Research Article

Efficacy of Antibacterial Activity of Antibiotics Ciprofloxacin and Gentamycin Improved with Anti Depressant Drug, Escitalopram

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ABSTRACT

Escitalopram oxalate was shown to inhibit the *in vitro* growth of microbial infections. Generally Escitalopram used in the treatment of depression, but having other pharmacological action of antimicrobial activity. A total of five strains were studied to determine whether the anti depressant drug Escitalopram used in combination with the conventional antibiotic Gentamycin and Ciprofloxacin synergistically augments the efficacy of the antibiotic *in vitro*. The effect of combination was evaluated by MIC determination by turbidity method and disc diffusion method Escitalopram was synergistic with Gentamycin and Ciprofloxacin against tested organisms Gram-positive clinical isolates like *staphylococcus aureus* NCIM 2079, Gram-negative organisms such as *pseudomonas aeruginosa* NCIM 2036, *klebsiella pneumonia* NCIM 2719, *proteus mirabilis, enterobator cloacae* NCIM 2164. From the results of MIC Escitalopram oxalate against *staph aureus* 1000 µg/ml, *enterococcus faecalis* is 1000 µg/ml, *pseudo flatulence*1000 µg/ml, *Klebsiella pneumonia* 250 µg/ml, *proteus mirabilis* 500 µg/ml whereas in combination there was two, three fold reduction of MIC. Thus, the ability of extended antibiotic therapy may be improved with the help of this synergistic drug pair in micro organisms. The Significance of such findings may indicate parallel administration of antidepressant with antibiotics against micro organisms.

Keywords: Antibacterial activity, Ciprofloxacin, Escitalopram oxalate, Gentamycin, Microbial infection.

INTRODUCTION

Antibiotics improved the quality of health related infectious diseases and it is one of our most significant arms in fighting bacterial infections. Though, more than the past few decades this health related infectious diseases are under risk as many regularly used antibiotics have develop into very less effective antibiotics against certain illnesses because many of them produce drug resistant bacteria. This emergence of drug resistant bacteria leads to cause the ineffective antibiotics. In that case it is essential to investigate newer drugs with lesser resistance. Methodological searching from various pharmacological compounds has exposed their different functions and thus may have useful activity in medical field.[1-4] Drugs belonging to different pharmacological classes such as anti psychotic agent Thioridazine,[5] Prochlorperazine,[6] anti hypertensive's methyl-DOPA,[7] Cardiovascular agent amlodipine,[8] oxyfedrine,[9] lacidipine[10] and anti inflammatory drugs, e.g., diclofenac [11-14] and aspirin[15] possess powerful antibacterial activity. Increased interest in NSAIDs, traditionally known as the analgesic-antipyretics came with the discovery of their anti-inflammatory properties. Escitalopram already reported to have anti microbial activity. But no definitive studies have demonstrated the detailed study of antimicrobial activity of Escitalopram with antibiotics. So in the present paper describes the detailed *in vitro* activity of such a non antibiotic Escitalopram with ciprofloxacin and Gentamycin against *staphylococcus aureus*, *pseudomonas aeruginosa*, *klebsiella pneumonia*, *proteus mirabilis, enterobactor cloacae*.

MATERIALS AND METHODS

Drugs used in this study were obtained as pure powders of pharmaceutical grade. The drugs Escitalopram, Ciprofloxacin, Gentamycin were obtained from Madras scientific, Tiruchirappalli. Drug are obtained in pure dry powder form and dissolved in distilled water, DMSO depending on their solubility, and kept at 4°C.

Bacteria

Gram-positive clinical isolates like *Staphylococcus aureus* NCIM 2079, Gram-negative organisms such as *Pseudomonas aeruginosa* NCIM 2036, *Klebsiella pneumonia* NCIM 2719, *Enterobactor cloacae* NCIM 2164, *Proteus mirabilis,* were used in this study. A total of 5 strains of bacteria were belonging to gram positive and gram negative. From this one microbe were of human isolates obtained from K.A.P. Visvanathan Medical College, Tiruchirappalli, and remaining four were obtained from National Collection of Industrial Microorganisms, Pune. They were maintained at 4°C as slant cultures of sterile nutrient agar for a maximum of 1 month.

Media

Liquid media used for this study were Muller Hinton Broth, Solid media were Mueller Hinton agar (MHA), obtained by solidifying the liquid media with 1.2% (w/v) agar.

Inoculum

The inoculum for each bacterial strain was prepared by taking four or five pure colonies from an overnight culture using a sterile inoculation loop. These colonies were

mixed in sterile normal saline. Gentle dilution was performed, till the turbidity was comparable visually to 0.5 to 1.0 McFarland turbidity standard.

Determination of minimum inhibitory concentration (MIC) of different drugs

The MIC of Ciprofloxacin, Gentamycin, and Escitalopram with respect to different test bacteria was determined both by broth and agar dilution methods. For broth dilution 0.1mL of standardized suspension of a strain (1Mcfarland standard) was added to each tube containing Escitalopram at concentrations of 1000 µg/ml serially diluted up to 1.95µg/ml in MHB and the same method was repeated with 0.5Mcfarland standard at 10,20,30,40 and 50µg/ml to calculate percentage inhibition. The tubes were incubated at 37°C for 24 hours, and looked for visible growth after vortexing the tubes gently. The optical densities were measured by determining the absorbance at 530 nm in spectrophotometer (UV-Thermoscientific BIOMATE 35) and from these values percentage of inhibition of microorganisms based on non antibiotic and antibiotics were calculated. The lowest concentration of Escitalopram in a tube or plate that failed to show any visible macroscopic growth was considered as its MIC. The MIC determination was performed in duplicate for each organism, and the experiment was repeated where necessary.[16]

In tube assay method, the percentage inhibition was determined from the following formula

Percentage inhibition =

Absorbance of positive control (without drug) - absorbance of test solution**/**Absorbance of positive control.

Table 1: The minimal inhibitory concentrations of therapeutic drugs and antibiotics with respect to microorganisms 1Mc far land standard

Name of the organism	Concentration in µg/ml				
	Ciprofloxacin	Gentamycin	Escitalopram Oxalate	Cip + E.o	Gen + E.o
Klebsiella pneumoniae	250	500	250	62.5	250
Proteus mirabilis	250	500	500	125	250
Enterobactor cloacae	500	500	1000	125	250
Staphylococcus aureus	500	1000	1000	62.5	500
Pseudomonas aeruginosa	500	1000	1000	250	500

Table 2: The Zone of inhibition of the organisms with respect to the antibiotics and drugs

Name of the organism	Type of the sample	Zone of inhibition in mm				
		Concentrations in µg/ml				
		10	12.5	15	17.5	20
Staphylococcus aureus	Ciprofloxacin	7.3±0.3	7.6±0.45	8±0.15	7.6±0.45	7.6±0.45
	Escitalopram oxalate	6.3±0.25	7.3±0.6	7±0.25	6.6±0.5	8.3±0.5
	Cipro(10µg/ml) + E.o	7.3±0.15	7.3±0.5	8±0.5	8±0.65	8.3±0.85
	Cipro(15µg/ml) + E.o	7.3±0.4	7.6±0.2	8±0.65	8.6±0.75	8.6±0.3
	Cipro(20µg/ml) + E.o	13.3±0.55	13.6±0.35	12±0.45	14.3±0.25	13.6±0.65
Staphylococcus aureus	Gentamicin	13.6±0.3	12.6±0.45	13.6±0.15	14.3±0.45	15±0.45
	Escitalopram oxalate	8.3±0.25	8.6±0.6	8.6±0.25	8.6±0.5	9±0.5
	Gen(10µg/ml) + E.o	14±0.15	15.6±0.5	16.3±0.5	19±0.65	19.3±0.85
	Gen (15µg/ml) +E.o	19.3±0.4	18.6±0.2	20.6±0.65	20.3±0.75	21.3±0.3
	Gen (20µg/ml) + E.o	22.6±0.55*	23±0.35*	23±0.45*	23±0.25*	23±0.65*

Values are compared with Gentamycin significant at P<0.05; Superscript indicate statistical significance when the values are compared with that of Ciprofloxacin and Gentamycin alone at the same concentration at P<0.05

Plate method by disc diffusion process

The prepared agar plate was inoculated by inoculating needle with different directions to get a uniform growth. The plates were allowed to dry for 5-10 minutes and then kept in incubator for 18 hours at 35 - 37°C. The required concentration of the non-antibiotic was taken in sterile discs by a micro pipette with different concentration of 10, 12.5, 15, 17.5 and 20µg/ml antibiotic and non antibiotics and it is soaked in refrigerator. The disc was then placed on the surface of the agar plate. Changing the tips of micro pipette, the process was repeated. All the discs were placed with same distance. The inoculated plates were kept inverted in incubator at 35°C for 18 hours in inverted position. The plates were viewed against a black background and zone of inhibition were measured by Kirby Bauer ruler.[17]

RESULTS AND DISCUSSION

In vitro determination of antimicrobial action of NSAID

Escitalopram exhibited the antimicrobial effect against *staphylococcus aureus*, p*seudomonas aeruginosa*, *klebsiella pneumonia, proteus mirabilis, enterobactor cloacae*. The MIC of ciprofloxacin, gentamycin was 250µg/ml to 1000µg/ml against five tested micro organisms. MIC of ciprofloxacin, Escitalopram was 250 µg/ml against *klebsiella pneumonia*. Whereas in combination the MIC of ciprofloxacin and escitalopram 62.5µg/ml which show the result of 3fold reduction of MIC of ciprofloxacin which was shown in Table 1. MIC of Gentamycin and Escitalopram against *staphylococcus aureus* showed 2 fold reduction of MIC of gentamycin than gentamycin alone. From the tube dilution method percentage inhibition was calculated and provides the information about the antimicrobial potency. The combined drugs were produced the synergistic or additive effect which was shown in figure 1 to 2. The Zone of inhibition of the selected three organisms with respect to the antibiotics and non antibiotics were determined and shown in Table 2. Gentamycin and ciprofloxacin were showed their additive, synergic effect against *staphylococcus aureus* with Escitalopram.

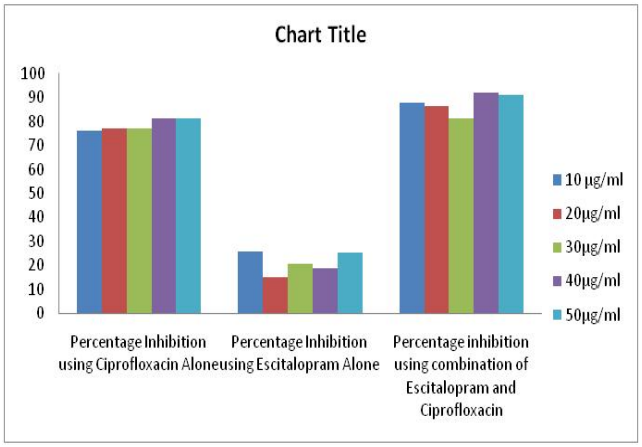

Figure 1: Percentage inhibition of Escitalopram oxalate on antibacterial activity of Ciprofloxacin against *Pseudomonas aeruginosa* 0.5 Mc far land standard

The anti depressant drug Escitalopram oxalate, which is regularly used to treat depression, has shown significant action against many bacteria *in vitro* against *Staphylococcus aureus*, *pseudomonas aeruginosa*, *klebsiella pneumonia*, *proteus mirabilis, enterobator cloacae*. Synergism was observed with Escitalopram oxalate in combination with ciprofloxacin and Gentamycin. The tricyclic phenothiazines in general possess moderate to powerful antimicrobial action observed from the earlier literatures.[18] The drug dobutamine, is having a benzene ring attached to another one, may be conceived to mimic a phenothiazine structure, thereby explaining its antibacterial property.[19] Escitalopram oxalate chemically S-(+)-1-[3-(dimethyl-amino)propyl]-1-(p-fluorophenyl)-5- oxalate. It consists of phthalancarbonitrile group and benzene ring attached to fluorine atom compared to the reported non antibiotics. Escitalopram oxalate also having the structure as like that of phenothiazine, this property may exhibit the antimicrobial property.

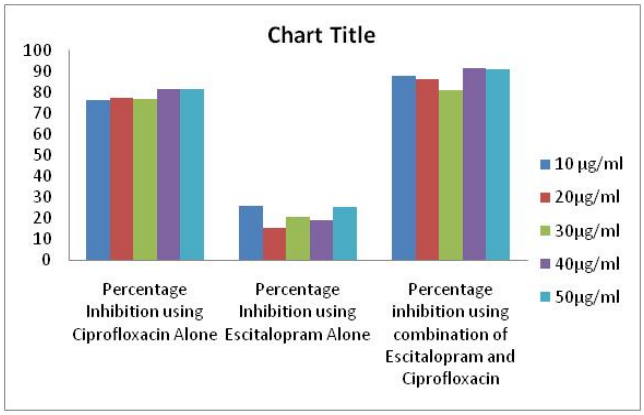

Figure 2: Percentage inhibition of Escitalopram oxalate on antibacterial activity of Gentamycin against *Staphylococcus aureus*0.5 Mc far land standard

CONCLUSION

The anti depressant drug Escitalopram that could be used as a model compound for the synthesis of next new antibacterial drug that might be free of adverse reactions of the drug. As this agent consists of a phthalan carbonitrile group and benzene ring attached to the fluorine atom structurally correlates with the reported non antibiotics. Further pharmacological studies are necessary to confirm our findings on the possible use of this drug to treat bacterial infections. To evaluate and confirm the *in vivo* synergistic effect of Escitalopram oxalate with gentamycin and ciprofloxacin and establish the ability of extended antibiotic therapy against *mycobacterium tuberculosis* resistancy.

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