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Comparative Evaluation of Fixed Dose Combination of Ofloxacin and Ornidazole Against Some Aerobic Bacteria

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Abstract: The present study is undertaken to evaluate the antimicrobial properties of a new Fixed Dose Combination (FDC) of Ofloxacin Ornidazole for infusion against some aerobic bacteria in comparison with Ofloxacin and Ornidazole individually. Antibiotic Susceptibility Test (AST) and Minimum Inhibitory Concentration (MIC) of FDC of Ofloxacin Ornidazole, Ofloxacin and Ornidazole for *P. aeruginosa*, *S. aureus*, *E. coli*, *K. pneumoniae*, *S. epidermis* and MRSA were determined by disc method and broth micro dilution method. In Antimicrobial Susceptibility Test, lytic zone of combination was found to be more than Ofloxacin or Ornidazole alone in all microbial strains under study. The MIC value of FDC of Ofloxacin Ornidazole was found to be higher than both Ofloxacin and Ornidazole. The data suggests that fixed dose combination of Ofloxacin and Ornidazole can be a good option for use in mixed microbial infection of aerobic bacteria, anaerobic bacteria and pathogenic protozoans.

Key words: Fixed dose combination, Ofloxacin, Ornidazole, mixed microbial infection

INTRODUCTION

Development of antibiotic resistance places a large burden on the health care system, with results in both increased costs and increased morbidity and mortality. Infection with resistant bacteria and multiple infection can significantly increase hospital length of stay and associated costs (French, 2005). The situation becomes worst in the case of mixed infection of aerobic bacteria, anaerobic bacteria and pathogenic protozoans. Fluoroquinolone group of antibiotics are widely used for the treatment of various infections and have efficient oral absorption, long serum elimination half-lives, good tissue distribution and a broad range of activities against aerobic pathogens (Hooper and Wolfson, 1991). Present trend to combat the resistance of organisms against antibiotics is to use various newer molecules and newer combination of drugs. This has resulted in the widespread use of drugs and the development of several new agents and combinations of antibiotics over the past decade.

The *in vitro* antibacterial activity of fluoroquinolones evident against Gram positive and negative bacteria including activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Biedenbach *et al.*, 1995; Masuda *et al.*, 1996; Khan *et al.*, 2008). Ofloxacin is one of the most effective and quinolones which is used in current clinical practices. When first introduced, Ofloxacin could only be administered orally, but recently a parenteral form has been developed with favorable pharmacokinetic properties, including high serum

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concentrations and a long terminal half-life, resulting in a higher area under the concentration-time curve (AUC) than comparative drugs such as ciprofloxacin (Farinotti *et al.*, 1988; Gruenberg *et al.*, 1988; Lode *et al.*, 1987).

Ornidazole is a nitro imidazole derivative with antiprotozoal and antibacterial properties. Ornidazole is used preferably in the treatment of abdominal or gynaecological anaerobic infection (Goldstein *et al.*, 1978). In some clinical conditions of mixed infection, combinations of antibacterial and antiprotozoal agents is required to increase the antimicrobial spectrum. The combination of antibiotics have also been attempted in order to overcome resistance development of microorganisms (Michael *et al.*, 1990).

There are some studies made to analyze serum bactericidal activities of Ofloxacin in combination of Ornidazole (Michael *et al.*, 1990). Some researchers have also established that the combination of ofloxacin and ornidazole is non toxic even at maximum prescribed individual drug level (Chaudhary *et al.*, 2009). Ornidazole has an established profile against protozoans and anaerobic bacteria whereas Ofloxacin can be used against aerobic microorganisms. The present study is undertaken to evaluate the antimicrobial properties of novel FDC of Ofloxacin Ornidazole infusion in some aerobic bacteria in comparison with Ofloxacin and Ornidazole individually.

MATERIALS AND METHODS

Bacterial Strains

Following strains obtained from Microbial Type Collection Center of Institute of Microbial Technology, Chandigarh, India were used for the study-*Pseudomonas aeruginosa* (MTCC No.-1688), *Staphylococcus aureus* (MTCC No.-737), *Escherichia coli* (MTCC No.-1687), *Klebsiella pneumoniae* (MTCC-109), *Staphylococcus epidermis* (MTCC No.-435). Methicillin Resistant *Staphylococcus aureus* (MRSA) was obtained from PGI, Chandigarh as a clinical isolate.

Antibiotics

Infusion of Ofloxacin Ornidazole fixed dose combination (700 mg 100 mL, in ratio of 1:2.5), Ofloxacin and Ornidazole used in study were provided by manufacturer (Venus Remedies Limited, India) for the study.

Medium

Mueller-Hinton (MH) broth supplemented with calcium (25 mg L⁻¹) and magnesium (1.25 mg L⁻¹) was used for susceptibility tests. Colony counts were determined with MH agar plates.

Efficacy Testing

Antibiotic Susceptibility Test (AST) and Minimum Inhibitory Concentration (MIC) of Ofloxacin Ornidazole combination, Ofloxacin and Ornidazole for *P. aeruginosa*, *S. aureus*, *E. coli*, *K. pneumoniae*, *S. epidermis* and MRSA were determined by disc method and broth micro dilution method as per the standard (NCCLS, 1997). Overnight MH broth cultures were used to prepare inocula of 10⁵ cfu mL⁻¹. The MIC was performed in Ofloxacin Ornidazole Infusion of fixed dose combination having the ratio of 1 : 2.5, Ofloxacin and Ornidazole. Thirty microgram of Ofloxacin and Ornidazole individually and 30 µg of Ofloxacin with 30 µg of Ornidazole in combination were taken on each disc for AST. Minimum inhibitory concentration and AST studies were performed at Microbiology and Biotechnology

Laboratory of Venus Medicine Research Centre, India. These studies were carried out from January 2009 to March 2009.

Statistical Analysis

One-way analysis of variance (ANOVA) with student-Newman-Keuls comparison test was used to determine statistical difference between different groups under AST study. p values < 0.05 were considered statistically significant.

RESULTS

MIC Studies

In case of *P. aeruginosa*, *S. aureus*, *E. coli*, *K. pneumoniae* and MRSA MIC value of Ofloxacin Ornidazole combination was found to be higher than Ofloxacin. In *S. Epidermis* MIC value was found to be similar to the combination. In case of Ornidazole, MIC value of 256 was found in *E. coli* and *P. aeruginosa*. Minimum inhibitory concentration of 512 $\mu\text{g mL}^{-1}$ was found in *S. aureus*. In all other organisms growth was found in all conditions (Table 1).

Susceptibility Studies

In Antimicrobial Susceptibility Test, lytic zone of combination was found to be more than Ofloxacin alone in all microbial strains under study. Lytic Zone less than 10 mm was found in *S. aureus*, *E. coli* and *P. aeruginosa* in Ornidazole whereas no other organism showed any lytic zone development with Ornidazole (Fig. 1).

Table 1: The MIC values of fixed dose combination of Ofloxacin Ornidazole, Ofloxacin and Ornidazole

Organism	Ofloxacin	Ornidazole	Ofloxacin ornidazole
	($\mu\text{g mL}^{-1}$)		
<i>S. aureus</i>	4	512	16
<i>S. epidermis</i>	8	-	8
MRSA	8	-	32
<i>E. coli</i>	1	256	8
<i>K. pneumoniae</i>	4	-	8
<i>P. aeruginosa</i>	8	256	32

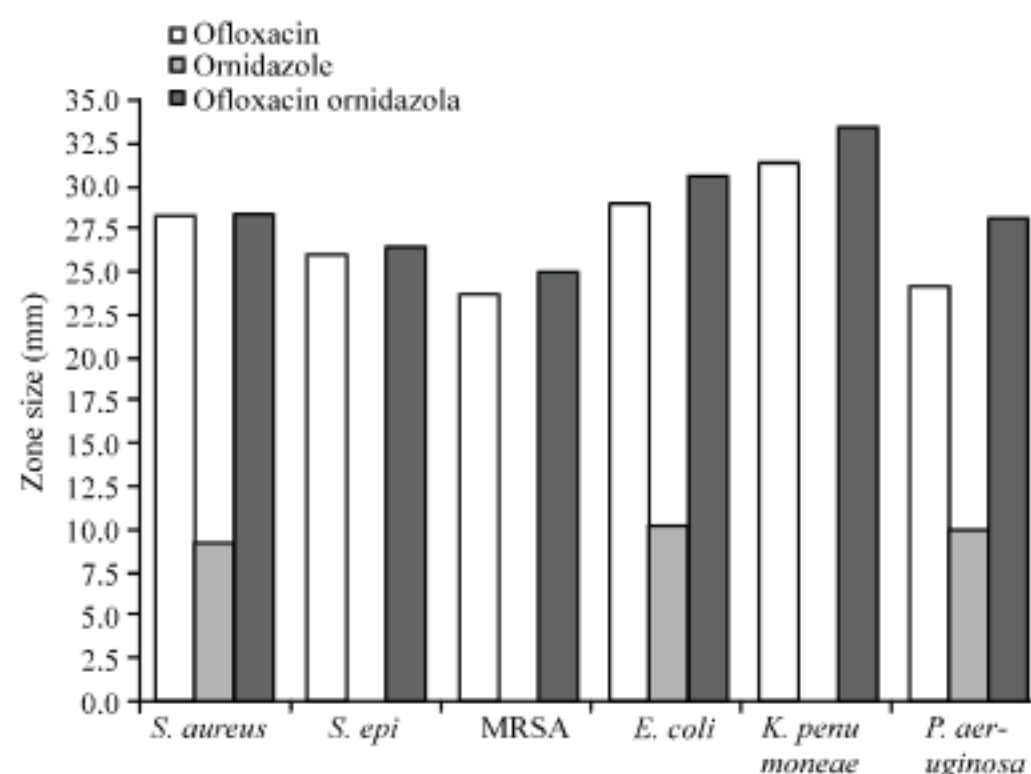


Fig. 1: Lytic Zone size (mm) in Antibiotic susceptibility test of fixed dose combination of Ofloxacin Ornidazole, Ofloxacin and Ornidazole

DISCUSSION

As the problem of antibiotic resistance is growing in mixed microbial infection, awareness of use of fixed dose combination for antibacterials has also increased. It is the present trend to evaluate interaction and efficacy of antibiotic combinations such as quinolones with other antibiotics like gentamicin to being used overcome the resistance in microorganisms (Iroha *et al.*, 2008). This concept has most firmly taken root in the Intensive Care Unit (ICU), where empiric antibiotic regimens have been developed not only for safety and efficacy of treatment, but also to prevent the rise of resistant organisms (Kollef, 2005). The traditional antimicrobial therapy of choice in any circumstance has been a regimen that is efficacious, safe and inexpensive. However, if that regimen induces antibiotic resistance then efficacy soon suffers. Ofloxacin is a fluoroquinolone with an indication for bacterial infection. It is well documented that Ornidazole is a potent antiprotozoal and also has antibiotic property against anaerobic bacteria (Merdjan *et al.*, 1985; Jokipii and Jokipii, 1977). There have been attempts to combine these antimicrobials into intravenous infusion for treatment of mixed microbial infection particularly gastric tract infection and diarrhea. It is also evident that pharmacokinetics of these groups of antibacterials do not interfere with each other (Michael *et al.*, 1990).

Present study was carried out with the hypothesis that fixed dose combination of Ofloxacin and Ornidazole act synergistically in aerobic bacteria apart from having proven individual anaerobic antibacterial and antiprotozoal properties. There has been increase in MIC value of combination by 2 to 8 folds in all bacteria other than *S. epidermis* under study, when compared with Ofloxacin alone. In case of *S. epidermis* MIC of the combination was found to be similar to Ofloxacin. The MIC of the combination was significantly lower when compared with Ornidazole alone in all organisms under study. Ornidazole practically no therapeutic significant MIC value in organisms under study. In AST, Zone of Ofloxacin alone was found to be similar to FDC with non significant variation. There is statistically significant increase of lytic zone of FDC when compared with Ornidazole alone in *S. aureus*, *E. coli* and *P. aeruginosa* under this study. The FDC has proven efficacy against aerobic bacteria and expected to have efficacy against anaerobic bacteria and pathogenic protozoans. There are earlier reports describing antimicrobial properties of Ofloxacin (Farinotti *et al.*, 1988; Gruenberg *et al.*, 1988) and Ornidazole (Goldstein *et al.*, 1978) individually. There is no earlier work done on antimicrobial efficacy of these drugs in combination, although there has been published reports of toxicological studies of this FDC (Chaudhary *et al.*, 2009).

In the present study, antimicrobial properties of FDC of Ofloxacin Ornidazole infusion is evaluated in some aerobic bacteria in comparison with Ofloxacin and Ornidazole individually. Minimum inhibitory concentration and AST results suggests that the FDC is more effective than Ofloxacin or Ornidazole alone in all organisms under study. It is already established that Ornidazole has got activity against anaerobic bacteria and pathogenic protozoans. It may be concluded that fixed dose combination of Ofloxacin and Ornidazole can have be a good potential for use in mixed microbial infection of aerobic bacteria, anaerobic bacteria and pathogenic protozoans.

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