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Sensitization of *Burkholderia cepacia* to antibiotics by cationic drugs

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Chlorpromazine and prochlorperazine have previously been shown to enhance the susceptibility of *Burkholderia cepacia* to aminoglycosides. To screen other non-antibiotic drugs containing similar amine (-N-CH_3) groups, we examined a range of such agents that are in current clinical use for the treatment of non-infectious diseases, in combination with antibiotics that are ineffective against *B. cepacia.* At a concentration of 0.2 mM, theobromine, theophylline, trifluoperazine, fluophenazine and coumarin-152 significantly reduced (by four-fold) the MICs of gentamicin and ceftazidime. Theobromine and theophylline also reduced the MICs of amikacin and azithromycin.

Introduction

Burkholderia cepacia infections in cystic fibrosis patients can be treated with only a few antibacterial agents, such as co-trimoxazole, chloramphenicol, ceftazidime and ciprofloxacin. Resistance is generally attributed to a highly impermeable and selective outer membrane. A self-promoting pathway whereby cationic compounds aid their own entry by disrupting the outer membrane structure[1] has been proposed for *Escherichia coli* and *Pseudomonas aeruginosa* in addition to porin permeability pathways. However, as *B. cepacia* is resistant to aminoglycosides and polymyxin B, the self-promoted pathway, as proposed for aminoglycosides in *P. aeruginosa*, is thought to be absent. Compounds such as diaminoacetone and methylglyoxal bis-guanylhydrazone,[2] some topical anaesthetics (e.g. lidocaine, procaine and dibucaine) and the antipsychotic drug chlorpromazine[3] significantly enhance the susceptibility of *E. coli* to various hydrophobic antibacterial agents. Chlorpromazine and prochlorperazine also reduce the MICs of aminoglycosides for *B. cepacia.*[4,5] In this study our aim was to investigate whether other compounds with similar cationic groups also influence the susceptibility of *B. cepacia* to antibacterial agents.

Materials and methods

B. cepacia ATCC 13945 was obtained from the American Type Culture Collection and stored at −70°C in glycerol. Mueller–Hinton agar (MHA) and Mueller–Hinton broth (MHB; Difco, Detroit, MI) were used to sustain the growth of *B. cepacia* for MIC determination. Gentamicin, amikacin, kanamycin, ampicillin, ceftazidime, theobromine, theophylline, trifluoperazine, fluphenazine, coumarin-152 (warfarin), desipramine, amitryptiline, pyrilamine, promethazone, ranitidine, famotidine, chlorcyclizine, chlorpheniramine, diphenhydramine, procaine, lidocaine, dibucaine and dacarbazine were obtained from Sigma–Aldrich (St Louis, MO, USA). Azithromycin was a kind gift from Pfizer Inc. (Groton, CT, USA) and was dissolved in a minimal amount of 95% ethanol and then made up to volume with water. A standard microtitre broth dilution method, as specified by the NCCLS,[6] in MHB without cation supplementation was used for MIC determination using a final inoculum of 10^5 cfu/mL of exponentially growing cells, and MICs were read after incubation for 24 h at 37°C in ambient air. MICs of gentamicin, amikacin, kanamycin, ampicillin, azithromycin and ceftazidime were determined in combination with the various cationic drugs (at 0.2 mM and 0.4 mM unless stated otherwise) to examine whether these drugs reduced the MICs of the antibiotics for *B. cepacia* ATCC 13945.

Results and discussion

Theophylline and theobromine significantly reduced the MICs of gentamicin, amikacin, azithromycin and ceftazidime, but not those of ampicillin or kanamycin (Table). Coumarin-152 reduced the MICs of gentamicin, azithromycin and ceftazidime. Famotidine reduced the MICs of ampicillin, gentamicin, kanamycin, azithromycin and ceftazidime. Diphenhydramine reduced the MIC of gentamicin only. Lidocaine reduced the MICs of

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Table. Effect of cationic compounds on the susceptibility of *B. cepacia* ATCC 13945 to antibiotics

	MIC (mg/L)					
	ampicillin	gentamicin	amikacin	kanamycin	azithromycin	ceftazidime
Control	>64	16	32	>64	128	8
Theophylline						
0.05 mM	>64	2	32	>32	16	8
0.2 mM	>64	0.5	4	>32	2	1
0.4 mM	>64	1	2	>32	4	4
Theobromine						
0.05 mM	>64	2	32	>32	16	8
0.2 mM	>64	0.5	0.5	>32	2	0.125
0.4 mM	>64	1	4	>32	4	0.5
Coumarin 152						
0.05 mM	>64	4	32	32	8	0.5
0.2 mM	>64	16	32	32	128	8
0.4 mM	>64	<0.25	32	32	4	<0.25
Famotidine						
0.05 mM	>64	>16	>32	>64	>128	>8
0.2 mM	>64	8	16	64	32	>8
0.4 mM	4	<0.25	ND	2	4	0.5
Diphenhydramine						
0.05 mM	>64	8	32	ND	16	0.5
0.2 mM	>64	4	32	ND	16	1
0.4 mM	32	2	32	ND	>128	4
Lidocaine 1.0 mM	32	<0.25	ND	0.5	4	2
Dibucaine 1.0 mM	2	1	32	2	32	0.5

ND, not done.

gentamicin, kanamycin, azithromycin and ceftazidime; dibuciane reduced the MICs of ampicillin, gentamicin, kanamycin, azithromycin and ceftazidime (Table).

Trifluperazine reduced the MICs of gentamicin, amikacin, kanamycin (at 0.4 mM only), azithromycin (at 0.4 mM only) and ceftazidime; fluphenazine reduced the MICs of gentamicin and ceftazidime only. Desipramine (at 0.2 mM) reduced the MICs of gentamicin and kanamycin only; chlorcyclizine (at 0.4 mM) reduced the MICs of amikacin and ceftazidime only; dacarbazine (at 0.2 mM) reduced the MIC of ceftazidime only; procaine (1.0 mM) reduced the MIC of gentamicin only. Amitryptiline, promethazine, pyrilamine and chlorpheniramine at 0.2 mM and at 0.4 mM had no effect on the MICs of any of the antibiotics tested (data not shown). Cultures of *B. cepacia* ATCC 13945 in MHB containing up to 4 mM theophylline, 4 mM theobromine, 4 mM coumarin-152, 2 mM famotidine (4 mM was inhibitory), 2 mM diphenhydramine, 4 mM lidocaine and 4 mM dibucaine were very turbid, suggesting that even these high concentrations of these agents were not close to the MICs for the *B. cepacia* strain used.

Several types of agent have been used in an attempt to alter the outer membrane permeability of Gram-negative bacteria, including EDTA, lysozyme, sodium hexametaphosphate,[7] detergents (e.g. SDS and deoxycholate), permeabilizing portions of antibiotics like polymyxin,[8] and other compounds containing positively charged amino groups, such as spermidine and lysine,[9] and diaminoacetone and methylglyoxal bis-guanylhydrazone.[2] *B. cepacia* is naturally resistant to the outer membrane permeabilizing action of EDTA, aminoglycosides and polymyxin B.[1] Outer membrane permeabilizers like gentamicin act by disrupting the otherwise intact and impermeable lipopolysaccharide (LPS) layer by cationic binding, as has been shown by experiments using magnesium ions to compete with antibiotics such as gentamicin. The inability of gentamicin and other such compounds to alter *B. cepacia* permeability suggests that the *B. cepacia* LPS differs significantly in composition, structure and/or function from that in *E. coli* and *P. aeruginosa*. The phenothiazines chlorpromazine and prochloperazine, both of which reduce aminoglycoside MICs for *B. cepacia*,[4,5] both contain a (-N-CH_3) group. Theophylline and theobromine each have two of these groups. Diphenhydramine contains (-N-$(CH_3)_2$), whereas famotidine has a (-N=C$(NH_2)_2$), lidocaine a (-N-$(C_2H_5)_2$) and dibuciane a (-N-$(C_2H_2)_5$) side chain (Figure). Coumarin-152 does not contain any similar side groups but did reduce the MICs of some antibiotics (Table). Agents containing methyl (-CH_3),

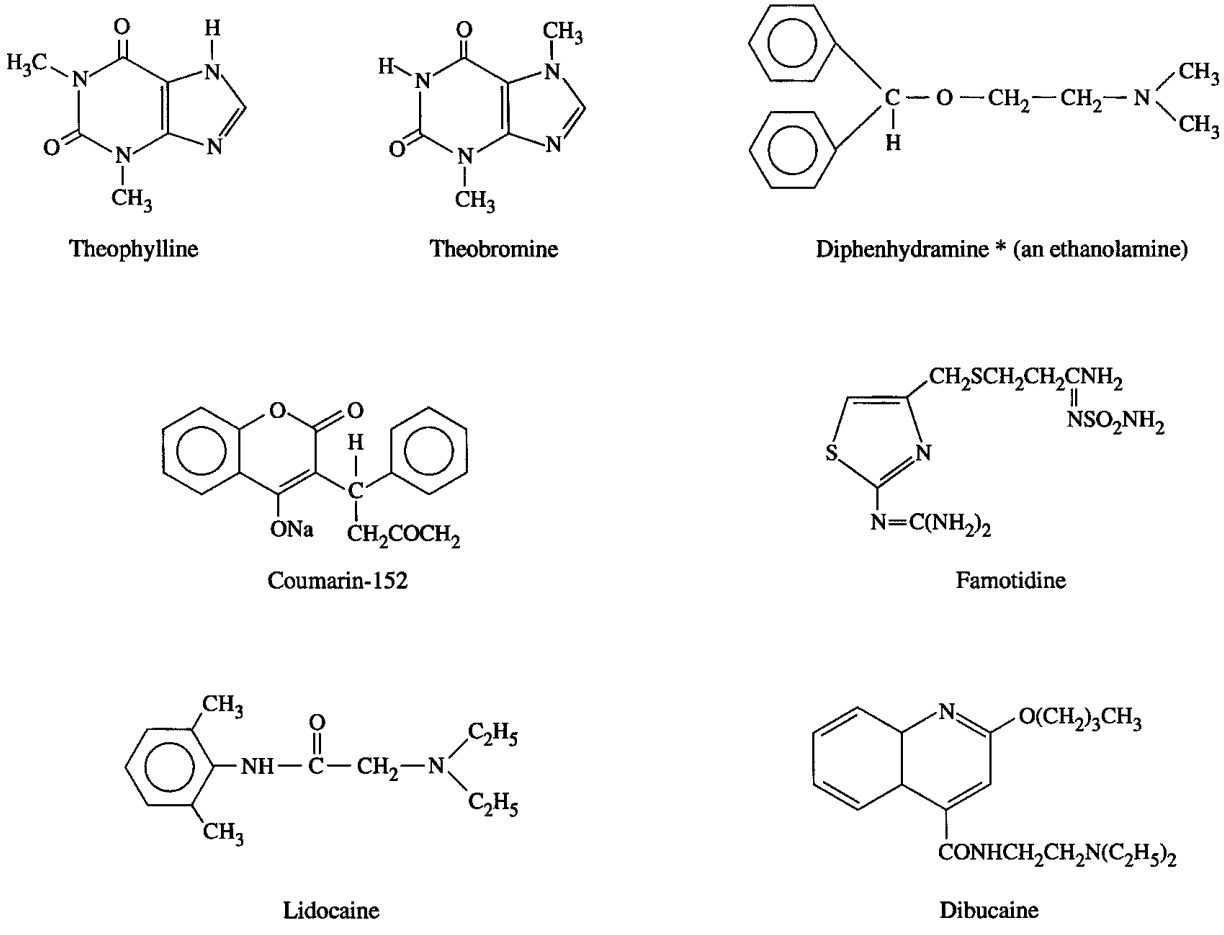

Figure. Structures of some of the cationic drugs tested in this study.

dimethyl (-$(CH_3)_2$), ethyl (-C_2H_5) and diethyl ((-$C_2H_5)_2$) groups attached to a nitrogen atom may also perturb the LPS layer, as such nitrogen atoms are highly electronegative and so would probably make the methyl, dimethyl and ethyl groups attached to them positively charged and able to compete for magnesium ions, and therefore destabilize the outer membrane. However, the MIC-reducing effect of coumarin-152 cannot be explained by this mechanism. The drugs that did not enhance the MICs all contain a methyl (chlorcyclizine) or dimethyl (chlorpheniramine, amitryptiline, pyrilamine and promethazine) group linked to a nitrogen atom in the side chains. The only apparent difference is that in these drugs, the methyl and dimethyl groups are attached to a nitrogen atom that is further away from ring structures, which may reduce the ability of the nitrogen atom to act as a proton attractor and as a mediator when it is situated very close to, for example, a benzene ring.

In *P. aeruginosa*, aminoglycosides severely disrupt cell structure, causing outer membrane wrinkling and blebbing of vesicles from the outer membrane.[10] Electron micrographs of chlorpromazine-treated *B. cepacia* ATCC 13945 cells showed no wrinkling or vesicle blebbing of the outer membrane.[5] Exposure to chlorpromazine induced a widening of the cell envelope, mainly the periplasmic space, as the cytoplasmic membrane was seen to peel inwards. The choice of concentrations of the drugs tested in this study was based on those concentrations that were found from our previous study to reduce the MIC of chlorpromazine and prochlorperazine. However, these concentrations may still be too high for therapeutic use and therefore are not of direct medical application. Existing or newly synthesized analogues of compounds such as theophylline and theobromine may show efficacy at therapeutic levels and hence be of significance in treatment of *B. cepacia* infections in the lung.

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