Explanation of Theophylline site of action in cells of skin pathogenic fungi by inhibition cascade hypothesis

AL-Janabi A. A.*

* University of Karbala, College of Pharmacy

Abstract

Background: The mode of action of theophylline has been determined in mammalian cells by three mechanisms, CAMP phosphodiesterase (PDE) inhibition, adenosine receptor antagonism and calcium mobilization, but after determined its activity against *Trichophyton mentagrophytes*, one of Dermatophytes fungi, therefore, it is needful to be known the effective site of action in the cells of fungi.
Methods: *Trichophyton mentagrophytes* was cultured in Sabouraud's glucose broth that contains 1, 3, and 5 mg/ml of theophylline. Morphological changes were noted in the treated cells when stained with trypan blue using light microscopy in addition to the measurement of the electrical conductivity to the medium which contains such cells.
Result: Morphological changes were noted in *Trichophyton mentagrophytes* cells after incubation in a media containing 3 mg/ml of theophylline. These changes included aggregation of protoplasmic materials at the center of fungal cells (spores and hyphi). Plasma membrane permeability was not affected by this compound after measurements of electrical conductivity and staining by trypan blue stain.
Inhibition cascade hypothesis, that it is proposed by the author, explained these morphological changes according to the supposed series of biochemical steps and depending on information of theophylline biochemical activities in fungal living cells.
Conclusion: Inhibition cascade hypothesis tries to explain the site of action of theophylline by series of biochemical steps according to the information about its mechanism of activities in the mammalian cells. Plasma membrane is not affected by theophylline compound.

Keywords: Theophylline; *Trichophyton mentagrophytes*; cascade; hypothesis

Introduction

Antifungal agents have variable sites of action in fungal cells, but most of the current agents bind avidly to ergosterol in the cell membrane and inhibit ergosterol synthesis. Some antifungals increase the permeability of cell membranes, causing cell lyses [(1)] such as amphotercin B, nystatin and azoles [(1, 2)]. Other antifungal agents like flucytosine inhibit DNA or RNA synthesis, whilst griseofulvin inhibits fungal growth by preventing of mitosis [(3)]*Trichophyton mentagrophytes* is considered as the most important species of Dermatophytes. It has the ability of infection on a wide range of animals and human. The main sites of infection are skin, nail and hair to form a superficial disease called Tinea infection or Dermatophytoses [(4, 5)].

In a previous study, antidermatophytes activity of theophylline was confirmed *in vitro* when it was noted to induce complete inhibition of some species of Dermatophytes at concentration 3 mg/ml. *In vivo* study was also performed with successful results about the use of topical theophylline in treatment of infected animals and human volunteers with Dermatophytoses [(6)]. This study aimed to propose the site of action of

theophylline in fungal pathogen cell, *Trichophyton mentagrophytes.*

Materials and Methods

Trichophyton mentagrophytes was isolated from old male patient aged 23 years suffering from Tinea corporis. Skin scales from an infected area (Hand) were cultured in Sabouraud's glucose cycloheximid-chloramphenicol agar. The media had been prepared from mixing: 20 gm glucose, 10 gm peptone, 15 gm agar, 0.04 gm chloramphenicol, and 0.5 gm cycloheximid in one liter of distill water [(7)]. Cultures had been incubated at 25-28 ˚ C for one week. Diagnosis was performed according to Rippon [(4)] and Emmons [(5)].

Theophylline (Arabic Drug Industry, Iraq) was mixed with Sabouraud's glucose broth (prepared from mixing 20 gm glucose with 10 gm peptone in one liter of distill water) in sterile conical flasks containing 30 ml of media in order to obtain various concentrations (1 mg/ml, 3 mg/ml and 5 mg/ml). One half milliliter of fungal cells (spores and hyphi) suspension containing 2.3 X 10^3 spores/ml counted by Haemocytometer were added to each concentration and incubated at 25-28 ˚ C. Cells were examined many times (30 mint, 1 hr, 2 hr, 3 hr, 6 hr) to check the morphological changes. Two controls were used in this experiment, griseofulvin (1mg/ml), as standard antifungal compound (3) and media free compound.

In order to determine plasma membrane viability, electrical conductivity of the media was measured more than one time by electrical conductivity instrument ALDA (AVD 890 F, China) after inoculation of *Trichophyton mentagrophytes* colony (4 gm) in a conical containing either 40 ml of Sabouraud's glucose broth mixed with 5 mg/ml of theophylline or the two controls (distilled water and media free compound).

Fungal cells viability was also examined after staining of treated cells with a drop of trypan blue stain (Fluka, Switzerland) for approximately 30 minutes using microscopy [(8)].

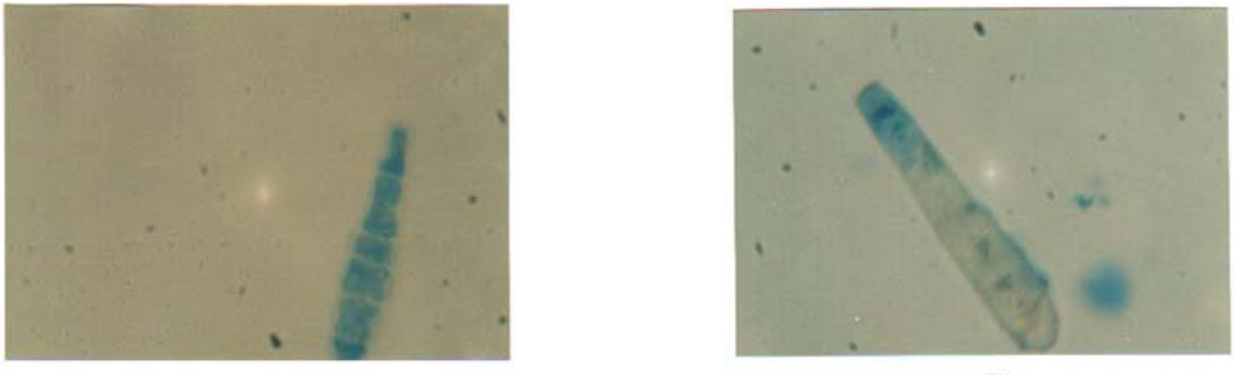
The experiments had been repeated three times with trireplicate of each concentration for statistical analysis by analytical variation (ANOVA) at ($P<0.01$).

Results

Growth of fungal cells in media containing theophylline showed clear morphological changes when it was examined by light microscope, especially at concentrations 3 mg/ml and 5 mg/ml after two or three hours of incubation.

The effect of theophylline on spore and hyphi morphology included aggregation of protoplasmic materials within the central region of cells, (figure1).

Griseofulvin also induced similar changes at concentrations of 1 mg/ml.



A B

Figure (1) :- Macroconidia of *Trichophyton mentagrophytes* staining by Lactophenol cotton blue. X 100.
A- normal macroconidia in Sabouroud's glucose broth .
B- after three days in Sabouroud's glucose broth containing theophylline (5 mg/ml).

None of the spores or hyphi that showed morphological changes were stained by trypan blue, this probably indicated that plasma membrane permeability could be not affected by theophylline. This result was further supported by the measurement of electrical conductivity that was not affected by theophylline when compared to the controls; however the electrical conductivity of media containing theophylline or controls were started to rise after 20 minutes and began to decline after 30 minutes and continued for 24 hours (table 1).

Table (1): Electrical conductivity (mmols/cm) of *T. mentagrophytes* colony in media containing 5 mg/ml of theophylline at various concentrations.

Time	control	Theophylline
Zero time	1061 ± 0.8	980 ± 2
20 mint	1085 ± 1	985 ± 1.2
30 mint	1121 ± 1.2	993 ± 0.3
1 hr	1116 ± 2	975 ± 0.5
2 hr	1124 ± 0.8	986 ± 1
3 hr	1080 ± 1.8	989 ± 0.8
4 hr	1091 ± 1.2	980 ± 1.2
24 hr	1080 ± 0.8	964 ± 0.9

Discussion

The mode of action and site of action of theophylline have been determined in mammalian cells, by three proposed mechanisms, these mechanisms are: cAMP phosphodiesterase (PDE) inhibition, adenosine receptor antagonism and calcium mobilization [9, 10]. There is limited information about theophylline activity against fungi, so the author tries to explain its site of action in fungal cells by supposed hypothesis called (inhibition cascade hypothesis). This is so called on the information in the literatures about theophylline activities. Plasma membrane could not be affected by theophylline according to the electrical conductivity measurement and the lack of treated cells to be stained by trypan blue.

Electrical conductivity is a good indicator for plasma membrane viability. Any defect in the function of plasma membrane will be discovered via measurement ions concentration out side the cells. Fungal colony occurred in broth media, so that any increase in ions concentration in medium will be explained as a disorder in the function of plasma membrane. Trypan blue is a vital stain which is used to differentiate between living and dead cells. Vital cells will take the blue color of the stain, while dead cells will not be stained [8].

Theophylline inhibition cascade hypothesis

Theophylline inhibits cAMP phosphodiesterases (PDEs) of fungal cells after contacts with the surface of the cells [11]. Intracellular cAMP concentration will increase and this will activate protein kinase enzymes that

phosphorylate cytoskeleton filaments proteins e.g. myosin & actin [(12)]. These biochemical events probably alter the contractility of mentioned filaments and lead to the aggregation of protoplasmic materials at the central region of fungal cells and prevented cytoskeleton filament to retain to the normal state.

Similar biochemical steps of this hypothesis were also noted by other study in tissue- culture of mammalian cells (AP-1 cells) treated with 10 μM of forskolin compound (an activator of adenylate cyclase) for
5 minutes at 37 º C and gave the same results by aggregation of protoplasmic materials after contractile of cytoskeleton filaments [(11)].

The increase in cAMP concentration in the amoeba form of fungus Dictyostelium discoidium changes its shape after phosphorylation of heavy & light myosin chains [(12)] and decreasing of branched hyphae of Neurospora crassa at concentration 10-30 mmol [(13)]. Griffin (1981) recorded that cAMP activated fungal protein kinase enzyme and that will affect on microfilments and microtubal behavior of fungal cytoskeleton filaments [(14)].

Mitosis spindle fiber was supposed by Raemaekers et al. (2003)[(15)] that it would be affected by theophylline, especially their associated proteins (Dynein, Dynactin, Y-tubuline and nucleolar spindle associated protein (NuSAP)) when they were phosphorylated by protein kinase to yield morphological changes as noted in Shizosaccharomyces pombe after its spindle fiber was affected.

The site of action of theophylline, according to this hypothesis, depended on structural changing in fungal cell with no effect in the plasma membrane.

References

1- Vanden-Bossche h, Marichal P, Gorrens J, Coene MC, Willemsens G, Bellens D, Roels I, Moereels H and Janssen PA: biochemical approaches to selective antifungal activity. Focus on azole antifungals. *Mycoses*. 1989. 1: 35-52.

2- Haller I: mode of action of clotrimazole: implications for therapy. AMJ Obstet-Gynecol. 1985. 152 (7): 939-44.

3- Gladwin M and Trattler B: Clinical microbiology: made ridiculously simple. Med Master, Inc. Miami. 1999.

4- Rippon, John W. (1988): Medical mycology. W.B. Saunders comp. Philadelphia.

5- Emmons,C.W.,Binford,C.H.&Utz, J.P. (1970): Medical mycology.
2 ed. Lea & Febiger. Philadelphia.pp128-129.

6- AL-Janabi Ali S: Treatment of Dermatophytoses by drugs containing some purine compounds. Ph.D. Thesis. 2004.AL-Mustansiryia unvi., college of science.Iraq (Arabic).

7- AL-Janabi, Ali S. 2005. Perfect media for isolation of fungi from
patients with Dermatophytosis. Journal of Karbala university. 3
(12): 18-21.

8- Prescott, L.M.; Harley, J.P.& Klein, D.A. 1990. Microbiology. Wm.C. Brown publish. U.S.A.

9- Barnes Peter J: Theophylline: New respective for an old drug. Am J respire Crit Car Med. 2003. 167: 813-818.

10- Sawynok J and Yaksh TL: Caffeine as an analgesic adjuvant: A review of pharmacology and mechanisms of action. Am Socit for pharmacology and experimental therapeutics. 1993. 45 (1): 43-85.

11- Szaszi K, Kurashima K, Kaibuchi K, Grimstein S and Orbwski J: Role of the cytoskeleton in mediating cAMP-dependent protein kinase inhibition of the Epithelial Na+ / H+ exchanger NHE3.J Bio Chem. 2001. 276 (44): 40761-40768.

12- Berlot CH, Spudich JA and Devreotes PN: Chemoattractant- elicited increases in myosin phosphorylation in *Dictyostelium.* Cell. 1985. 43 (1): 307-14.

13- Reissig JL and Kinney SG: Calcium as a branching signal in *Neurospora crassa.* J Bacteriol. 1983. 154 (3): 1397-402.

14- Griffin DH: Fungal physiology. John Wiley & sons. New York. 1981.

15- Raemaekers, T; Ribbeck, K; Beaudouin, J; Annaert, W; Van Camp, M; Stockmans, I; Smets, N; Bouillon, R; Ellenberg, J and Carmeliet, G. 2003. NuSAP, a novel microtubule-associated protein involved in mitotic spindle organization. The journal of cell Biology. 162 (6): 1017-1029.