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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Vitamin D_3 a new drug against *Candida albicans*

Vitamine D_3 un nouveau médicament contre Candida albicans

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KEYWORDS
Candida albicans;
Vitamin D_3;
Minimum fungistatic concentrations (MFC_s);
Minimum fungicide concentrations (MFC_c);
Fungicidal activity

Summary
Objective. — In this study, we demonstrate that vitamin D_3 had fungicidal activity against *Candida albicans*.
Material and methods. — The susceptibility of the yeast strain to the vitamin D_3 was investigated by the antimicrobial screening using modified agar diffusion method, minimum fungistatic concentrations (MFC_s) and minimum fungicide concentrations (MFC_C) of the vitamin D_3 were determined by the broth dilution method.
Results. — The antifungal activity indicted that 100 μg/ml of vitamin D_3 had a power inhibition in the growth of *C. albicans* with zone of inhibition 12.5 mm and CMF_C and CMF_s were 1.58 ± 0.0764 μg/ml.
Conclusion. — These values indicate that vitamin D_3 can be considered to have fungicide activity. This antifungal effect may be due to the large lipsolubility of vitamin D_3 changing the integrity of the cell membrane.

MOTS CLÉS
Candida albicans ;
Vitamine D_3 ;
Concentrations

Résumé
Objectif. — Dans cette étude, on a démontré que la vitamine D^3 a une activité fongicide contre *Candida albicans*.
Matériel et méthodes. — La sensibilité antifongique de la souche de levure à la vitamine D^3 a été étudiée à l'aide de la méthode de diffusion en gélose, les concentrations minimales

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minimales fongistatiques (MFC_s) ;
Concentrations minimales fongicide (MFC_c) ;
Activité fongicide

fongistatiques (MFC^s) et minimales fongicides (MFC^C) de la vitamine D^3 ont été déterminées par la méthode de dilution en bouillon.
Résultats. — L'activité antifongique montre que 100 µg/mL de vitamine D^3 a eu une inhibition sur la croissance de *C. albicans* avec une zone d'inhibition de 12,5 mm et CFM^C et CMF^s ayant été 1,58 ± 0,0764 µg/mL.
Conclusion. — Ces valeurs indiquent que la vitamine D^3 a une activité fongicide. Cet effet antifongique peut être du à la grande liposolublité de la vitamine D^3, changeant l'intégrité de la membrane cellulaire.

Introduction

Candidiasis is an infection caused by fungi of the genus *Candida*, being also the most prevalent fungal infections in humans. Candidiasis can manifest both topically and systemically and may involve many different sites in the human body [1].

Candida species are commensal microorganisms with a presence that ranges from 20% to 50% of the microorganisms in the oral cavity of the healthy dentate population [2] *Candida albicans* is regarded as the most pathogenic *Candida* species in all forms of human candidosis [3]. In recent reports, *Candida* spp. remains the leading fungal cause of central line-associated blood stream infections [4].

Although *C. albicans* remains the major species responsible for disseminated candidiasis, a large number of reports have documented infections caused by other *Candida* species [5].

Prolonged prophylaxis or treatment with antifungal agents has increased the incidence of clinical isolates resistant to one or more antifungals in previously susceptible strains [6]. *C. albicans* and *C. tropicalis* were for a long time regarded as species largely susceptible to fluconazole and amphotericin B, but reports over the few last years have shown development of resistance to fluconazole in some centers and clinical therapy failure [7]. The emergence of azole resistance in *Candida* species makes necessary the development of new effective antifungal strategies against drug-resistant strains [8]. It is increasingly recognized that vitamin D_3 plays an important role in pulmonary defense, immunity, and inflammatory processes [9]. Vitamin D_3 insufficiency is associated with an increased risk for several medical conditions, including osteoporotic fractures [10], falls [11], cancer [12], diabetes [13] and hypertension [14]. Immune effects of vitamin D_3 include increased secretion of the antimicrobial peptide cathelicidin, decreased chemokine production, inhibition of dendritic cell activation, and alteration of T-cell activation [15]. But there are no studies on the direct activity of vitamin D_3 on microorganisms, including *C. albicans*.

In the current study, we sought to test this hypothesis by determining the direct sensitivity of *C. albicans* via vitamin D_3.

Materials and methods

Anti-candidal susceptibility testing

Vitamin D_3 provided by Laboratory of Bouchara-Recordati, ampoule of 1 ml contained 200,000 UI, such as 1 µg equivalent of 40 UI of vitamin D_3. In this study the vitamin D_3 was individually tested against a yeast *C. albicans* ATCC 10231, provided by University of M'sila. The susceptibility of the yeast strain to the vitamin D_3 was investigated for the antimicrobial screening using two modified methods agar diffusion method [16] and the method of Hayes and Markovic [17].

To prepare the inoculate, yeast cell suspensions were adjusted to match the turbidity of a 0.5-McFarland standard (10^7 CFU/mL) spread on agar plate into Sabouraud dextrose (SDA). Sterile 6-mm filter paper discs (whatman No. 1) were impregnated with 20 µL of the crude vitamin D_3 and were deposited at equal distances on the surface of the inoculated agar Saburaud. Control was used, a negative control with 20 µL of DMSO and an antifungal 5Fluorocytosin (5FC10 µg/ml) as a positive control. Plates were left in at 4 °C for 4 hours before incubation to ensure a good diffusion of the vitamin D_3 in the agar. The diameter of inhibition was measured after 48 h of incubation at 37 °C. The essay was carried out in triplicate.

Determination of minimum fungistatic concentrations (MFC_s) and minimum fungicide concentrations (MFC_C)

(MFC_S) and (MFC_C) of the vitamin D_3 were determined by the broth dilution method reported by Bajpai and Kang [18] derived from the original method of Murray et al. [19]. Vitamin D_3 was first diluted in DMSO, from this solution, 2 ml was introduced into a tube containing 18 ml of Sabouraud Dextrose Broth (SDB), containing 0.5% Tween 80. A dilution series were made to the final concentrations (6500 µg/ml to 0,79 µg/ml). 20 µL of yeast suspension (10^7 CFU/mL) tested strain was inoculated in test tubes containing the SDB medium at different concentrations. The mixture, forming the SDB, yeast suspension and the vitamin D_3 is homogenized and incubated at 37 °C for 48 hours. Tubes control containing the middle of SDB, were inoculated with the yeast suspension only. In parallel, the tubes with DMSO and tween 80 (0.5%) were used as negative control. After 48 hours of incubation, the first tubes with total inhibition, compared to the control are reseeded in the boxes containing 20 ml of the SDA culture medium. Growth is tracked for 1—4 days at a temperature of 37 °C. When there is a resumption of growth, concentration is said fungistatic (MFC_S), on the other hand, if there is no growth; it is called fungicide (MFC_c). The vitamin D_3 is said to be fungicide when the (MFC_c)/(MFC_s) ratio is less than 4, however, when this ratio is higher or equal to 4, it is said to be fungistatic[20].

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Statistical analysis

The analysis of variance was determined by one-way ANOVA and differences among the means were determined for significance at $P < 0.05$ using a T test.

Results and discussion

The antifungal activity indicted that 100 μg/ml of vitamin D_3 had a power inhibition in the growth of *C. albicans* with zone of inhibition 12.5 mm and CMF_C and CMF_s were 1.58 ± 0.0764 μg/ml. These values indicate that vitamin D_3 had a good antifungal activity. On the basis of the obtained results, the vitamin D_3 can be considered to have fungicide activity. The obtained MFC_c and MFC_s which was 1 (less than 4) are similar to those of Derwich et al. [20].

Our results showed that vitamin D_3 demonstrated higher antifungal activity against *C. albicans*. According to Neumann et al. [21] Amphotericin B, the golden standard of antifungal treatment, exhibits higher activity toward ergosterol-rich fungal membranes, which permits its use against systemic mycoses; however, the selectivity for fungal membranes is far from satisfactory leading to severe side effects. The general mechanism of action of vitamin D_3 can be explained by its steroid nature, so any effect disrupts membrane sterols can cause cell lysis. Thus sterols have direct biological activities on sterols of eukaryotic membrane cells including the fungal cells containing ergosterol. This antifungal effect may be due to the large liposolibility of vitamin D_3 changing the integrity of cell membrane. In addition, due to the development of drug resistance as well as side effects of certain antifungal agents, the search for novel antifungal agents has become that more urgent [22]. Most available antifungal agents have fungistatic activities [23]. Thus, discovery of novel antifungal agents with fungicidal activity may be important in developing future treatments [22]. This is the first study on direct contact of vitamin D_3 with *C. albicans* in vitro furthermore we have demonstrated the antifungal effect of vitamin D_3.

Conclusion

The present results implicate vitamin D_3 as a potential antifungal agent. The above results suggest that vitamin D_3 can be proposed as a new antifungal drug. Details concerning the mechanism of the antifungal activity of vitamin D_3 on *C. albicans* growth require more.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgments

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