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Research Article

ENHANCEMENT OF THE EFFICACY OF ANTIBIOTICS CIPROFLOXACIN AND GENTAMYCIN AGAINST GRAM POSITIVE AND GRAM NEGATIVE MICRO ORGANISM WITH NON ANTIBIOTIC NIMESULIDE

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ABSTRACT

Objective: Current trend in treatment of microbial infections different types of antibiotics and some other combinational therapy are used to treat the microbial infections. In the present study non-antibiotics was taken to study the effect of anti microbial activity.

Methods: Nimesulide is used in the treatment of Non steroidal anti inflammatory drug was screened for possible antimicrobial property determined by tube dilution method and disc diffusion method. against two clinical isolates and standard micro organism belonging to various gram-positive organism like *Staphylococcus aureus* NCIM 2079, Gram-negative organisms such as *Pseudomonas aeruginosa* NCIM 2036, *Klebsiella pneumonia* NCIM 2719 , *Enterobactor cloacae* NCIM 2164, *Proteus mirabilis, Pseudomonas fluoroceae*

Results: Nimesulide were shown to have moderate antimicrobial activity from the *in vitro* study. The minimal inhibitory concentration of nimesulide was determined by broth dilution method which ranged from 125µg/ml to 250µg/ml in most of the strains, while some strains inhibited at even lower concentrations. But when it was combined with antibiotics Ciprofloxacin and Gentamycin it showed significant enhancement of the activity. In agar diffusion method the increase in diameter of zone of inhibition was observed with ciprofloxacin and nimesulide.

Conclusion: Nimesulide may possibly used for the synthesis of more active novel agents with free of side-effects as like a lead compound

Keywords: Non-antibiotics, Ciprofloxacin, Gentamycin, Cyclooxygenase-2 Inhibitor, Zone Of Inhibition, Nimesulide, Microbial resistance.

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 hypertensives methyl-DOPA[7], Cardiovascular agent amlodipine[8], oxyfedrine[9], lacidipine[10] and anti inflammatory drugs, e.g., diclofenac [11,12,13,14] 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. Nimesulide already reported to have anti microbial activity. But no definitive studies have demonstrated the detailed study of antimicrobial activity of nimesulide with antibiotics. So in the present paper describes the detailed in vitro activity of such a non antibiotic nimesulide with ciprofloxacin and gentamycin against staphylococcus aureus, pseudomonas aeruginosa, pseudomonas fluoroceae, klebsiella pneumonia, proteus mirabilis, enterobactor cloacae.

MATERIALS AND METHODS

Drugs used in this study were obtained as pure powders of pharmaceutical grade. The drugs Nimesulide, 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, E*nterobactor cloacae* NCIM 2164, *Proteus mirabilis, Pseudomonas fluoroceae* were used in this study. A total of 6 strains of bacteria were belonging to gram positive and gram negative. From these 2 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 Nimesulide 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 Nimesulide 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 Nimesulide 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. [16,17,18].

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.

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 [19]

RESULTS

In vitro determination of antimicrobial action of NSAID

Nimesulide exhibited the antimicrobial effect against *staphylococcus aureus*, p*seudomonas aeruginosa*, *pseudomonas fluoroceae, klebsiella pneumonia, proteus mirabilis, enterobactor cloacae*. The MIC of ciprofloxacin, gentamycin was 31.25µg/ml to 500µg/ml against six micro organisms and MIC of Nimesulide 500µg/ml. Whereas in combination the MIC of ciprofloxacin and Nimesulide 3.906 µg/ml which show the result of 5 fold reduction of MIC of ciprofloxacin against *pseudomonas fluoresceae* which was shown in Table 1. MIC of Gentamycin and Nimesulide against *staph aureus* and *pseudomonas aureuginasa* was 31.25µg/ml which was 4 fold reduction of MIC of 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 3. 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 *proteus mirabilis, staphylococcus aureus and pseudomonas aerungiunosa* with Nimesulide.

Table 1: The minimal inhibitory concentrations of non-antibiotic and antibiotics with respect to microorganisms 1Mcfarland standard

S. No.	Name of the Micro organism	MIC in µg/ml				
		Cip*	Gen*	Nim*	Cip+Nim	Gen+Nim
1.	*Pseudomonas fluoroceae*	31.25	125	250	3.906	62.5
2.	*Klebsiella pneumoniae*	31.25	125	250	15.65	125
3.	*Proteus mirabilis*	31.25	125	250	15.65	125
4.	*Enterobactor cloacae*	31.25	500	250	31.25	125
5.	*Staphylococcus aureus*	250	500	250	31.25	31.25
6.	*Pseudomonas aeruginosa*	250	125	250	62.5	31.25

Cip – Ciprofloxacin, Gen – Gentamycin, Nim-Nimesulide

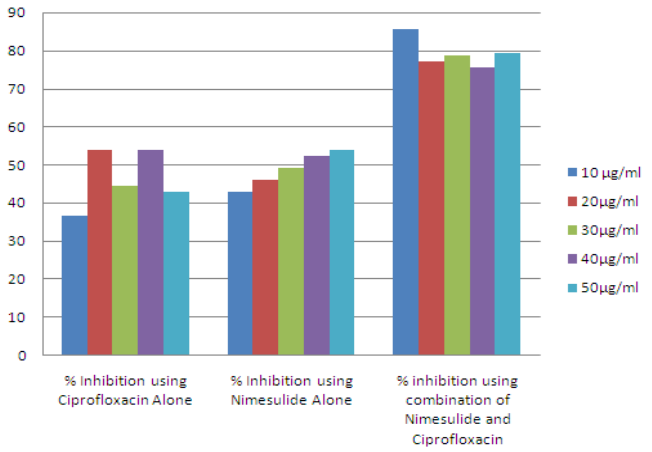

Fig. 1: Percentage inhibition of Nimesulide on antibacterial activity of Ciprofloxacin against *Pseudomonas fluoroceae 0.5Mcfarland standard*

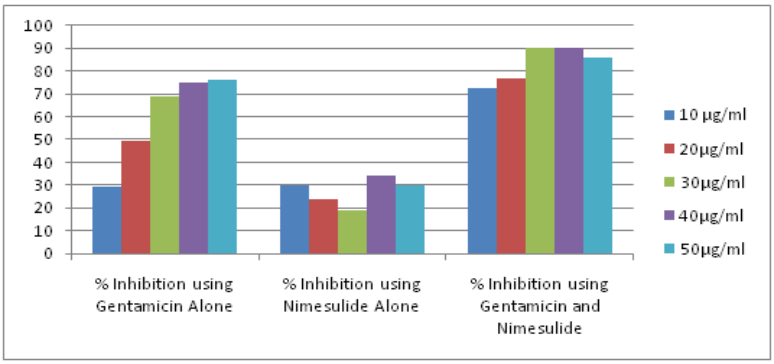

Fig. 2: Percentage inhibition of Nimesulide on antibacterial activity of Gentamicin against *Staphylococcus aureus*

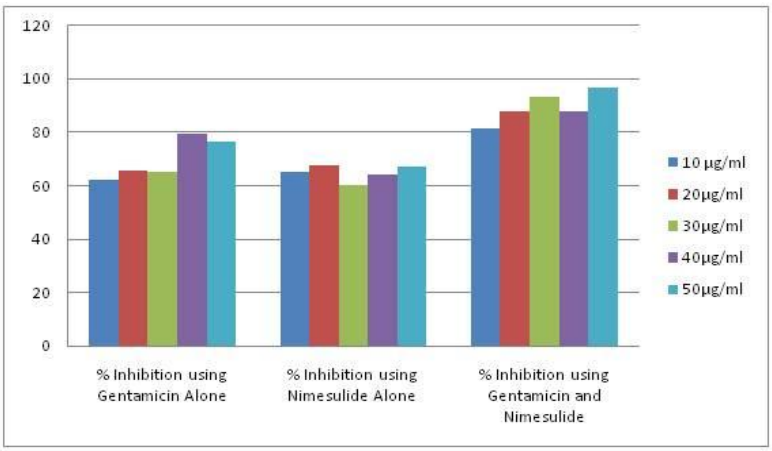

Fig. 3: Percentage inhibition of Nimesulide on antibacterial activity of Gentamicin against *Pseudomonas aeruginosa*

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

S. No.	Name of the organism	Name of the drug	Zone of inhibition in mm				
			Concentrations in µg/ml				
			10	**12.5**	**15**	**17.5**	**20**
1.	*Proteus mirabilis*	Ciprofloxacin	17±0.30	18±0.45	19±0.15	20±0.45	20±0.45
		Nimesulide	9±0.25	9±0.60	9±0.25	9.2±0.50	9.5±0.50
		Cipro(10µg/ml) + Ni	28*±0.55	24*±0.35	22±0.45	25±0.25	26±0.65
2.	*Staphylococcus aureus*	Gentamicin	8.5±0.30	9.3±0.45	9.3±0.15	9.6±0.45	10.3±0.45
		Nimesulide	6.3±0.25	6.3±0.60	7±0.25	7.4±0.5	7.6±0.5
		Gen(10µg/ml) + Ni	14±0.50	18±0.35	20*±0.5	20*±0.25	18*±0.65
3.	*Pseudomonas aeruginosa*	Gentamicin	22±0.30	20.3±0.45	19±0.15	26.6±0.45	27.6±0.45
		Nimesulide	9.6±0.25	9.6±0.60	8.3±0.25	8.3±0.5	9±0.5
		Gen (10µg/ml) + Ni	27±0.55	26.6±0.35	27*±0.5	29±0.25	28±0.65

* Values are compared with Ciprofloxacin and 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

DISCUSSION

The NSAID drug Nimesulide, which is regularly used to treat inflammation, has shown significant *in vitro* action against many bacteria *Staphylococcus aureus*, *pseudomonas aeruginosa*, *pseudomonas fluoroceae*, *klebsiella pneumonia*, *proteus mirabilis, enterobator cloacae*. Nimesulide was shown to exhibit their enhanced inhibitory effect against microbes due to synergism and additive antimicrobial activity with ciprofloxacin and gentamycin. This observation was similar to the previous literatures search among various classes of pharmacological agents have revealed that the tricyclic phenothiazines in general possess moderate to powerful antimicrobial action phenothiazine exhibit reversal of resistances activity [20]. The reduction of microbial growth may be due to antimicrobial resistance was reduced when it was combined with nimesulide. Nimesulide chemically N-(4-Nitro-2-phenoxy phenyl) methane sulfonamide which consists of sulfonamide group and two benzene rings may exhibit the antimicrobial property.

CONCLUSION

Nimesulide may possibly used for the synthesis of more active novel agents with free of side-effects as like a lead compound. As this agent consists of a phenoxy phenyl methane sulfonamide, a sulfonamide group and a phenyl ring. Additionally study about the pharmacological activity of drug is necessary to confirm our findings on the possible use of this drug to treat bacterial infections. With appropriate compound modifications, it may be possible to obtain compounds with greater antimicrobial actions, thereby, creating a new generation of potential non antibiotic drugs. Our next target would be to evaluate the *in vivo* synergistic effect of Nimesulide with gentamicin and ciprofloxacin and establish the ability of extended antibiotic therapy for clinical use as a helper compound in the potential management of *mycobacterium tuberculosis* resistancy.

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