Efficacy of 2-(3,4-Dimethyl-2,5-Dihydro-1H-Pyrrole-2-yl)-1-Methylethyl Pentanoate in a Murine Model of Invasive Aspergillosis


Institute of Biomedical Sciences, Bundelkhand University, Jhansi-284128, India; Department of Biochemistry, Kurukshetra University, Kurukshetra-136119, India; Institute of Genomics and Integrative Biology, Mall Road, Delhi University Campus, Delhi-110007, India; and Department of Microbiology, Bundelkhand University, Jhansi-284128, India

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2-(3,4-Dimethyl-2,5-dihydro-1H-pyrrole-2-yl)-1-methylethyl pentanoate, an antifungal compound, was found to be nontoxic to RAW cells up to a concentration of 312.5 μg/ml, whereas amphotericin B was lethal to all cells at 37.5 μg/ml. The treatment of Aspergillus fumigatus-infected mice with a dose of 200.0 mg of compound/kg of body weight increased their survival rate by 60%, with a decrease in CFU in organ tissues. The protection afforded by the compound against experimental aspergillosis was found to be dose dependent.

Synthetic and natural-product-based drugs are currently available to treat fungal infections. However, none of these drugs is ideal. The treatment of invasive mycosis is much more complicated because of the limited efficacies of available drugs. Further, the development of resistance in fungi against most drugs has become an increasingly serious problem (8). In view of the need for new antifungal formulations, studies were carried out earlier to investigate the antiymycotic properties of Datura metel, and the active molecule 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrole-2-yl)-1-methylethyl pentanoate was characterized (2). The current study was undertaken to evaluate the molecule for its cytotoxicity and in vivo efficacy.

**Compound.** The purified antifungal compound 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrole-2-yl)-1-methylethyl pentanoate was used throughout the studies (2, 3).

**Pathogen.** Clinical isolates of Aspergillus fumigatus were grown for 3 days at 37°C on Sabouraud dextrose (SD) agar procured from E. Merck (2). The conidia were harvested by flushing the culture plates with 5.0 ml of 0.025% Tween 20 in sterile saline and counted by using a hemocytometer.

**Cytotoxicity.** In vitro cell cytotoxicity was carried out as described earlier, by use of RAW cells (7). The percent toxicity with respect to the negative control was calculated and plotted against log concentrations of the compound to determine the dose cytotoxic to 50% of the cells.

**Animals.** Six-week-old BALB/c mice of either sex, weighing 18 to 20 g, housed in microbarrier cages on sterile bedding and fed ad libitum water and food, were used. Ethical clearance was obtained from an institutional animal ethics committee. Mice were treated with 100.0 mg of cortisone acetate/kg of body weight subcutaneously on days 0, 2, 4, and 6 before infection. On day 0, mice were anesthetized by using diethyl ether, and a suspension of 10⁷ conidia/20 μl of saline was slowly applied onto the nostrils. The animals were held upright until the suspension was completely inhaled and normal breathing resumed (6). The mice thus challenged were divided into six groups of 18 to 20 animals each.

**Treatment of animals.** The dosing of animals with the compound 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrole-2-yl)-1-methylethyl pentanoate in different groups was initiated the next day after challenge with A. fumigatus conidia. The mice of groups I, II, III, IV, and V were treated orally with 0.0, 25.0, 50.0, 100.0, and 200.0 mg of compound/kg/day, respectively, for 7 days. The animals of group VI were kept as controls, and seven doses of 3.0 mg of amphotericin B/kg/day (A9528; Sigma-Aldrich) were administered intravenously. An additional control group (group VII) consisting of normal, uninfected mice was also included in the study.

**Survival rate.** The animals were housed in properly labeled cages and kept under close watch for weight loss and mortality, if any. The survival rate or any extension in survival time over a period of 15 days after infection was determined. Statistical comparisons were made by using the log rank test and the Wilcoxon test of life table with a P of <0.05 for determining the significance (1).

* Corresponding author. Mailing address: Institute of Genomics and Integrative Biology, Mall Road, Delhi University Campus, Delhi-110007, India. Phone: 91-517-2321180. Fax: 91-517-2320761. E-mail: drglsharma@hotmail.com.
Quantification of CFU. The CFU, indicating fungal loads, in various organs of animals were determined. The organs of the mice (five or six moribund or dead animals in each group) were removed aseptically on day 10 after treatment and homogenized in sterile phosphate-buffered saline (pH 7.2). Dilutions (10-fold) of organ homogenates were plated on Sabouraud dextrose agar containing 0.05% Triton X-100, and the CFU were counted after incubation at 37°C for 48 h. The colony counts obtained from animals of treated groups were compared with those of control groups by using Student’s t test for levels of significance.

The cytotoxicity of the compound 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrole-2-yl)-1-methylethyl pentanoate was studied by use of RAW cells, and it was found to be nontoxic up to a concentration of 312.5 μg/ml. Amphotericin B was lethal to all the cells at a concentration of 37.5 μg/ml. The dose cytotoxic to...
50% of the cells of the compound was found to be 889.0 \mu g/ml (Fig. 1). There was no apparent adverse effect of the compound in animals treated with a dose of 400.0 mg/kg of body weight, indicating better tolerability than for amphotericin B (data not shown). To study the in vivo efficacy of the compound, the experiments were conducted by use of BALB/c mice. The survival rate in the treated mice increased with the increase in the dose of compound. The effective dose for the survival of 50% of the animals was calculated by plotting probit values of percent survival against the log dose of the compound. It was found to be 162.18 mg/kg of body weight. A dose of 50.0 mg of itraconazole/kg of body weight could afford protection in 45.45 percent of the mice (4). Sixty percent of the mice treated with a dose of 200.0 mg of compound/kg of body weight survived up to 15 days after infection, compared to none in the control group (Fig. 2), the difference being significant ($P < 0.082$). The compound, therefore, had in vivo efficacy against experimental aspergillosis. Amphotericin B was reported to have high activity in vivo (5); however, its high toxicity (50% lethal dose, 2.3 mg/kg of body weight) has been the major disadvantage (10).

The treatment of animals with the compound decreased the fungal burden in body organs. The results indicated that all the doses of compound higher than 100.0 mg/kg of body weight decreased the fungal colony counts in the lung, liver, and kidney tissues. The total number of CFU in the liver was significantly less than those obtained from lung and kidney. These observations were in accordance with those reported by Wallace et al. (9). The dose of 200.0 mg of compound/kg of body weight reduced the fungal burden significantly on the 10th day in lungs, liver, and kidneys compared to controls (Fig. 3). The nystatin was shown to reduce CFU in liver by 44 percent at a dose of 8.0 mg/kg of body weight; however, the point of concern has been the twofold increase in the number of CFU in kidneys on the fifth day of treatment with nystatin at the same dose (9).

Although the compound 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrole-2-yl)-1-methylethyl pentanoate reported here had limited efficacy against experimental infection with A. fumigatus, it had much less cytotoxicity and better tolerability than amphotericin B; therefore, it can be used as a model compound to synthesize novel series of antifungal molecules with better antifungal activity.

REFERENCES