Article I

The in-vitro antimicrobial effect of non-antibiotics and putative inhibitors of efflux pumps on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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Abstract

The anti-microbial activity of six non-antibiotics (one amino-ethylchloride, three phenothiazines, two tricyclic antidepressives) were tested on 20 clinical isolates of *Pseudomonas aeruginosa*, one clinical isolate of *Klebsiella pneumoniae*, 2 ATTC strains and 14 clinical isolates of *Staphylococccus aureus*, using the plate dilution method. The effects on *P. aeruginosa* were independent of antibiotic resistance pattern and the species *Stenotrophomonas maltophilia* was found to be the most susceptible to the non-antibiotics, with MIC values as low as 20 mg/l for some of the substances. The 16 *S. aureus* strains tested were all particularly susceptible to the anti-microbial effects of the putative inhibitors of efflux pumps thioridazine and trifluoperazine with MIC values of ≤16 mg/l independently of the methicillin resistance profile of the strains. Because phenothiazines are well known to inhibit efflux pumps our results may indicate the existence of such pumps. Current works in progress are attempts at reversing the antibiotic resistance of selected bacterial strains using specific non-antibiotics and their stereo-chemical isomers.

Keywords: Non-antibiotics; Putative inhibitors of efflux pumps; Antibiotic resistance

1. Introduction

During the past 70 years sporadic reports have suggested that neuroleptics, especially the phenothiazines and chemically related compounds employed today for the management of psychosis, exhibit additional properties such as antimicrobial activity against a wide array of microorganisms [1]. The anti-microbial activity of phenothiazines have generated particular interest since the molecular active site(s) of these compounds may contribute to modern concepts of pharmacology, such as stereoisomeric relationships and specificity of targets [2].
The antimicrobial activity of neuroleptics might eventually have a place in the armamentarium of antimicrobials especially with regard to the management of infectious diseases caused by highly resistant organisms where the possibility for adequate treatment is problematic. It was therefore of interest to investigate the effect of selected phenothiazines, some of which are known to inhibit efflux pumps and chemically related compounds on highly antibiotic resistant humans pathogens such as multi-drug resistant (MDR) *Staphylococcus aureus*, *Pseudomonas* species and *Stenotrophomonas maltophilia*.

2. Materials and methods

2.1. Materials

2.1.1. Reagents

Promazine (PZ), amitriptyline (ATT), thioridazine (THIO), trifluopromazine (TFPZ), 2-dimethyl-amino-ethylchloride (2-DMAE) and imipramine (IMI) were kindly donated by Professor Hendrik Keyser of California State University, Los Angeles.

2.1.2. Bacterial strains

Strains of *S. aureus* employed were strains obtained from clinical material, isolated in Sønderborg (Denmark), Kiel (Germany) and Athens (Greece). *S. aureus* ATCC strains 33591 and 25923 served as control for methicillin-resistance and -susceptible, respectively. Strains of *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *S. maltophilia, Aerogenes hydrophilia* and *Serratia marcesens* were all clinical isolates from Sønderborg Sygehus, Denmark.

2.1.3. Culture media

Mueller Hinton agar was purchased from Becton Dickson, adjusted to pH 7.1 and employed for studies involving *S. aureus*; Oxoid Iso-Sensitest agar was purchased from Oxoid, and adjusted to pH 7.3 for studies of *P. aeruginosa*, *K. pneumoniae*, *S. maltophilia* and *S. marcensens*.

2.2. Methods

2.2.1. MIC determination

MIC's were determined by the agar dilution method [3] and utilising the Kärber analysing system [4].

3. Results

All seven strains of methicillin-resistant *S. aureus* (MRSA) and strain ATCC 33591 (MRSA control) had an MIC of oxacillin of >256 mg/l and penicillin MIC of >256 mg/l. With the seven methicillin-susceptible strains (MSSA), the MICs of oxacillin ranged from 0.125 to 0.75 mg/l. The MIC of the control MSSA (ATTC 25923) strain was 0.25 mg/l. With penicillin, the MICs for MSSA varied from 0.016 to 0.75 mg/l with ATCC strain having a MIC of 0.05 mg/l.
The MICs of promazine (PZ), amitriptyline (ATT), thioridazine (THIO), trifluopromazine (TFPZ), 2-dimethyl-amino-ethylchloride (2-DMAE) and imipramine (IMI) against MSSA and MRSA strains is summarised by Table 1. It was noted that although the responses of all of the strains tested were essentially similar for each of the compounds tested, the most effective compounds which demonstrated greatest activity against MSSA and MRSA were thioridazine and trifluopromazine (MICS were of the order of 16 mg/l). It is important to note that these concentrations cannot be achieved in a patient.

Table 1. MICs (in mg/l) of promazine (PZ), amitriptyline (ATT), thioridazine (THIO), trifluopromazine (TFPZ), 2-dimethyl-amino-ethylchloride (2-DMAE) and imipramine (IMI) against methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) strains of *Staphylococcus aureus*

	MRSA 7 clinical strains	MRSA ATTC 33591	MSSA 7 clinical strains	MSSA ATTC 25923
PZ	64–128	128	64–128	128
ATT	128	128	64–128	128
THIO	16	16	16	16
TFPZ	16	16	16	16
2-DMAE	> 256	> 256	> 256	> 256
IMI	128–256	128	128–256	128

The MICs of the above compounds were also determined for a number of other bacteria.

The data presented by Table 2 indicate that of the 16 *Pseudomonas* strains investigated only *Pseudomonas* spp. 25879 was the most sensitive to the thioridazine and trifluopromazine. 2-DMAE was most effective against the two strains of *S. maltophilia*. No correlation between the antibiotic resistance profiles and the relative inhibition of any of the bacterial strains was found (data not shown).

Table 2. MICs (in mg/l) of promazine (PZ), amitriptyline (ATT), thioridazine (THIO), trifluopromazine (TFPZ), 2-dimethyl-amino-ethylchloride (2-DMAE) and imipramine (IMI) against *Pseudomonas* spp., *S. maltophilia, A. hydrophilia, K. pneumoniae and S. marcesens*

	P. aeruginosa (N=15 strains)	*Pseudomonas* spp. 25879	*S. maltophilia* FR	*S. maltophilia* 79408	*A. hydrophilia* 67985	*K. pneumoniae* 33573	*S. marcescens* 32343
PZ	>256	N/D	81	81	81	162	>256
ATT	>256	N/D	162	81	162	> 256	>256
THIO	>256	40	162	81	> 256	> 256	>256
TFPZ	>256	10	> 256	162	> 256	> 256	>256
2-DMAE	>256	N/D	20	40	> 256	> 256	>256
IMI	>256	N/D	162	162	162	> 256	>256

N/D, not determined.

4. Discussion

Management of *S. aureus* infections is a major problem due to wide spread resistance to beta-lactams and glycopeptides [5]. In our study, phenothiazine derivatives thioridazine and trifluopromazine exhibited consistent antimicrobial activity against strains of *S. aureus* regardless of their susceptibility to oxacillin. It is important to note that although these in vitro concentrations of thioridazine are impossible to achieve in human, they are readily achieved and effective against *S. aureus* that has been phagocytosed by human macrophages [6].
Phenothiazines have been shown to inhibit efflux pumps of MDR *S. aureus* that confer resistance to fluoroquinolones [7 and 8] as well those present in *Escherichia coli* [9]. In our current study, thioridazine was effective against only one strain of *P. aeruginosa. Pseudomonas*, as is the case for most Gram-negative rods, is very resistant to thioridazine as well as to chlorpromazine. This resistance is possibly related to a relatively impermeable outer membrane [10 and 11]. *Pseudomonas* is known to have a number of efflux pumps, some of which are associated with antibiotic resistance [12]. Our finding that one *Pseudomonas* strain demonstrated relatively high susceptibility to thioridazine affords the opportunity to conduct studies which compare this strain to others which are relatively resistant to phenothiazine, perhaps defining a relationship between this compound and any efflux pump of thioridazine-susceptible and resistant-strains of this bacterial species.

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