Antifungal Activities of R-135853, a Sordarin Derivative, in Experimental Candidiasis in Mice

Yasuki Kamai,1* Masayo Kakuta,1 Takahiro Shibayama,2 Takashi Fukuoka,1 and Shogo Kuwahara3

Biological Research Laboratories1 and Drug Metabolism and Pharmacokinetics Research Laboratories,2 Sankyo Co., Ltd., Shinagawa-ku, and Toho University School of Medicine, Ohta-ku,3 Tokyo, Japan

Received 10 June 2004/Returned for modification 1 August 2004/Accepted 8 September 2004

The activities of R-135853, a novel sordarin derivative that possesses a 1,4-oxazepane ring moiety, were evaluated in vitro and in vivo. R-135853 exhibited potent in vitro activities against Candida albicans (fluconazole-susceptible strains), Candida glabrata, Candida tropicalis, and Cryptococcus neoformans, with MICs at which 90% of isolates were inhibited at 0.03, 1, 0.5, and 0.5 μg/ml, respectively. R-135853 also exhibited potent activities against fluconazole-susceptible dose-dependent and fluconazole-resistant strains of C. albicans, with MICs ranging from 0.03 to 0.06 μg/ml. However, R-135853 exhibited weak or no activity against Candida parapsilosis, Candida krusei, and Aspergillus spp. R-135853 exhibited dose-dependent efficacy against experimental murine hematogenous candidiasis induced by C. albicans when it was administered by both the subcutaneous and the oral routes and reduced viable cell counts in the kidneys significantly when it was administered at 50 mg/kg of body weight/dose (administration three times a day). In this model, R-135853 also exhibited dose-dependent efficacy by single oral administration. Subcutaneous administration of R-135853 exhibited dose-dependent efficacy against experimental murine esophageal candidiasis induced by fluconazole-resistant C. albicans, against which fluconazole at 50 mg/kg/dose was ineffective, and reduced viable cell counts in the esophagus significantly when it was administered at 10 and 50 mg/kg/dose. R-135853 eradicated C. albicans from the esophagi of one and four of five mice when it was administered at 10 and 50 mg/kg/dose, respectively. These results suggest that R-135853 is promising for the treatment of disseminated or mucosal candidiasis, including fluconazole-refractory infections.

The risk of opportunistic fungal infections is greatly increasing in patients who are immunocompromised due to cancer chemotherapy, organ or bone marrow transplantation, or human immunodeficiency virus infection (18). Candida albicans is the organism most often associated with both mucosal and hematogenously disseminated infections (4, 6, 33). Recently, azole-resistant C. albicans has become a clinical problem in AIDS patients with oropharyngeal candidiasis (OPC) and esophageal candidiasis (11, 24, 31, 34, 35); and other patients with AIDS (12). This multiple interaction may explain the high degree of selectivity of this class of compounds between fungal and mammalian cells (12). Some pharmaceutical companies have reported that sordarin derivatives exhibit potent antifungal activities with relatively broad-spectrum activities in vitro and that some compounds exhibit good efficacies in vivo (2, 7, 15, 16, 17, 25, 26). We also synthesized sordarin derivatives from zofimarin, a sordarin-related natural product which was isolated from the fungus Zopfella marina (32). Chemical modification efforts led to R-135853 (Fig. 1), which was selected as a candidate for further evaluation (1, 21, 22). In the present study, we investigated the in vitro antifungal activities of R-135853 against pathogenic fungi and its in vivo activities against experimental candidiasis in mice.


MATERIALS AND METHODS

Antifungal agents. R-135853 was synthesized by Sankyo Co., Ltd., for both the in vitro and the in vivo studies. For the in vitro study, fluconazole (FLC) and itraconazole (ITC) were extracted from commercial preparations purchased from Pfizer Pharmaceuticals, Inc. (Tokyo, Japan), and Janssen-Kyowa Co., Ltd. (Tokyo, Japan), respectively. Amphotericin B (AMB) was obtained com-
VOL. 49, 2005 ANTIFUNGAL ACTIVITIES OF R-135853 53

Candidiasis, R-135853 was uniformly suspended in 0.5% sodium carboxymethyl cellulose (CMC; Kanto Chemical Co., Inc., Tokyo, Japan); for subcutaneous administration, R-135853 was dissolved in 0.1 N NaOH (Kanto Chemical Co., Inc.)–25 mM Na2CO3 (Iwai Chemicals Company, Tokyo, Japan) and FLC was dissolved in physiological saline (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan). For the pharmacokinetic analysis, R-135853 was uniformly suspended in 0.5% CMC–10% DMSO for oral administration and was dissolved in 1% Tween 80 (Kanto Chemical Co., Inc.)–10% DMSO for intravenous administration.

Organisms. In the present study, we used C. albicans ATCC 24433, C. glabrata ATCC 90030, C. tropicalis ATCC 750, C. parapsilosis ATCC 29199, C. krusei ATCC 6258, and C. guilliermondii ATCC 9390, C. neoformans ATCC 90112, Aspergillus fumigatus ATCC 26430, A. fumigatus SANK10569, and Aspergillus flavus ATCC 9643 for the investigation of antifungal spectrum of R-135853. We also used C. albicans SANK51486 and 2035B for the in vivo studies. The ATCC and TIMM strains were obtained from the American Type Culture Collection and Teikyo University Institute of Medical Mycology, respectively. The SANK strains had been stored at Sankyo Co., Ltd. C. albicans 2035B was provided by Scott G. Filler of Harbor-University of California, Los Angeles, Research and Education Institute. We also used clinical isolates of C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, and C. neoformans provided by T. Oguri of Juntendo University Hospital, S. Kohno of Nagasaki University School of Medicine, and Scott G. Filler for the investigation of the MICs of R-135853. For the in vitro study, Candida spp. and C. neoformans were cultured on Sabouraud dextrose agar (SDA; Eiken Chemical Co., Ltd., Tokyo, Japan) and Aspergillus spp. were cultured on potato dextrose agar (Eiken Chemical Co., Ltd.). For the in vivo study, C. albicans SANK51486 and 2035B were cultured in YPG medium, which consisted of 0.5% yeast extract (Difco Laboratories,Detroit, Mich.), 1% peptone (Difco Laboratories), and 2% glucose (Wako Pure Chemical Industries, Ltd.) and then cultured on the SDA plates.

In vitro susceptibility testing. The MICs for the test organisms were determined by the broth microdilution method described in NCCLS document M27-A2 (28) for Candida spp. and C. neoformans and NCCLS document M38-A (29) for Aspergillus spp. The MICs of R-135853 were defined as the lowest concentration that resulted in slight growth (approximately 90% inhibition) or the absence of growth at 48 h.

Animals. Specific-pathogen-free male ddY mice (age, 4 weeks) were purchased from Japan SLC, Inc., Shizuoka, Japan, and were used for both the experimental candidiasis and the pharmacokinetic analyses. The mice were used for the experiments after an acclimation period of 6 days. In the experimental study of murine esophageal candidiasis, the mice were immunosuppressed by subcutaneous injection of 4 mg of cortisone acetate (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) per mouse 2 days before, on the day of, and 3 days after inoculation. During the period of immunosuppression, the mice were given tetracycline hydrochloride (0.5 mg/ml in drinking water; Achromycin V;Wyeth Lederle Japan, Ltd., Tokyo, Japan) for the prevention of bacterial infection. Mice were given food and water freely throughout the experiments. All animal experiments were carried out according to the guidelines provided by the Institutional Animal Care and Use Committee of Sankyo Co., Ltd. The experiments used five mice per group.

Efficacy against experimental murine hematogenous candidiasis. We used C. albicans strain SANK51486 in the experimental study of murine hematogenous candidiasis. The inoculum suspension of 2.5 × 10⁶ cells/ml was prepared with sterile physiological saline. The mice were inoculated with 5.0 × 10⁶ cells via the tail vein. For subcutaneous therapy, R-135853 was administered subcutaneously at 2, 5, and 8 h postinoculation at 2, 10, and 50 mg/kg of body weight/dose. For oral therapy, R-135853 was administered orally at 2 h postinoculation (single administration) or at 2, 5, and 8 h postinoculation (administration three times a day) at 2, 10, and 50 mg/kg/dose. The control group received 0.2 ml of the vehicle at 2, 5, and 8 h postinoculation by the same route by which R-135853 was administered. Efficacy was evaluated by determination of the viable cell counts in the kidneys. The mice were sacrificed at 24 h postinoculation, the kidneys were excised and homogenized in sterile physiological saline, and serial dilutions were cultured on SDA containing 10 μg of chloramphenicol per ml at 35°C for 2 days. The viable cell counts in the kidneys were determined by counting the colonies on the SDA plates.

Efficacy against experimental murine esophageal candidiasis. Experimental murine esophageal candidiasis was induced by the same procedure by which experimental murine oropharyngeal candidiasis was induced, as reported previously (20). In this experiment, we used C. albicans strain 2035B. The inoculum suspension of 1.0 × 10⁶ cells/ml was prepared with sterile physiological saline. Before inoculation, the mice were anesthetized by intraperitoneal injection of 27.5 μg of dimorpholamine (Therapique; Eisai Co., Ltd., Tokyo, Japan), 219 μg of xylazine (Bayer Yakuhin, Ltd., Osaka, Japan), and 1.28 mg of pentobarbital sodium (Nembutal; Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) per mouse. R-135853 was administered subcutaneously three times a day for 2 days starting at 3 days postinoculation (70, 73, 76, 94, 97, and 100 h postinoculation) at 2, 10, and 50 mg/kg/dose. For the reference group, FLC was administered subcutaneously once a day for 2 days starting at 3 days postinoculation (70 and 94 h postinoculation) at 50 mg/kg/dose. The control group received 0.2 ml of the vehicle at 2, 5, and 8 h postinoculation by the same route by which R-135853 was administered. Efficacy was evaluated by determination of the viable cell counts in the esophagi. The mice were sacrificed at 5 days postinoculation, the esophagi were excised and homogenized in sterile physiological saline, and serial dilutions were cultured on SDA containing 10 μg of chloramphenicol per ml at 35°C for 2 days. The viable cell counts in the esophagi were determined by counting the colonies on SDA plates.

Pharmacokinetic analysis. Mice received R-135853 orally at 20 mg/kg or intravenously at 2 mg/kg. Mice (n = 3) were killed at 5, 15, 30, 45, 60, 120, and 180 min postadministration and blood samples were collected. R-135853 levels in plasma were measured by liquid chromatography-mass spectrometry; and the pharmacokinetic parameters of half-life in plasma, area under the concentration-time curve (AUC) from time zero to infinity (AUC₀–∞), total body clearance, volume of distribution, the maximum concentration in plasma (Cmax), and the time to Cmax (Tmax) were calculated by using WinNonlin software (Scientific
In vitro antifungal activity. Table 1 shows the spectrum of activities of R-135853 and other reference agents against various fungal strains and also shows the MICs of R-135853 for the strains used for the in vivo studies. R-135853 exhibited potent activities against C. albicans, C. glabrata, C. tropicalis, C. guilliermondii, and C. neoformans, with MICs ranging from 0.016 to 0.5 μg/ml. However, R-135853 exhibited weak activity against C. parapsilosis and no activity against C. krusei or Aspergillus spp. Table 2 shows the MICs of R-135853 and other reference agents for clinical yeast isolates. Table 2 shows the results for C. albicans separately for FLC-susceptible (FLC-S) strains (FLC MICs, ≤8 μg/ml) and FLC-susceptible dose-dependent (FLC-S-DD) and FLC-resistant (FLC-R) strains (FLC MICs, ≥16 μg/ml), according to the guidelines of NCCLS document M27-A2 (28). R-135853 exhibited potent activities against C. albicans (FLC-S), C. glabrata, C. tropicalis, and C. neoformans, with MICs at which 90% of isolates are inhibited (MIC90s) of 0.03, 1, 0.5, and 0.5 μg/ml, respectively, but weak activity against C. parapsilosis, with an MIC90 of 128 μg/ml. R-135853 also exhibited potent activities against the FLC-S-DD and FLC-R strains of C. albicans (MIC range, 0.03 to 0.06 μg/ml), which were comparable to those against the FLC-S strains.

Efficacy against experimental murine hematogenous candidiasis (subcutaneous therapy). Figure 2 shows the efficacy of subcutaneous administration of R-135853 against experimental murine hematogenous candidiasis induced by C. albicans strain SANK51486. R-135853 exhibited dose-dependent efficacy against the infection and at 50 mg/kg/dose significantly (P < 0.01) reduced the viable cell counts in the kidneys.

Efficacy against experimental murine hematogenous candidiasis (oral therapy). In the study for oral therapy for experimental murine hematogenous candidiasis, we examined the efficacy of dose administration once and three times a day (Fig. 3). As was the case with the results of the study of subcutaneous therapy, the oral administration of R-135853 exhibited dose-dependent efficacy against the infection after administration both once and three times a day. R-135853 at 50 mg/kg/dose significantly (P < 0.001) reduced the viable cell counts in the kidneys by administration three times a day.

Efficacy against experimental murine esophageal candidiasis induced by FLC-resistant C. albicans. Figure 4 shows the efficacy of subcutaneous administration of R-135853 against experimental murine esophageal candidiasis induced by FLC-resistant strain C. albicans 2035B. R-135853 exhibited dose-dependent efficacy against the infection and reduced the viable cell counts in the esophagi significantly when it was administered at 10 and 50 mg/kg/dose (P < 0.05 and P < 0.001, respectively). Notably, R-135853 eradicated C. albicans from the esophagi of one and four of five mice at 10 and 50 mg/kg/dose, respectively. On the other hand, FLC did not reduce the
viable cell counts significantly, even when it was administered at 50 mg/kg/dose.

Pharmacokinetics. Table 3 shows the values of the pharmacokinetic parameters after oral administration of R-135853 at 20 mg/kg and intravenous administration of R-135853 at 2 mg/kg.

DISCUSSION

Sordarins belong to a new class of antifungal agents with a novel mechanism of action (9, 10). In this study we evaluated the in vitro and in vivo activities of R-135853, a novel sordarin derivative possessing a 1,4-oxazepane ring moiety. R-135853 exhibited potent in vitro activities against C. albicans, including FLC-resistant strains; C. glabrata; C. guilliermondii; and C. neoformans. On the other hand, R-135853 exhibited weak or no activity against C. parapsilosis; C. krusei; and Aspergillus spp. These in vitro characteristics of R-135853 are similar to those of other sordarins reported previously (16, 17). It is remarkable that R-135853 exhibited potent activities against C. albicans, whether or not the strains showed resistance to FLC. C. albicans is a key yeast pathogen, and FLC-resistant C. albicans has become a clinical problem in AIDS patients with OPC (24, 31, 34, 35). Therefore, we focused on C. albicans for the in vivo evaluation of R-135853.

We examined the in vivo efficacy of R-135853 against experimental murine hematogenous candidiasis. R-135853 exhibited good dose-dependent efficacy against the infection when it was administered subcutaneously, and similarly, it also exhibited good efficacy when it was administered orally. It was demonstrated that R-135853 was highly absorbed by oral administration in mice (63% at 20 mg/kg). The good in vivo efficacy reflected this high level of oral bioavailability. The fact that R-135853 showed efficacy when it was administered orally suggests that R-135853 could be used in formulations for both oral and parenteral use. This is an important attribute, because R-135853 can be used for parenteral-to-oral step-down therapy. In reports regarding other sordarins, it was shown that drugs of this class have short half-lives in plasma in experimental animals (2, 3, 12). It was demonstrated that the half-life of R-135853 in plasma was similarly short in mice (1.1 and 0.47 h after administration at 20 mg/kg orally and 2 mg/kg intravenously, respectively). However, we demonstrated that R-135853 exhibits dose-dependent efficacy even after a single administration, although the efficacy was somewhat weaker than that by administration three times a day. Aviles et al. (2) reported that the AUC is the parameter most predictive of the efficacy of sordarins in a pharmacokinetic-pharmacodynamic study with mice. A pharmacokinetic-pharmacodynamic study with R-135853 is also needed to investigate the relationships between the pharmacokinetic-pharmacodynamic parameters and efficacy.

We next examined the in vivo efficacy of R-135853 against experimental murine esophageal candidiasis induced by FLC-resistant C. albicans. FLC did not exhibit significant efficacy in this model, even when it was administered at 50 mg/kg/dose. Although FLC was administered once daily in this experiment, it was previously demonstrated (19) that this FLC dosing regimen is a proper means of achieving a good correlation between in vitro activity and efficacy in the murine OPC model, which is very similar to the esophageal candidiasis model used in the present study. In this model, R-135853 also exhibited good dose-dependent efficacy and eradicated C. albicans from the esophagi of one and four of five mice when it was administered at 10 and 50 mg/kg/dose, respectively. In the experimental models of hematogenous candidiasis and esophageal candidiasis, the efficacy of R-135853 was markedly higher than that by administration three times a day. Aviles et al. (2) reported that the efficacy of sordarins in a pharmacokinetic-pharmacodynamic study with mice is also needed to investigate the relationships between the pharmacokinetic-pharmacodynamic parameters and efficacy.

**FIG. 4.** Efficacy of R-135853 against experimental murine esophageal candidiasis induced by FLC-resistant C. albicans. Each circle represents the result for an individual mouse. Closed circles, results above the detection limit; open circles, results below the detection limit. Each bar represents the mean value (n = 5). *P < 0.05 versus the results for the vehicle-treated control group by the nonparametric Dunnett test; ***P < 0.001 versus the results for the vehicle-treated control group by the nonparametric Dunnett test.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Oral dose (20 mg/kg)</th>
<th>Intravenous dose (2 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-life (h) in plasma</td>
<td>1.1</td>
<td>0.47</td>
</tr>
<tr>
<td>AUCmax (µg · h/ml)</td>
<td>3.19</td>
<td>0.509</td>
</tr>
<tr>
<td>Total body clearance (liters/kg)</td>
<td>—</td>
<td>3.93</td>
</tr>
<tr>
<td>Distribution volume (liters/kg)</td>
<td>—</td>
<td>1.84</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.25</td>
<td>—</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>2.32</td>
<td>—</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>62.7</td>
<td>—</td>
</tr>
</tbody>
</table>

*a* — not determined.
candidiasis, as the intervals between the time of administration of the last dose and the time of killing were somewhat prolonged (16 to 24 h), it is possible that differences in the viable cell counts between the groups might be obscured to some extent by regrowth after administration of the last doses. However, we think that such an interval has little influence on the interpretation of the results, because the growth of the organisms was relatively slow in these infection models (results of a preliminary study; data not shown).

In conclusion, the results of the present study suggest that R-135853 is a promising compound, in particular, for the treatment of disseminated or mucosal infections induced by C. albicans, including FLC-resistant strains. The development of R-135853 is in the preclinical stage; and further investigations, such as pharmacokinetic-pharmacodynamic and toxicity studies, are needed.

ACKNOWLEDGMENTS

We thank T. Oguri of Juntendo University Hospital, S. Kohno of the Nagasaki University School of Medicine, and Scott G. Filler of the Harbor-University of California, Los Angeles, Research and Education Institute for providing us with clinical yeast isolates.

REFERENCES

ERRATUM

Antifungal Activities of R-135853, a Sordarin Derivative, in Experimental Candidiasis in Mice

Yasuki Kamai, Masayo Kakuta, Takahiro Shibayama, Takashi Fukuoka, and Shogo Kuwahara

Biological Research Laboratories and Drug Metabolism and Pharmacokinetics Research Laboratories, Sankyo Co., Ltd., Shinagawa-ku, and Toho University School of Medicine, Ohta-ku, Tokyo, Japan

Volume 49, no. 1, p. 52–56, 2005. Page 53: Figure 1 should appear as shown below.