The High Therapeutic Effect of Thiabendazole on Chytridiomycosis in *Xenopus laevis*

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Abstract

Chytridiomycosis, caused by *Batrachochytrium dendrobatidis,* is involved in serious population declines of many amphibian species in four continents. A drug treatment test for this disease was conducted with the fungicide thiabendazole in *Xenopus laevis,* which is a disease mediator and is reared in many laboratories all over the world. The therapeutic effect of thiabendazole was found to be similar to or greater than that of benzalkonium chloride, which is conventionally used for the treatment for chytridiomycosis. The eradication of chytridiomycosis with this drug is considered to be possible at a laboratory level population.

Keywords: chytridiomycosis, *Batrachochytrium dendrobatidis, Xenopus laevis,* thiabendazole, drug treatment

Introduction

Chytridiomycosis in amphibians, is a disease caused by a fungus, *Batrachochytrium dendrobatidis* which colonizes on the keratinized skin cells[1]. It infects more than 350 amphibian species in four continents, and is involved in population declines or extinction of more than 200 species[1-6]. Frequently used as experimental animals, *Xenopus laevis* is distributed in laboratories worldwide and is a mediator of *B. Dendrobatidis.* Defense against infection of wild amphibians by laboratory animals is an important issue.

Thiabendazole is a drug which exhibits antibacterial and antifungal activities by binding to β-tubulin and inhibiting mitosis. This drug is used as a post-harvest agricultural chemical, industrial fungicide, and anthelmintic for animals.

We unexpectedly found a possibility that thiabendazole, which had been given to *Xenopus laevis* for nematode extermination[7], showed a great therapeutic effect on chytridiomycosis. Benzalkonium chloride is already known as a drug for the treatment of chytridiomycosis[8, 9]. Thus, we compared the therapeutic effect of thiabendazole on chytridiomycosis, with that of benzalkonium chloride. The results showed that the effect of thiabendazole is similar to or greater than that of benzalkonium chloride.

Materials and methods

Drug treatment of chytridiomycosis

Following method for the use of thiabendazole which is employed for the nematode extermination[7] and that for the use of benzalkonium chloride[8, 9], we comparatively treated adult *X. laevis* infected with *B. Dendrobatidis.* In the former method, the frogs were immersed overnight in a 0.1g / L solution of thiabendazole (Tokyo Chemical Industry Co., Ltd.). The next day, frogs were thoroughly washed in a water bath, and kept for three days in another tank filled with a 5mg / L solution of oxytetracyclin (Wako Pure Chem. Instr. Ltd.). Then, the frogs were transferred to another tank filled with fresh water. After two weeks, the same procedure was repeated.

In the method using benzalkonium chloride, the frogs were immersed in a 1mg / L solution of this reagent (Wako Pure Chem. Instr. Ltd.), for one hour per day on the 1st, 3rd, 5th, 9th, 11th, and 13th days. After immersion, the frogs were thoroughly washed with water and managed in another tank.

In both methods, the frogs were treated with chemical solution in batches, and each batch was thereafter managed separately in independent tanks for 2 months. Individual animals were tested for chytridiomycosis using polymerase chain reaction (PCR).

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Swab sampling and testing for chytridiomycosis

For each frog subjected to testing, a sample was taken from the back of a frog's thighs by rubbing with a cotton swab, soaked with TE buffer. The liquid squeezed from the cotton swab was heated at 98°C for 10 minutes, and 1 μL of it was used as a PCR template. Detection of *B. dendrobatidis* using PCR was carried out following the reported method[10]. This method detects a band of approximately 300 bp in size for a portion of the rDNA cassette of *B. dendrobatidis* (Figure 1, lanes 2 and 6).

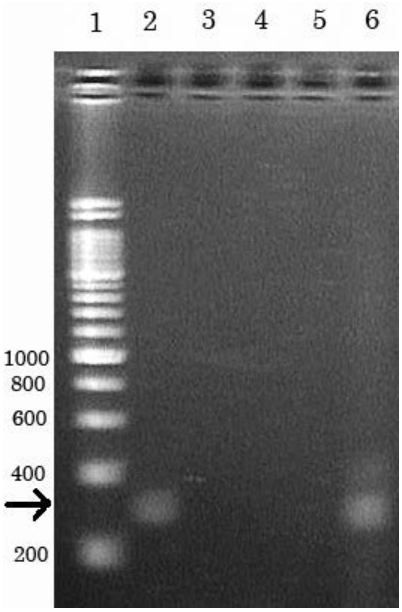

Figure 1. An example of a frog that tested positive for chytridiomycosis using PCR. About 300 bp of the band indicated with the arrow detects *Batrachochytrium dendrobatidis* and the frog as positive for chytridiomycosis (lane 2). This band was not seen in swab samples from other frogs (lanes 3-5), showing these frogs are negative for chytridiomycosis. Size markers and a positive control were loaded in lanes 1 and 6, respectively.

Results and discussion

We compared the therapeutic effects of thiabendazole and benzalkonium chloride on chytridiomycosis, and the results are shown in Table 1.

In the treatment with thiabendazole, 24 animals were tested in two batches. A total of seven animals were selected from the two batches, and were subjected to testing for chytridiomycosis using PCR. All seven of the animals were negative for chytridiomycosis, as the band for *B. dendrobatidis* was not detected in any swab sample.

In the treatment with benzalkonium chloride, 20 animals were treated in three batches. A total of five (one female and four male) animals from the three batches were tested using PCR, and three (one female and two male) animals, one animal out of each batch, showed a chytridiomycosis-positive band.

The difference in the results between the two drugs is great enough to be significant if sex is ignored ($P < 0.05$ Fisher's exact probability test). The difference in the therapeutic effect of both drugs between sexes is unclear from this experiment. It seems that there was a risk in the investigation that the larger the frog population in a tank after the chemical treatment, the higher the incidence of positive frogs by reinfection from any still-infected animals. The average frog population per tank was 12 animals in the thiabendazole treatment, compared to 6.7 animals in the benzalkonium chloride treatment. The above results do indicates that the therapeutic effects of thiabendazole on chytridiomycosis is similar to or higher than that of benzalkonium chloride.

Certain precautions should be taken when using thiabendazole. Feeding should be avoided when frogs are kept in thiabendazole or oxytetracyclin solution because oral intake of these drugs with feed may be fatal. A tank used for thiabendazole treatment will not be suitable for future breeding of frogs, because trace amounts of thiabendazole causes abnormal development in embryos. In albino *X. laevis* and *X. borealis,* half strength thiabendazole solution should be used, as full strength may be fatal.

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Table 1. Comparison of the therapeutic effect of thiabendazole and benzalkonium chloride on chytridiomycosis.

Treatment batch	Drugs	Treated frogs		Numbers in PCR test		
		Numbers	Sex	Total	Positive	Negative
1	Thiabendazole	7	♀	2	0	2
2	Thiabendazole	17	♀, ♂	5	0	5
3	Benzalkonium chloride	5	♀	1	1	0
4	Benzalkonium chloride	2	♂	1	1	0
5	Benzalkonium chloride	13	♂	3	1	2

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