INFLUENCE OF POTASSIUM CITRATE OR ASCORBIC ACID ON THE IN-VITRO ANTIBACTERIAL ACTIVITY OF SOME ANTIBIOTICS

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ABSTRACT

Bacteriostatic activity of each of four antibiotics was determined singly and in combination with either potassium citrate or ascorbic acid employing three bacterial genera as test organisms.

Results obtained in the study showed that presence of ascorbic acid with gentamicin or tetracycline potentiated bacteriostatic activity of each of these antibiotics against Pseudomonas aeruginosa and Escherichia coli respectively. While combination of potassium citrate tremendously enhanced in-vitro activity of ampicillin, tetracycline and cefuroxime against Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.

It was also observed that potassium citrate improved the sensitivity of some strains of Pseudomonas aeruginosa hitherto resistant to ampicillin.

INTRODUCTION

It is the usual practise in the treatment of bacterial infection to employ antibiotics singly or in combination with other antibacterial agents. In the treatment of urinary-tract infections however, alkalinisation of urine with potassium citrate or acidification with ascordic acid has been known to enchance antibacterial efficacy of some antibiotics.

One worker[1] reported the potentiating action of citrate on inhibitory activity of trace amounts of penicillin while another set of researchers[2] observed that antibacterial action of gentamicin was influenced to a great extent by the pH of the medium depending on the bacterial species. It was reported in a study[3] where ascorbic acid was found to have no bactericidal effect on Pseudomonas aeruginosa but potentiated the activity of erythromycin, sulphamthoxazole, trimethoprim and chloramphenicol against the same organism.

This study was therefore designed to investigate the effect of either potassium citrate or ascorbic acid on the in-vitro activity of some antibiotics. The study is to detect if such combination will be beneficial or detrimental to the antibacterial activity of the antibiotics. This study was also designed to investigate the effect of such combination on the improvement of sensitivity of hiterto resistant bacteria to antibiotics.

MATERIALS AND METHODS

ORGANISMS

The following bacterial species served as test organisms in this study: Escherichia (E) coli 14SZ5 Pseudomonas (Ps) aeruginosa NCTC 6750, Staphylococcus (Staph) aureus NCTC 6572 all obtained from the Department of Pharmacy, University of Strathclyde, Glasgow and three strains of Ps. aeruginosa 17145, 18855 and 18864 obtained from the Department of Medical Microbiology, University College Hospital, Ibadan.

MEDIA

Culture media used in this study included nutrient broth no 2 pH 7.4 and nutrient agar pH 7.4 products of Oxoid Laboratories, England.

DRUGS

The drugs tested in this study were, ampicillin sodium injection (Beecham Laboratories, England) cefuroxime injection (Glaxo Laboratories England) gentamicin injection (Eupharma Laboratories, India) tetracycline hydrochloride injection (Oftalmiso Laboratories, Spain) ascorbic acid powder (Hopkin and Williams Ltd., England) and potassium citrate powder (British Drug Houses, England).

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION.

Bacteriostatic activity of each antibiotic was determine in the presence or absence of potassium citrate using the broth dilution method[4]. Serial 2-fold dilution of each antibiotic was carried our in single strength nutrient broth to which was added a known concentration of sterile potassium citrate solution. Test organism was added to each tube to contain 10 colony forming units per ml. The inoculated tubes of nutrient broth were incubated at 37° for 48 hours after which the lowest concentration inhibiting growth was taken as the MIC.

BACTERIAL GROWTH IN THE PRESENCE OF ANTIBIOTICS

Effect of drug combination was also studied by monitoring bacterial growth in the presence of some antibiotics and potassium citrate or ascorbic acid. Each test organism was diluted 1 in 100 in nutrient broth and incubated at 37°c in a Gallenkamp Water bath with shaking at 100 throws per minute. Growth of each organism was recorded at 1 hour interval by measuring the optical density on a spectrophotometer spectronic 21 at 560nm wavelength. After $2\frac{1}{2}$ hours of incubation known concentrations of

antibiotics and in combination with known concentrations of potassium citrate or ascorbic acid were added to the growing culture. There were control flasks to which antibiotics were not added and others to which only potassium citrate or ascorbic acid was added. Incubation with shaking continued for another $3\frac{1}{2}$ hours after which bacterial growth was terminated. Bacterial growth in percentage was determined and plotted against time of incubaction on a linear graph.

RESULTS

In both figures 1 and 2, 10mg/ml of potassium citrate and 30 um/ml of ascorbic acid did not produce any antibacterial activity on their own. However in figure 1^A ascorbic acid was observed to potentiate antibacterial activity bof gentamicin against Ps. aeruginosa while potassium citrate antagonised antibacterial activity of gentamicin against the same organism. The presence of potassium citrate with tetracycline and ampicillin as seen in figure 1^B, 1^C produced synergistic effects against Ps. aeruginosa. There was no significant difference observed in the antibacterial activity of tetracycline when combined with either potassium citrate or ascorbic acid against E. coli as shown in figure 2^A though bacterial growth was slightly more depressed in the presence of ascorbic acid. Both ascorbic acid and potassium citrate could be seen in figure 2^B to have potentiated the antibacterial activity of ampicillin against E. coli but the potentiation was more pronounced in the presence of potassium citrate.

Effects of graded concentrations of potassium citrate on bacteriostatic activity of both ampicillin and cefuroxime against certain organism are shown in Tables 1 and 2. In both Tables, the MIC of each antibiotic decreased generally with increase in concentration of potassium citrate where the presence of potassium citrate produced an enhanced effect.

In Table 3 presence of potassium citrate could be seen to have decreased the MIC of ampicillin 2- fold against two strains of Ps. aeruginosa.

DISCUSSION

In the treatment of urinary – tract infection such as cystitis, it is a common practice to recommend potassium citrate mixture to be diluted with water primarily to alkalinise the urine in addition to antibacterial agents. It was predicted in 1984 that the management of cystitis may have to depend on alkalinisation of th urine before antibiotic administrations[5]. The BNF[6] reported that potassium citrate in water may help increase the excretaion of salicylate and phenobarbitone as well as the efficacy of aminoglycosides, cephalosphorisns, clindamycin and linchomycin while acidification of urine with ascorbic acid may help increase elimination of amphetamine and enchance the effects of cloxacillin and tetracycline. Rawal and Mckay[3] observed that ascorbic acid which has no batericidal effect on Ps. aeruginosa acted synergistically with drugs such as co-trimoxazole, ampicillin and erythromycin which on their own have no activity on Ps. aeruginosa. This study has therefore confirmed the observations of previous workers on the effect on combing potassium citrate or ascorbic acid with antibiotics.

It is significant to observe that presence of potassium citrate improved the sensitivity of some resistant strains of Ps. aeruginosa to ampicillin though the improve sensitivity was very minimal. It may not be out of place therefore to explore this type of drug combination to reduce incidence of bacterial drug resistance.

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Table 1
Minimum Inhibitory Concentration of Ampicillin in the Presence of Potassium Citrate

Conc. of Pot. Cit. (mg/ml)	M. I. C. (ug/ml) E. coli	Staph. aureus
0	16	0.125
2.5	8	0.0625
5	4	0.0625
10	4	0.0625

Table 2
Minimum Inhibitory Concentration of Cefuroxime in the Presence of Potassium Citrate

Conc. of Pot. Cit. (mg/ml)	M. I. C. (ug/ml) E. coli	Staph. aureus	Ps. aeruginosa
0	16	8	128
2.5	16	4	128
5	16	2	128
10	16	2	64

Table 3
Sensitivity of Ampicillin Resistant Strains of Pseudomonas aeruginosa in the Presence of Potassium Citrate

Pseudomonas Isolate	M. I. C. ug/ml Ampicillin	Ampicillin with 10mg/ml Pot. Cit.
17145	2000	1000
18855	1000	1000
18864	2000	2000

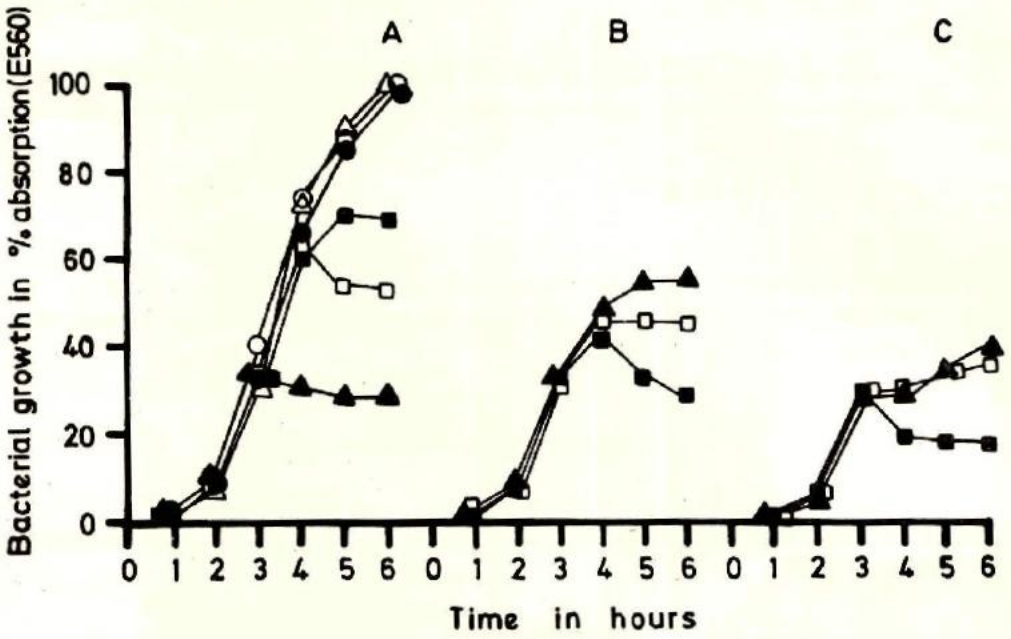

FIGURE 1

EFFECT OF DRUG COMBINATION ON GROWTH OF Pseudomonas aeruginosa

A.
- △ Organism alone
- ● 10mg/ml Pot Cit.
- ○ 30µg/ml Ascorbic acid
- □ 2µg/ml Gentamicin
- ▲ 2µg/ml Gentamicin + Ascorbic acid
- ■ 2µg/ml Gentamicin + Pot Cit.

B.
- □ 50µg/ml Tetracycline
- ▲ 50µg/ml Tetracycline + Ascorbic acid
- ■ 50mg/ml Tetracycline + Pot Cit.

C.
- □ 250µg/ml Ampicillin
- ▲ 250µg/ml Ampicillin + Ascorbic acid
- ■ 250µg/ml Ampicillin + Pot Cit

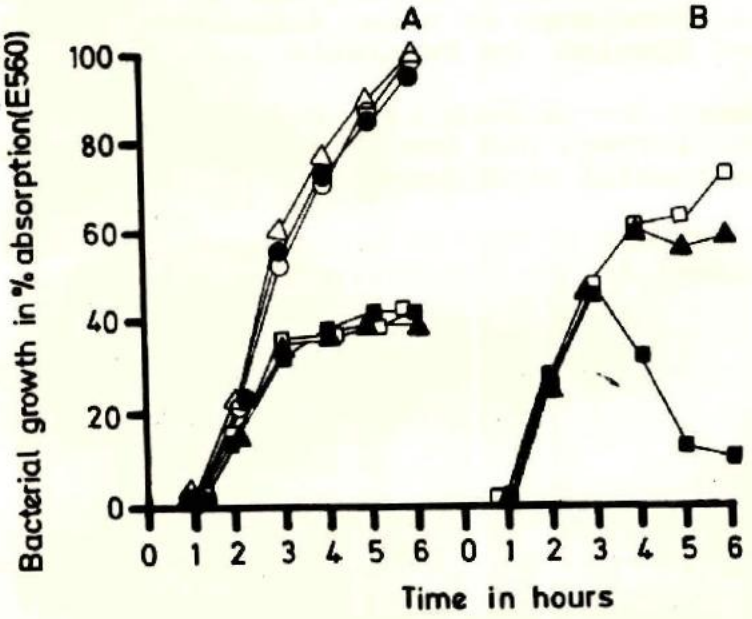

FIGURE 2

EFFECT OF DRUG COMBINATION ON GROWTH OF Escherichia coli

A.
- △ Organism alone
- ● 10mg/ml Pot Cit.
- ○ 30µg/ml Ascorbic acid
- □ 25µg/ml Tetracycline
- ▲ 25µg/ml Tetracycline + Ascorbic acid
- ■ 25mg/ml Tetracycline + Pot Cit.

B.
- □ 5µg/ml Ampicillin
- ▲ 5µg/ml Ampicillin + Ascorbic acid
- ■ 5µg/ml Ampicillin + Pot Cit