The Antimycotic Activity of Rosuvastatin

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ABSTRCT

Background: The steady increase in microbial resistance is a global health problem as a result of uncontrolled use of antimicrobial drugs, In order to overcome the challenge, there is a continuous need to search for new antimicrobials by either investigate novel compounds or repurposing drugs i.e. identifying new clinical use for existing approved agent thus saving time and cost required to develop a new antimicrobial therapy.
Aim: This study aimed to assess the *in vitro* antimycotic activity of different concentrations of rosuvastatin against 4 different fungi isolated from patients with malignancies 1 mold of genus *Aspergillus*, of 3 spices (*A. flavus, A.niger*, and *A. fumigatus*) and 3 yeast of genus *Candida* of 2 species (*C. albicans* , and *C. glabrata*), one genus and species from each *Saccharomyces cerevisiae* , and *Rhodotoryoa rubra* by disc diffusion method.
Materials and Methods: Sputum was taken from 30 patients with malignant disease, different micro and macroscopical tests were used to identified the 14 isolated fungi from them 1 genus from mold of *Aspergillus*, and 3 genus from yeast, from genus *Aspergillus* 3 species recognized as tailed, *A. flavus*, *A. niger*, and *A.fumigatus*. On the other hand from yeast of genus *Candida*, *C.albicans* and *C.glabrata*, whereas from genus *Saccharomyces*, and *Rhodotoula*, *Saccharomyces cerevisiae* and *Rhodotoula rubra* were recognized.
Results: Antimycotic susceptibility test exhibited zone of inhibition against yeast ranged from (30-20), (30-15), (30-15), and (25-15) against *C.albicans*, *C.glabrata Saccharomyces cerevisiae* and *Rhodotoula rubra* respectively, while mold show zone of inhibition ranged from (25-10), (10-0), and (30-10) against *A.flavus*, *A.niger*, and *A.fumigatus* respectively, although rosuvastatin show an antifungal activity only at dose 0.6 mg/ml against *A. niger*.
Conclusion: Rosuvastatin has antifungal activity apart of its pleiotropic activity of statins.

Keywords: Mold, Rosuvastatin, Yeast, Zone of inhibition.

النشاط المضاد للفطريات للرسوفاستاتين

الخلاصة:

المدخل: تعد الزيادة الطردية في مقاومة الميكروبات مشكلة صحية عالمية نتيجة للاستخدام غير المنضبط للأدوية المضادة للميكروبات، ومن أجل التغلب على هذا التحدي، هناك حاجة مستمرة للبحث عن مضادات جديدة للميكروبات إما عن طريق التحقيق في مركبات جديدة أو إعادة استخدام الأدوية، أي تحديد سريري جديد. باستخدام العامل المعتمد الحالي وبالتالي يؤدي الى توفير في الوقت والتكلفة اللازمين لتطوير علاج جديد مضاد للميكروبات
الهدف: هدفت هذه الدراسة إلى تقييم النشاط المضاد للفطريات خارج جسم الانسان لتراكيز مختلفة من رسوفاستاتين على 4 فطريات معزولة من مرضى مصابين بأورام خبيثة منهم 1 من جنس العفنيات، من 3انواع وهي (*A. flavus*، *A.niger*، *A. fumigatus*) و 3 اجناس من الخميرة من جنس المبيضات من نوعين (*C. albicans* و *C. glabrata*) ، وجنس واحد ونوع واحد من كل *Saccharomyces cerevisiae* و *Rhodotoryoa rubra* بطريقة الانتشار بالقرص والطبق.

المواد وطرق العمل: تم أخذ البلغم من 30 مريضًا يعانون من مرض خبيث ، واستخدمت اختبارات عينية وميكروسكوبية مختلفة للتعرف على 14 نوعًا من الفطريات المعزولة منهم ، جنس واحد من العفنيات ، و 3 جنس من الخميرة ، من جنس العفنيات تم تشخيص 3 انواع وهي ، *A. flavus* ، *A.niger* ، و *A.fumigatus*. من ناحية أخرى تم التعرف على الخميرة من جنس المبيضات ، *C.glabrata, C.albicans* بينما من جنس *Saccharomyces* و *Rhodotoula* ، تم التعرف على *Saccharomyces cerevisiae* و *Rhodotoula rubra*.
النتائج: أظهر اختبار الحساسية لمضادات الفطريات أن منطقة التثبيط ضد الخمائر تراوحت بين (30-20) ، (30-15) ، (30-15) ، (25-15) ضد *C.albicans*،*C.glabrata, Saccharomyces cerevisiae* و *Rhodotoula rubra* على التوالي ، بينما تظهر منطقة التثبيط للعفن بين (25-10) ، (10-0) ، (30-10) ضد*A.flavus , A.niger* و*A.fumigatus* على التوالي ، على الرغم من أن الرسوفاستاتين يظهر نشاط مضاد للفطريات فقط عند تناول جرعة. 0.6 ملغ / مل ضد *A. niger*.
الملخص: يحتوي رسوفاستاتين على نشاط مضاد للفطريات بصرف النظر عن النشاط المتعدد الاتجاهات للستاتينات.

الكلمات المفتاحية: عفنيات, رسوفاستاتين, خمائر, منطقة التثبيط.

INTRODUCTION

Today's a steady increase in microbial resistance is a global health problem as a result of uncontrolled use of antimicrobial drugs, thus the trent nowadays is to use an alternative measures of repurposition drugs to find other indications of an approved non antibiotic drugs. searching for drugs with antimicrobial activity is approach pharmaceutical companies in order to save time and cost required to develop a new antimicrobial therapy (1).

Rosuvastatin is one of the most commonly used of stain group, it act as compelling 3-hydroxy-3-methylglutaryl-CoenzymeA (HMG)-CoA reductase inhibitors, In addition, rosuvastatin possess a pleiotropic activity unconnected to its lipid lowering effect, latest studies implies the diverse extrahepatic pharmacological activities allied with statins class, especially rosuvastatin, such off target effect include antioxidant activity, increase in cytokines concentration, impede some markers associated with inflammation, which owing to inhibit biosynthesis of imperative isoprenoid intermediates then tracked by reduction in signaling molecules similar to Rho, Ras, and Rac (2,3).

Moreover, Rosuvastatin have been shown to assess antibacterial and antifungal action, diverse theories have been suggested regarding its antimicrobial mechanism of action however, the most conventional one related to the being there of pyrimidine ring in rosuvastatin structures, in addition to its pleiotropic activity which is promising and several effects are being speculated (4).

Fungal infection was the furthermost mutual cause of morbidity and mortality especially in cancer patients, the most isolated fungi from such patients was yeast frequently from genus *Candida*, tracked by *Aspegillus*, then rare fungi, the most mutual encountered species from genus *Candida* was *Candida albicans*, *Candida glabrata*, *Candida krusei*, and *Candida parapsillosis*, in the other hand the most mutual isolated species of genus *Aspergillus* was, *A.flavus*, *A. niger*, *A. fumigatus* and *A. terrus* (5). Fungal resistance have a clinical implication which conduct a surveillance that led to heightened interest in the study of antifungal resistance from different angles and search for new class of existing drugs with antifungal potential(6).

Furthermore, researcher shift the way toward the non -antimicrobial medications with various pharmacological activity, epidemiological results highlighted that individuals using statins are less in danger to bacterial and fungal infection (7). Also a countless *in vivo* and *in vitro* studies identified the antibacterial and antifungal activity of statin.

Accordingly, the aim of the present study was to assess *in vitro* antimycotic activity to different concentrations of rosuvastatin in contrast to isolated 4 fungi from patients with malignancies from them 1 mold of genus *Aspergillus*, of 3 spices (*A. flavus, A.niger*, and *A. fumigatus*) and 3 yeast of genus *Candida* of *2* species (*C. albicans* , and *C. glabrata*), one genus and species from each *Saccharomyces cerevisiae , and Rhodotoryoa rubra* by disc diffusion method.

MATERIALS and METHODS

Patients

Thirty patients with malignant disease were included in this study. They were attending out patients clinics (from March –May 2022) with sign and symptoms of chest infection, Sputum was collected. microbiology laboratory testing for isolation and identification.

Isolation and identification

Sputum was inspected by direct microscopical investigation by Gram stain for presence of any conidial head morphology for mold or budding cell for yeast, on the other hand sputum was cultured on double plate of cezpek's agar for mold investigation, double plates of Brain- Heart infusion blood agar supplemented with chloramphenicol was used for yeast. The inoculated plates for mold incubated for 5-7 days at 28-30 °C, however plates for yeast incubated for 2-3 days at 37°C. One genus of mold were identified by macroscopical inspected by perceive their colony morphology by distinguish their pigmentation, wooly and velvety, On the other hand, microscopical inspection by mixing portion of mold colony with lactophenol mount and examine under 40X light microscope, one genus of *Aspergillus* of three species that include *A.flavus*, *A.niger*, and *A.fumigatus* were identified (8, 9).

Although yeast colony inspected macroscopically by existence of colored pasty colony, while microscopical inspected by mixing portion of the colony with Gram stain and inspected under 100X light microscope for presence of budding yeast cell, however from yeast 3 genus were identified which include *Candida*, *Saccharomyces*, and *Rhodotorula*. From genus *Candida* 2 species were recognized *C. albicans*, and *C. glabrata*, however *C. albicans* was identified by germ tube test, and chlamydospre positive test, while *C.glabrata* was identified by API C 10 system. *Saccharomyces cerevisiae* and *Rhodotorula rubra* were recognized from genus *Saccharomyces* and *Rhodotorulla*, by colony morphology and lactophenol mount. After uniqueness the isolates were reserved in stock bottle culture for later use in susceptibility test (10,11).

This study intended to detect the antimycotic activity of rosuvastatin in contrast to isolated mold and yeast. In this study Rosuvastatin

tablet 20mg/day (Crestor, AstraZeneca) obtained from local pharmacy was used.

Susceptibility test

Muller-Hinton agar was used to assess the antimycotic activity of rosuvastatin by disc diffusion method, inoculum from every stock isolate was prepared in concentration equal to 0.5 McFarland (1.5 x 10^8 CFU/ml). Stock solution of rosuvastatin was prepared by dissolving rosuvastatin (2.5 mg) in 1ml of 1% DMSO (dimythyl sulfoxide) then serial dilution to accomplish the following concentration (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/ml) (12, 13).

***In vitro* antifungal activity**

One hundred filter papers in 6 mm diameter were socked in each concentration of rosuvastatin solution for overnight, then each tested fungal isolate was spread uniformly on the surface of Muller-Hinton agar by sterile cotton swab, discs of rosuvastatin in different concentration was placed on the surface of sensitivity medium, in addition to fluconazole disc (50ug/disc) by sterile forceps as a control. Inoculated plates incubated at 37 °C, then the mean diameter of inhibition zone were measured as a clear zone around the disc after 24,48and 72 hr. sensitivity test was repeated in triplicate for each isolate to ensure the accuracy of the technique, fluconazole disc use as a positive control, however DMSO use as a solvent control DMSO with this concentration has no antifungal activity (14).

RESULTS

From thirty malignant patients with chest infection,14 isolated fungi were identified, from them 5 isolates from mold and 9 isolate from yeast, from mold one genus of *Aspergillus* were isolated from them 3 species, there were 3 from *A. flavus*, and 1 from each *A. niger* and *A. fumigatus*. While from yeast three genus were isolated from them 7 from genus *Candida* there were 6 from *C. albicans* and 1 from *C. glabrata* in addition to 1 isolate from each *Saccharomyces cerevisiae* and *Rhodotorula rubra* as shown in Table 1and Table 2.

Table 1. Number of isolated mold from malignant patients.

Mold	**No. of isolate**
Aspergillus flavus	3
Aspergillus niger	1
Aspergillus fumigatus	1

Table2. Number of isolated yeast from malignant patients.

Yeast	**No. of isolate**
Candida albicans	6

Candida glabrata	1
Saccharomyces cerevisiae	1
Rhodotoryoa rubra	1

In this study, the antifungal activity of rosuvastatin was tested against the recognized isolates of mold and yeast, from the result of inhibition zone as shown in Table3, Table4, and Figure 1, Figure 2 , the maximum activity of rosuvastatin was detected with the highest concentration of 0.6 mg /ml concentration the inhibition zone decrease with manner of concentration thus the effect of rosuvastatin is concentration-dependent, In addition, the results of inhibition zone showed that the antifungal activity of rosuvastatin against yeast was higher than mold.

Table3. Antifungal activity of rosuvastatin in relation to zone of inhibition against mold.

Fungi	Rosuvastatin concentration disc in mg/ml					
	0.6	0.5	0.4	0.3	0.2	0.1
	Mean zone of inhibition in mm					
A. flavus	25	25	25	20	20	10
A. niger	10	0	0	0	0	0
A. Fumigatus	30	30	25	25	25	10

Table4. Antifungal activity of rosuvastatin in relation to zone of inhibition against yeast.

Fungi	Rosuvastatin concentration disc in mg/ml					
	0.6	0.5	0.4	0.3	0.2	0.1
	Mean zone of inhibition in mm					
C. albicans	30	30	25	25	20	20
C. glabrata	30	25	25	20	20	15
R. rubra	30	30	25	25	15	15
S. cerevisiae	25	25	20	20	15	15

A.flavus *A.niger* *A. fumigatus*

Figure 1. The antifungal activity of rosuvastatin against mold.

C.albicans *C.glabrata* *R.rubra* *S.cerevisiae*

Figure 2. The antifungal activity of rosuvastatin against yeast.

DISCUSSION

Fungi class containing various members. Antifungal resistance is an inclusive issues of countless health problem in different, The resistance to antifungal drugs was recorded especially in immunocompromised patients which shift the way to discover the antimicrobial activity in the existing drugs, many studies designed for detection a new antibiotic class of drug sources, so that certain non- antibiotic with antimicrobial therapy could be used as a supportive or in combination with antifungal drugs (15).

In this study *Candida* spp. then followed by *Aspergillus* species, then rare fungi of each *Saccharomyces cerevisiae* and *Rhodotorula rubra* were the foremost isolated fungi from patients with malignancies. Fungal infection inspection among cancer patients to *Candida* species, then followed by *Apergillus* species with variance in frequency rendering to the countries (16).

Researchers noticed that patients on statin containing drugs for lipid lowering remedy had a lower risk to get fungemia or even septicemia this antimicrobial activity could be attributed to the pleiotropic influence of statins (17).

The result of the current study which was designed to examine the antimycotic activity

of rosuvastatin against isolated fungi 1 of them were mold and 3 yeast by using disc diffusion method. All the isolated yeast and mold showed zone of inhibition at the highest concentration of rosuvastatin at 0.6 mg/ml. the inhibition zone was decreasing in dose dependent manner.

Rendering to inhibition zone of rosuvastatin which exert a powerful antifungal activity at all concentration against genus *Aspergillus* of species *A. fumigatus* and *A. flavus*, However, rosuvastatin show an antifungal activity only at dose 0.6 mg/ml in contrast to *A. niger*.

Similarly, rosuvastatin has antifungal activity in all concentration against yeast that include *Candida* spp., *Saccharomyces cerevisiae* , and *Rhodotoryoa rubra*.

Although the Antifungal activity of rosuvastatin still poorly understood with respect to its antimicrobial mechanism, It is quite possible that antifungal activity to be connected with its ability to inhibit synthesis of isoprenoids, which affect several important biosynthesis of ergosterol, the inhibition in turn result in modulation of G-protein actions, and activation of Ras and Ras-like signaling, the latter result in destruction of fungal cell wall, and reduction of new fungal cell formation (18).

Galgoczy and Coworkers (2011) (19) studied the intrinsic activity of rosuvastatin as antifungal medication on *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Aspergillus flavus* and *Aspergillus fumigatus* their results showed that rosuvastatin inhibitory dose ranged from 0.25 - 23 µg/ml. On the other hand, Jayalekshmi and Coworkers (2020) (20) showed that the forceful antifungal inhibitory concentration of rosuvastatin against yeast was 128 µg/ml, and 8 µg/ml against mold (2). However, rosuvastatin assess an extensive spectrum of antifungal activity against (*Candida* spp., *Aspergillus* spp., *Saccharomyces cerevisiae*, and *Dermatophytes*), in addition to boost the activity of antifungal medications (2,122).

CONCLUSION

In summary, this study concluded that rosuvastatin possess a good antimycotic activity against different genus of isolated fungal species, which documented by suppress the fungal growth by susceptibility disc diffusion method, the result of the current study augmented the antifungal activity of rosuvastatin. more studies showed be done to examine the effects of other statin members on fungal growth and to explore the molecular mechanism of this inhibition.

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CONFLICT OF INTEREST: no conflict of interest.

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