Oral administration of itraconazole solution has superior efficacy in experimental oral and oesophageal candidiasis in mice than its intragastric administration

Hiroko Ishibashi*, Katsuhisa Uchida, Yayoi Nishiyama, Hideyo Yamaguchi and Shigeru Abe

Teikyo University Institute of Medical Mycology, 359 Otsuka, Hachioji, Tokyo 192-0395, Japan

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Objective: The therapeutic activities of cyclodextrin-associated itraconazole oral solution (itraconazole OS) by two administration routes in experimental oral and oesophageal candidiasis in mice were examined and compared.

Methods: Using experimental oral and oesophageal candidiasis models in ICR mice, we investigated the efficacy of oral and intragastric administration of itraconazole OS and checked the concentration of itraconazole and its metabolite hydroxyitraconazole (OH-itraconazole) in tongues or oesophagus tissue after administration of itraconazole OS.

Results: Oral administration of itraconazole OS at doses of 0.8, 4.0 or 20 mg/kg/day clearly decreased the number of viable Candida albicans cells in the oral cavity of mice with oral candidiasis in a dose-dependent manner at 3 days after infection. Intragastric administration of itraconazole OS at doses of 4 and 20 mg/kg/day once a day were also effective but to a lesser degree than that of oral administration. In the oesophageal candidiasis model, oral administration of itraconazole OS displayed superior therapeutic efficacy to the intragastric route. In coincidence with the greater efficacy, itraconazole was detected in lesional tissues after oral administration of itraconazole OS.

Conclusions: Oral administration of itraconazole OS displayed therapeutic efficacy against murine oral and oesophageal candidiasis superior to that achieved by intragastric administration. This can be explained by there being higher concentrations of itraconazole in tongues or oesophagus tissues after administration of the suspension by the oral route.

Keywords: mucosal infections, intestinal tracts, antifungal chemotherapy, drug delivery

Introduction

As clinical treatment for oropharyngeal and oesophageal candidiasis, oral administration of azole antifungals including itraconazole oral solution (itraconazole OS) is recognized as first choice. But pharmaceutical analysis of mechanisms of therapeutic actions of orally administered itraconazole OS in vivo has been very limited.1–3

Using murine oral and oesophageal candidiasis models, we evaluated the efficacy of itraconazole OS administered by oral or intragastrical routes on this condition and assessed the concentration of itraconazole and its metabolites in the tongue and oesophagus tissues after that administration.

Materials and methods

Oral and oesophageal candidiasis in mice

Experimental procedures of the oral and oesophageal candidiasis models were described previously.4,5 All animal experiments were performed according to the guidelines for the care and use of animals approved by Teikyo University. Six-week-old female ICR mice (Charles River Japan, Inc., Kanagawa, Japan) were used for all animal experiments. Immunosuppressed mice were induced by subcutaneous treatment with a dose of 100 mg/kg of prednisolone (Mitaka Pharmaceutical Co., Tokyo) 1 day prior to oral or oesophageal infection. Tetracycline hydrochloride (Takeda Shering Purau Animal Health Co., Tokyo, Japan) in drinking water at a dose...
of 0.08% was given to the animals, beginning 1 day before infection. Ten minutes before Candida infection, the mice were anaesthetized by intramuscular injection in the foot with 100 µL of 0.25% chlorpromazine chloride (Wako Pure Chemical Industries Ltd, Osaka, Japan) and their sedated condition continued for about 3 h.

In the oral candidiasis model they were orally infected with ~2 × 10⁶ cells/mL viable cells of Candida albicans TIMM2640 in 2.5% FCS-RPMI 1640 medium. Oral infection was performed using a cotton swab (baby cotton buds; Johnson & Johnson Co., Tokyo) rolled over all the areas of the mouth.

In the oesophageal candidiasis model the anaesthetized mice were intraoesophageally infected with C. albicans TIMM2640. The infection was performed by intraoesophageal inoculation of 4 × 10⁷ viable C. albicans cells in 0.2 mL of 2.5% FCS-RPMI 1640 medium by a round-head needle (39 mm length, Fuchigami, Ltd, Kyoto) with a syringe. When the C. albicans cells were injected the needle head was placed at a site just under the pharynx.

Antifungal treatment

Itraconazole OS and beta-cyclodextrin were provided by Janssen Research Foundation, Beerse, Belgium. Itraconazole OS was administered to mice by two different routes throughout the experiment (once a day from 3 h after inoculation to day 2 post-infection). Oral administration was performed by spreading the drug in oral cavity by using a round-head needle with a syringe as described above, and intragastric dosing was done by the conventional method using the same type of needle. Doses of itraconazole OS for oral and intragastric administration were 0.8, 4 or 20 and 4 or 20 mg/kg/day, respectively, that were adopted in an effective range suggested by results of preliminary therapeutic experiments. Control groups of mice received the same volume of 40% beta-cyclodextrin on a time schedule the same as that of itraconazole OS. Therapeutic efficacy was evaluated on day 3 post-infection. The cfu of C. albicans recovered from oral cavity were determined and the white patch of each tongue and squamous disorder was scored as described previously. At the end of oesophageal candidiasis experiments, the oesophageal tubes were obtained and homogenized in 2 mL saline, and the homogenates were cultured to determine the cfu.

Pharmacokinetics

Murine tongues or oesophageal tubes were obtained from the infected mice on the last day in the intraoesophageal TOS-treated mice with oral or oesophageal candidiasis model. In other experiments, itraconazole OS was administered once to normal mice and their blood was collected until 8 h after administration. Concentrations of itraconazole and hydroxy itraconazole (OH-itraconazole), in plasma and tissues were measured by a method described previously.

Results

Efficacy of itraconazole solution administered through oral cavity or intragastrically for oral and oesophageal candidiasis

We confirmed that the MIC of itraconazole is 0.016 mg/L for C. albicans TIMM2640 by the microbioassay method described by NCCLS. The therapeutic effects of itraconazole OS given by two routes against murine oral candidiasis were examined. As shown in Figure 1(a), oral administration of itraconazole OS at doses of 0.8, 4.0 or 20 mg/kg/day clearly decreased the number of the viable C. albicans cells in the oral cavity in a dose-dependent manner. On the other hand, when itraconazole OS was administered directly to the stomach, a dose of 20 mg/kg/day significantly lowered the number of viable C. albicans cells but 4 mg/kg/day did not. Figure 1(b) shows that the symptom scores of the tongues of the mice were improved in all groups of itraconazole OS-administered mice. It was notable that tongue lesions of the mice given 4 mg/kg/day of itraconazole OS intragastrically showed only partially improved symptoms.

We also examined the therapeutic effect of itraconazole OS given by the two routes for oesophageal candidiasis. Figure 1(c) shows that oral administration at doses of 0.8, 4.0 or 20 mg/kg/day clearly lowered C. albicans cell number in oesophagus tubes with dose-dependence. But when was administered to the stomach, only a dose of 20 mg/kg/day decreased the cell numbers but 4 mg/kg/day did not.

These results indicate that administration of itraconazole through the oral cavity, more effectively protected the mice from oral and oesophageal candidiasis than that given intragastrically.

Concentration of itraconazole and OH-itraconazole in tongues, oesophageal tissues or plasma after itraconazole OS administration by two different routes

In oral candidiasis experiments performed by the protocol described above, tongue samples were collected to estimate concentration of itraconazole and its metabolite, OH-itraconazole 3 days after infection, that is, 24 h after the last administration of itraconazole OS. The concentration (average ± SD) of itraconazole of the tongue tissues of the groups of mice given 4 or 20 mg/kg/day of itraconazole OS orally was 7.31 ± 2.47 or 53.5 ± 47.8 ng/g, but when given intragastrically, levels were below the limit of detection (<2.5 ng/g). OH-itraconazole in all samples tested was not detectable (<10 ng/g).

We also examined the concentration of itraconazole in the oesophageal tubes of the mice in the oesophageal candidiasis similar to the case of oral candidiasis. Detectable levels of itraconazole (8.66 ± 5.17 ng/g), but no OH-itraconazole, were measured in the oesophageal tissues of the groups given 20 mg/kg/day of itraconazole OS orally, but not intragastrically (data not shown).

To investigate the pharmacokinetics of itraconazole after intraoesophageal OS administration, early change of blood concentration of itraconazole after a single intraoesophageal OS administration to normal mice was evaluated. As shown in Figure 2, the maximal concentrations of itraconazole in the plasma of the mice given itraconazole OS by oral and intragastric routes, detected 1 h after itraconazole OS administration, were 2200 and 500 ng/mL, respectively. Comparison of pharmacokinetics between the two groups of mice revealed a higher concentration of itraconazole and OH-itraconazole in the group receiving itraconazole OS intragastrically.

Discussion

Here, we showed that oral administration of itraconazole OS elicited therapeutic responses superior to intragastric administration against murine oral and oesophageal candidiasis.
Corresponding to these therapeutic efficiencies, concentration of itraconazole in tongues and oesophageal tissues remained at a detectable level even 24 h after the last oral administration of itraconazole OS, but not after intragastric administration. Therefore, it can be concluded that oral administration of itraconazole OS allowed a high distribution of itraconazole in oral and oesophageal lesions and achieved greater therapeutic efficiency.

The concentration profiles for itraconazole and OH-itraconazole in plasma of normal mice after itraconazole OS administration indicated that intragastric delivery increased plasma concentration of both compounds to higher levels than oral administration. These suggest that itraconazole in lesional tissues, which was detectable after oral administration of itraconazole OS, was not supplied from circulating blood.

Superior efficacy of oral intake of itraconazole OS can also be explained from another aspect: oral administration must increase the chance for interaction between C. albicans cells in situ and a high concentration of itraconazole for relatively short periods. This possible interaction of itraconazole OS to C. albicans cells or lesional tissue may allow rapid absorbance of itraconazole by the tissues, because highly lipophilic itraconazole is reported to be accumulated in cell membrane. Garcia et al. and our group reported that the growth of Candida cells, when pretreated with a relatively high concentration of itraconazole, was suppressed even in conditions without an antifungal agent.

We also showed that administration of itraconazole OS at a dose of 20 mg/kg/day into stomach by gastric gavage results in therapeutic activity against oral and oesophageal candidiasis.

Figure 1. Efficacies of itraconazole OS against oral and oesophageal candidiasis. (a) Efficacies of itraconazole OS by two routes against oral candidiasis. Y-axis: cfu recovered from oral cavity of the mice infected orally with C. albicans 3 days before. ND: Not detected (<100 cfu). (b) Score of white patches on the tongue of mice with oral candidiasis. Y-axis: symptom score of tongues at 3 days after C. albicans infection. (c) Efficacies of itraconazole OS by two routes against oesophageal candidiasis. ND: Not detected (<40 cfu). The designated dose of itraconazole OS was administered orally or intragastrically at 3 h, 1 day and 2 days after Candida infection. *P < 0.05, **P < 0.01 versus placebo control (a and c: Tukey test, b: Mann–Whitney U-test). The dashed line shows a detection limit value. The line for each sample shows the mean.

Figure 2. Change in blood concentration of itraconazole and OH-itraconazole after single administration of itraconazole OS. Itraconazole OS (20 mg/kg/day) was administered orally (open circles) or intragastrically (closed circles) to normal mice, and blood samples were collected to measure the concentration of itraconazole (a) and OH-itraconazole (b). Mean and standard deviation for three or four mice per group.

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We also showed that administration of itraconazole OS at a dose of 20 mg/kg/day into stomach by gastric gavage results in therapeutic activity against oral and oesophageal candidiasis.
This suggests that a sufficient dose of itraconazole OS can elicit its anti-*C. albicans* activity without direct contact with lesional *C. albicans* cells, perhaps through transportation through the blood flow, although the possibility that a part of the itraconazole in gastric cavity may regurgitate to the *C. albicans*-infected lesions cannot be completely excluded.

Finally, we wish to discuss about the optimal manner of intake of itraconazole OS to assure obtaining the maximal therapeutic effect against oral or oesophageal candidiasis. Assuming our results obtained here can be applied to human case, itraconazole OS should be retained as long as possible in the mouth and swallowed slowly, a little at a time.

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**Transparency declarations**

None to declare.

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