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Short Communication

Effect of EDTA on the activity of ciprofloxacin against *Shigella sonnei*

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Ethylenediamine Tetraacetic Acid (EDTA) is a compound predominantly known for its chelating ability. It has also been found that in combination with antibacterial agents like benzakolium and chlorocresol, they were able to kill resistant bacteria. EDTA was used in combination with ciprofloxacin, a fluoroquinolone, against *Shigella sonnei*. This was done by determining their respective and combined Minimum Inhibitory Concentration (MIC) and using their obtained MIC in combination to obtain their fractional inhibitory concentration (F.I.C) in order to determine if their interaction is synergistic, antagonistic or additive. Results obtained using the checkerboard technique showed that combinations of EDTA with ciprofloxacin showed synergy at ratios of 9:1, 8:2, 7:3 and 6:4, and additive effect at ratios of 5:5, 4:6, 3:7 and 2:8.

Key words: EDTA, ciprofloxacin, *Shigella sonnei*, synergy.

INTRODUCTION

Several reasons have been advanced to justify the use of combination of two or more antibiotic treatments (Esimone et al., 2006b; Ibezim et al., 2006). For many years now; combination of two or more antibiotics has been recognized as an important method for, at least, delaying the emergence of bacterial resistance (Chambers, 2006). Besides, antibiotic combinations may also produce desirable synergistic effects in the treatment of bacterial infections (Zinner et al., 1981).

However, methods have been developed to quantify the effect of antimicrobial combinations on bacterial growth *in vitro*. Two very distinct traditional methods of testing *in vitro* antibiotic interaction are the checkerboard technique and the time killing curve method (Eliopoulos et al., 1988).

Ciprofloxacin is a broad-spectrum fluoroquinolone and possesses good activity against *Escherichia coli (E. coli)* and *Staphylococcus aureus.* It is active *in vitro* against *Citrobacter* spp., *Serratia* spp., *Klebsiella* spp., *Salmonella* spp., *Shigella* spp., etc. Recently, there have been reports of resistance to this, hitherto effective group of antibiotics, by efflux mechanism described in *S. aureus* (De Chene et al., 1990).

It has been shown that chelating agents such as Ethylenediamine Tetraacetic Acid (EDTA) destabilize the outer membrane of gram negative bacteria by sequestering the stabilizing divalent cations. Such destabilization leads to the release of substantial (up to 40%) lipopolysaccharides, release of periplasmic enzymes and cell membrane associated proteins and phospholipids. Thus an EDTA-treated bacterium becomes susceptible to agents that do not normally penetrate the outer membrane and as a consequence do not affect the bacteria (Vaara, 1992). This phenomenon is often referred to as permeabilization.

MATERIALS AND METHODS

Culture media

The media used in the study included nutrient broth (Merck Germany), deoxycholate citrate agar (lab M, England) and nutrient agar (Merck Germany).

Test microorganism

Clinical isolates of *Shigella sonnei* were obtained from a patient

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Table 1. Table of the combined activity of EDTA and ciprofloxacin against *Shigella sonnei*.

Drug ratio EDTA:Cipro 10:0	Mic. Mic EDTA: Cipro 150…	Fic. Fic EDTA: Cipro	FIC index	Activity index	Effect
9:1	33.75:12.5	0.225:0.025	0.25	-0.602	SYN
8.2	30:25	0.200:0.050	0.25	-0.602	SYN
7:3	52.5:75	0.35:0.15	0.50	-0.301	SYN
6:4	30.0:150	0.20:0.30	0.50	-0.301	SYN
5:5	90:200	0.60:0.4	1.0	0	ADD
4.6	75:250	0.50:0.50	1.0	0	ADD
3:7	45:350	0.3:0.70	1.0	0	ADD
2:8	30:400	0.2:0.80	1.0	0	ADD
1:9	15:450	0.1:0.90	1.0	0	ADD
0:10	…. : 500				

Key: SYN = synergism; ADD = addictive; CIPRO = ciprofloxacin; Activity index = Log $_{10}$(FIC index).

with dysentery in the Medical Center, University of Nigeria, Nsukka.

Isolation and identification of test microorganisn

Samples of microorganism on Desoxycholate citrate agar (DCA) incubated in air at 37°C for 24 h formed pale pink colonies of 1 - 2 mm in diameter. This test was used to identify *S. sonnei*.

Antimicrobial agents and disc

Ethylenediaminetetra acetic acid, dipotassium salt (sigma chemicals, USA) and sample of ciprofloxacin hydrochloride extracted from the tablets dosage form (Orange Drugs, Nigeria) were used. These were used to prepare the antibiotic disc using Whatman No. 1 filter paper in accordance with the NCCLS standards (1990).

Preparation of culture media

All culture media were prepared according to the manufacturer's specifications.

Maintenance and standardization of test microorganism

The microorganisms were maintained by weekly subculturing on nutrient agar slants stored at 4°C after previous 24 h incubation at 37°C. Prior to each experiment, the microorganisms were activated by successive subculturing and incubation. 24 h old cultures of the test organism were always used. Standardization of test microorganism was according to previously reported method (Chinwuba et al., 1994; Esimone et al., 1999).

Sensitivity of test microorganism

The sensitivity of test microorganism to EDTA and ciprofloxacin hydrochloride was evaluated by determining the minimum inhibitory concentration (MIC) of the antibiotics using the two-fold broth dilution technique previously described (NCCLS, 1990; Esimone et al., 1999).

Evaluation of combined effects of EDTA and ciprofloxacin

Stock solutions of EDTA (300 mg/ml) and ciprofloxacin (500 mg/ml) prepared in double-strength nutrient broth and autoclaved at 121°C for 15 min, were employed. Thereafter varying proportions of the EDTA and ciprofloxacin (cipro) were prepared according to the continuous variation checkerboard method previously described (NCCLS, 1990). Each proportion of the EDTA/ ciprofloxacin combination was serially diluted (2 fold), inoculated with 0.1 ml of 10^6 CFU/ ml culture of test microorganism and then incubated for 24 h at 37°C. Interaction was assessed algebraically by determining the fractional inhibitory concentration (FIC) indices according to the equations:

$$FIC_{index} = FIC_{EDTA} + FIC_{Cipro} \quad (1)$$

FIC_{EDTA} = Fractional inhibitory concentration of EDTA

$$= \frac{\text{MIC of EDTA in combination with ciprofloxacin}}{\text{MIC of EDTA}} \quad (2)$$

FIC_{cipro} = Fractional inhibitory concentration of ciprofloxacin

$$= \frac{\text{MIC of ciprofloxacin in combination with EDTA}}{\text{MIC of ciprofloxacin alone}} \quad (3)$$

RESULTS AND DISCUSSION

The combined effects of EDTA and ciprofloxacin against *S. sonnei* are presented in Table 1.

Combined drug use is occasionally recommended to prevent resistance emerging during treatment and to achieve higher efficacy in the treatment of infections and diseases. The combination is hoped to achieve a synergistic effect in this study. Results of the systematic and scientific evaluation of the in vitro effects of EDTA and Ciprofloxacin have been presented in this paper.

In the checkerboard technique, the interactions between EDTA and ciprofloxacin again*st S. sonnei*

indicate additivity at some combination ratios and synergy at other ratios as reflected in Table 1. FIC $_{index}$ values < 1 were considered as synergy and the degree of synergy increases as the value tends towards zero. FIC $_{index}$ values of 1 indicate additive effect, values greater than 1, but less than 2 represent indifference while values greater than 2 show antagonism (Vaara, 1992; Esimone et al., 1999).

Based on these, synergistic effect was obtained by combination of EDTA and ciprofloxacin against in the ratios (9:1, 8:2, 7:3, 6:4) while others (5:5, 4:6, 3:7, 2:8, 1:9) showed additive effect.

These results show that there is minor therapeutic advantage in the use of these agents (EDTA and ciprofloxacin) in combination therapy against infections due to *S. sonnei*. However, further work can be done using other chelating agents/quionolone combinations to see if there will be greater therapeutic benefits.

In conclusion, it may be stated that there is a favorable interaction between EDTA and Ciprofloxacin against *S. sonnei* in some given combination.

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