In Vitro Evaluation of the Interactions between Chloramphenicol and Lamivudine against *Escherichia coli* and Nystatin and Lamivudine against *Candida albicans*

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Abstract

Escherichia coli (E. coli) and Candida albicans account for 30 % of organisms that are responsible for opportunistic infections which occur at the early and late stages of HIV infection. An in vitro study was carried out on the interaction between chloramphenicol and lamivudine, nystatin and lamivudine using sterile paper strips against hospital isolates of E. coli and C. albicans respectively. Sensitive strains of E. coli and C. albicans were used. The combined drugs showed higher effects than when the antibiotics were used singly. Chloramphenicol showed greater effect in combination with lamivudine against E. coli than when nystatin was combined with lamivudine against C. albicans.

Keywords: Chloramphenicol, nystatin, lamivudine, interactions, combinations, *E. coli, C. albicans*

Introduction

The human immunodeficiency virus (HIV) is a member of the retroviral family of viruses commonly called retroviruses and classified in the subfamily of the lentivirus (Dechercg, 1990). Infection with the HIV virus in humans results in a complex clinical disease known as the Acquired Immune Deficiency Syndrome (AIDS) which may take up to ten years or more to manifest. HIV infects human cells by binding its envelope glycoproteins GP 120 to the CD_4 on the surface of the cells of the T-lymphocytes (Redfield and Burk, 1988). Upon fusion it uses the replication machinery of the host cells to replicate producing new HIV particles which is released by budding from the cell surface taking a piece of the cell membrane as their envelope. HIV infection can readily and directly kill CD_4 (T_4 lymphocyte) cells. The loss of these cells paralyses the immune system and is one mechanism by which HIV infection causes AIDS (Redfield and Burk, 1988). At this point opportunistic infections such as those caused by *E. coli, C. albicans, Pneumocystis carinii, Mycobacterium avium,* cytomegalovirus, herpes virus, Epstein Barr virus, etc, occur. This is because the immune system can no longer protect the body against microorganisms normally found in our environment (Timbury, 1986). Thus the treatment of HIV infection often involves the use of an anti-HIV drug and those drugs used in the treatment of one or more of these opportunistic infections. This study is aimed at evaluating the interaction between the anti-HIV drug, lamivudine with the antibacterial drug chloramphenicol against *E. coli* and with the antifungal drug nystatin against *C. albicans.*

Material and Methods

Materials: The culture media used in this study were nutrient agar (Merck, Germany) and Sabouraud dextrose agar (Oxoid, England). Chloramphenicol (Eurogen); nystatin (Varhman Export, India) and lamivudine (Ranbaxy Lab. Ltd., India) were obtained from commercial sources.

Hospital isolates of *Escherichia coli* and *Candida albicans* were obtained from laboratory diagnostic unit of Bishop Shanahan Hospital, Nsukka.

Sterilization of Materials: The Petri dishes, test tubes, plugged with cotton wool and pipettes packed in metal canisters were loaded appropriately and sterilized in a hot air oven (Model OV- 335 Hereaus) at 170 °C for 1 h at each sterilization cycle. Sterilization of the culture media were done by autoclaving at 121 °C for 15 min in the Gallenkamp autoclave.

Preparation of Culture Media; All culture media used in this study were prepared according to manufacturers' specifications.

Sensitivity Test Using Nutrient Agar: A 20 ml aliquot of molten nutrient agar in sterile Petri dish was seeded with 0.1 ml of *E. coli,* gently swirled round to distribute and allowed to stand on a horizontal plane. Fresh solution of 25 mg/ml of chloramphenicol and another containing 15 mg/ml of lamivudine respectively were prepared. A paper strip was dipped into the antibiotic solution and the excess solution was drip-dried in the air. The soaked paper strip was placed on the surface of the seeded agar plate. Then another paper strip was dipped into the lamivudine alone and placed perpendicularly to the first containing the chloramphenicol. This was incubated at 37 °C for 24 h, after which the plate was examined visually and the zone of inhibition at the point of contact measured and at the far end for chloramphenicol alone.

Determination of antifungal effects using Sabouraud dextrose agar: Exactly 20 ml of molten nutrient agar was poured on sterile Petri dish. This was seeded with 0.2 ml of *C. albicans*, well distributed by swirling it round clockwise and anticlockwise three times each and allowed on a horizontal plane. Paper strips were socked in a solution of 16.67 mg/ml and 15 mg/ml for nystatin and lamivudine respectively. They were placed at right angles to each other and touching at one end as described above for chloramphenicol. The plate was incubated at 37 °C for 24 h after which the plate was examined visually and the zones of inhibition at the points of contact were measured and also at the far end for nystatin alone.

Determination of the MIC of Chloramphenicol in the Presence of Lamivudine: A 20 ml sterile nutrient agar plate was prepared. A concentration of chloramphenicol containing 25 mg/ml but containing lamivudine 15 mg/ml was prepared. Cups (4 per plate) were made in the sterile nutrient agar using 8 mm cork borers. Two drops of the combined drugs were placed in each well and allowed to soak into the medium before incubating. The plate was incubated at 37 °C for 24 h after which it was examined visually and the zones of inhibition measured.

Determination of the MIC of Nystatin in the Presence of Lamivudine: A 20 ml volume of sterile Sabouraud's dextrose agar plate was prepared. A solution containing 16.67 mg/ml of nystatin and 15 mg/ml of lamivudine was prepared. Wells (4 per plate) were made in the sterile agar using 8 mm cork borers. This was incubated at 37 °C for 24 h, after which it was examined visually and the zones of inhibition measured.

Results

Table 1 shows the effects of the drugs on the selected organisms both singly and in combination with lamivudine.

Table 1: Effects of the drugs singly and in combination

Drug	*Chloram. alone (IZD mm)	Chloram. +Ωlam. (IZD mm)	Nystatin alone (IZD mm)	Nystatin +lam. (IZD mm)
E. coli	22	28	N/A	N/A
C. albicans	N/A	N/A	24	24

*Note: * Chloram. =chloramphenicol; Ωlam. = lamivudine; IZD = inhibition zone diameter*

From the results it seems that there was a slight increase in the antimicrobial effect of chloramphenicol against *E. coli* in the presence of lamivudine. This was not the case with nystatin. The lamivudine showed no antimicrobial effect and hence no result is presented for it.

The results of the minimum inhibitory concentrations deduced from Figs. 1 and 2, however, show that there were differences in the antimicrobial actions of the antimicrobial agents in the presence and absence of lamivudine. The lamivudine decreased the MIC of chloramphenicol from 3.981 µg/ml to 0.398 µg/ml while it reduced that of nystatin from 3.68 µg/ml to 2.57 µg/ml. Thus the effect of lamivudine was more effective against *E. coli* in combination with chloramphenicol than it was with nystatin.

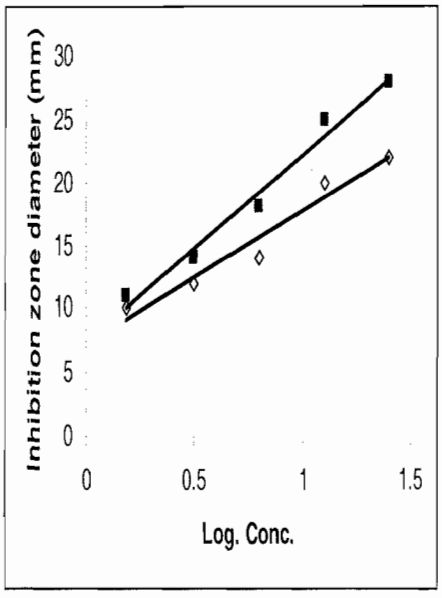

Fig. 1: Minimum inhibition determination of chloramphenicol alone and in the presence of lamivudine: ▌Chloramphenicol alone; ⁞⁞ *chloramphenicol* +lamivudine

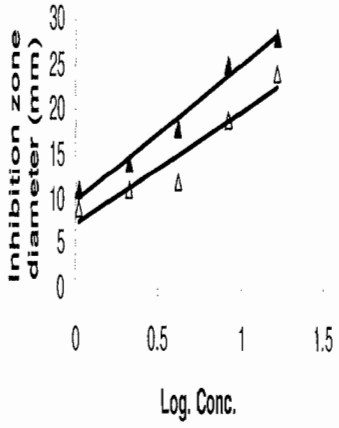

Fig. 2: Minimum inhibition determination of nystatin alone and in the presence of lamivudine: ▲nystatin alone; △ nystatin+lamivudine

Discussion

The simultaneous action of two drugs can result in an effect which could be much greater than that of either the first drug or the second drug alone. The combination may also produce effect which is lower

that of both when used singly. The effects may also be indifferent (Okore, 2005). Thus it is essential to study the effect of combination of drugs to evaluate whether there could be enhanced or decreased effect when used together in chemotherapy.

E. coli is Gram negative motile rod shaped bacterium which can be sensitive or resistant to antimicrobial agents depending on the strain. It is implicated in various forms of diarrhoea in AIDS patients. Different strains have been implicated. They could be enterotoxigenic or enteroinvasive. From this study *E. coli* strain which was of hospital origin, was sensitive to chloramphenicol. When the two drugs were combined the effect of the chloramphenicol was potentiated. *E. coli* was sensitive to chloramphenicol because the drug is able to bind to the 50s unit of the ribosome thereby blocking or inhibiting the protein synthesis by preventing growth of peptide on the ribosome thus inhibiting the organism completely (Okamoto and Suzaki, 1965).

Lamivudine has no effect against *E. coli* and *C. albicans* because the organisms do not have the receptor site where the drug can bind. Lamivudine is an antiviral drug that has affinity for mostly viruses and from its mode of action it works as a DNA-chain terminator (Lorian, 1991; Redfeild and Burk, 1988). It is most used in immune-compromised persons and effective in HIV patients because its helps to revalidate the down graded immune system of patients with serious infections. E. coli does not recognize the envelope glycoprotin GP 120 cells as a receptor site which HIV viruses bind to and because of this cannot bind to the human receptor cells which are the receptors which lamivudine attacks to reduce the rate in which the GP_4 cells of the T- lymphocytes and other co-receptors are destroyed (CDC, 1992).

The result obtained from the combination of nystatin and lamivudine was not significant, when tested statistically. The nystatin alone however, was effective against the fungus *C. albicans* which is a major opportunistic fungus that afflicts AIDS patients. Nystatin is a polyene antifungal antibiotic which acts mainly by interfering with the permeability of the cell membrane by binding to sterols. When this happens it destabilizes the integrity of the cell membrane and thus increases the leakage of cell material components.

The *in vitro* susceptibility patterns of many antimicrobial combinations against various micro organisms have been demonstrated, though the *in vitro* studies do not always correlate with the *in vivo* studies. Interactions that occur *in vitro*, therefore, might not be applicable *in vivo*. The actual establishment of synergism between two antimicrobial agents in the laboratory is, however, very important because the effect of antimicrobial combination from observation is both ratio and isolate dependent.

Conclusion: In conclusion the combination of chloramphenicol and lamivudine against *E. coli* could give a successful therapeutic value because of the expected enhanced activity. Nystatin and lamivudine combination in clinical condition against *Candida albicans* may not have serious therapeutic advantage.

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