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A new function of Aminophylline as antidermatophytic agent

الوظيفة الجديدة للامينوفيلين كعامل مضاد للفطريات الجلدية Dermatophytes

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Abstract :

Aminophylline (theophylline derivative) is an important drug compound which is generally used to treat bronchoconstriction diseases for a long period of times. Dermatophytoses is the most common disease on human skin caused by many species of dermatophytes.

minophylline was tested against three species with two variants of *Trichophyton* (one genus of dermatophytes) using two methods. Those species were *Trichophyton mentagrophytes var. mentagrophytes, Trichophyton mentagrophytes var.interdigitale, Trichophyton rubrum and Trichophyton simii*. Aminophylline was revealed a high activity against all species of dermatophytes at concentration of 5 mg/ml with a completely inhibited of colony growth on the bases of colony diameter and dry weight measurements. The efficiency of aminophylline to inhibit fungal growth was less than theophylline. Both of aminophylline and theophylline indicated no significant differences value ($P<0.01$) compared with griseofulvin (0.5 mg/ml).

Minimal inhibitory concentration (MIC) of aminophylline was also determined in this study. The MIC value of aminophylline against two species of dermatophytes was observed in 4 mg/ml.

الخلاصة :

يعتبر الامينوفيلين من المركبات الدوائية المهمة المستخدمة لفترة طويلة في علاج أمراض تضييق القصبات . ولكون أمراض الفطريات الجلدية Dermatophytoses من أكثر الأمراض شيوعا" على جلد الإنسان والتي تسببها مجموعة من الفطريات الجلديةDermatophytes ، فقد فحصت قابلية الامينوفيلين باستخدام طريقتين لتثبيط ثلاثة أنواع (مع ضربين لاحدهما) التابعة للفطر *Trichophyton* (احد أجناس مجموعة Dermatophytes) وشملت الانواع:

Trichophyton mentagrophytes var. mentagrophytes, Trichophyton mentagrophytes var.interdigitale, Trichophyton rubrum and Trichophyton simii.

اظهر الامينوفيلين عند تطبيق طريقة قياس قطر المستعمرة ووزنها الجاف قابلية تثبيط كاملة لنمو جميع الفطريات المدروسة عند التركيز ٥ ملغم/مل ولكن بكفاءة تثبيطية اقل من الثيوفيلين والكريزوفيولفين (٠,٥ ملغم /مل)، وان اقل تركيز مثبط للامينوفيلين حدد عند التركيز ٤ ملغم/مل.

Introduction :

Aminophylline is one of theophylline derivative that is formed by mixing theophylline with ethylenediamine (2:1)[1]. It has more improved pharmaceutical efficiency comparable to that of theophylline which is represented by high water solubility of aminophylline. This advantage character gives aminophylline more ability to dissolve in body fluids and in liquid preparations. In general, the pharmacological activities of aminophylline in human body are myocardial stimulation, diuretic action and bronchodilation [2]. Over the years, the use of aminophylline in treatment of acute asthma has fallen from favor [3]. Early studies demonstrated that aminophylline is an inefficient bronchodilator in contrast to adrenergic agonists given by injection or inhalation [4]. Therefore, aminophylline agent would constitute an option only for patients where all other modalities drugs had failed (β-agonist, corticosteroids, intravenous sulbutamol, etc.) [5]. Aslaksen, *et al*.(1981) [6] did not find any significant differences in the pharmacokinetic parameters or protein binding between aminophylline and theophylline. Their demonstration was based on the results of comparing between theophylline (250 mg) and aminophylline (390 mg) preparations that were taken orally by eight healthy volunteers in a randomized cross-over study. Dermatophytes are a specialized group of pathogenic fungi that are usually infected keratinous tissues of humans,

including skin, nail and hair. These pathogenic organisms have acquired, in the evolutionary process, the ability to digest keratin protein (protein resistant to most other micro-organisms) by attacking tissue enrichment with such type of protein. Concerning dermatophytes nutrient source, they produce a wide variety of disease mainly called dermatophytosis with a special clinical characters on their infectious sites [7]. Dermatophytic infections have been progressively increased in recent years due to several factors, such as immunosuppressive host status [8], excessive miscues of systemic/local corticosteroids, environmental pollution and poor hygienic conditions [9]. Development of microorganism's resistance to antifungal agents is a growing problem that possesses a challenge to clinician. Research efforts have been frequently directed towards the development of new antimicrobial agents that can overcome the problems with resistance and for discovered new sources for antifungal drugs.There are several groups of antidermatophytic compounds that are ordinary used today in the treatment of dermatophytic infections. Synthetic azole group, such as itraconazole, ketoconazole, miconazole, clotrimazole, and voriconazole showed highly efficiency to prevent the growth of more than 24 species of dermatophytes [10]. Synthetic allylamine derivatives, including terbinafine and naftifine, have also been confirmed to have antifungal activity mainly against dermatophytes [11]. To our knowledge, no published information has been submitted consisting the use of aminophylline as antimicrobial agent, especially against pathogenic organisms, such as dermatophytes. We can find only high available information about theophylline and aminophylline pharmacological functions in treatment of bronchoconstriction diseases [1, 2, 3]. In previous study, theophylline demonstrated to have antidermatophytic effects after investigated at *in vitro* and *in vivo* levels [11]. The goal of the present study was to evaluate the activity of the most commonly theophylline derivative (aminophylline) against some species of dermatophytes.

Materials and Methods :

Isolation of dermatophytes was performed from patients who have clinical feature of dermatophytosis at Morjan Hospital – Hilla city by scraping of infected skin lesion. After direct microscopically examination with 20 % KOH, positive scales had been cultured on Sabouraud's glucose cycloheximid-chloramphenicol agar (20 gm glucose, 10 gm peptone, 15 gm agar, 0.04 gm chloramphenicol, 0.5 gm cycloheximid and one liter of D.W) [12].

Cultures had been incubated at 25-28 º C for one week. Three species of *Trichophyton* with two variants were diagnosed according to the criteria established by Rippon (1988) [13] and Emmons (1970) [14]:- *Trichophyton mentagrophytes var. mentagrophytes*, *Trichophyton mentagrophytes var.interdigitale, Trichophyton rubrum and Trichophyton simii.*

Two types of methods had been used to determine the antifungal activity of aminophylline when it was mixed in various concentrations with the media.

1-Colony diameter measurement

Colony diameter method, which was described by Özgönen *et al.*(2001) [15] was used to determine the antidermatophytes activity of aminophylline. Briefly, different concentrations of aminophylline were mixed separately with melting Sabouraud's glucose agar in sterilized conical flask. Mixed media was poured in sterilized Petri dishes. A disk (9 mm) of old fungal culture, grew at 25-28 º C for one week, was formed using cork pore. In the center of Petri with mixing media, one well (9 mm) was performed. A disk of each species of fungi was embedded in a well. Cultures had been incubated at 25-28º C for one week. Average of two perpendicular diameters (mm) of growth colony was measured.

2- Dry weight measurement

Dry weight of fungal colonies growth in Sabouraud's glucose broth was performed as described by previous method [16]. Briefly, various concentrations of aminophylline were separately dissolved in Sabouraud's glucose broth at sterilized conical flask (250 ml). The 2 X 10^3 spore/ml of

old fungal growth was inoculated in each conical. Cultures had been incubated at 25-28 º C for one week. Grown colonies were filtered through filter paper (Whatman No.1). Isolated colonies were dried in oven at 60 º C for two hours. Dry colonies (gm) were measured as dry weight using sensitive balance (Sartorius, BL-2105, West Germany). Minimal inhibitory concentration (MIC) of highly effective tested compounds was determined by decreased those compounds until they reached to the minimum effective concentration by mingling that compound in Sabouraud's broth media. Media containing compound was inoculated with 0.1 ml of fungal culture (2 X 10^3 cell ml^{-1}). Culture had been incubated at 25-28 º C for one week. The absence of growth in media after incubation was recorded as MIC. Three controls were used in this study, including 0.5 mg/ml of griseofulvin (A.D.I. Iraq), theophylline (A.D.I. Iraq) and media without any compounds (control).

Statistical analysis

All experiments are separately repeated three times with three replicate of each concentration.
Data were subjected to the analysis of variance (ANOVA) and means were compared at 0.0 property level.

Results

Recently, the number of infections caused by dermatophytes has increased considerably, causing particular concern when they infect immunocompromised patients where a typical manifestations and more sever, extensive lesions can be produced. Detection of aminophylline activity against tested species of dermatophytes using colony diameter measurement was revealed much better results. The growth of *Trichophyton* species that was cultured on a media with 5 mg/ml of aminophylline was completely inhibited. An inhibitory effect of aminophylline has significantly been shown at concentration of 3 mg/ml compared with colony growth on control medium (free compounds) (figure 1). Theophylline containing media also completely inhibited all of tested fungi at concentration of 3 mg/ml and 5 mg/ml with significant differences value ($P<0.01$) at 1 mg/ml (Table 1). Other employment assay for antifungal activity of aminophylline was dry weight measurement. This method gave more satisfactory distinction results when 1 mg/ml and 3 mg/ml of aminophylline indicated significant differences ($P<0.01$) after comparing with the values of dry weight of fungi grown on free compound media (control) (figure 2). As they are shown the result above, aminophylline action is particularly also less than theophylline activity based on the susceptibility of fungi to completely inhibited by these drugs (Table 2).Among all species of *Trichophyton*, two variants of *T. mentagrophytes* revealed high susceptibility to inhibit by aminophylline or theophylline on the bases of MIC determination. *T. mentagrophytes var. mentagrophytes* showed much more sensitive than that other species towed the minimal inhibitory concentration of theophylline compound (2 mg/ml) (table 3). The measurement values of aminophylline MIC against all dermatophytes, except *T. rubrum*, had been determined at 4 mg/ml, whereas *T.rubrum* affected by 4.4 mg/ml of aminophylline. Theophylline inhibited fungal growth as MIC at fewer concentrations than aminophylline. Statistical analysis of our results illustrated in table 1 and 2 indicated that each of aminophylline and theophylline have been shown no significant differences value ($P<0.01$) contrarily to the action of griseofulvin.

Discussion

In contrast to bacterial infections, fungal infections are particularly difficult to treat and target with novel drugs because fungal diseases result in maladies caused by an organisms have occasionally similar cellular structure to host cells with a few differences [17]. In recent years, several studies on the *in vitro* susceptibility of dermatophytes to antifungal drugs have been done and the results have shown several considerable variation. The antifungal agents that specialist against dermatophytes can be grouped depending upon two main categories; structure and mechanism of action. Based on structural variation, these agents have two principle effective pharmacologic groups; azoles, eg. Clotrimazole and econazole and allylamines, including naftifine

and terbinafine [18]. The main target sites in fungal cells for effective agents were also variable. The antifungal activities of azole derivatives arise from a complex multimechanistic processes initiated by the inhibition of cyclochrome P450 that involved in the biosynthesis of ergosterol [19]. R126638, new triazole derivative, was exhibited inhibitory action on ergosterol synthesis in the cell wall of dermatophytes at nanomolar concentrations [20]. Terbinafine, allylamine derivative, interferes specifically with dermatophytic sterol biosynthesis at an early step and finally leads to a deficiency in ergosterol synthesis that results in abolish of fungal growth [11]. Topical therapy is commonly used for most dermatophytic infections. Cure rates are higher and treatment courses are shorter with topical fungicidal allylamines contrarily to fungistatic azoles [21]. Progressive development of fungal resistant to antifungal drugs stimulated authors to investigate for more efficiency compounds in order to replacement old resistance drugs by new synthesis or discovered agents. The main three factors that influence on appearance of fungal resistant were summarized by White *et al.* (1998) [22]. These factors are: Immune state of the patient with fungal infection, such as infected with immunodeficiency diseases, antineoplastic and corticosteroid therapy; replacement of a susceptible fungal strain by a more resistant strain or species; and the most frequent resistance mechanisms include reduction in the import of the drug into the cell, such as alteration in target enzyme to drug in host cell. Dermatophytes resistant to common drugs that use to treat dermatophytosis were observed during last years. The results obtained by the broth macrodilution procedure revealed that the values of terbinafine MICs and MFCs (minimal fungicidal concentrations) for sequential isolates of *Trichophyton rubrum* apparently increased during the course of therapy [23]. Aminophylline has since been widely used in the treatment of asthma [11]. It takes several pharmaceutical formulas, such as tablets and syrup as a suspension form [24]. In many industrialized countries, the frequency of side effects and the relatively low efficacy of theophylline and its derivative (aminophylline) have led to reduced usage [25]. Aminophylline is usually reserved now for the rare patients with sever exacerbations who do not responded adequately to other therapy [3]. Because of the problems with the side effects of theophylline, pharmacologists have been attempts to improve on the pharmaceutics properties of such type of drug. The results revealed that aminophylline (5 mg/ml) was completely inhibited growth of four species of *Trichophyton* according to colony diameter method. At the same time, lower concentrations (3 mg/ml) of theophylline have been given the same result of colony inhibition. Each of aminophylline and theophylline has no significant differences ($P<0.01$) in inhibiting of fungal growth in comparable to that of griseofulvin at 0.5 mg/ml.

In general, Aminophylline exhibited antifungal activity at high concentrations in contrast to theophylline. This observation could be explained by the fact that aminophylline contains low concentration of theophylline in their synthetic formula. One molecular of aminophylline is equivalent to approximately 79% of theophylline [24].

The normal growth of colonies of many dermatophytes species could be increased toward vertical direction with accumulation of great amount of fungal mass in restricted growing area of culture media. Thus, we can see a limitation in diameter of grown colony [14]. In this instance, other than colony diameter methods, such as dry weight measurement will be useful to determine fungal growth. Dry weight of *Trichophyton* colonies gives supporting to the results of colony diameter when both of them were revealed the more sensitive of fungi to theophylline concentrations than those of aminophylline. However, if we concentrated on the effects of aminophylline against dermatophytes, we can be found that both of above methods have been given good results about aminophylline antifungal activity which may be adding another function of this compound beside of known bronchodilation action. The ability of aminophylline to completely inhibit different species of dermatophytes required high doses in contract to griseofulvin. This ability did not prevent the new idea about application of aminophylline as antidermatophytic agent to establish a recent line in the antifungal world.

Restriction use of aminophylline in treatment of bronchoconstriction diseases could be reduced after confirming new function of it as antifungal agent by extensive scientific works to complete our

results. Theophylline and aminophylline have more than encouraged characters to make them enable to have the winner side among other drugs in the same pharmacological application. Theophylline is not expensive drug [25] when relative cost of different therapies has been an important factor in bringing about therapeutic diversity. Furthermore, theophylline action in immune system has also been demonstrated to reduce 22% of inflammatory cells and declined chemotaxis ability of neutrophils [26]. However, all known antidermatophytic agents could be stimulated uncomfortable side effects consisting hypersensitivity and gastrointestinal symptoms, including nausea, vomiting, abdominal discomfort and diarrhea [1, 2].

The mode of action of theophylline or aminophylline in mammalian cells is proposed by three mechanisms: cAMP phosphodiesterase inhibition [27], adenosine receptor antagonism [28] and induction of histone deacetylase activity [29]. Theophylline previously demonstrated to cause morphological changes in hyphi and spore of dermatophytes. These changes had been explained by proposing a hypothesis depending upon activity of theophylline to inhibit cAMP phosphodiesterase enzyme [30].

In conclusion, aminophylline was significantly inhibited growth of all species of isolated dermatophytes by using two different methods. The alteration of the usually usage of theophylline in the treatment of asthma or obstructive pulmonary disease to antimicrobial agent need more than one work and the researcher tries initially in this study and followed by series other studies to confirm this idea after testing the compound against different types of micro-organisms.

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Table (1): Average of fungi colony diameter (mm) grown on Sabouraud's glucose agar containing Aminophylline with inclusion of inoculation disk (9 mm).

Compound	Conc. mg/ml	*T. ment. var. mentagrophytes*	*T. ment.var. interdigitale*	*T. rubrum*	*T. simii*
Control		56 ± 1	54 ± 0.9	29 ± 0.8	45 ± 1
Theophylline	0.5	26 ± 1.5	28 ± 1	16 ± 2	24 ± 2
	1	22 ± 2 *	23 ± 2 *	14 ± 2	21 ± 1 *
	3	Zero *	Zero *	Zero *	Zero *
	5	Zero *	Zero *	Zero *	Zero *
Aminophylline	0.5	52 ± 2	42 ± 0.8	27 ± 2	45 ± 2
	1	39 ± 1	39 ± 0.9	19 ± 1	34 ± 1
	3	12 ± 2 *	16 ± 0.8 *	12 ± 0.8 *	14 ± 2 *
	5	Zero *	Zero *	Zero *	Zero *
Griseofulvin	0.5	14 ± 0.7 *	15 ± 0.8 *	13 ± 0.5 *	12 ± 1 *

Mean ± SE

*** Significant differences (P<0.01) between compound and control.**

Table (2): Average of fungi colony dry weight (gm) grown on Sabouraud's glucose broth containing Aminophylline.

Compound	Conc. mg/ml	*T. ment. var. mentagrophytes*	*T. ment.var. interdigitale*	*T. rubrum*	*T. simii*
Control		3.59 ± 0.9	3.31 ± 1	2.10 ± 2	3.50 ± 0.8
Theophylline	0.5	0.61 ± 1 *	0.73 ± 2 *	0.52 ± 2 *	0.68 ± 0.9 *
	1	0.49 ± 0.9 *	0.53 ± 1 *	0.30 ± 2 *	0.43 ± 0.8 *
	3	0.043 ± 1 *	0.041 ± 1 *	0.04 ± 1 *	0.04 ± 1 *
Aminophylline	0.5	3.23 ± 0.3	2.73 ± 1	2.43 ± 1	3.08 ± 0.9
	1	0.68 ± 1 *	0.70 ± 2 *	0.15 ± 1 *	0.60 ± 2 *
	3	0.04 ± 0.5 *	0.060 ± 2 *	0.044 ± 3 *	0.04 ± 1 *
Griseofulvin	0.5	0.081 ± 1 *	0.085 ± 0.9 *	0.060±0.8*	0.060 ± 0.9 *

Mean ± SE

*** Significant differences (P<0.01) between compound and control.**

Table (3): Minimum inhibitory concentration (MIC) of theophylline, and aminophylline.

Compound	Conc. mg/ml	*T. ment. var. mentagrophytes*	*T. ment.var. interdigitale*	*T. rubrum*	*T. simii*
Theophylline	1.4	+	+	+	+
	1.6	+	+	+	+
	1.8	+	+	+	+
	2	+	+	+	+
	2.2	-	+	+	+
	2.4	-	-	-	-
Aminophylline	3.4	+	+	+	+
	3.6	+	+	+	+
	3.8	+	+	+	+
	4	-	-	+	-
	4.2	-	-	+	-
	4.4	-	-	-	-

+ : Positive growth
- : Negative growth

Figure (1): Comparison between colony diameter of fungi grown on media containing aminophylline and control (free media).

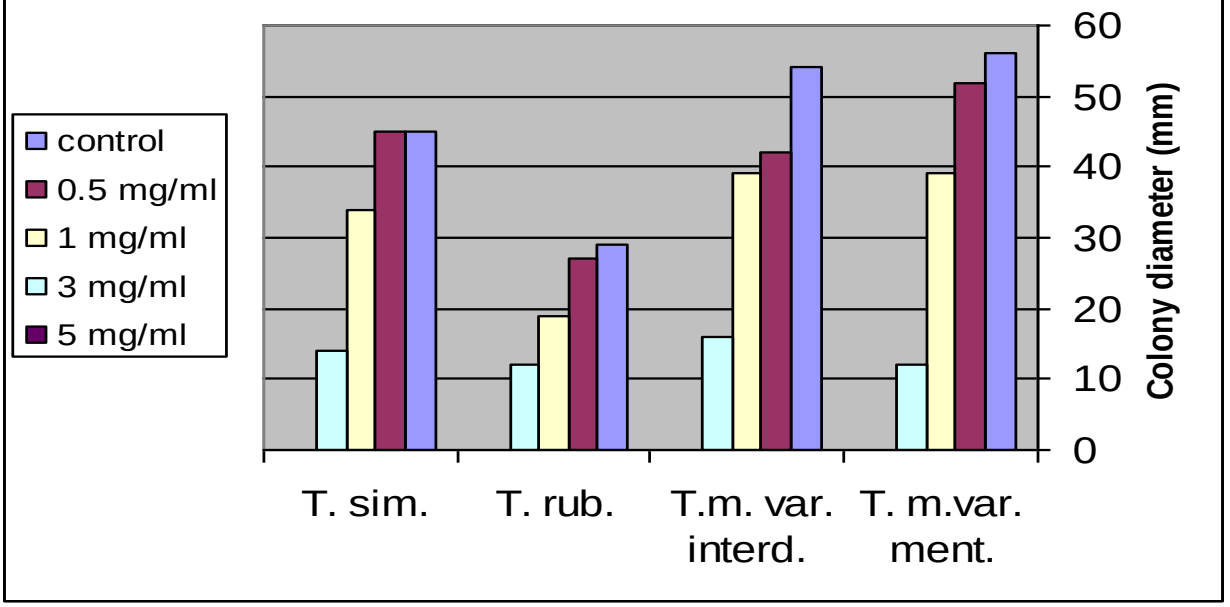
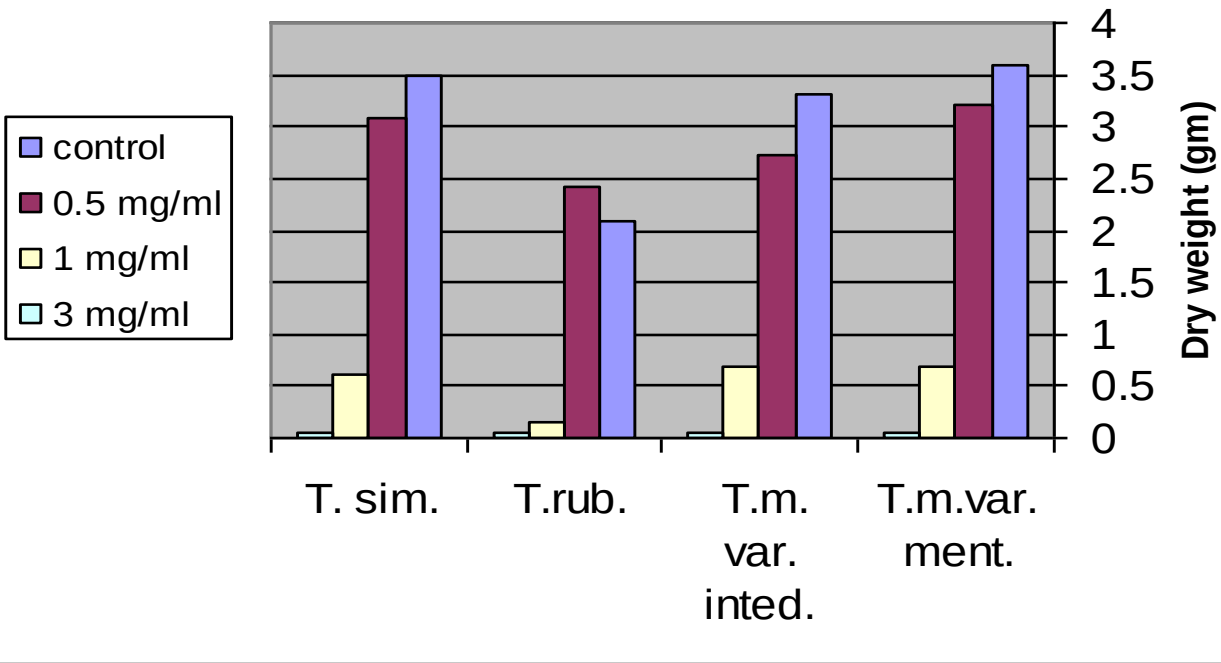
Figure (2): Comparison between dry weight of grown fungi in liquid media containing aminophylline and control (free media).