No: _____________________ Date: ______________________

Title

IN VITRO EFFECTS OF VORICONAZOLE AGAINST ASPERGILLUS FUMIGATUS IN PRESENCE OF CURRENT QUINOLONES AND SIMVASTATIN

Name of Candidate: Sonia Qureshi

Name of Supervisor: Associate Prof. Dr. Niaz Ali

Co-Supervisors:
1. __________________________________________________
2. __________________________________________________
3. __________________________________________________

Duration of Project: 6 months

Institute: Institute of Basic Medical Science, Khyber Medical University, Peshawar.

Budget Required: Rs 62500

Name & Signature of Student/Scholar: Sonia Qureshi

Name & Signature of the Supervisor: Associate Prof. Dr. Niaz Ali

Name & Signature of Head of Institute: Prof. Dr. Jawad Ahmed
1. TITLE:

In vitro Effects of Voriconazole against Aspergillus fumigatus In Presence of Current Quinolones and Simvastatin

2. INTRODUCTION:

Patients, sometime, suffer from mix infections like invasive mycosis and bacterial infections. This is very common in pulmonary disease and pelvic inflammatory diseases (PIDs), where a combination of antifungal and antibacterial agents is in practice (1). There are reports of in vitro pharmacodynamics interactions of fluoroquinolones and antifungal agents like voriconazole and fluconazole (2, 3).

The condition becomes more complex when our patients are suffering from other concomitant illness or taking other lifelong drugs. Hypercholestremia is a condition associated with increased level of cholesterol in the blood, thus patient take lipid lowering drugs lifelong (4). When hypercholestremia patients suffer from condition of PIDs and different clinical conditions with suspected mycoses. This makes the condition more complex as the patients are already managed for hypercholestremia with lipid lowering therapy and they require a combination of antibacterial agents, most frequently fluoroquinolones, and antifungals like fluconazole and voriconazole. This leads to practice of polypharmacy which makes the situation more complex as it is sometimes associated with poor prognosis or shatters the quality of life of patients in shape of possible adverse drug reactions(5, 6). Statins are 3-hydroxy-3- of taryl-coenzyme A (HMG-CoA) reductase inhibitors(7). Statins are the most commonly advised agents in condition like hypercholestremia in order to lower the LDL level in blood(8, 9). The reason for their most frequent recommendation to be used against hypercholestremia is their excellent tolerability and appreciable safety profile(10). So far we have observed that statins affect the entry of calcium across the membrane (our unpublished data), and whereas, yeast requires calcium for their growth. Whereas, patients who are already on statins if require antifungal and antibacterial agents, on the analogy of above clinical conditions, hence we suspect that in vitro pharmacodynamics interactions may exist which may ultimately affect the therapeutic outcomes.
3. OBJECTIVE(S):

Thus we set our objectives to:

1. To determine the effects of Simvastatin on voriconazole antimicrobial activity in presence of current quinolones against *Aspergillus fumigatus*.

4. OPERATIONAL DEFINITIONS:

**Antibiotic:** A substance produced by one microorganism that are selectively inhibits the growth or kill the microorganism at low concentration.

**Synergism:** The interaction or cooperation of two or more organizations, substances, or other agents to produce a combined effect greater than the sum of their separate effects.

**Antagonism:** where the involvement of multiple agents reduces their overall effect.

5. HYPOTHESIS (If required)

Statins may affect the efficacy of voriconazole in presence of current fluoroquinolones.

6. MATERIALS AND METHODS:

**6a. Study Design:** Experimental study

**6b. Study Settings:** Khyber Medical University, IBMS Peshawar

**6c. Study Duration:** 6 months
7. SAMPLE SELECTION:

7a. Inclusion Criteria:

1) Pure culture media is used for each test.

7b. Exclusion Criteria:

1. Contaminated culture media.
2. Expired culture media.

8. DATA COLLECTION PROCEDURE:

This study will be conducted at Pharmacology department of IBMS, Khyber medical university Peshawar. The isolates of *Aspergillus fumigatus* will be collected from Lady Reading hospital Peshawar preferably from ICU or pulmonology ward. Alternatively preserved isolates of *Aspergillus fumigatus* may be used. The isolates will be preserved at -70 degree centigrade using potato dextrose agar slants, isolates will be cultured on Saboraud dextrose agar in the laboratory, Maize agar medium can be used alternatively. Aspergillus suspensions will be prepared using respective solvents with support of spectrophotometer at 530nm where its transmittance will be adjusted between ranges of 755 to 775. These suspensions will be subsequently diluted two times to give a final inoculum within range of 5*10^2—2.5*10^3 cfu/ml (cfu colony forming unit)

**Preparation of drug solutions:**

The ranges of anti-fungal voriconazole concentration will be around its MIC (0.03-2mg/L). concentration of quinolones and statins will be as per their respective plasma concentration following their average current doses. Agar tube dilution assay will be used for assessment of effects of statins and current fluoroquinolones on antifungal activity of voriconazole using the following groups.

- Group 1: Negative control
- Group 2: Voriconazole
- Group 3: Voriconazole+ Levofloxacin
- Group 4: voriconazole + Ciprofloxacin
- Group 5: Voriconazole+ Moxifloxacín
- Group 6: Voriconazole +simvastatin
- Group 7: Voriconazole+ Levofloxacin+simvastatin
- Group 8: Voriconazole+ciprofloxacin+simvastatin
- Group 9: Voriconazole+moxifloxacín+smvastatin
Following steps will be involved in agar tube dilution assay

1. Test samples as per above groups will be dissolved in sterile DMSO that will serve as stock solution.
2. Saboraud dextrose agar will be prepared by mixing Saboraud 4% glucose agar in distilled water.
3. It will be then stirred with magnetic stirrer so that all material will be equally suspended, then will be transferred into capped test tubes. Test tube containing media will be autoclaved at 121 degree centigrade for 15 minutes.
4. Tubes will be allowed to cool down to 50 degree centigrade, and test samples of desired concentration will be pipette from stock solution to non-solidified Saboraud agar media. Tubes will be allowed to solidify in slanting position at room temperature. Each tube will be then inoculated with 4mm diameter piece of inoculums removed from seven day old culture of fungi.
5. All culture containing tubes will be inoculated at their optimum temperature (28 to 30 degree centigrade) for a growth period of seven to ten days. Humidity will be maintained using a pan of water in the incubator.
6. Cultures will be examined at least twice a week during the incubation period.
7. After the incubation of 7 to 10 days, test tubes with no visible growth of microorganisms will be considered to represent Minimum inhibitory concentration (MIC).

The observed MIC of different groups will be compared using ANOVA.

9. DATA ANALYSIS:

Data will be analyzed using micro soft excel 2007 and graph pad prism for construction of graphs. ANOVA at 95% of confidence level, \( p \leq 0.05 \) will use.
10. BIBLIOGRAPHY:


ANNEXE:

Annexure I: Data Collection Instrument

Annexure II: Any other Relevant Material (if applicable)

Budget:

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