J. Microbiol. Biotechnol. (1999), **9**(5), 672–674

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Immunosuppressive Activity of Cepacidine A, a Novel Antifungal Antibiotic Produced by *Pseudomonas cepacia*

LEE, CHUL-HOON[1]*, JUNG-WOO SUH[2], AND YOUL-HEE CHO[1]

[1]*Department of Medical Genetics, College of Medicine, Hanyang University, Seoul 133-791, Korea*
[2]*R&D Center of Bioscience Institute of Science and Technology, Cheiljedang Corp., Ichon, Kyunggi-Do 467-810, Korea*

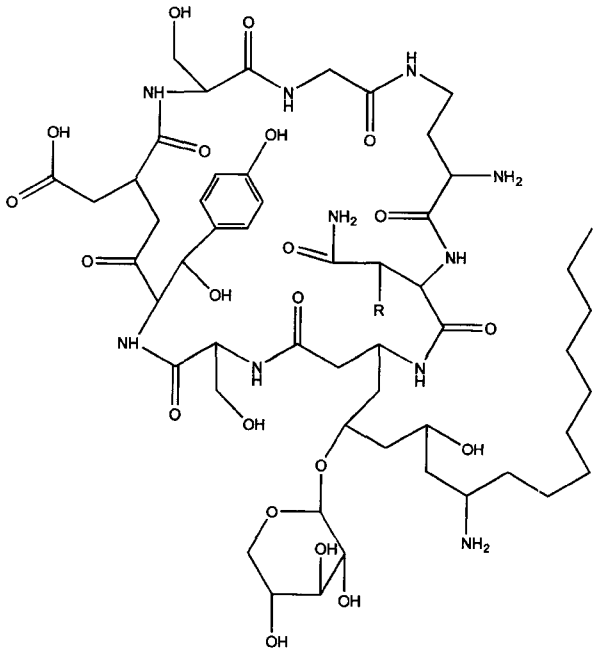
Received: May 29, 1999

Abstract Cepacidine A was first identified as a novel antifungal antibiotic which was isolated from the culture broth of *Pseudomonas cepacia* AF2001. It showed a potent *in vitro* antifungal activity against various pathogenic fungi, but did not show any activity against bacteria. Recently, the immunosuppressive action of cepacidine A was discovered using an *in vitro* screening system involving inhibition of the proliferation of murine lymphocytes stimulated by 2 mitogens, and also by *in vivo* mouse models involving inhibition of delayed type hypersensitivity and SRBC hemagglutination. Cepacidine A showed a significant activity of cellular immunosuppression (ED_{50}) at concentration levels of 1-3 mg/kg, i.p.. Unfortunately, the delayed toxicity at a dose of above 3 mg/kg i.p. was apparent.

Key words: Antifungal antibiotic, cepacidine A, immunosuppressive activity

In the course of screening for new antifungal substances of microbial origin, cepacidine A, a novel glycopeptide, was found in the fermentation broth of a strain of *Pseudomonas cepacia* AF2001 [4, 6]. This strain was deposited in the Korean Federation of Culture Collections, Seoul, Korea, with a registration number of KFCC 10773. Cepacidine A is a mixture of two closely related compounds of cepacidine A_1 and A_2 (Fig. 1).

Some immunosuppressive substances such as FK-506 (tacrolimus), cyclosporin A, and rapamycin were first screened as antifungal antibiotics, and then they were found to have potent immunosuppressive activities after extensive immunopharmacological studies [2, 7, 10]. Therefore, we also tried to test the possible immunosuppressive activity of cepacidine A by using immunopharmacological studies, such as the oxazolone-induced delayed type hypersensitivity, sheep red blood cell hemagglutination, and proliferation of



Cepacidine A_1 R=OH
Cepacidine A_2 R=H

Fig. 1. The structure of cepacidine A_1 and A_2.

murine lymphocytes stimulated by concanavalin A for the T-cell and lipopolysaccharide for the B-cell. The degree of each type of lymphocyte stimulation by mitogen may be assayed, by measuring the amount of [^{3}H] thymidine which was incorporated into newly synthesized DNA. In the present paper, the results obtained from the above-mentioned studies are presented.

Mitogen-Induced Proliferation of the Murine Lymphocytes

B-lymphocytes were isolated from the ICR mouse spleen and T-lymphocytes from the mouse thymus by standard conditions [1, 5, 8], and they were suspended in Dulbecco's

*Corresponding author
Phone: 82-2-2290-8289; Fax: 82-2-2298-4857;
E-mail: chhlee@email.hanyang.ac.kr

Table 1. The influence of cepacidine A on mitogen-induced blastogenesis.

Functional assay	Activity (ED_{50}, μM)		
	Cepacidine A	Cyclosporin A	Cyclophosphamide
Suppression of the B-cell proliferation + LPS	0.3	0.3	>10
Suppression of the T-cell proliferation + Con-A	1	0.01	>10

modified Eagle's medium (DMEM). To assess suppression of the cell proliferation, 10^6 cells/ml were incubated overnight at 37°C in the presence of 10 μg/ml lipopolysaccharide for B-cells and 3 μg/ml concanavalin A for T-cells. Cepacidine A, dissolved at 10, 1, 0.3, 0.1, and 0.01 μM in 0.1% DMSO, was evaluated under these conditions. Cyclophosphamide and cyclosporin A in 0.1% DMSO were used in each case as positive reference substances. After an overnight incubation, 2 μCi [^{3}H] thymidine was added to each well. Cells were then harvested after an additional 48-h incubation, and thymidine incorporation was assessed by a liquid scintillation counting. As shown in Table 1, an effective concentration of immunological suppression of LPS-induced B-cell proliferation (ED_{50}) with cepacidine A is known to be 0.3 μM. This result revealed that cepacidine A has a potential for the potent immunosuppressant which is comparable to cyclosporin A. However, in the case of T-cell proliferation in response to Con-A, the ED_{50} value of cepacidine A is about 3 times higher (1 μM) than that of B-cell proliferation. These results indicated that cepacidine A significantly suppressed the activation of B lymphocyte, but can suppress T-lymphocyte activation only moderately, although suppression of cepacidine A in T-cell activation was much lower than that of the cyclosporin A. Interestingly, cyclosporin A suppressed the response to Con-A much more than to LPS. According to these results, it is concluded that cyclosporin A strongly affects T-cell function [9]. In addition, cepacidine A seems to affect mainly the B-cell activation. Unfortunately, under the condition, we could not determine an applicable level of cepacidine A as a therapeutic agent. Therefore, the mouse *in vivo* assays described below were the only experiments that were carried out to detect any functional activity of cepacidine A for immunosuppression.

Cellular Immunosuppression: Oxazolone-Induced Delayed Type Hypersensitivity

One type of cell-mediated immunity is known as a delayed type hypersensitivity (DTH). It is caused by injecting antigen (5% oxazolone) into the skin of a mouse which was previously immunized by the same antigen. The reaction is characterized by a reddening of the skin and a localized inflammation.

The shaved abdominal surfaces of a group of 5 male ICR mice (23-27 g) were sensitized by applying 0.1 ml of 5% oxazolone. One hous later, cepacidine A dissolved in 0.1% DMSO at initial doses of 0.1, 1, 3, and 10 mg/kg was first administered i.p., and then daily for 5 consecutive doses were given. After an additional 4 days, animals were challenged by applying 25 μl of 5% oxazolone to the right ear. Twenty-four hours later, ear thickness was measured with a Dyer Model micrometer gauge [3].

Table 2. Cellular immunosuppressive activity of cepacidine A on oxazolone-induced DTH.

(n=5 mice/group)

Compound	Route	Dose	Inhibition (%)[d]
Vehicle control (0.1% DMSO)	i.p.	20 ml/kg×5 days[c]	0
Cepacidine A	i.p.	10 mg/kg×5	-[a]
		3 mg/kg×5	50[b]
		1 mg/kg×5	49
		0.1 mg/kg×5	36
Cyclosporin A	i.p.	50 mg/kg×5	60

[a]1/5 died on day 2, 4/5 died on day 4, totally 5/5 died in the study.
[b]2/5 died on day 3, 1/5 died on day 4, totally 3/5 died in the study.
[c]Administered once a day for 5 consecutive days.
[d]Percent inhibition of the swelling in oxazolone-treated ears versus control ears.

In general, 50% or greater decrease in oxazolone-treated ears versus control ears was considered to have a significant immunosuppressant activity. As a positive reference substance, a single dose (50 mg/kg) of cyclosporin A in 0.1% DMSO was used.

As shown in Table 2, the ED_{50} value of *in vivo* cellular immunosuppressive activity of cepacidine A was shown at the concentration of 1-3 mg/kg i.p.. Before beginning the above described experiment, a comparison of the acute toxicity of cepacidine A with ICR mice was made and then evaluated. All of the mice survived the p.o. administration of 200 mg/kg cepacidine A, but half of the mice died when injected intravenously with 12.5 mg/kg (LD_{50}, data not shown). Due to the potent toxicity of cepacidine A when i.v. injected, we decided to identify *in vivo* immunosuppressive activity by an i.p. route. But unfortunately, the immunosuppressive activity of cepacidine A induced the delayed toxicity of cepacidine A at a dose of above 3 mg/kg i.p.. On the other hand, only in the aspect of certain activity, cepacidine A showed a significant reaction of the cellular immunosuppression.

Humoral Immunosuppression: Sheep Red Blood Cell (SRBC) Hemagglutination

Group of 6 female ICR mice (23-27 g) were sensitized by the i.v. injection of 0.2 ml of 2% SRBC suspension. After

Table 3. Humoral immunosuppressive activity of cepacidine A on SRBC hemagglutination.

(n=6 mice/group)

Compound	Route	Dose	Inhibition (%)[d]
Vehicle control (0.1% DMSO)	i.p.	20 ml/kg×3 days[c]	32
Cepacidine A	i.p.	10 mg/kg×3	-[a]
		3 mg/kg×3	8[b]
		1 mg/kg×3	32
		0.1 mg/kg×3	64
Cyclophosphamide	i.p.	20 mg/kg×3	2

[a]3/6 died on day 2, 3/6 died on day 3, totally 6/6 died in the study.
[b]3/6 died on day 3, totally 3/6 died in the study.
[c]Administered once a day for 3 consecutive days.
[d]Reciprocal serum dilution, exhibiting a complete hemolysis.

9 days of sensitization to SRBC, blood samples were taken from the orbital sinus, and equal parts of complement inactivated serum from the group of 6 mice were pooled to yield a single 0.25 ml sample. Serial 2-fold dilutions were then carried out 10 times in the presence of added complement. Serum titer was expressed as the reciprocal of that dilution which exhibited a complete hemolysis. Serum titers of less than 16 were, in general, considered to be significant, which indicated a possible immunosuppressant activity.

Cepacidine A dissolved in 0.1% DMSO at initial doses of 0.1, 1, 3, and 10 mg/kg was administered i.p. to mice for 3 consecutive days beginning 2 h after sensitization to SRBCs. As a positive reference substance, cyclophosphamide (30 mg/kg) in 0.1% DMSO was used.

As shown in Table 3, cepacidine A showed a significant humoral immunosuppressive activity at a concentration of 3 mg/kg i.p. It should be mentioned here that we could not achieve our goal mainly because of the delayed toxicity of the compound, which seems to have a positive immunosuppressive activity without any sign of toxicity. Further studies on the chemical modification of the cepacidine A structure should be delineated to separate its positive immunosuppressive activity from their toxicity.

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