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Effect of Salicylate on Antibacterial Activity of Different Antibiotics

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Abstract □ Susceptibility of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* to gentamicin and cefotaxime was affected by salicylaye. In presence of salicylaye (15 mM) and gentamicin (1.0 ug/m*l*), log efficiency of plating (log E.O.P.s) for the tested bacteria were −1.24, −2.17 and −1.66 respectively. The activity of cefotaxime against *Bacillus subtilis* was reduced (log E.O.P.=1.33). The highest potentiating effects of salicylaye were shown when using gentamicin against *Staphylococcus aureus*, cefotaxime against *Ps. aeruginosa*, log E.O.P.s were −3.0, and −2.4, respectively. On the other hand, no significant effects were detected with cefotaxime against *Staphylococcus aureus* (log E.O.P.=−0.04). No significant killing was shown in presence of gentamicin or salicylaye alone. There was no significant effect for salicylaye on MICs (By broth dilution) could be observed except in case of gentamicin against *Staphylococcus aureus*, which was reduced from 0.02 ug/m*l* to 0.0012 ug/m*l*. These results raise the concern that high concentrations of salicylaye in patients might interfere with antibiotic therapies.

Keywords □ Salicylate, efficiency of plating, gentamycin, cefotaxime

Salicylate affects greatly the antibacterial activity of some antibiotics against *Escherichia coli*[1,2]. In general, the more positively charged aminoglycosides showed greater enhancement of activity by salicylaye. However, salicylaye induced a phenotypic resistance in *Escherichia coli* to ampicillin, chloramphenicol, nalidixic acid, and tetracycline that have dissimilar structures, targets and mode of actions[3]. It was found that salicylate decreased the rate of permeation of cephaloridins through the membrane of *Escherichia coli* by three to five folds[1]. Sawai *et al.*[4] found that the OmpF content of the outer membrane was greatly reduced in cells grown in salicylate. Since OmpF forms a major porin channel for the antibiotics mentioned above, its absence can explain, at least in part, the increased resistance of salicylate-grown cells.

The report describes different effects of salicylate on the susceptibility of *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* to gentamicin and cefotaxime.

MATERIALS AND METHODS

Bacteria

A clinical strain of each of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated and identified according to Hugh and Gilardi[5]. In addition, a standard strain of *Bacillus subtilis* ATCC 6633 was used.

Chemicals

Chemicals used (and sources) were; sodium salicylate, tris hydrochloride, gentamicin sulphate (Sigma chemical Co.), and sensidisk (BBL Microbiology System Cockeysville, Md USA) of amikacin (30 ug), tobramycin (10 ug), streptomycin (10 ug), cefotaxime (10 ug) and ampicillin (10 ug).

Media

Tryptone broth (TB) contained the following, per liter; 10 g of tryptone (Difco laboratories Detroit, Michigin USA) and 5 g sodium chloride, the pH

was adjusted to 7.4 with sodium hydroxide. It was supplemented with 1.1% Bacto agar (Difco) for plates and 0.6% agar for top agar. The plates were made by combining 1 volume of 40 mM Tris hydrochloride (filter sterilized), 1 volume of 4.4% molten agar with 2 volumes of double-strength TB and kept at 55℃ while appropriate supplements of antibiotics or other chemicals were added as indicated. Each plate contained 32 m*l* that was dispensed with a pipette. Dilutions of cells were made in TMG buffer (10 mM tris hydrochloride [pH 7.4], 10 mM magnesium sulphate and 0.01% gelatin).

***Determination of E.O.P.*[1]**

Fresh overnight cultures of the tested bacteria, which were grown at 37℃ in TB (pH 7.4), were diluted in TMG buffer and placed in 2.5 m*l* of TB top agar on the indicated plates. The plates were incubated at 37℃ for at least 24 h. In case of low growth because of high amounts of antibiotics, salicylate or both, incubation was continued until there was no increase in the number of colonies (usually no more than 6 days). At that time, the final counts reported here were made. The efficiency of plating (E.O.P) was the titer of CFU obtained from the test plates divided by the titer obtained from the control plates lacking both antibiotic and salicylate.

***Agar double diffusion tests*[1]**

About 10^6 bacteria (from fresh overnight cultures in TB at 37℃) were plated in 2.5 m*l* of TB top agar on TB plates (pH 7.4). A sterile paper disk (diameter 0.5 in.) was placed at the center of the plates after being impregnate with sodium salicylate solution (15 mM). Antibiotic disks were placed at a suitable distance from central disk. After overnight incubation at 37℃, the plates were examined for the effects of salicylate. Synergistic (or antagonistic) effet was indicated by asymmetric zones of inhibition surrounding the antibiotic containing disk with more (or less) inhibition on the side facing the cental disk than on the side aways from the central disk.

Determination of minimal inhibitory concentration (MIC)

The broth dilution technique[5] was adopted. It includes the preparations of final concentrations of bacteria (about 10^6 CFU/m*l*) in double strength TB. Incubated at 37℃ for 2 h, then added the specified concentrations of salicylate. Broth cultures containing salicylate were distributed (1 m*l* portions) in tubes that were containing 1 m*l*-volumes of serial dilutions of the tested antibiotics in TM-buffer pH 7.4, incubated at 37℃ for 24 h. The MIC was determined as the least concentration that inhibits growth.

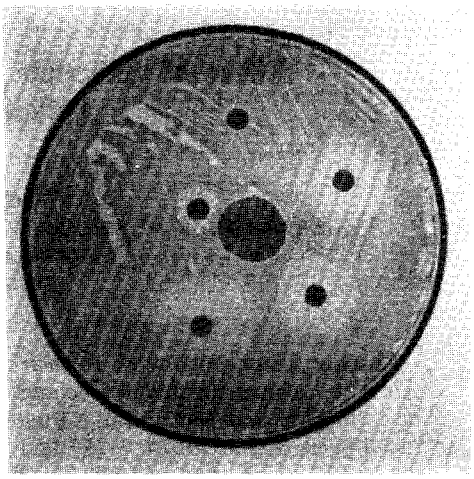

Fig. 1. Antagonizing effect of salicylaye on cefotaxime (Ctx) and its potentiating effect on streptomycin (S) and gentamicin (Gm) activities against *Bacillus subtilis* ATCC 6633.

RESULTS

To determine the effect of salicylate on resistance of the tested antibiotics, double-diffusion agar tests with the test strains (*Pseudomonas aeruginosa, Staphylococcus aurea* and *Bacillus subtilis*) were performed. It was observed that the zones of growth inhibition surrounding the disks containing each of the tested aminoglycosides (amikacin, gentamicin, tobramycin and streptomycin) were much greater on the side facing the containing salicylate than on the other side. Thus, in the areas where subinhibitory concentrations of both salicylate and aminoglycosides were present, there was increased inhibition of growth. In contrast, the inhibitory zones surrounding the disks containing betalactam antibiotics (cefotaxime and ampicillin) were asymmetrically smaller on the side facing salicylate, indicating less susceptibility to betalactams in the presence of salicylate (Fig. 1).

These observations were extended by quantitative plating experiments of the tested bacterial strains

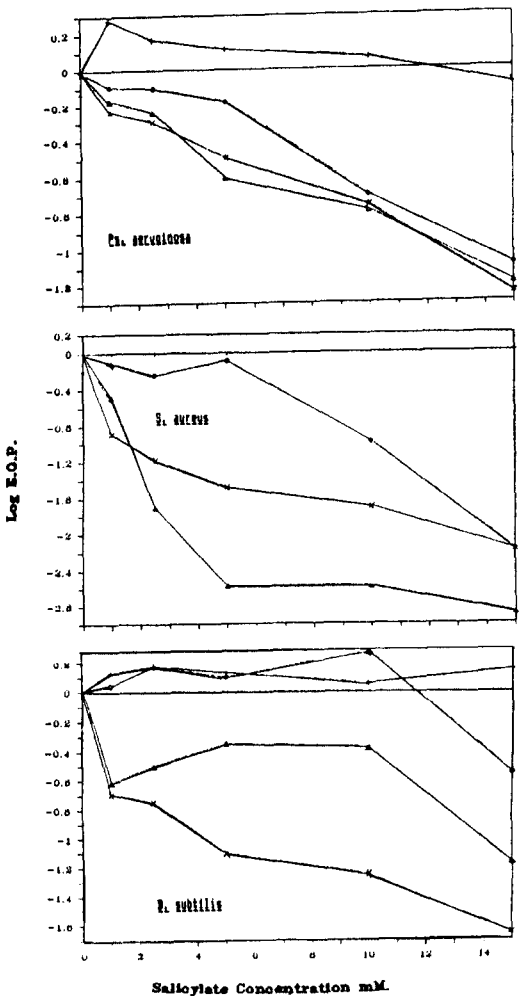

Fig. 2. EOPs of tested bacterial strains on TB agar plates (pH 7.4) with 0(+), 0.25(◇), 0.50(△) and 1.0 (×) ug of gentamicin per m*l* as a function of sodium salicylate concentration.

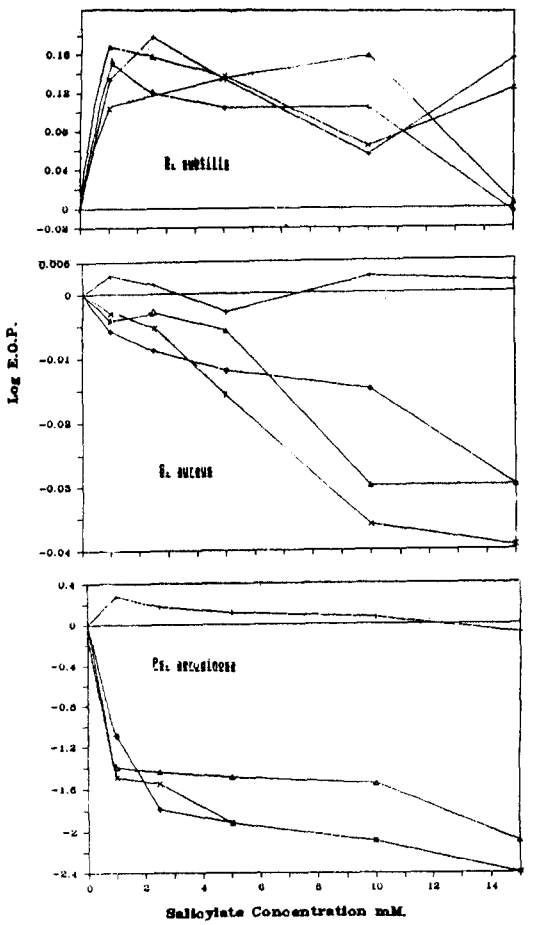

Fig. 3. EOPs of tested bacterial strains on TB agar plates (pH 7.4) with 0(+), 0.25(◇), 0.50(△) and 1.0 (×) ug of cefotaxime per m*l* as a function of sodium salicylate concentration.

on TB agar containing different concentrations of salicylate (1.0, 2.5, 5.0, 10.0 and 15.0 mM) and antibiotics (0.25, 0.5 and 1.0 ug/m*l*). Salicylate alone, even up to 15 mM, did not affect the E.O.P. however, in the absence of salicylate, no concentration of the tested antibiotics resulted in significant killing, the E.O.P. were about 0.2.

Effect of salicylate on gentamicin activity

Generally, increasing concentrations of both salicylate and gentamicin resulted in a strong synergistic effect, *Staphylococcus aureus* was highly affected by salicylate/gentamicin combinations, followed by *Bacillus Subtilis and Pseudomonas aeruginosa*, the E.O.P. were reduced to −3.0 and −1.70, respectively (Fig. 2).

The minimal inhibitory concentration (MIC) of gentamicin against *Staphylococcus aureus* was reduced 16 times in the presence of 5.0 and 10.0 mM of salicylate (Table I).

Effect of salicylate on cefotaxime activity

High synergistic effect was observed against *Psudomonas aeruginosa* (Fig. 3). Increasing salicylate concentrations potentiated the antipseudomonal activity of cefotaxime Log E.O.P. was reduced greatly

Table I. Effect of different concentrations of salicylaye (sal) on MICs of the tested antibiotics using the broth dilution method

Antibiotic	Sal//mM	MIC (ug/ml) *Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*
Gentamicin	0.0	5.0	0.0195	0.166
	1.0	2.5	0.0195	0.166
	5.0	5.0	0.0012	0.166
	10.0	2.5	0.0012	0.313
Cefotaxime	0.0	25.0	2.50	0.313
	1.0	25.0	2.50	0.313
	5.0	25.0	2.50	0.313
	10.0	25.0	2.50	0.156

to −2.4. On the contrary, the activity against *Bacillus subtilis* was antagonized by salicylate since log E.O.P. was increased up to 0.18. On the other hand, no significant potentiation of antistaphylococcal activity of cefotaxime/salicylate combinations was detected, log E.O.P. values ranged between −0.005 and −0.04.

DISCUSSION

Salicylate, a medically significant drug, was found to potentiate greatly the activity of aminoglycosides against *Escherichia coli* (2). In the present investigation, similar effects for salicylate on the bactericidal activities of gentamicin and cefotaxime against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis* could be reported.

Being a weak acid, salicylate increases the membrane potential of cells at low pHs[6,7]. The increased membrane potential could be the basis of this synergism[8-12]. Normally uncoupling of oxidative phosphorylation would be expected to decrease the susceptibility to aminoglycosides by blocking the energy-dependent uptake steps[8]. Bryan[13], Bryan and Kwan[14] and Taber *et al.*[15] have argued that, in addition to a requirement for membrane potential, a quinone or related part of the electron transport system is needed as an anionic transporter for aminoglycosides. Conceivably, salicylate could also increase such respiratory chain-related activity.

Salicylate can act −as EDTA− to chelate divalent cations that are antagonistic to aminoglycoside activity on *Psudomonas aeruginosa*[9]. Perhaps salicylate can have similar effects on *Staphylococcus aureus* and *Bacillus subtilis*. However, the salicylate structure may have a regulatory effect[10,12] on the activity of some other cellular element involved in aminoglycoside uptake or target susceptibility e.g. the ribosomes.

On the other hand, it was observed that salicylate reduced the susceptibility of *Bacillus subtilis* to cefotaxime (Fig. 3). Other authors[1,3,16-18] have reported that salicylate antagonized the activity of many antibiotics such as ampicillin, chloramphenicol, nalidexic acid and tetracycline against *Escherichia coli*. These antibiotics differ both in their mode of action and in their chemical structures. It seems plausible that the resistance was caused by an effect of weak acids on antibiotic uptake. However, salicylate/cefotaxime combinations were synergistic against *Pseudomonas aeruginosa* and indifferent against *Staphylococcus aureus*.

It may be anticipated that, when administered together, salicylates and related compounds could improve the therapeutic action of aminoglycosides and other related positively charged antibiotics. It should be noted however, that salicylates reduce the susceptibility to a number of betalactams and so would be contraindicated when these antibiotics are administered.

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