Effect of Amphotericin B Dilution with Various Beverages on the Survival of Candida albicans Cells

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Abstract

Amphotericin B (AMPH) has been generally used for prophylaxis or treatment of specific fungal diseases in immunocompromised patients. However, because it is difficult for children to ingest, mainly because of its bitter taste, it is often diluted with soft drinks. We therefore investigated the effect of dilution of AMPH with various beverages on its antifungal activity in vitro. Candida albicans cells were exposed for 30 min to AMPH diluted twofold with each of six commercially available beverages or distilled water, and percent survival was determined. The results showed 60% survival in the dilution with distilled water and higher survival when diluted with Yakult (136%; p<0.01), orange juice (104%; p<0.01), and coffee-milk (92%; p<0.01). By contrast, lower survival was obtained when diluted with gum-syrup (54%), sweet cider (76%), and shaved-ice syrup (52%) with no significant differences from distilled water (60%), suggesting that these three beverages may be useful for diluting AMPH. The results of this study are a warning to medical workers that some methods of making AMPH more palatable considerably decrease its antifungal activity and may have a negative effect on host defenses against infectious diseases.

Introduction

Protecting patients who are immunocompromised by chemotherapy for malignant tumors from contracting infectious diseases is a significant problem, and diseases caused by fungal infection, such as candidosis, progress rapidly and often lead to death11-30. Therefore, antifungal agents are usually administered to immunocompromised patients, as prophylaxis, to treat specific infections, or as empirical or preemptive therapy11. Continuous oral administration of antifungal agent, such as amphotericin B (AMPH)206, is required to treat such patients, in addition to general countermeasures such as hand-washing by the medical personnel and gargling by the patients.

However, it is sometimes difficult for children to ingest AMPH because of its appearance, bitter taste, and unpleasant smell, and oral administration seems to exacerbate the suffering of these patients, who
have already experienced exhaustion and vomiting during the course of chemotherapy. Therefore, attempts are generally made to reduce the discomfort by chilling or diluting AMPH with beverages, such as orange juice. However, the antifungal activity of AMPH may be greatly affected by conditions within the oral cavity or by the method used to make it palatable (such as dilution with beverages). We therefore investigated the effects of dilution of AMPH with various commercially available beverages on its antifungal activity in vitro, using Candida albicans as a representative causative species of opportunistic fungal infections.

**Materials and Methods**

Strain and culture conditions. C. albicans M1012 serotype A that had been maintained in our laboratory and identified by serological methods with Candida Check was used as a representative causative strain of opportunistic infections. It possesses marked ability to adhere to human buccal epithelial cells or to various tissue cells. The cells were grown in Sabouraud glucose liquid medium or on Sabouraud glucose agar plates containing streptomycin (50 mg/l).

Antifungal agent and preparation of test samples. Amphotericin B (AMPH), whose proprietary name is FUNGIZONE SYRUP (Bristol-Myers Squibb, Munich, Germany), was used as the antifungal agent in this study. Yakult (a Lactobacillus product; Yakult Honsha Co. Ltd., Tokyo), 100% orange juice (Orange juice; GOLD PACK Co. Ltd., Tokyo), coffee-milk (Snow Brand Milk Products Co. Ltd., Tokyo), gum-syrup (KEY COFFEE INC., Tokyo), sweet cider (Asahi Soft Drinks Co. Ltd., Tokyo), and shaved-ice syrup (MEIDI-YA Co. Ltd., Tokyo) were used in this study. AMPH, 100 mg (potency)/ml, was diluted twofold with each of these beverages or with distilled water and used as the test solutions.

Determination of antifungal activity. A 0.2 ml portion of a C. albicans cell suspension (1.1×10⁷/ml) was added to 2 ml of each of the test solutions described above, and they were incubated for 30 min at room temperature. After 500-fold dilution, a 0.1 ml sample was immediately plated onto Sabouraud glucose agar and incubated at 30°C for 48 hr. The number of yeast cells after the 30-min incubation was determined by counting the colonies on agar plates. The sample in which the test solution was replaced by distilled water (containing no AMPH or beverage) was used as a control. The ratio of the number of colonies with the test solution to the number of colonies in the control (averaged 200 colonies/plate) times 100 was used as the percent survival. Experiments with each sample were replicated at least three times. In this protocol, the “washing” step to remove traces of AMPH transferred onto each agar plate by 0.1 ml plating (10 μg/20 ml of plate) was omitted based on the results of the following experiment. To test the effect of traces of AMPH on percentage survival, immediately after the 30-min drug exposure cells were washed 1–3 times with 5 ml of distilled water (DW) by centrifugation, then suspended in 2 ml DW, and diluted 500-fold with DW (“washed sample”), or the cells exposed to the drug for 30-min were directly diluted 500-fold without washing (“unwashed control sample”). Thereafter, both of the 0.1 ml “washed” and “unwashed” samples were subjected to colony counting as described. The percentage survival, 62.0% for 1 wash, 66.7% for 2 washes, and 56.7% for 3 washes (averages of 3 experiments), did not significantly differ from the 60.3% in the unwashed control by the Student’s t test, showing that traces of AMPH had no effect on percentage survival, and thus that the “washing” step could be omitted.

Statistical analysis. The statistical significance of the data was determined by Student’s t test. A P value of less than 0.05 was considered significant.
Results

We investigated the effects of dilution of AMPH with various beverages on its antifungal activity in vitro (Fig. 1). The results showed that percentage survival after dilution with distilled water was 60%, and that it was significantly higher in Yakult (136%; $p<0.01$), orange juice (104%; $p<0.01$), and coffee-milk (92%; $p<0.01$). By contrast, a markedly lower level of percentage survival was obtained with gum-syrup (54%), sweet cider (76%), shaved-ice syrup (52%), none of which was significantly different from the percentage survival with distilled water (60%) (indicated by stars in Fig. 1), suggesting that these three drinks may be useful for diluting AMPH.

We also examined the direct effect (without AMPH) of the six beverages used in the above experiments on the survival of C. albicans cells. The cells were exposed for 30 min to each of the beverages in

![Fig. 1 Effect of AMPH dilution with various beverages on percentage survival of Candida albicans cells. To AMPH, which was diluted twofold with distilled water (DW), Yakult (YK), orange juice (OR), coffee-milk (CF), gum-syrup (GS), sweet cider (CD), and shaved-ice syrup (IS), C. albicans cells were added, and the cells were incubated for 30 min. Yeast cell survival was then determined as a percentage of the control (CO) (incubated 30 min solely with distilled water without AMPH) and expressed as means ± standard errors (100% survival corresponds to an average of 200 colonies/plate). The bar with the solid star indicates that the percentage survival with these beverages was not significantly different from that for AMPH diluted with distilled water (open star: ca. 60% survival) by Student’s $t$ test.](image1)

![Fig. 2 Direct effect of various beverages on the survival of C. albicans cells. C. albicans cells were treated for 30 min with each of the six beverages (the same beverages and abbreviations as those used in the experiments shown in Fig. 1). After treatment, the percentage survival of yeast cells compared to the control (exposed for 30 min to distilled water alone) (CO) was determined. The bar with a solid circle indicates that the percentage survival with these beverages was found to be significantly higher than with for the control (open circles) by Student’s $t$ test.](image2)
the absence of AMPH, and survival as ratios of the control cells (100% survival) exposed to distilled water alone were compared (Fig. 2). The results showed significantly increased percentage survival with Yakult (144%; p<0.01), orange juice (148%; p<0.01), and coffee-milk (122%; p<0.05) (indicated by the solid circles in Fig. 2), indicating cell division-promoting activity of these drinks. (Although Yakult contains living Lactobacillus cells, none of these cells were observed in the agar plates with streptomycin [see Materials and Methods]). By contrast, the survival ratio was not greatly affected by the other three beverages (gum-syrup, sweet cider, and shaved-ice syrup).

Discussion

For many years AMPH has been used as a systemic antifungal agent. It is a polyene macrolide that binds to sterol components of the fungal cell membrane, altering membrane permeability and permitting cytoplasmic constituents to leak out of the cell. In clinical investigations, AMPH has been reported to bind to serum lipoproteins. The results of our in vitro experiment shown in Fig. 1 are indicative of retention of the antifungal effect of AMPH when diluted with certain beverages, such as gum-syrup, whose main ingredient is sugar. By contrast, dilution of AMPH with beverages containing lipids, such as Yakult or coffee-milk, markedly reduced its antifungal effect against C. albicans, suggesting an interaction between AMPH with some of these compounds.

While most nurses have emphasized the comfortable use of antifungal agents clinically, especially when administering them to children, they have not always sufficiently checked their effect on antifungal activity. The results of this study indicate that dilution of AMPH with certain beverages markedly reduces its antifungal activity. Our study therefore may warrant a warning to medical workers that some attempts to make AMPH more palatable cause a marked decrease in antifungal activity, and thus have a negative effect on host defense against serious infectious diseases.

In addition, the results of our experiment on the direct effects of the six beverages used in the above experiments on the survival of C. albicans cells revealed a significant increase in percentage survival with some of them, such as Yakult (Fig. 2). This indicates that some beverages may possess cell division-promoting activity and suggests that gargling immediately after their ingestion should be performed to prevent fungal infections in immunocompromised patients.

The results of this study suggest that some beverages, such as Yakult, have the ability to markedly reduce the antifungal activity of AMPH (Fig. 1) and may also possess cell division-promoting activity (Fig. 2), however, it remains unclear whether such effects also occur in vivo. Further investigations, such as in experimental animals or etiological studies in immunocompromised patients, are required to apply the results from the basic study to clinical fields.

References

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Candida albicans に対する amphotericin B の抗菌活性に及ぼす薬剤希釈法の影響

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小児の真菌感染予防及び治療に使用される amphotericin B シロップ（以下，AMPH）は，内服が困難であるため，ジュースで稀釈する等の工夫がされている。そこで，AMPH を稀釈する際の稀釈液の違いが抗菌活性に与える影響を試験管内（in vitro）で検討した。ヤクルト等 6 種の希釈液，または蒸留水により AMPH を各々 2 倍希釈したものに，Candida albicans 菌液を添加，30 分間放置後の残存生菌率を測定した。その結果，蒸留水（60%）と比べ，ヤクルト 136%（P<0.01），オレンジジュース 104%（P<0.01），コーヒー牛乳 92%（P<0.01）と有意に高値であった。一方，ガムシロップ，炭酸ジュース，かき氷シロップの 3 種では，各々 54%，76%，52% と蒸留水と有意差を認めず，抗菌活性が保持されることから，これら 3 種が稀釈液として有効であることが示唆された。今回の結果から，臨床の現場で患児に AMPH を安楽に内服させようとする行為が，方法によっては抗菌効果を低下させ，感染予防という本来の目的に逆行してしまう可能性が強く示唆された。

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