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Comparison of the Therapeutic Efficacy of Oral Doses of Fluconazole and Griseofulvin in a Guinea Pig Model of Dermatophytosis

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Fluconazole (FLCZ) developed by Pfizer Ltd. in the United Kingdom, is a triazole antifungal agent with a broad spectrum of activity. In both oral and intravenous dosing forms, FLCZ is widely used for the treatment of candidiasis, cryptococcosis and several other deep-seated mycoses[1,2]. In addition, orally administered FLCZ has been reported to be useful for a systemic therapy of dermatophytoses, oropharyngeal candidiosis and other superficial mycoses[3~6].

Methods for the susceptibility test of yeast to antifungal agents have been standardized by the National Committee for Clinical Laboratory Standards[7], while the standard method has not yet been established for the filamentous fungi. Furthermore, the *in vitro* activity of azole antifungal agents is known to be affected by various assay conditions[8~12]. For these reasons, measuring the *in vivo* activity of azole antifungal agents in suitable animal models is essential for predicting the clinical efficacy of those agents against mycoses caused by filamentous fungi.

In the present study, the therapeutic efficacy of orally administered FLCZ was studied in a guinea pig model of tinea corporis that has been successfully used in our laboratory for evaluating the *in vivo* activity of several antifungal agents on dermatophytes[13~16]. Griseofulvin (GRF) which has long been clinically used for a systemic therapy of dermatophytosis was chosen as a reference drug.

Materials and Methods

Antifungal Agents

FLCZ (Pfizer Pharmaceuticals Inc.) and GRF (Sigma) preparations of known potency were used. The agents were suspended at their specified concentrations in a sterile aqueous 2% methyl cellulose (15CP; Wako Pure Chemical Industries, Ltd.) solution containing 0.5% Tween 80.

Animals

Female, 5-week-old, specific pathogen-free Hartley guinea pigs (Japan SLC Inc.) were used. The animals were kept, one to a cage, at a temperature of 23±2°C and humidity of 55±5%, and were permitted free access to feed pellets (CG-7; Oriental Yeast Co., Ltd.) and drinking water.

Fungal and Inocula

Trichophyton mentagrophytes TIMM 1189 used in this study was a stock culture maintained in Teikyo University Institute of Medical Mycology. The 50% inhibitory concentrations of FLCZ and GRF against this strain determined with an ATP bioluminescence assay[17] were 1 and 0.25 μg/ml, respectively.

The testing strain was grown on modified 1/10 Sabouraud dextrose agar and conidia were prepared as described in our previous paper[15].

Infection Method

The guinea pigs were infected with the inoculum using a previously described method[13~16]. Hair was removed from the entire surface of each animal's back with electric hair clippers, and a circular spot of 2 cm in diameter in the center of the back was designated the inoculation site. Then a procedure in which tape was stuck on the inoculation site and forcefully removed was repeated several times. The short hairs remaining after that were pulled out with tweezers. When the hairs were pulled out locally in the inoculation site, the upper stratum corneum of the epidermis was also removed. Then 60 μl (1.1×10^6 conidia) of inoculum was applied to each spot.

On the 5th day after infection, at which time lesions had clearly developed at the inoculation sites, 10 infected animals each were randomly allocated to the following 6 groups: (1) untreated control group, (2) FLCZ 1 mg/kg/day group (FLCZ 1-mg group), (3) FLCZ 4 mg/kg/day group (FLCZ 4-mg group), (4) FLCZ 16 mg/kg/day group (FLCZ 16-mg group), (5) GRF 25 mg/kg/day group (GRF 25-mg group), and (6) GRF 100 mg/kg/day group (GRF 100-mg group). The antifungal solution was orally administered to the animals by gastric tube once a day for 14 successive days.

On the 4th day after the final drug administration, culture

Table 1. The therapeutic efficacy of oral doses of fluconazole and griseofulvin in the guinea pig model of dermatophytosis.

Treatment with		Number of culture negative animal / total number of infected animal (%)	Average culture score (mean±SD)	Statistical analysis
None (Control)		0 /10 (0)	+9.9 ± 0.32	
FLCZ	1 mg/kg	0 /10 (0)	+8.4 ± 3.03	
	4 mg/kg	0 / 9 (0)	+9.3 ± 1.66	
	16 mg/kg	2 / 9 (22)	+1.8 ± 1.48	
GRF	25 mg/kg	2 /10 (20)	+2.5 ± 2.17	
	100 mg/kg	1 / 6 (17)	+2.3 ± 1.86	

NS ; no significant ; *, P<0.05 ; **, P<0.01

study was performed according to the method as described previously[15].

The therapeutic efficacy in each group was assessed based on the culture study results for each animal overall and those for the fragments of skin tissue from each animal. Two indices were used to make this assessment: (1) the negative culture rate for each group (the percentage of animals in the group whose tissue fragments were all culture-negative), and (2) the mean fungal burden for each group (the mean number of culture-positive tissue fragments for 10 tissue fragments in the group).

To compare the therapeutic efficacy among different animal groups, a multiple comparison analysis of the infection intensity results was performed. First, the Kruskal-Wallis test was done. Then a multiple comparison (nonparametric) using the Bonferroni inequality was performed for groups in which a significant difference ($P<0.01$) was shown by the Kruskal-Wallis test. Six animals that died during the study were excluded from the statistical analysis. Three of the animals (one each in the FLCZ 4-mg and 16-mg groups, and one in the GRF 100-mg group) probably died because of dosing errors, and the other three (all in the GRF 100-mg group) probably because of weakness caused by gastrointestinal symptoms.

Results and Discussion

Once a day treatment with FLCZ or GRF was begun on the 5th day after the animals in the 6 groups (control, FLCZ 1 mg, FLCZ 4 mg, FLCZ 16 mg, GRF 25 mg, GRF 100 mg) were infected with *T. mentagrophytes* and was continued for 14 days. Therapeutic efficacy was assessed by culture study performed at the end of the observation period (the 18th day post infection). The results are shown in Table 1.

In the control group, skin lesions were confirmed on the 4th or 5th day post infection. The lesions gradually worsened, and crusts appeared on the 9th to 11th day post infection. The pathological signs persisted until almost the end of the observation period. Consistent with the development of the lesions, they yielded a high culture score of +9.9.

Although there were some intragroup differences between individual animals in all the FLCZ and GRF groups, the highest FLCZ dose and both GRF doses resulted in significantly decreased mean values of culture score. In the FLCZ 1-mg and 4-mg groups, none of animals was culture-negative, and the mean culture scores were as high as +8.4 and +9.3, respectively. Moreover, neither of these groups showed a significant therapeutic effect compared to the control group. In the FLCZ 16-mg group, however, 2 of the 9 animals became culture-negative (mycologically cured), and the mean culture score was only +1.8, which was significantly low when compared not only with the value for the control group ($P<0.01$) but also with the values for the FLCZ 1-mg group ($P<0.05$) and 4-mg group ($P<0.01$). As for the GRF groups, the mean culture scores were significantly lower than that of the control group; the score was +2.5 ($P<0.01$) for the GRF 25-mg group and +2.3 ($P<0.05$) for the GRF 100-mg group.

There was no significant difference in the mean culture score between the FLCZ 16-mg group and either of the 25-mg or 100 mg GRF group.

The incidence of dermal fungal infections is markedly higher than that of deep-seated mycoses, and dermatophytoses are the most common fungal skin infections[18]. Dermatophytosis is normally treated locally with topical agents, but systemic therapy with oral antifungal agents is needed in several superficial types of dermatophytosis diseases, such as tinea unguium, hyperkeratotic-type tines pedis or tines manus, as well as in several deep-seated types of trichophytosis such as tinea capitis, kerion celsi and sycosis because it is difficult for topical agents to penetrate the affected area in those diseases[19,20].

Although many antifungal agents have become available for the topical treatment of dermatophytosis, there are still few oral antifungals that can be used for systemic therapy. Two triazole antifungal agents FLCZ and itraconazole (ITCZ) were successfully developed in recent years. These triazoles are better tolerated and active against a broader range of pathogenic fungi than GRF, whose activity is almost limited to dermatophytes[21]. In particular, FLCZ is in the widest use, mainly for a treatment of disseminated candidiasis, cryptoccosis and some other deep-seated mycoses because of its high safety and excellent pharmacokinetic characteristics.

To evaluate the potential usefulness of oral FLCZ for the treatment of dermatophytosis at the preclinical stage, the therapeutic efficacy of oral FLCZ was compared with that of oral GRF in a guinea pig model of dermatophytosis. This model has been used to evaluate the *in vivo* effect of a variety of antifungal agents on dermatophytosis[13~16]. The favorable reliability and objectiveness of this evaluation system appear to be due to partly, at least, introducing a sequantitative mycological assessment criterion.

The results of the present study show that oral administration of FLCZ in the 16-mg group had a significant ($P<0.01$) therapeutic effect compared to the control group, and that the effectiveness obtained in the FLCZ 16-mg group was approximately equal to that in the GRF 25-mg and 100-mg groups ($P>0.05$). RICHARDSON *et al.* reported that a significant mycological therapeutic effect was seen after once-a-day oral administration of FLCZ at a daily dosage of 5 mg/kg for 10 days in a guinea pig model of dermatophytosis similar to that used in the present study[22]. These results cannot be directly compared with ours because duration of administration and the assessment criteria were different in RICHARDSON's study. Nevertheless, the results of the two studies seem to be basically the same, even though the significantly high therapeutic effect was only seen at a higher dose in our study.

The excellent pharmacokinetic characteristics of FLCZ probably account in large part for the *in vivo* efficacy of FLCZ seen in RICHARDSON's study and ours. Despite the lower *in vitro* anti-dermatophytic activity of FLCZ than that of GRF, the *in vivo* efficacy of oral FLCZ shown in experiments conducted in several different animal models is much higher than one would expect on the basis of FLCZ's *in vitro* activity[2]. In contrast to other antifungal agents, FLCZ is characteristic of relatively high hydrophilicity, low protein binding, high tissue penetration, favorable bioavailability, and metabolic stability. These characteristics of FLCZ are thought to be the principal explanation for its high *in vivo* efficacy[2]. There have also been reports showing high transfer of FLCZ to skin and related tissues including the stratum corneum, epidermis-dermis, and nails[23~25]. FAERGEMANN reported that when 50-mg dose of FLCZ once a day was orally administered to humans for 12 days, the FLCZ concentration in the stratum corneum of skin on the back was greater than that in serum, and the drug concentration in the stratum corneum 4 and 7 days after the final administration was still 19.9 μg/g and 5.8 μg/g, respectively[23].

Considering a good efficacy in animal models, along with an excellent pharmacokinetics and low toxicity in humans, orally administered FLCZ is expected to have a high clinical usefulness in a treatment of dermatophytosis and further clinical studies are warranted.

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