400 mg/l of mafenide. Under these experimental conditions, however, pABA appeared to be a better inhibitor of *E. coli* than mafenide.

Effect of mafenide and KCl on dihydropteroate synthase

Mafenide did not inhibit the synthesis of dihydropteroate by dihydropteroate synthase in extracts of either *P. aeruginosa* or *E. coli* when used in mafenide:pABA ratios (mol/mol) of 100:1 and 1000:1 (Table II). When a mol/mol ratio of 10,000:1 of mafenide:pABA was used, however, 37% and 24% inhibition of dihydropteroate synthesis by dihydropteroate synthase from *P. aeruginosa* and from *E. coli* respectively was noted. As a control, sulphadiazine, which is a well-documented inhibitor of dihydropteroate

Table II. Effect of mafenide and KCl on dihydropteroate synthase of *P. aeruginosa* and *E. coli*

Additions (final conc.)	<i>P. aeruginosa</i>		<i>E. coli</i>	
	enzyme activity ^a	per cent inhibition	enzyme activity ^a	per cent inhibition
0.02 mM pABA plus				
Nil else	0.209; 0.214; 0.216 ^b		0.136	
0.2 mM sulphadiazine	0	100	0	100
2.0 mM mafenide	0.200	4	ND	ND
20.0 mM mafenide	0.200	4	ND	ND
200.0 mM mafenide	0.137	37	0.103	24
200.0 mM KCl	0.191	11	ND	ND
601.35 mM KCl	0.083	61	ND	ND

^aEnzyme activity = nmol dihydropteroate produced min⁻¹ mg protein⁻¹.

^bThe three enzyme activity base values cited were derived from separate experiments; and the per cent inhibition values were calculated using the base value which was appropriate for that particular experiment. ND, Not done.

synthesis, was used and at a mol/mol ratio of 10:1 of sulphadiazine:pABA, it completely inhibited the synthesis of dihydropteroate by extracts of both *P. aeruginosa* and *E. coli* (Table II).

A 200 mM solution of mafenide, which is a 10,000:1 (mol/mol) ratio with pABA, is a 4.45% solution (wt/vol) and in order to determine whether the inhibition of dihydropteroate synthesis by mafenide at this concentration was due to a 'salt effect' on dihydropteroate synthase, KCl was substituted for mafenide. The results are shown in Table II. A 10,000:1 (mol/mol) ratio of KCl:pABA resulted in 11% inhibition of dihydropteroate synthesis by an extract of *P. aeruginosa*, but a 4.45% solution (wt/vol) of KCl (i.e., 601.35 mM KCl) resulted in 61% inhibition. From this we concluded that the inhibition of dihydropteroate synthesis by the same high percentage concentration of mafenide was due to a salt effect on dihydropteroate synthase and not to competition with pABA.

Discussion

At the intact cell level, our data showed that *P. aeruginosa* was more susceptible to the inhibitory effects of mafenide than *E. coli* (thus confirming past clinical observations), that pABA did not reverse, or prevent, the inhibitory effects of mafenide and that pABA itself was inhibitory. In the case of the latter observation, pABA was found to be more inhibitory toward *E. coli* than *P. aeruginosa*. Under our experimental conditions, the MIC of pABA in BSG medium was 200 mg/l for *E. coli* and 500 mg/l for *P. aeruginosa*. Thus, pABA was more effective against *E. coli* under our conditions than mafenide.

The inhibitory concentrations of pABA would be high for systemically administered chemotherapeutic purposes. Nevertheless it seems probable that pABA could be useful for topical application, such as on burn wounds. Our experimental data, however, did not permit us to determine the mechanism of antimicrobial action of pABA. At the enzyme level, our experimental data confirmed that mafenide did not inhibit dihydropteroate synthase. Thus, although structurally similar to the sulphonamides, mafenide appeared not to exert its inhibitory effects in the same manner. Our experimental data did not permit us to discover the actual mechanism of antimicrobial action of mafenide.

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