Original Article

A Human Case of Protothecosis Successfully Treated with Itraconazole

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Abstract

Cutaneous protothecosis developed in a 63-year-old Japanese female. The patient had a long history of steroid use for bronchial asthma. A tender, swollen, erythematous plaque with white papules covered the dorsal aspect of the patient's right hand. Histopathological examination showed typical protothecosis with numerous "mulberry-like" sporangia positive for periodic acid-Schiff stain. The pathogen was identified as *Prototheca wickerhamii*. After a 6-week course of 150–200 mg/day itraconazole (ITCZ) therapy, the patient's culture was negative for *P. wickerhamii*. No recurrence was seen after 12 weeks of ITCZ therapy. This case of protothecosis and those of 16 patients are summarized. All the patients had lived in mid- to southern Japan.

Only 12 cases of protothecosis treated with ITCZ have been reported in the literature; 8 of these cases were cured with ITCZ therapy alone. In cases treated with ITCZ at no more than 100 mg/day, medication was typically needed for 2 months; most of the cases treated with ITCZ at 200 mg/day were successfully cured within 2 months. A 2-month course of ITCZ at 200 mg/day appears to be adequate treatment for human protothecosis.

Key words: protothecosis, *Prototheca wickerhamii*, itraconazole

Preface

Prototheca is a genus of microbes that fall between fungi and achlorophyllous mutants of the green alga *Chlorella*. Such microbes were first found in the slime flux of a deciduous tree. Protothecosis, infection by *Prototheca*, is quite rare in humans; only about 100 cases have been described in the literature. No standard therapeutic regimen for protothecosis has been established. We report a case of cutaneous protothecosis and review itraconazole (ITCZ) therapy for treatment of this disease.

Case report

A 63-year-old Japanese housewife was referred to our hospital on April 17, 1997. She experienced painful swelling on the dorsal aspect of her right hand. The swelling had first appeared 4 weeks earlier, and spontaneous pain, tenderness,

and a localized burning sensation had gradually progressed in spite of treatment with systemic antibiotics and antiallergic agents.

The patient had a long history of receiving systemic steroids for bronchial asthma and showed signs of steroid-induced diabetes and glaucoma. Long-term steroid use had also caused skin atrophy and scarring on the patient's forearms and on the dorsal aspects of both hands.

Physical examination revealed a large erythematous plaque covering the dorsal aspect of the patient's right hand, including the fingers (Fig. 1). Several white papules were found on the upper surface of the plaque (Fig. 2). Purulent discharge drained from the papules when they were pricked with a fine needle.

Laboratory examination revealed leukocytosis, C-reactive protein, and increased levels of lactate dehydrogenase, alkaline phosphatase, and total cholesterol. A direct examination in potassium hydroxide preparation of the scales on the plaque found no hyphae or spores.

A sample of a white papule on the plaque was removed for histological examination and

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Fig. 1. A large erythematous plaque on the dorsal aspect of the right hand, including the fingers.

Fig. 3. Histopathological examination of a biopsy specimen from the plaque showed granuloma formation with a central necrotized area and moderately dense polymorphous infiltration. ($\times 40$, Hematoxylin and eosin)

Fig. 5. Colony on Sabouraud's dextrose agar medium. The wet creamy white colony resembles that of *Candida*, a genus of yeast-like fungi.

Fig. 7. Appearance of the patient's right hand on the sixth week of treatment. The plaque and papules have been resolved and only scars remain.

Fig. 2. Several white papules on the upper surface of the plaque. White papules may represent small, deep abscesses.

Fig. 4. High magnification of samples after periodic acid-Schiff (PAS) staining. The organisms are PAS-positive "mulberry-like" sporangia. ($\times 400$)

Fig. 6. Lactophenol cotton blue staining of a cultured colony shows the endospore characteristic of the genus *Prototheca*. ($\times 400$)

culturing on both Sabouraud's dextrose agar medium and mycobacterium medium. Staining with hematoxylin and eosin showed granuloma formation with a central necrotized area surrounded by moderately dense infiltration with lymphocytes, neutrophils, and rare eosinophils (Fig. 3). The inflammatory cell infiltration extended from the papillary dermis to the deep dermis. The overlying epidermis appeared normal. Periodic acid-Schiff (PAS) stain and Grocott methenamine silver (GMS) stain revealed organisms among the infiltrating cells in the dermis

(Fig. 4). The internal structure of the organisms was clearer in the samples colored with PAS stain than in those colored with GMS stain because the organisms deeply absorbed the GMS stain. The organisms seen were spores of various sizes and several “mulberry-like” sporangia.

Many wet creamy yellow-white colonies similar to those of *Candida*, a genus of yeast-like fungi, grew on the Sabouraud’s dextrose agar medium (Fig. 5). No colonies grew on the mycobacterium medium.

Microscopic examination of a cultured colony stained with lactophenol cotton blue showed numerous spores and mulberry-like sporangia (Fig. 6) identical to those found in the PAS-stained specimen. The organism was identified as a species of *Prototheca* on the basis of the morphological findings.

Sugar assimilation tests were performed by API 20C Auxanogram (BIO MERIEUXS S.A., France). Assimilation was positive for glucose, galactose, and trehalose, but not for n-propanol. On the basis of these results, the organism was identified as *Prototheca wickerhamii*.

Treatment

After the biopsy procedure, the patient was given 200 mg/day minocycline for 9 days without improvement. Once the organism was identified as a species of *Prototheca*, the patient was given 100 mg itraconazole (ITCZ) twice a day, beginning May 14. When digestive irritation developed a week later, the dosage was changed to 50 mg three times a day, which the patient tolerated better.

By the third week of ITCZ treatment, most of

the symptoms had resolved, the plaque was flat and no longer erythematous, and the white papules had transformed into scabs. The follow-up culture taken in the sixth week of ITCZ treatment was negative for *P. wickerhamii* in the needle aspiration specimen, the scale, and the crust. The ITCZ was discontinued after 12 weeks. The disease did not recur in the next 10 months.

Discussion

The genus *Prototheca* is a unicellular achlorophyllous algae with a life cycle and microstructures similar to those of the green alga *Chlorella*. These microbes were first found in the slime flux of a deciduous tree. Several species of *Prototheca* have been described but only three are currently recognized: *P. wickerhamii*, *P. zophii* and *P. stagnora*¹⁾. Virtually all of the protothecal infections in humans are caused by *P. wickerhamii*, which has the largest spores in the *Prototheca* species, ranging from 1.3 μm to 16 μm in diameter.

The natural habitats of *Prototheca* species are sewage systems and the slime flux of trees. *Prototheca* can also be found in: soil, lakes, and ponds; dogs, cats, and cattle; cow’s milk; and even human digestive tracts²⁾.

Table 1. Results of sugar assimilation tests. On the basis of these results, the pathogen was identified as *Prototheca wickerhamii*

	Sucrose	Trehalose	Propanol
<i>P. stagnora</i>	+	—	—
<i>P. wickerhamii</i>	—	+	—
<i>P. zophii</i>	—	—	+
Present Case	—	+	—

Table 2. Summary of protothecosis reported in Japan

Year	Reference	Prefecture	Age	Sex	Region	Culture Result
1 1983	Shibata M (4)	Nagoya	42	F	Cheek	<i>P. wickerhamii</i>
2 1987	Maeda K (5)	Nagasaki	62	F	Lower Limb	—
3 1988	Matsuda T (6)	Fukuoka	80	M	Upper Limbs	<i>P. wickerhamii</i>
4 1990	Matsuda T (6)	Fukuoka	13	M	Intestine-Liver	—
5 1989	Otoyama K (7)	Ibaraki	62	M	Back	<i>P. wickerhamii</i>
6 1990	Koura S (8)	Kagoshima	52	M	Limbs	—
7 1992	Uoi M (9)	Osaka	40	F	Limbs	<i>P. wickerhamii</i>
8 1992	Fukumitsu K (10)	Kagoshima	79	M	Upper Limb	<i>P. wickerhamii</i>
9 1993	Hachisuka H (11)	Fukuoka	71	M	Upper Limb	<i>P. wickerhamii</i>
10 1994	Hatano Y (12)	Ehime	66	M	Hand Finger	—
11 1994	Fujita H (13)	Shizuoka	66	F	Upper Limb	—
12 1994	Hirata A (14)	Hyogo	68	F	Larynx	<i>P. wickerhamii</i>
13 1996	Matsumoto Y (15)	Nagoya	64	F	Cheek	<i>P. wickerhamii</i>
14 1998	Saruta T (16)	Kohchi	71	M	Face-Neck	—
15 1998	Miyazaki K (17)	Chiba	75	M	Face	<i>P. wickerhamii</i>
16 1999	Ono K (18)	Ibaraki	78	F	Chest	—
17 1997	Present Case	Kanagawa	63	F	Hand-Finger	<i>P. wickerhamii</i>

Since the first case in Sierra Leone in 1964 described by Davies *et al.*³⁾, more than 60 cases of human protothecosis have been reported in the literature. In Japan, 16 cases of human protothecosis have been reported⁴⁻¹⁸⁾ (Table 2), and of these 16, 14 were cutaneous protothecosis. The cases were equally distributed between the sexes and most often found in elderly people. Protothecosis has been reported in temperate and tropical areas around the world; in Japan, all of the patients with protothecosis lived in the mid- to southern portion of the country.

Human protothecosis has been classified into three types: cutaneous, olecranon bursitis, and systemic⁶⁾. More than half the number of reported cases of human protothecosis are cutaneous. Most investigators consider protothecosis to be an opportunistic infection because prototheca can be easily cultured from the environment and protothecosis often develops in patients with immunosuppression resulting from circumstances such as topical or systemic steroid therapy and diabetes mellitus¹⁹⁾.

Cutaneous protothecosis commonly develops on exposed body surfaces, such as the face and limbs³⁾. The prototheca is believed to enter the body through an open wound²⁰⁾. Because the wound site is typically small, as it was in our patient, the incubation period of protothecosis is not known. The appearance of the protothecosis lesion may vary from indurating erythema to papulo-pustular eruption, yet localized tenderness is a common feature of the disease. A biopsy of the lesion is required for making a diagnosis. Typical histopathological findings are polymorphous infiltration or granulomatous dermatitis and PAS-positive mulberry-shaped sporangia which are pathognomonic of the disease.

Because protothecosis is an extremely rare disease, no standard therapy for its treatment has

been established. Reported cases have been treated with a variety of agents, including amphotericin B, ketoconazole, and tetracycline, with or without surgery. In most cases, tetracycline has been used in combination therapy with amphotericin B or surgical excision. In spite of their successful results, amphotericin B also causes renal toxicity and ketoconazole also causes hepatotoxicity²¹⁾, limiting their usefulness. Recently, fluconazole and itraconazole have also been used to treat protothecosis; in one reported case, fluconazole successfully treated protothecosis after ITCZ treatment was unsuccessful²²⁾. Surgical excision remains important for managing large abscess formation in protothecosis.

We have identified in the literature 12 cases of cutaneous protothecosis treated with oral ITCZ^{15, 17, 18, 22-27)}. Of these 12, 11 showed at least some positive response to the ITCZ and none showed severe side effects. In 8 cases, protothecosis was completely cured with ITCZ alone. In one case, the patient's culture was positive for *Prototheca* even after a 4-month course of ITCZ at 50 mg/day, suggesting that this dosage may be too low to be effective. In cases treated with ITCZ at 100 mg/day, at least 2 months of administration was required to obtain negative culture results. On the other hand, most of the cases treated with ITCZ at 200 mg/day received the medication for less than 2 months before a negative culture was obtained. In our case, the patient received ITCZ at 150~200 mg/day and her culture was negative after 6 weeks of treatment, though we continued the therapy for a total of 12 weeks. A 2-month course of ITCZ at 200 mg/day appears to adequately treat protothecosis in humans.

Itraconazole also appears to be the drug of choice because it is effective yet less hepatotoxic than ketoconazole.

Table 3. Summary of protothecosis treated with itraconazole

	Year	Reference	Itraconazole dosage mg/day	Duration	Combination Therapy	Outcome
1	1995	Boyd AS (23)	200	2 wk		Cured
2	1996	Matsumoto Y (15)	50	4 mo		Improved
3	1995	Tang WY (24)	200	3 wk	Surgery	Improved
4	1990	Pierard GE (25)	200	2 mo		Cured
5	1996	Kim ST (22)	200	8 wk		No change
6	1993	Ravisse P (26)	200	30 d		Cured
7	1997	Kim JA (27)	100	10 d + 11 w		Cured
8	1997	Kim JA (27)	200	6 w		Cured
9	1997	Kim JA (27)	200	12 + 4 w	Topical antimycotics	Cured
10	1998	Miyazaki K (17)	unknown		Fluconazole	Cured
11	1999	Ono K (18)	100	60 d		Cured
12		Present Case	200-150	12 wk		Cured

References

- 1) Tsubaki K: A review of the genus *Prototheca*. Jpn J Med Mycol **29**: 155-160, 1988 (in Japanese).
- 2) Pore RS, Barnett EA, Barnes WC Jr, Walker JD: *Prototheca* ecology. Mycopathologia **81**: 49-62, 1983.
- 3) Davies RR, Spencer H, Wakelin PO: A case of human protothecosis. Trans R Soc Trop Med Hyg **58**: 448-451, 1964.
- 4) Shibata M, Ohhashi M, Mori T, Tanaka K: Protothecosis no honpou dai 1 rei. Jpn J Dermatol **93**: 779-781, 1983 (in Japanese).
- 5) Maeda K, Nishimoto K: A case of protothecosis. Jpn J Med Mycol **31**: 75-78, 1990.
- 6) Matsuda T, Matsumoto T: Protothecosis: a report of two cases in Japan and a review of the literature. Eur J Epidemiol **8**: 397-406, 1992.
- 7) Otoyama K, Tomizawa N, Higuchi I, Horiuchi Y: Cutaneous protothecosis —a case report—. J Dermatol **16**: 496-499, 1989.
- 8) Koura S, Matsumoto T, Nishimoto K: Hifu protothecosis. Jpn J Clin Dermatol **35**: 285-288, 1993 (in Japanese).
- 9) Uoi M, Hosokawa H, Isei T, Asada Y, Tsuji K, Hirohara J, Inoue K, Toyazaki N: Steroid ryouchouchuu no SLE kanja ni hasshou sita Protothecosis. Jpn J Clin Dermatol **37**: 697-700, 1995 (in Japanese).
- 10) Fukumitsu K, Kuru H, Setoyama M, Nomoto S, Nishimoto K: Protothecosis no 1 rei. Jpn J Med Mycol **33**(Supple): 153, 1992 (in Japanese).
- 11) Hachisuka H, Kusahara M: A case of protothecosis. Jpn J Med Mycol **34**: 373-379, 1993 (in Japanese).
- 12) Hatano Y, Yamamoto S, Okano S: A case of cutaneous protothecosis. Jpn J Clin Dermatol **50**: 215-218, 1996 (in Japanese).
- 13) Fujita H, Kurosawa S, Imaizumi S, Oku T, Uno A, Kobayashi H: A case of human cutaneous protothecosis. Acta Dermatol-Kyoto **89**: 273-276, 1994.
- 14) Hirata A, Tamagawa M, Urakawa K, Kakimaru T, Ohbayashi C: Protothecosis at the larynx. Jpn J Med Tech **46**: 1465-1467, 1997 (in Japanese).
- 15) Matsumoto Y, Shibata M, Adachi A, Ohashi M, Kanbe T: Two cases of protothecosis in Nagoya, Japan. Australas J Dermatol **37**: S42-43, 1996.
- 16) Saruta T, Uchida K, Naoe S: Protothecosis no 1 rei. Kouchishi Ishi **3**: 81-85, 1998 (in Japanese).
- 17) Miyazaki K, Tanaka A, Kato T, Hotchi M, Shinoda T: Protothecosis no 1 rei. Jpn J Dermatol **108**: 1087, 1998 (in Japanese).
- 18) Ono K, Koyake S, Naito S: Protothecosis no 1 rei. Jpn J Dermatol **109**: 1372, 1999 (in Japanese).
- 19) Laeng RH, Egger C, Schaffner T, Borisch B: Protothecosis in an HIV-positive patient. Am J Surg Pathol **18**: 1261-1264, 1994.
- 20) Woolrich A, Koestenblatt E, Don P, Szaniawski W: Cutaneous protothecosis and AIDS. J Am Acad Dermatol **31**: 920-924, 1994.
- 21) Pegram PS Jr, Kerns FT, Wasilauskas BL, Hampton KD, Scharyj M, Burke JG: Successful ketoconazole treatment of protothecosis with ketoconazole-associated hepatotoxicity. Arch Intern Med **143**: 1802-1805, 1983.
- 22) Kim ST, Suh KS, Chae YS, Kim YJ: Successful treatment with fluconazole of protothecosis developing at the site of an intralesional corticosteroid injection. Br J Dermatol **135**: 803-806, 1996.
- 23) Boyd AS, Langley M, King LE Jr: Cutaneous manifestations of prototheca infections. J Am Acad Dermatol **32**: 758-764, 1995.
- 24) Tang WY, Lo KK, Lam WY, Fung KS, Koehler A, Cheng AF: Cutaneous protothecosis: report of a case in Hong Kong. Br J Dermatol **133**: 479-482, 1995.
- 25) Pierard GE, Rurangirwa A, Arrese Estrada J, Pierard-Franchimont C: Cutaneous protothecosis treated with itraconazole. Ann Soc Belg Med Trop **70**: 105-112, 1990 (in French).
- 26) Ravisse P, Bievre C, Magalhaes MC, Ramos I, Huerre M: Cutaneous protothecosis cured by itraconazole: a new case and general review on human protothecosis. J Mycol Med **3**: 84-89, 1993 (in French).
- 27) Kim JA, Moon SE, Chun HS, Yoon JI, Song KY: Cutaneous protothecosis: Unique histopathological findings with crystal violet staining and the therapeutic effect of itraconazole. Jpn J Med Mycol **38**: 75-80, 1997.