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6-[(N-2,3-Dichlorophenyl)amino]-7-Chloro-5,8-Quinolinedione Treatment of Candidiasis in Normal Mice

Chung-Kyu Ryu[1], Dong-Hyun Kim[2], Do-Hee Kim[1], In-Kyung Lee[1] and Sung-Hee Kim[1]

[1]College of Pharmacy, Ewha Womans University, Seoul 120-750 and [2]College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea

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6-[(N-2,3-Dichlorophenyl)amino]-7-chloro-5,8-quinolinedione (RCK11) was tested for *in vivo* antifungal activities in the treatment of systemic infection with *Candida albicans* in normal mice compared with ketoconazole. The therapeutic potential of RCK11 had been assessed by evaluating their activities (survival rates) against systemic infections in normal mice. ED_{50} of intraperitoneally administered RCK11 was 0.10±0.01 mg/kg but that of ketoconazole had 8.00±0.73 mg/kg respectively. When RCK11 was administered intravenously at the ED_{50} (0.10 mg/kg), the colony counts of *Candida albicans* in the liver after 7 days and 14 days were reduced as likely as ketoconazole at the ED_{50} (8.00 mg/kg), and the better survival rates than ketoconazole were achieved after 14 days. The results suggest that RCK11 may be a potent antifungal agent.

Key words : 6-[(N-2,3-dichlorophenyl)-amino]-7-chloro-5,8-quinolinedione, *Candida albicans*, Candidiasis, *In vivo* antifungal activity

INTRODUCTION

Fungal infections have become increasingly frequent owing to the increasing number of patients who receive treatment with antibiotics and chemotherapeutic agents or who are immunocompromised (Clark *et al.*, 1992, Georgiev *et al.*, 1988, Georgopapadakou *et al.*, 1994, Rex *et al.*, 1993, Sheehan *et al.*, 1993). The emerging magnitude of fungal infections has generated a renewed interest in aspects of antifungal drugs, including development of new antifungal agents (Dupouy-Camet *et al.*, 1991, Sternberg *et al.*, 1994).

The 5,8-quinolinedione ring is the pharmacophore for development of antifungal agents. 6-(Substituted)-7-chloro-5,8-quinolinedione derivatives had potent antifungal (Jeschke *et al.*, 1992, 1993, Ryu *et al.*, 1994a, 1995), antibacterial (Roberts *et al.*, 1978) and antimalarial (Bowman *et al.*, 1973) activities. The 5,8-quinolinedione derivatives were selective and potent inhibitors of pyrimidine biosynthesis (Hudson *et al.*, 1992) due to blockade of mitochondrial electron transport in fungi and malaria (Bowman *et al.*, 1973, Roberts *et al.*, 1978). In the previous paper (Ryu *et al.*, 1994a, 1994b), newly prepared 6-(N-(halophenyl)amino) 7-chloro-5,8-quinolinediones(RCKs, Fig. 1) were tested for *in vitro* antifungal activities against *Candida* spp., *Aspergillus niger and Trichophyton mentagrophytes*. 6-[(N-2,3-Dichlorophenyl)amino]-7-chloro-5,8-quinolinedione (RCK11) showed not only the most potent antifungal activities among these RCK derivatives, but also more potent antifungal activities than ketoconazole.

For the continuing evaluation on antifungal activities of RCK11, the *in vivo* antifungal activities were tested in the treatment of systemic infection with *Candida albicans* in normal mice compared with ketoconazole. The therapeutic potential of RCK 11 had been assessed by evaluating their activities (survival rate). We performed this study in an attempt to determine the ability of RCK11 to prolong survival and decrease colony counts of *Candida albicans* in the kidneys and liver in established models (Fisher *et al.*, 1989, Mcginnis *et al.*, 1991, Ryu *et al.*, 1995, Sugar *et al.*, 1994, Viscoli *et al.*, 1991) of murine disseminated candidiasis.

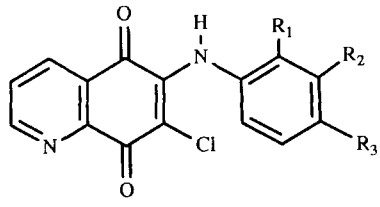
MATERIALS AND METHODS

Materials and apparatus

The RCK11 (Fig. 1) prepared previously (Ryu *et al.*, 1994a) was used for *in vivo* antifungal activity tests.

Sabouraud Agar and Brain Heart Infusion (BHI)

Correspondence to: Chung-Kyu Ryu, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea



RCKs : R_1, R_2, R_3 = F, Cl, Br
RCK11 : R_1 = H, R_2 = R_3 = Cl

Fig. 1. Chemical structure of 6-[(N-halophenyl)amino]-7-chloro-5,8-quinolinedione

broth were purchased from Difco Lab (U.S.A.) Tween 20 was obtained from Aldrich Chemical Co. (U.S.A.), and ethanol from Shinyo Pure Chemicals Co.(Japan). Other chemicals such as ketoconazole and saline were reagent grade commercially available.

UV spectrophotometer from Shimadzu UV-120-02 (Japan) was used. The microorganisms were incubated in incubator bath from Vision Scientific Co. (Korea).

Mice

ICR male mice, 3 to 4 weeks old, were purchased from Daehan Experimental Animal Center (Korea). At the start of each experiment, the mice weighed 20.0 g. Six mice were housed per cage in filter-topped cages and were fed standard mouse chow and water *ad libitum*. Each group of survival experiments consisted of eight mice. Additional cohorts of mice were infected for studies of kidneys and liver colony counts.

Fungi

Candida albicans used in this study was recent clinical isolates from Kyung Hee University hospital (Korea), which was maintained on Sabouraud dextrose agar slants at 4°C. A large loopful of yeast cells were suspended in fresh Sabouraud dextrose broth and incubated for 24 hrs at 37°C. Blastoconidia were harvested and washed twice in sterile buffered saline (pH 7.4) by centrifugation. Cells were counted in a hemacytometer, and the concentration was adjusted to 10^7 cells per ml. The number of yeast cells administered to the mice was determined by planting the same inoculum on Sabouraud dextrose agar plates. Colonies were counted 24 to 48h later.

Antifungal agents

The RCK11 and ketoconazole were suspended in saline with 0.25% Tween 20 and were administered by the intravenous injection of 0.1 ml.

Table I. Efficacy of RCK11 against systemic infection with *Candida albicans* in normal mice

Compound	Mean $ED_{50}\pm SD$ ($n\geq 5$) in normal mice (mg/kg)
RCK11	0.10±0.01
Ketoconazole	8.00±0.73

[1]Dose range; RCK11 0.025, 0.1, 0.5, 2.0, 10.0 mg/kg; ketoconazole 0.2, 1.0, 2.0, 10.0, 40.0 mg/kg
[2]Drugs were administered intraperitoneally at 1, 4 and 24 hrs postinfection.
[3]ED_{50} at 2 days postinfection

Systemic infection with *Candida albicans* and evaluation of in vivo Antifungal activities

The evaluation of *in vivo* antifungal activities was determined by the modified established methods (Fisher *et al.*, 1989, Ryu *et al.*, 1995, Sugar *et al.*, 1994, Viscoli *et al.*, 1991).

ED_{50}, Efficacy of RCK11 and ketoconazole: Acute infection in normal mice was produced by intraperitoneal injection (via the lateral tail vein) with 0.1 ml of sterile saline calculated containing 10^6 blastospores of *Candida albicans*, a dose uniformly lethal for placebo-treated animals within 48 hrs. Groups of six animals received one of the following dose range; RCK11 0.025, 0.1, 0.5, 2.0, 10.0 mg/kg; ketoconazole 0.2, 1.0, 2.0, 10.0, 40.0 mg/kg. Appropriate doses in 0.1 ml of diluent were administered intravenously at 1, 4 and 24 hrs postinfection. The 50% effective dose (ED_{50}) at 2 days postinfection was calculated by fitting survival data to logistic dose response model (Table I).

Survival experiments and study of colony counts: The Groups of seven mice were infected by injection with 0.1 ml of sterile saline containing 2×10^4 blastoconidia in a lateral tail vein. Each groups received the following treatment. The fresh suspension of RCK 11 and ketoconazole were prepared daily in sterile saline with 0.25% Tween 20 and administered intravenously by the injection of 0.1 ml. Therapy was begun from 4 days after infection of mice with *C. albicans* and was continued for a total of 14 days. The control group was injected with the sterile saline with 0.25% Tween 20 daily. Cages were observed twice daily for deaths (Fig. 2).

Randomly selected mice in each group were sacrificed at designated intervals, and the kidneys and liver were removed aseptically. The kidneys and liver were homogenized in small volume of saline, and 10-fold dilutions were plated onto Sabouraud dextrose agar. The plates were incubated for 24 to 48 hrs at 37°C, and then the colonies were counted (Table II).

Student's t test was used to compare the means of colony counts in the kidneys and liver. Significance was defined as $P<0.05$.

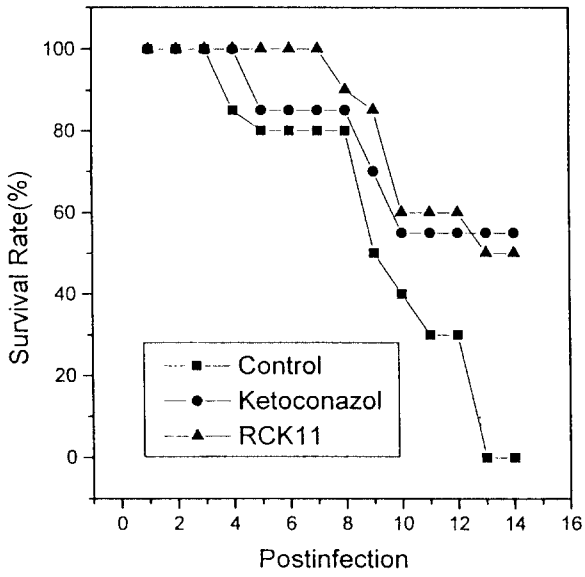


Fig. 2. Survival of *C. albicans* systemically infected mice treated with RCK11 and ketoconazole. Treatment was begun from 4 days after infection and continued for a total of 14 days. Mice (6 per group) received intravenous therapy once daily. Data for groups given RCK11 at the ED_{50} (0.1 mg/kg/day) and ketoconazole at the ED_{50} (8.0 mg/kg/day). ▲, RCK11: ●, Ketoconazole: ■, Control (saline with 0.25% Tween 20)

RESULTS AND DISCUSSION

RCK11 was tested for determination of in vivo antifungal activities against pathogenic *Candida albicans* by the modified known techniques (Fisher *et al.*, 1989, Ryu *et al.*, 1995, Sugar *et al.*, 1994, Viscoli *et al.*, 1991). The results are given in Fig. 2, Table I and Table II, compared with ketoconazole. The control groups showed no antifungal activities against the pathogenic *Candida albicans*.

The 50% effective doses (ED_{50}) of RCK11 and ketoconazole when administered intraperitoneally to mice infected with *Candida albicans* are summarized in Table I. RCK11 was approximately 80 times more potent than ketoconazole against the infection in normal mice. Placebo-treated mice were dead by 2 days postinfection.

Analysis of a typical experiment revealed that any control (untreated) animals did not survive after receiving an inoculum of 2×10^4 *C. albicans* cells per mouse (Fig. 2). Intravenously administered RCK11 at the ED_{50}, (0.10 mg/kg of body weight) per day prolonged survival compared with the control.

In spite of lower ED_{50} of RCK11 (0.10 mg/kg), better results were observed than higher ED_{50} of ketoconazole (8.00 mg/kg), including the prolonged survival length and increase of survival ratio after 7 day treatment, but at the end of the 14-day experiment the level of mortality was the same, as seen in Fig. 2.

Table II. Colony counts of *Candida albicans* recovered from kidneys and liver of systemically infected mice

Organ	Agent & Dosage (ED_{50}, mg/kg)	Mean $\log_{10}$ CFU/g of tissue±S.E.	
		7-Day Rx[a]	14-Day Rx
Liver	Control[b]	4.90±0.59	4.95±0.78
	Ketoconazole (8.00)	3.37±0.70*	4.00±0.77*
	RCK11 (0.10)	3.28±0.60**	2.63±0.81**
Kidney[c]	Control	≥5.8	≥6.0
	Ketoconazole (8.00)	≥5.8	≥6.0
	RCK11 (0.10)	5.8	6.0

[a]Rx; Drug treatment, intravenously administered
[b]Control; saline with 0.25% Tween 20
[c]Mean for right and left kidneys
*P< 0.05, **P<0.01

Liver colony counts obtained at the end of therapy for 7 days and 14 days showed substantial decreases in numbers of Candida organisms in mice treated with RCK11 at ED_{50} as well as ketoconazole. But both RCK11 and ketoconazole didn't reduce significantly *Candida albicans* colony counts in the liver (Table II).

The results presented in this paper indicate that RCK11 possesses superior activity to that of ketoconazole in a range of animal models of systemic fungal infection. Continuing interest is to demonstrate that other 6-(N-(halophenyl)amino)-7-chloro-5,8-quinolinediones (RCKs, RCK3, 11, 20) were also more protective than ketoconazole in the same murine cadidiasis model (Ryu *et al.*, 1995, Park *et al.*, 1996). The results suggest that RCKs may be potent antifungal agents. Further studies to continuously explore the *in vivo* antifungal activities and safeties of various new RCKs are required.

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