Biomedicine & Pharmacotherapy 88 (2017) 770–777

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Efficacy of vitamin B complex as an adjuvant therapy for the treatment of complicated vulvovaginal candidiasis: An in vivo and in vitro study

Mei-Guo Sun[a], Ying Huang[b], Yuan-Hong Xu[b], Yun-Xia Cao[a,*]

[a] Department of Gynecology and Obstetrics, The First Affiliated Hospital of Anhui Medical University, Hefei 230031, PR China
[b] Department of Clinical Laboratory, The First Affiliated Hospital of Anhui Medical University, Hefei 230031, PR China

ARTICLE INFO

Article history:
Received 5 October 2016
Received in revised form 20 December 2016
Accepted 1 January 2017

Keywords:
Complicated vulvovaginal candidiasis
Vitamin B complex
Adjuvant therapy
In vivo
In vitro

ABSTRACT

Objective: This study aimed to explore the efficacy of vitamin B complex as an adjuvant therapy for the treatment of complicated vulvovaginal candidiasis (VVC) in vitro and in vivo.
Methods: One-hundred fifty-eight complicated VVC patients were randomly divided into group A (treated with suppository + oral antifungal agents), group B (treated with suppository + vaginal cream), and group C (treated with suppository + vaginal cream + oral vitamin B complex). A mouse model of VVC was established. Eighty VVC mice were randomly divided into 4 groups according to the dose of vitamin B complex (20 mice in each group): V1 group (injected with 150 μL normal salin), V2 group (injected with 50 μL vitamin B complex solution + 100 μL normal saline), V3 group (injected with 100 μL vitamin B complex solution + 50 μL normal saline), and V4 group (injected with 150 μL vitamin B complex solution). After 4 weeks of treatment, the vaginal secretion was obtained for microscopic smear examination. HE stainning was performed to observe histopathological changes of vaginal tissues. The expressions of inflammatory factors were detected by ELISA. Meanwhile, VVC model of vaginal epithelial cells was established. The effects of different concentrations of vitamin B complex on anti-fungal effect of fluconazole were detected in vitro.
Results: After the treatment, complicated patients in the group C had significantly higher effective rates than those in the group A and group B. After the intra-gastric administration, the microscopic smear examination found that obvious pseudohypha in cluster with a lot of blastospores can be seen in the vaginal secretions of mice in the V1 group under the microscope. There was significant difference between mice treated with different dosages of vitamin B complex. The inflammatory response of mice in the V1 group was significantly higher than those in other groups and the inflammation response reduced with the increase of vitamin B complex dosage. The vitamin B complex elevated the curative effects of fluconazole on VVC model of vaginal epithelial cells and significantly increased the anti-fungal effect of fluconazole.
Conclusions: Our findings suggest that vitamin B complex could be an effective adjuvant therapy for complicated VVC.

1. Introduction

Vulvovaginal candidiasis (VVC) is a kind of opportunistic fungal infections caused by Candida species, especially Monilia Albicans [1]. VVC, the second major cause of vaginitis in women only after bacterial vaginitis, is also known as fungal vaginitis or Candida vaginitis [2]. About 75% premenopausal women are infected with VVC at least once in a lifetime, among which 5% women will develop into recurrent VVC, that is, 4 times a year or even more [3]. The clinical manifestations of VVC include frequent micturition, dysuria, dyspareunia, leucorrhea, vulva and vaginal burning, itching, etc. [4]. Pregnant women infected with VVC may also end up with adverse pregnancy outcomes such as abortion, premature delivery, premature rupture of membranes and neonatal infections, leading to a huge physical and psychological pain for a majority of patients [5]. Common drugs for the clinical treatment of VVC usually are antifungal agents like nystatin and fluconazole, miconazole, but the complexity of complicated VVC contributes to different degrees of drug resistance in the clinical course of treatment [6,7]. Therefore, the combined use of vitamins

* Corresponding author at: Department of Gynecology and Obstetrics, The First Affiliated Hospital of Anhui Medical University, No. 218, Jixi Road, Shushan District, Hefei 230031, Anhui Province, PR China.
E-mail address: caoyunxia6@126.com (Y.-X. Cao).

http://dx.doi.org/10.1016/j.biopha.2017.01.001
0753-3322/

and anti-fungal agents is proposed to improve the cure rate of VVC. For example, vitamin B is necessary for the synthesis of specific vaginal structural proteins, such as cytokeratin, and its deficiency will lead to structural integrity loss and desquamation of vaginal epithelium [8]. Hence, this paper proposed vitamin B complex as an adjuvant therapy for complicated VVC.

Vitamin B is water-soluble and vitamin B complex mainly contains vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, and calcium pantothenate [9]. As indispensable vitamins in human body, vitamin B plays an important role in the metabolism of carbohydrate, fat, and protein, and respiration [10,11]. It has been reported that vitamin B complex combined with allicin can treat acetic acid-induced toxic reaction in mice, making blood and biochemical parameters return to normal state [12]. Vitamin B metabolites can be used as therapeutic target agents for the treatment of Plasmodium falciparum [13]. Besides, the supplement of high dose of B vitamins to healthy population can improve their psychological quality, ease their pressure, and effectively adjust their cognitive ability under mental stress [14]. Intake of vitamin B2 and B6 can reduce the risk of colorectal cancer in postmenopausal women [15]. Additionally, vitamin B complex supplementation has a positive effect on the control of adult depression [16]. Furthermore, it has been demonstrated clinically that B vitamins can slow down the development of diabetic nephropathy and prevent vascular complications [17]. However, there are few researches about the application of B vitamins in the treatment of fungal infections. Therefore, this paper carried out in vivo and in vitro study to investigate the efficacy of vitamin B complex as an adjuvant therapy for complicated VVC.

2. Materials and methods

2.1. Ethical statement

All animals were raised and treated in accordance with the *Guide for the Care and Use of Laboratory Animals* by National Institutes of Health of the U.S.A. This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University, and informed consent was obtained from each patient, or guardians prior to study.

2.2. Study subjects

The study included 158 patients with complicated VVC diagnosed in the First Affiliated Hospital of Anhui Medical University. The pathological tissue sections were identified after separation and purification, showing positive results in germ tube test and spore formation experiment. The diagnosis was strictly in accordance with criteria of VVC [18]. The patients were 21–50 years old (mean age of 35.39 ± 4.37 years) with disease course of 15–127 d (mean duration of 46.45 ± 17.87 d). The patients were married and their major manifestations included different degrees of burning pain of vulvar with bean curd residue-like secretions, vaginal mucosal hyperemia, increasing leucorrhoea and edema, white lumps attached to the inner side of labium minus and swelling membrane. Pregnant patients with VVC, severe VVC and recurrent VVC were all complicated types. Exclusion criteria were as follows: (1) patients with simple VVC; (2) patients with liver dysfunction; (3) patients with renal failure; (4) patients allergic to drugs; (6) patients complicated with other types of vaginitis.

2.3. Grouping and treatment regimes

The 158 patients with complicated VVC were randomly divided into three groups: the group A included 49 patients aging 24–50 years old (mean age of 36.10 ± 4.84 years) treated with local vaginal suppository for 1 week and oral antifungal agents for 4 weeks (1 tablet miconazole every night + 150 mg fluconazole once for a week), with the disease course of 17–109 d (the mean duration of 50.02 ± 20.80 d); the group B enrolled 54 patients aging 21–48 years old (mean age of 35.04 ± 4.58 years) treated with local vaginal suppository for 1 week and vaginal cream for 4 weeks (1 tablet miconazole every night + compound ketoconazole ointment every night), with the disease course of 18–127 d (the mean duration of 45.52 ± 19.05 d); the group C consisted of 55 patients aging 25–47 years old (mean age of 35.11 ± 4.06 years) treated with local vaginal suppository for 1 week, vaginal cream for 4 weeks, and oral vitamin B complex for 2 weeks (1 tablet miconazole every night + compound ketoconazole ointment every night + 2 tablets vitamin B complex twice a day), with the disease course of 15–102 d (the mean duration of 47.35 ± 17.37 d). During the period of treatment, sexual life was prohibited.

2.4. Follow-up and efficacy evaluation

After treatment, patients would come back regularly to the Department of Gynecology in the First Affiliated Hospital of Anhui Medical University for outpatient or follow-up by telephone was conducted. In order to facilitate follow-up and timely feedback, phone numbers of follow-up worker will be given to patients so that psychological counseling can be provided at any time. Recovery meant that all symptoms and signs disappeared within 6 months after the treatment and the test result of Candida albicans was negative. Marked effective meant that symptoms and signs were significantly improved within 6 months after treatment and the test result of Candida albicans was negative. Improvement indicated that symptoms and signs were somewhat improved within 6 months after treatment, but the test results of Candida albicans were still positive. Ineffective meant that there was no improvement in symptoms and signs with 6 months after treatment and the test results of Candida albicans were still positive [19].

2.5. Establishment of a mouse model of VVC

One hundred and one female mice of ICR strains (aging 8–10 weeks and weighing 22.31 ± 3.26 g) were purchased from Better Biotechnology Co., Ltd, (Nanjing, Jiangsu, China). The mice were fed with standard diets for more than a week to reduce the stress response caused by environmental change. Subcutaneous injection of estradiol benzoate was conducted to cause fake estrous state of mice (one injection in every two days with 0.1 mg each time). Isolated and purified bacterial strain were inoculated into medium containing 1% peptone and 0.1% glucose, and cultured in oscillating water at 25 °C for 16–18 h. At the same time, bacteria at the density of 2.5×10^6/L were dissolved in 20 uL phosphate buffer saline (PBS) bacterial suspension, indicating that spores of Candida albicans were 5×10^4. On the 6th day after injection, the fluid of bacterial was injected into absorbable hemostatic sponge and the sponge was inverted into the vagina of mice. After the injection, each mouse was maintained inverted for 5 min. On the 2nd, 4th 7th and 21st day after inoculation, 5 mice were selected for vaginal lavage with 100 μL PBS stored in sterilized 1.5 mL Eppendorf (EP) tube. Then the mice were killed by cervical dislocation and vaginal tissues were obtained.

2.6. Identification of VVC mouse model

Lavage fluid was mixed thoroughly and lotion was (40 uL) added to dilute 1 mL sterile saline. Sabouraud Dextrose Agar medium was modified for inoculation and culture at 30 °C for 72 h before CFU counting (colony-forming unit, colonies/L). Gradient dilution of the