In Vitro Study of the Interaction between Amoxicillin and Norfloxacin with Lansoprazole

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Abstract

The disc diffusion method (DDM) was used to evaluate the in vitro interaction of amoxicillin and norfloxacin with lansoprazole against selected bacterial isolates (Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi). Minimum inhibitory concentration (MIC) of the drugs was determined separately and in combination with lansoprazole (0.6 mg/ml), against S. aureus, Ps. aeruginosa and S. typhi. The result of the study revealed that lansoprazole produced an increase in the MIC of amoxicillin and norfloxacin against the test organisms. Amoxicillin had no effect on Ps. aeruginosa and S. typhi.

Key words: Interaction study, amoxicillin, norfloxacin and lansoprazole.

Introduction

In rational drug therapy, the concurrent administration of two or more anti-microbial agents is often essential and sometimes mandatory in order to achieve the desired therapeutic aim or to treat co–existing disease. However, drug interaction may have different effects on the host as well as the infecting organism and can decrease potency, increase adverse effect or toxicity (Harry *et al* 1998, Brooks *et al* 1998 and Jawetz 1987). Treatment of mixed infections, therapy of severe infections in which a specific causative organism is known, enhancement of antibacterial activity in the treatment of specific infections and prevention of the emergence of resistant micro-organism have been a problem militating against combined anti-microbial therapy (Aguwa 1986, Hugo *et al* 1993). As a result, it is highly expedient that the *in vitro* interaction of combination of anti-microbial be evaluated using suitable test microorganisms before such combination are clinically used (Weisser *et al* 1966).

Antibiotics have been used in the treatment of ulcers likewise amoxicillin and norfloxacin in the treatment of typhoid fever (Altman 1998). Intractability (lack of response to H_2-blockers or antacids) of peptic ulcers remains a problem among older people and cigarette smokers. Alternative pharmacological approach has been switching to a proton-pump inhibitor (Altman 1998). Concern has arisen that long term use of highly effective antisecretory drugs may increase the risks of gastric neoplasia and consequently prolonged hypochlorhydria which favours the colonization of stomach by bacteria that have the potential to convert ingested nitrates into carcinogenic nitrosamines in rats. Although this is not proven a health hazard but certainly unlikely with short-term use e.g. a daily dose of 30 mg of lansoprazole for 8 weeks (Lawrence et al 1997). Patients who are demonstrated to have *H. pylori,* intractability warrants a trial of combination of antibiotics and bismuth (Altman 1998). Amoxicillin, like all beta lactam antibiotics inhibit bacterial growth by interfering with the cross-linking of peptidoglycan strands in bacterial cell wall synthesis (Chambers *et al* 1998; Rodringuez *et al* 1987). Norfloxacin is fluorized quinolone particularly suited for complicated and therapy resistant urinary tract infections (Van Der Auwera 1992). Norfloxacin blocks bacterial DNA synthesis by inhibiting bacterial topoisomerase II (DNA gyrase) and topoisomerase IV thus prevents relaxation of positively supercoiled DNA that is required for normal transcription and replication. Lansoprazole is a benzimidazole proton pump inhibitor in that it blocks the final step of acid production in the gastric parietal cell. The aim of this research is to evaluate the clinical suitability of the use of combinations of amoxicillin-lansoprazole and norfloxacin-lansoprazole against *S. aureus* and against resistant strains of *S. typhi* and *Ps. aeruginosae.*

Materials and methods

Bacterial isolates of *S. aureus, Ps. aeruginosa* (Department of Pharmaceutics, University of Nigeria, Nsukka). Multi-drug resistant strains of *Salmonella spp* (Bishop Shanahan Hospital, Nsukka). Nutrient broth, Nutrient agar (International Diagnostics Group, UK), amoxicillin sodium (Evans Pharmaceutical PLC), norfloxacin (Ranbaxy), lansoprazole (Elbe Pharmaceuticals Ltd.). All other solvents and reagents were of analytical grades.

Isolation and purification of test microorganism: All isolates after culturing were Gram stained. A sterile wire loop was used to collect a little of each stock and this was then streaked on sterile nutrient agar plates. Subsequent culturing in selective media for the different organism was carried out.

Standardization of Microbial Isolates: A 10 ml volume of sterile water was added to the agar slant containing a 24 h old culture of the desired microorganism and shaken carefully to harvest the organism. Subsequently, dilutions were carried out

Table 1: MIC of the antibiotics and their combinations with the test organisms

Organisms	Antibiotics	MIC (mg/ml)	
		Alone	With Lansoprazole
Staphylococcus aureus	Amoxicillin	0.1496	0.1660
Pseudomonas aeruginosa	Norfloxacin	0.0513	0.1445
Salmonella typhi	Norfloxacin	0.2344	0.3090
Salmonella typhi	Norfloxacin	0.0750	0.0955

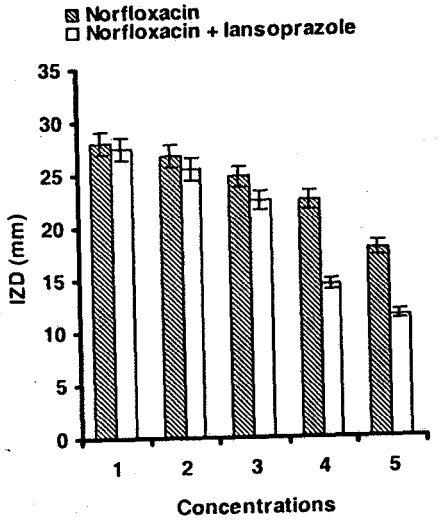

Fig. 1: Statistical representation of the IZD of norfloxacin alone and norlfoxacin-lansoprazole combination against S. aureus (1=4mg/ml; 2=2mg/ml; 3=1mg/l; 4=0.5mg/ml; 5=0.25mg/ml)

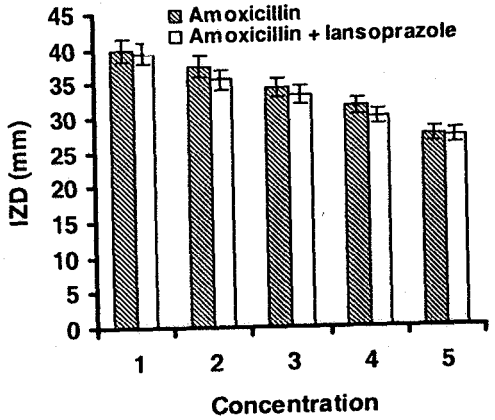

Fig. 2: Statistical representation of the IZD of amoxicillin alone and amoxicillin-lansoprazle combination against S. aureus (1 = 5 mg/ml; 2 = 2.5 mg/ml; 3 = 1.25 mg/ml; 4 = 0.625 mg/ml; 125 mg/ml)

to get a microbial population of 10^5 cfu/ml by comparing with Mcfarland 0.5 standard.

Preparation of drug stock solution: Stock solutions of amoxicillin, norfloxacin and lansoprazole were prepared on each occasion by weighing and subsequent dissolution in a calculated volume of double strength nutrient broth to get a desired, drug concentration. Aseptic conditions were maintained in each case. Sterile ten-fold dilutions of the stock were carried out to get the appropriate drug concentrations in 10 ml single strength nutrient broth contained in test tube

Determination of the minimum inhibitory concentration of the antibacterials (amoxicillin and norfloxacin) and their combinations with lansoprazole against *S. aureus, S. typhi* and *Ps. aeruginosa*: A 0.02 ml volume of the inoculum prepared from the cultures was used. For *S. aureus*, amoxicillin and lansoprazole solutions were prepared in sterile distilled water to give initial starting concentrations of 5 mg/ml and 0.6 mg/ml respectively. Also for the same organism, norfloxacin and lansoprazole solutions were prepared in distilled water to give initial starting concentrations of 4 mg/ml and 0.6 mg/ml respectively. For *Ps. aeruginosa* and *S. typhi*, the initial concentrations of norfloxacin and lansoprazole were also 4 mg/ml and 0.6 mg/ml.

Using the strip agar diffusion method, ten empty sterile petri dishes were marked. An inoculum of the culture, *S. aureus* and that for *Ps. aeruginosa*, were added to the tubes containing molten nutrient agar (45 °C). The content was poured into the sterile dishes and mixed thoroughly to ensure an even distribution of the organism. These agars were allowed to set in about 10 min and dried. With wax pencil, perpendicular lines crossing the middle of petri dish were drawn giving two sections. Using a flamed forceps, one paper disc was dipped into the first dilutions of the amoxicillin and norfloxacin solutions, which were 5 mg/ml and 4 mg/ml respectively and drained in air to remove the excess solution. By the aid of sterile loop, the soaked disc was placed on the surface of the inoculated agar plate. The above steps were carried out for the different dilutions of amoxicillin, norfloxacin and their combinations with lansoprazole. Replicates of each dilution were made for accurate results. The filter paper disc inside the lid of each plate were allowed at room temperature to diffuse for 2 h and incubated in an upright portion for 24 h at 37 °C. The diameters of the zones of inhibition produced by the various concentrations of the antibacterials and their combinations were measured.

Analysis of the data: The interaction data was analysed by statistically representing the effects produced by the lansoprazole – drug interaction on a multiple bar chart.

Results and discussion

There was no increase in MIC observed for amoxicillin with lansoprazole against *Ps. aeruginosa*. This may partly be due to the more complex cell of *Ps. aeruginosa* (Wiblin 1997; Wick *et al* 1990; Passador *et al* 1995). The Gram-negative bacteria, *Ps. aeruginosa* has been shown to exhibit strong resistant properties against most penicillin like the ampicillin and its congeners. Concanon *et al* (1986) studied the effect of temperature on the stability of penicillins, which showed that ampicillin and amoxicillin degraded faster as temperature increased from 37 – 42 °C. It may be inferred that the increase in MIC of norfloxacin on combination with lansoprazole against *Ps. aeruginosa* was due to resistance offered by the bacteria probably as a result of β-lactamase production; various modification of the penicillin binding proteins on their cell wall and decrease in drug permeability; and also because of degradative tendencies that may have occurred during the long hours of incubation at the temperature of 37 °C.

There was no observed increase in MIC for amoxicillin with lansoprazole against *S. typhi*. This may be due to the aforementioned complex cell of *S. typhi*, which has been shown to exhibit resistant properties against most penicillin like ampicillin and its congeners (Finley *et al* 1992; Baine *et al* 1978; Giannella *et al* 1973).

As soon as the bacterial cells were inoculated into the nutrient agar, physiological processes leading to the growth of the cells began and consequently cell growth. Also, as soon as the antibiotic disc was introduced on the surface of the agar, diffusion of the drug into the agar commenced. In this study, the two events proceeded concurrently. A zone of inhibition is produced when a bacteriostatic concentration of the antibiotic reaches a certain distance in the agar just before visible growth develops. The MIC of each of the antibiotics increased when combined with lansoprazole (Table 1). The increase in the MIC of amoxicillin and norfloxacin on combination with lansoprazole may be attributed to the decreased solubility and stability provided by lansoprazole solution medium.

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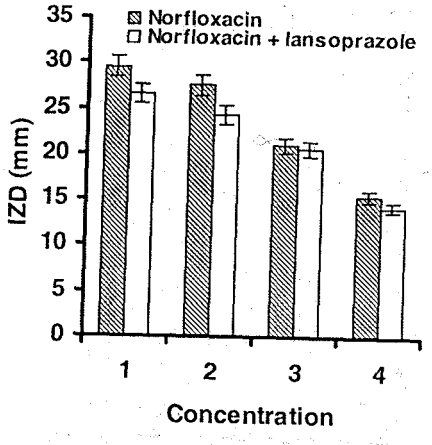

Fig. 3: Statistical representation of the IZD of norfloxacin alone and norfloxacin-lansoprazole combination against *Ps. aeruginosa* (1 = 4 mg/ml; 2 = 2mg/ml; 3 = 1 mg/ml; 4 = .5mg/ml;)

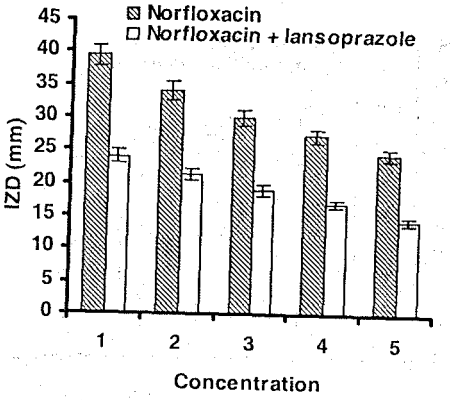

Fig. 4: Statistical representation of the IZD of norfloxacin alone and norfloxacin-lansoprazole combination against *S. typhii* (1 = 4mg/ml; 2 = 2 mg/ml; 3 = 1 mg/ml; 4 = 0.5 mg/ml; 5 = 0.25 mg/ml)

Conclusion: The results so far show that lansoprazole possess some antimicrobial activity against the test organisms. This probably gives an insight to the possibility of concurrent use of these antibiotics and lansoprazole especially in peptic

ulcer. It is anticipated that the result of this *in vitro* study be extrapolated to *in vivo* action. But since all the combinations produced increased MIC as against the antibiotics alone, they should therefore, not be administered concurrently.

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