Involvement of Cytomegalovirus Infection in the Ileal Lesions of the Patient with Behçet's Disease

To the Editor:

Considerable attention has been paid to cytomegalovirus (CMV) infection as one of the exacerbating factors of ulcerative colitis and Crohn's diseases refractory to conventional therapies.[1–4] However, the involvement of CMV infection in the exacerbation of intestinal Behçet's disease (BD) has not been reported to date. We herein reported the first case of Behçet's ileocolitis associated with CMV infection, which was successfully treated with antiviral therapy. This case suggests that the importance of concomitant CMV infection should be kept in mind in patients of Behçet's ileocolitis treated with immunosuppressant as well as patients with inflammatory bowel diseases.

Case

A 43-year-old man with an 8-year history of refractory ocular involvement of BD was admitted to our hospital for anemia and hematochezia. He fulfilled the international study group criteria for diagnosis of BD. He had been treated with prednisolone (PSL) and oral cyclosporine (CyA). Physical examination demonstrated severe tenderness in the right lower abdomen. Laboratory data showed that hemoglobin was 7.1 g/dL. Upper endoscopic examination did not detect any significant lesion. Colonoscopic examination demonstrated deep and discrete ulcers with exposed vessels at the terminal ileum (Fig. 1a). Histological examination of biopsy specimen from ileal ulcerated lesion showed nonspecific inflammation with granulocyte infiltration and mild fibrous changes, but there was no granuloma and obvious inclusion body. Based on these findings, we initially diagnosed ileocecal involvement of BD. However, since endoscopic findings of ileocecal ulcers are so deep and large in addition to the immunosuppressed condition, we speculated on the involvement of CMV infection in the ulcerated lesion of this patient. CMV DNA was detected in the biopsy sample of ileal lesions by real-time polymerase chain reaction (PCR). Moreover, the immunohistochemistry of the biopsy specimen demonstrated positive staining of CMV antigen in endothelial cells. These findings suggested that these ileal lesions were Behçet's ileocolitis with concomitant CMV infection. We discontinued the administration of CyA and started a 10 mg/kg dose of gancyclovir for 2 weeks. After starting antiviral therapy, right lower abdominal tenderness subsided. Colonoscopic findings demonstrated that ileocecal ulcerations were improved (Fig. 1b). CMV DNA in the biopsy sample also became negative following antiviral therapy. In April, 2006, he was doing well, and colonoscopic examination demonstrated no recurrence of ileal ulcerations.

BD is characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis, and skin lesions.[5] Generally, gastrointestinal involvement seems to be rare, but ileocecal involvement is relatively more common in Japanese patients with BD.[6] Intestinal BD is intractable and medical treatment for intestinal BD has not been fully established.[5,7] However, drugs such as PSL or immunosuppressants are administered to many patients with BD. Therefore, these patients, who have been treated by PSL or immunosuppressants, are usually in an immunosuppressed condition. Generally, CMV is a clinically important pathogen in immunocompromised hosts because the involvement of CMV infection in intestinal mucosa leads to severe gastrointestinal lesions. Various endoscopic findings have been reported in the intestinal CMV infection but there is no typical endoscopic appearance. To prove whether CMV infection is involved in intestinal lesions it is necessary to investigate the existence of CMV in the gastrointestinal tract by PCR or immunohistochemistry. However, it is difficult to judge whether CMV infection is involved in ileocolitis of BD or CMV enterocolitis in a patient with ocular BD. Finally, the former diagnosis was done because endoscopic findings demonstrated scar formations in the ileum, suggesting the existence of previous ileal lesions associated with BD.

When abdominal symptoms in patients with intestinal BD deteriorate, we consider a dose increase of PSL or adding immunosuppressants. However, if CMV infection is involved in the exacerbation of intestinal lesions in patients with BD, such as we encountered, additional immunosuppressive therapies make intestinal lesions worse. Therefore, we should investigate whether CMV infection is present in the intestinal lesion of patients with BD prior to making a decision regarding additional immunosuppressive therapies.

Sakae Mikami, MD
Hiroshi Nakase, MD
Satoru Ueno, MD
Minoru Matsuura, MD
Takaki Sakurai, MD†
Tsutomu Chiba, MD
*Department of Gastroenterology and Hepatology
Graduate School of Medicine
Kyoto University
Kyoto, Japan
†Laboratory of Diagnostic Pathology
Kyoto University Hospital
Kyoto, Japan

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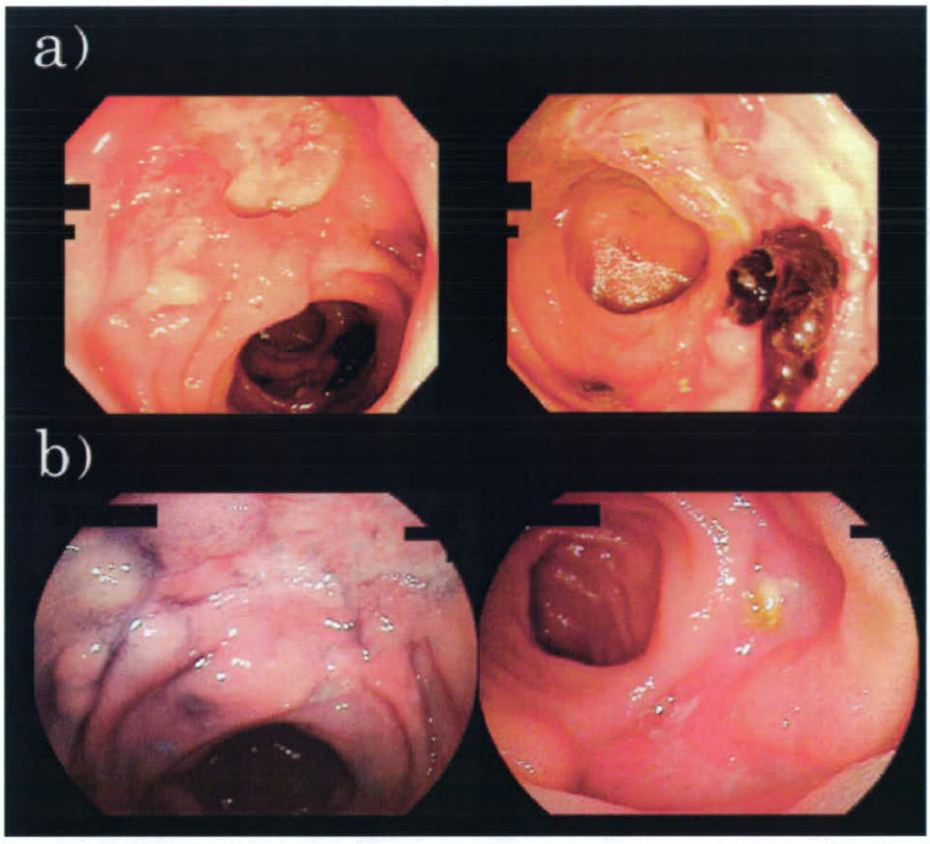

FIGURE 1. a: Endoscopic findings of the terminal ileum showed deep and large ulcers with exposed vessels on the deformed ileal mucosa accompanied by ulcer scars. b: Endoscopic picture of terminal ileum 3 weeks after antiviral treatment showed the improvement of ileal ulcerations. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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Crohn's Disease in a Patient With Chronic Renal Failure

To the Editor:

Crohn's disease may present with extraintestinal manifestations [1] but is not usually associated with renal disease. A 47-year-old man from Nigeria presented with a 3-day history of generalized central abdominal pain associated with vomiting and diarrhea. The patient was on renal dialysis three times weekly for the past 3 years for end-stage renal disease caused by hypertension; he was normally anuric.

The patient was clearly in pain but was not septic or toxic. On examination the abdomen was diffusely tender, tense, and distended, but without true peritonitis. On plain radiography, there was no free air under the diaphragm or distended bowel loops. There was a slight neutrophilia with a raised C-reactive protein (CRP) (50 mg/L) increased to 199 mg/L over 4 days and hypoalbuminemia (29 g/L). Computed tomography (CT) revealed markedly thick-walled distal and terminal ileum, diverticular change throughout the large bowel, bilateral atrophic kidneys, and a large amount of ascitic fluid within the abdomen

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ORIGINAL ARTICLE

Usefulness of Quantitative Real-time PCR Assay for Early Detection of Cytomegalovirus Infection in Patients with Ulcerative Colitis Refractory to Immunosuppressive Therapies

Takuya Yoshino, MD, Hiroshi Nakase, MD, PhD,* Satoru Ueno, MD,* Norimitsu Uza, MD,* Satoko Inoue, MD,* Sakae Mikami, MD,* Minoru Matsuura, MD, PhD,* Katsuyuki Ohmori, MD, PhD,† Takaki Sakurai, MD, PhD,‡ Satoshi Nagayama, MD, PhD,§ Suguru Hasegawa, MD, PhD,§ Yoshiharu Sakai, MD, PhD,§ and Tsutomu Chiba, MD, PhD**

Background: Studies suggest that cytomegalovirus (CMV) infection exacerbates ulcerative colitis (UC) refractory to immunosuppressive therapies. Early and accurate diagnosis of CMV infection is important for the treatment of UC. We evaluated the usefulness of quantitative real-time polymerase chain reaction (PCR) for detecting CMV infection in inflamed colonic mucosa of patients with UC refractory to immunosuppressive therapies.

Methods: From 2003 to 2006, 30 patients (mean age: 41 ± 18 years; 14 men, 16 women) with UC refractory to immunosuppressive therapies were enrolled in the study. We evaluated CMV infection by CMV antigenemia, histologic examination, and quantitative real-time PCR for CMV using colonic mucosa and investigated the clinical outcomes of antiviral therapy.

Results: CMV-DNA was detected only in the inflamed colonic mucosa in 17 (56.7%) of 30 patients. Of the 17 CMV-DNA-positive patients, 4 were positive for CMV antigenemia or inclusion bodies on histologic examination; of the 13 CMV-DNA-negative patients none was positive for CMV antigenemia or inclusion bodies. Of the 17 CMV-DNA-positive patients, 12 (70.6%) were treated with ganciclovir for 2 weeks and 10 patients went into remission. Two other patients required colectomy after antiviral therapy. In contrast, of the 13 CMV-DNA-negative patients 12 (92.3%) achieved remission after intensifying their immunosuppressive therapies.

Conclusions: Quantitative real-time PCR assay for detecting CMV-DNA is useful for early, accurate diagnosis of CMV infection in patients with UC refractory to immunosuppressive therapies, enabling prompt and appropriate treatment.

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Key Words: ulcerative colitis, cytomegalovirus, real-time PCR

Cytomegalovirus (CMV) infection is an important exacerbating factor in patients with ulcerative colitis (UC).[1–3] Because CMV infection occurs often in immunocompromised hosts,[4] CMV infection in patients with UC refractory to immunosuppressive therapies must always be considered a possibility. If CMV infection is not recognized at an early stage, appropriate treatment is not promptly initiated and the prognosis of patients with UC complicated by CMV infection is generally poor.[5,6] Thus, an accurate and rapid diagnosis of CMV infection is critical in UC patients refractory to immunosuppressive therapies.

Several modalities are currently used for detecting CMV infection.[7–9] For diagnosis of CMV infection in the gastrointestinal tract, combined CMV antigenemia assay and detection of CMV inclusion bodies in biopsy specimens from the gastrointestinal mucosa by either hematoxylin and eosin (H&E) staining or immunohistochemistry (IHC) using anti-CMV monoclonal antibodies has been proposed.[1] It is often difficult, however, to accurately diagnose CMV infection in patients with UC,[10] even when using this combined diagnostic method. Several recent studies indicate that the real-time polymerase chain reaction (PCR) assay allows for sensitive and rapid detection of CMV-DNA in clinical samples, and is more useful and beneficial for diagnosing CMV infection than CMV antigenemia assay or histologic examination.[11,12]

From the *Department of Gastroenterology & Hepatology, Graduate School of Medicine, Kyoto University, †Department of Clinical Laboratory, Kyoto University Hospital, ‡Laboratory of Diagnostic Pathology, Kyoto University Hospital, §Department of Surgery, Kyoto University Hospital, Kyoto, Japan.

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Reprints: Hiroshi Nakase, MD, PhD, Department of Gastroenterology & Hepatology, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, 606-8507, Japan. (e-mail: hiropy-n@kuhp.kyoto-u.ac.jp).

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TABLE 1. Endoscopic Index of Rachmilewitz[16]

Items	Scores			
	0	1	2	4
1. Granulation scattering reflected light	No	—	Yes	—
2. Vascular pattern	Normal	Faded/disturbed	Completely absent	—
3. Vulnerability of mucosal	None	—	Slightly increased (contact bleeding)	Greatly increased (spontaneous bleeding)
4. Mucosal damage (mucus, exudates, erosions, ulcer)	None	—	Slight	Pronounced

Total score is sum of the item scores.

A conventional PCR assay, however, might also detect a latent CMV infection that has nothing to do with the deterioration of UC.[11] We recently applied quantitative real-time PCR for detecting CMV in patients with UC refractory to immunosuppressive therapies and found that the CMV-DNA copy number is higher in inflamed colonic mucosa than in noninflamed mucosa in these patients, suggesting the usefulness of this method to accurately diagnose active CMV infection.[13,14]

In the present study we further examined the usefulness of the quantitative real-time PCR assay using colonic mucosa for diagnosing active CMV infection in patients with UC. An accurate diagnosis of CMV infection might enable a more effective treatment for patients with UC refractory to immunosuppressive therapies.

MATERIALS AND METHODS

Patients

Among 93 patients with UC (55 men, 38 women) that visited Kyoto University Hospital from October 2003 to October 2006, 30 patients with UC refractory to immunosuppressive therapies, including steroids and immunomodulators, were studied retrospectively. The diagnosis of UC was based on clinical, endoscopic, radiologic, and histologic parameters. Fecal bacterial culture yielded no specific pathogens in any of the patients. All patients had been treated with immunosuppressive therapies, and had active UC defined as moderate to severe using the disease activity index (DAI) criteria,[15] with a score greater than 6 points.

Assessment of Endoscopic Severity

Endoscopic severity of UC was assessed using the DAI score,[15] Matts grade,[16] and the Endoscopic index of Rachmilewitz.[17] Endoscopic findings were scored from 0 to 3 according to DAI scores as: normal = 0, mild friability = 1, moderate friability = 2, and spontaneous bleeding = 3, and also scored from 1 to 4 according to Matts grade as: normal = 1, mild granularity and edema = 2, marked granularity and edema, and spontaneous bleeding = 3, severe ulceration = 4. The endoscopic index of Rachmilewitz is shown in Table 1.

Histopathology

Colonic biopsies were fixed in formalin, embedded in paraffin, stained with H&E, and IHC was performed using anti-CMV monoclonal antibodies (Dako Cytomation, Kyoto, Japan).[13] These sections were evaluated for characteristic cytomegalic cells and "owl's-eye" nuclear inclusion bodies.

CMV Antigenemia

The antigenemia assay was performed using a monoclonal antibody (C7HRP or C10C11) against a CMV structural protein of the 65 kDa lower-matrix phosphoprotein (pp65).[7,8]

Quantitative Real-time PCR

DNA for real-time PCR assay was extracted from the colonic tissues obtained from patients at endoscopic examination using QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. The assay was performed using an ABI Prism 7700 Sequence Detector System (Perkin Elmer Applied Biosystems, Foster City, CA) as described previously.[18] The oligonucleotide primers used for CMV-DNA amplification were constructed to detect the immediate early gene. The upstream primer was 5′-GACTAGTGTGATGCTGGCCAAG-3′ and the downstream primer was 5′-GCTACAATAGCCTCTTCCTCATCTG-3′. The 6-carboxyfluorescein-labeled probe was 5′-AGCCTGAGGTTATCAGTGTAATGAAGCGCC-3′. The PCR conditions were incubation at 95°C for 10 minutes, 50 cycles of 95°C for 15 seconds, followed by incubation at 62°C for 1 minute. Cases in which the CMV-DNA copy number was over 10 copies/μg DNA were defined as positive for CMV infection.

Diagnosis of CMV Infection

Cases that were detected as CMV infection by at least one of these methods (histopathology, CMV antigenemia, and quan-

titative real-time PCR) were defined as positive for CMV infection.

Statistical Analysis

Categorical and continuous data were compared using a 2-tailed Fisher exact test and Mann–Whitney *U*-test. CMV-positive patients were compared with CMV-negative patients for different parameters (age, DAI score, extent of disease, endoscopic score of DAI, Matts grade, endoscopic index of Rachmilewitz, ratio of patients undergoing colectomy, and treatment). A *P*-value <0.05 was considered statistically significant.

RESULTS

The clinical characteristics of the 30 patients are summarized in Table 2. The mean age of the 30 patients was 40.8 ± 17.6 years (range 16–73 years), and the mean DAI score was 9.5 ± 1.4. The extent of the disease was proctitis (3.3%), left-sided colitis (23.3%), and pancolitis (73.3%). The mean endoscopic DAI score was 2.3 ± 0.7, the mean Matts grade was 3.0 ± 0.8, and the mean endoscopic index score was 9.2 ± 2.4 (Table 2).

Of the 30 patients, 23 (76.7%) had been treated with corticosteroids, 6 (20.0%) with azathioprine, 7 (23.3%) with tacrolimus, and 2 (6.7%) with leukocytapheresis when visiting our institution. Six patients (20.0%) received colectomy during the observation period (Table 2, Fig. 1).

CMV-DNA was detected in the colonic tissues of 17 patients (56.7%) (4 with left-sided colitis, 13 with pancolitis) (Table 3). Notably, in all positive cases CMV-DNA was detected only in the inflamed colonic mucosa and not in the noninflamed mucosa. As a control, we examined CMV-DNA in the inflamed mucosa of 4 patients with UC who were in clinical remission with immunosuppressive therapies. CMV-DNA was not detected in the inflamed mucosa of any of these patients (data not shown). On the other hand, CMV antigenemia and histologic examination were positive in only 3 (17.6%) and 1 (5.9%) of the 17 patients positive for CMV-DNA in the colonic mucosa, respectively, and none of the patients negative for CMV-DNA in the colonic mucosa was positive for either CMV antigenemia or histologic examination.

A comparison of differences in age, DAI score, disease extent, ratio of patients undergoing colectomy, and the endoscopic score between CMV-DNA-positive and -negative patients revealed no significant differences between the 2 groups, although the number of patients who received colectomy tended to be greater in CMV-DNA-positive patients than in -negative patients (Table 4).

Moreover, a comparison of difference in treatment between CMV-DNA-positive and -negative patients also revealed no significance difference between the 2 groups. However, the number of patients treated with corticosteroid tended to be greater than in CMV-DNA-positive patients than in -negative patients (Table 5).

Of the 17 CMV-DNA-positive patients, 12 (70.6%) were treated with ganciclovir daily for 2 weeks (Fig. 1). CMV-DNA in the colonic mucosa became negative in all patients that received antiviral therapy. Four patients (33.3%) went into remission following antiviral therapy only. Although 7 patients (58.3%) were improved after antiviral therapy, the underlying UC remained active. These patients were treated with additional granulocytapheresis (GCAP) using an Adacolumn (Japan Immunoresearch Laboratories, Takasaki, Japan) or additional tacrolimus after their CMV-DNA became negative. Six (50.0%) of them went into remission, but 1 (8.3%) patient did not and therefore received a colectomy.

TABLE 2. Clinical Characteristics of 30 Patients with UC Refractory to Immunosuppressive Therapies

Age (mean ± SD)		40.8±17.6
Sex (M/F)		14/16
DAI score		9.5 ± 1.4
Extent of disease	Proctitis	1 (3.3)[a]
	Left-sided	7 (23.3)[a]
	Pancolitis	22 (73.3)[a]
Endoscopic score of DAI		2.3 ± 0.7
Matts grade		3.0 ± 0.8
Endoscopic index of Rachmilewitz		9.2 ± 2.4
Treatment on admission	Corticosteroid (CS)	23 (76.7)[a]
	Corticosteroid alone	14 (60.9)[b]
	With azathioprine	5 (21.7)[b]
	With tacrolimus	1 (4.3)[b]
	With LCAP	2 (9.1)[b]
	With GCAP	1 (4.3)[b]
	Azathioprine (AZA)	6 (20.0)[a]
	Azathioprine alone	1 (16.7)[c]
	With corticosteroid	5 (83.3)[c]
	Tacrolimus	7 (23.3)[a]
	Tacrolimus alone	5 (71.4)[d]
	With corticosteroid	1 (14.3)[d]
	With infliximab	1 (14.3)[d]
	LCAP	2 (6.7)[a]
	With corticosteroid	2 (100.0)[e]
Ratio undergoing colectomy		6 (20.0)[a]

Number of patients is shown. Age, DAI Score, Endoscopic Score of DAI, Matts Grade, and Endoscopic Index of Rachmilewitz are presented as mean ± SD. LCAP, leukocytapheresis

[a] Values in parentheses are percentages of all 30 patients.
[b] Values in parentheses are percentages of patients treated with CS.
[c] Values in parentheses are percentages of patients treated with AZA.
[d] Values in parentheses are percentages of patients treated with tacrolimus.
[e] Values in parentheses are percentages of patients treated with LCAP.

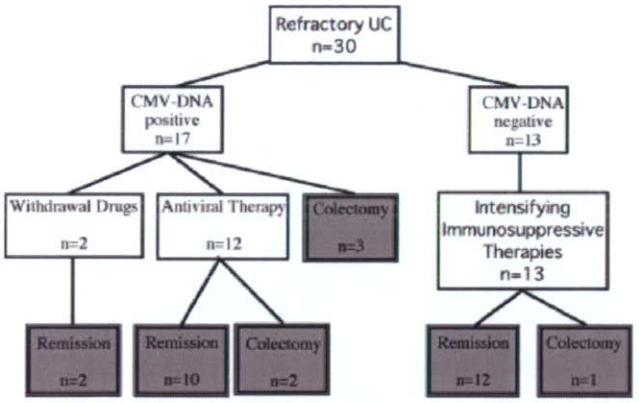

FIGURE 1. Clinical course of 30 patients with UC refractory to immunosuppressive therapy. CMV-DNA in inflamed mucosa was positive in 56.7% (17/30) of patients with UC, and negative in 43.3% (13/30) of patients with UC. Shaded rectangular columns with double lines show final outcomes.

Eventually, 10 (83.3%) of the 12 patients that received antiviral therapy went into remission and the remaining 2 patients (16.7%) received a colectomy. Three (17.6%) of the remaining 5 patients positive for CMV-DNA required urgent colectomy without receiving antiviral therapy, and 2 (11.8%) achieved remission by withdrawal of the immunosuppressive drugs.

Of the 13 CMV-DNA-negative UC patients, 12 (92.3%) went into remission after treatment with more intense immunosuppressive therapies. Only 1 patient (7.7%) was refractory to additional immunosuppressive therapies and finally required a colectomy.

DISCUSSION

In the present study, we applied quantitative real-time PCR for the diagnosis of active CMV infection in the colonic mucosa of 30 patients with UC refractory to immunosuppressive therapies. Using this method the detection rate of CMV

TABLE 3. Detection Rate of CMV-DNA in the Colonic Mucosa of UC Patients Refractory to Immunosuppressive Therapies

	CMV-DNA in Inflamed Mucosa	CMV-DNA in Noninflamed Mucosa	Antigenemia	IHC
CMV infection				
Postitive	17 (56.7%)	0	3 (17.6%)	1 (5.9%)
Negative	0	0	0	0
Total	17	0	3	1

Number of patients is shown. Values in parentheses are percentages of the total number of patients (n = 30).

TABLE 4. Comparison of Clinical Parameters between UC Patients with and without Detectable CMV-DNA in the Inflamed Mucosa

	CMV-DNA-positive N = 17	CMV-DNA-negative N = 13	P-value
Age	44.1 ± 16.3	36.5 ± 18.9	0.247
DAI score	9.8 ± 1.2	9.2 ± 1.6	0.206
Extent of disease			
Proctitis	0 (0.0)	1 (7.7)	0.245
Left-sided	4 (23.5)	3 (23.1)	0.977
Pancolitis	13 (76.5)	9 (69.2)	0.657
Endoscopic score of DAI	2.4 ± 0.7	2.1 ± 0.6	0.194
Matts grade	3.1 ± 0.8	2.9 ± 0.8	0.687
Endoscopic index of Rachmilewitz	9.5 ± 2.4	8.8 ± 2.4	0.444
Ratio undergoing colectomy	5 (29.4)	1 (7.7)	0.196

Number of patients is shown. Age, DAI Score, Endoscopic Score of DAI, Matts Grade, and Endoscopic Index of Rachmilewitz are presented as mean ± SD. Values in parentheses are percentages of the total number of patients either positive or negative for CMV-DNA in the inflamed mucosa.

TABLE 5. Comparison of Treatment between UC Patients with and without Detectable CMV-DNA in the Inflamed Mucosa

Treatment	CMV-positive n = 17	CMV-negative n = 13
Corticosteroid (CS)	15 (65.2)[a]	8 (34.8)[a]
Corticosteroid only	10	4
With azathioprine	3	2
With tacrolimus	1	0
With LCAP	1	1
With GCAP	0	1
Azathioprine	3 (50.0)[b]	3 (50.0)[b]
Azathioprine alone	0	1
With corticosteroid	3	2
Tacrolimus	3 (42.9)[c]	4 (57.1)[c]
Tacrolimus alone	1	4
With corticosteroid	1	0
With infliximab	1	0
LCAP	1 (50.0)[d]	1 (50.0)[d]
With corticosteroid	1	1

Number of patients is shown. There is no significant difference in treatment between CMV-DNA-positive and -negative patients.
[a] Values in parentheses are percentages of patients treated with CS.
[b] Values in parentheses are percentages of patients treated with AZA.
[c] Values in parentheses are percentages of patients treated with tacrolimus.
[d] Values in parentheses are percentages of patients treated with LCAP.

infection tended to be higher than when using other conventional methods such as CMV antigenemia and histologic examination. Moreover, a high remission rate was achieved in UC patients refractory to immunosuppressive therapies by applying either antiviral therapy or modulating immunosuppressive therapies according to the results of the quantitative real-time PCR for CMV-DNA in the inflamed colonic mucosa. Thus, our real-time PCR method for detecting CMV-DNA appears to be more useful than conventional modalities for diagnosing active CMV infection in patients with UC refractory to immunosuppressive therapies.

Quantitative real-time PCR revealed that CMV-DNA was positive in the inflamed colonic mucosa of 56.7% (17/30) of our UC patients, whereas CMV antigenemia and histologic examination were positive in only 17.6% (3/17) and 5.9% (1/17) of the patients positive for CMV-DNA, respectively. Thus, the detection rate of CMV infection by quantitative real-time PCR far exceeded that by CMV antigenemia and histologic examination. Several methods are used to diagnose CMV infection, including histologic examination, CMV antigenemia, and PCR assay.[7–9] Among them, CMV antigenemia and PCR assay using whole blood potentially reflect the reactivation of CMV in the whole body, but does not necessarily indicate CMV infection in the colonic mucosa. Indeed, there are several reports of reactivation of CMV in the plasma of patients with collagen disease and AIDS without gastrointestinal involvement of CMV infection.[7,19] Reactivation of CMV in the plasma does not reflect the involvement of CMV infection in UC.[20] Histologic examination is often considered the "gold standard" for diagnosing CMV infection in the gastrointestinal tract.[21] Its sensitivity for diagnosis, however, ranges from 10%–87%, and moreover, 37.5% of patients with gastrointestinal CMV disease fail to demonstrate any inclusions.[21] To overcome such low sensitivity, IHC with monoclonal antibodies was developed. The sensitivity for detecting CMV infection with IHC ranges from 78%–93%.[21] The sensitivity and specificity of CMV antigenemia for detecting CMV infection are 60%–100% and 83%–100%, respectively.[21] The present data, however, indicate that the detection rates of CMV infection by those established methods are lower than previously reported. In contrast, we found a significantly higher detection rate of CMV-DNA in the inflamed colonic mucosa by our quantitative real-time PCR system than by conventional modalities such as histologic examination and CMV antigenemia. These data strongly suggest that our quantitative real-time PCR for detecting CMV-DNA in the inflamed mucosa is very useful for diagnosing active CMV infection in patients with UC refractory to immunosuppressive therapies. It should be emphasized that none of the patients that were positive for CMV-DNA in the inflamed colonic mucosa were positive for CMV-DNA in the noninflamed mucosa. Thus, the high sensitivity of our method for detecting CMV infection is likely to be due to the sampling of the inflamed mucosa for the assay.

CMV is present in its latent form in most healthy subjects.[18] Therefore, we might expect low specificity for diagnosing active CMV infection when using a sensitive PCR method, because it is possible that sensitive PCR will detect CMV-DNA in subjects with a latent CMV infection. In this respect, we observed that CMV-DNA was detected only in the inflamed colonic mucosa and not in the noninflamed colonic mucosa. Moreover, by using biopsy specimens from the inflamed colonic mucosa, 12 of our 17 CMV-DNA-positive patients achieved remission by either antiviral therapy or withdrawing immunosuppressive therapies, whereas 12 of 13 CMV-DNA-negative patients achieved remission by intensifying the immunosuppressive therapies. In addition, none of the patients negative for CMV-DNA were positive based on either histologic examination or CMV antigenemia. Taken together, these findings suggest that both the sensitivity and specificity of our quantitative real-time PCR for diagnosing active CMV infection are high, and indeed, the findings were useful for making an appropriate decision regarding whether the immunosuppressive therapies should be intensified or tapered.

Endoscopy is a useful modality for diagnosing CMV infection when the characteristic findings such as deep ulceration are observed.[22] The endoscopic findings in CMV-positive colitis, however, vary,[21,23] and thus it might be difficult to distinguish CMV infection from severe UC. Sakamoto et al[24] reported that no specific endoscopic findings were observed in UC patients with concomitant CMV infection. We also evaluated whether endoscopic findings was useful for early detection of CMV infection using endoscopic score in the present study. Our data revealed no significant difference in endoscopic score according to 3 different indexes between the CMV-DNA-positive and -negative patients. Based on both the previous reports and the present report, the significance of endoscopic findings for diagnosing CMV in patients with UC remains unclear. Hommes et al[25] proposed a mechanism of CMV replication and activation in the intestinal tissue during active inflammatory bowel disease and classified the findings into 3 stages (initiation, reactivation, and consolidation). According to their proposal, the stage at which we detected CMV-DNA in the inflamed colonic mucosa of patients with UC might correspond to the initiation or reactivation stage prior to the occurrence of characteristic endoscopic findings in CMV colitis. Thus, one reason for the lack of a significant difference in the endoscopic score between the CMV-DNA-positive and -negative patients in our study might be due to the detection of CMV infection at an early stage.

An interesting observation in our study is that, as noted above, CMV-DNA was detected only in the inflamed colonic mucosa, and not in the noninflamed mucosa by quantitative

real-time PCR. Hahn et al[26] reported that proinflammatory cytokines such as interferon-γ and tumor necrosis factor-α induce the reactivation of CMV. Hommes et al[25] also reported that those proinflammatory cytokines induce the migration of monocytes to the inflammatory sites of the colonic mucosa and promote their differentiation into macrophages, which have a role in supporting active replication of CMV as CMV reservoir cells. Thus, it might be that, in patients with UC, CMV is more easily reactivated in the inflamed mucosa than in the noninflamed mucosa.

The therapeutic strategy for UC patients with concomitant CMV infection is a very important issue. In this study, 10 (83.3%) of the 12 CMV-DNA-positive patients went into remission after applying antiviral therapy and modulating immunosuppressive therapies. Of the 13 CMV-DNA-negative UC patients, moreover, 12 (92.3%) went into remission after treatment with more intense immunosuppressive therapies. At present, in UC patients refractory to immunosuppressive therapies we first perform quantitative real-time PCR using inflamed mucosa. In CMV-DNA-positive cases, antiviral therapy should be applied promptly and immunosuppressive therapies should be tapered. After CMV-DNA became negative, immunosuppressive therapies could be intensified. On the other hand, in CMV-DNA-negative cases immunosuppressive therapies could be intensified. Thus, clinical outcome in this study revealed that our quantitative real-time PCR using inflamed mucosa was useful for making a decision of treatment for patients with UC refractory to immunosuppressive therapies.

In conclusion, our use of quantitative real-time PCR for detecting CMV-DNA in inflamed mucosa was very useful for the early and accurate diagnosis of active CMV infection in patients with UC refractory to immunosuppressive therapies, enabling prompt and appropriate treatment. Further studies are required to determine whether this method will contribute to improving the prognosis of UC complicated by active CMV infection.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

The Effect of Medical Treatment on Patients with Fistulizing Crohn's Disease: A Retrospective Study

Norimitsu Uza, Hiroshi Nakase, Satoru Ueno, Satoko Inoue, Sakae Mikami, Hiroyuki Tamaki, Minoru Matsuura and Tsutomu Chiba

Abstract

Background Fistulas are a major complication of Crohn's disease (CD), but the treatment strategy for fistulizing Crohn's disease is controversial. The aim of this study is to analyze the efficacy of medical therapy for fistulizing Crohn's disease.

Methods Therapeutic regimens and clinical outcome of medical therapy were evaluated in 10 patients with fistulizing Crohn's disease (6 with external fistulas, 4 with internal fistulas). Complete response was defined as fistula closure with complete arrest of drainage in cases of external fistula, and disappearance of the fistula demonstrated by imaging studies in cases of internal fistula. Clinical remission was defined as a Crohn's disease activity index of less than 150 points.

Results Complete responses were observed in all 6 patients with external fistulas (4 patients treated with a combination of antibiotics and immunomodulators, and 2 also treated with infliximab). In contrast, fistula closure was observed in only 1 of 4 patients with internal fistulas. Clinical remission of CD was achieved in all patients with external fistulas, whereas there was no significant difference in the CD activity index before and after medical therapy in patients with internal fistulas.

Conclusions External fistulas were more responsive to medical therapy than internal fistulas in patients with CD. Combined treatment with antibiotics and immunomodulators might be a suitable initial therapy for CD patients with external fistulas, and infliximab can be used as an additional therapy in cases refractory to this combination therapy. However, randomized controlled studies will be required to investigate what kinds of therapies are optimal for CD patients with fistulas.

Key words: Crohn's disease, fistulizing Crohn's disease, medical therapy

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Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease of unknown etiology characterized by fissuring ulcers and segmental transmural inflammation of the gastrointestinal tract. Fistulas are a major complication in patients with CD, occurring in approximately one-third of patients (1). The cumulative risk for any fistula, as demonstrated by a population-based study, is 33% after 10 years and 50% after 20 years (2). Fistulas, based on their location and connection with contiguous organs are classified as internal if they terminate in adjacent organs, or external if they terminate on the skin. Perianal fistulas occur most commonly and decrease the patient's quality of life (3). Various medical therapies, including antibiotics, immunomodulators, and total parenteral nutrition, have been tried and effectively achieve fistula closure to some degree. Most of the previous studies, however, have been performed in an uncontrolled manner (4). Infliximab, a chimeric monoclonal antibody against tumor necrosis factor-α, was recently introduced and is the first agent shown to be efficacious in a randomized controlled trial for both the induction and maintenance of fistula closure (5, 6). Infliximab produces rapid fistula closure in approximately two-thirds of patients (5). Nevertheless, some patients with fistulizing Crohn's disease (FCD) are refractory, even to this new agent. Therefore, combination therapies using current and new options have been tried

Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, Kyoto
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Correspondence to Dr. Hiroshi Nakase, hiropy_n@kuhp.kyoto-u.ac.jp

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in such patients (7, 8). Currently, few studies have examined the clinical effects of medical therapy in Japanese patients with FCD. The present study focuses on a medical treatment in Japanese patients with FCD.

Patients and Methods

Patients

Ten patients with FCD that visited the Department of Gastroenterology at Kyoto University Hospital between August 2003 and October 2005 were included in the study. Six patients had an external fistula and 4 patients had an internal fistula. A diagnosis of FCD was established by clinical assessment, radiologic and endoscopic findings, and histology. The presence of an abscess was diagnosed using computed tomography or magnetic resonance imaging before starting medical therapy. Antibiotics were administered if an abscess was present.

Methods

To assess the efficacy of medical therapy for patients with FCD, the following data from the initial visit were evaluated: demographic information, including Crohn's disease activity index (CDAI) score, disease duration, disease phenotype, type and number of fistulas, previous medical therapy before starting treatment for external and internal fistulas, and prior surgical procedures, including perianal surgery. The therapeutic regimen, clinical outcome, and side effects of the medical therapy were also evaluated. Withdrawal rates were assessed in patients treated with corticosteroids or setons.

Medical protocol

FCD patients were treated with both 5-aminosalicylic acid (5-ASA) and antibiotics (metronidazole [MTZ] or ciprofloxacin [CPFX]) as the initial therapy, which is the standard of practice at Kyoto University Hospital. The initial dose of antibiotics was 500 mg/day MTZ or 300 mg/day CPFX. Following this initial approach, immunomodulators (azathioprine [AZA] or tacrolimus) were added in combination with the antibiotic therapy for patients who were refractory to 5-ASA plus antibiotics. The initial dose of AZA was 25 mg/day. This dose was adjusted to maintain a white blood cell count between 3,000 and 5,000/μl. Tacrolimus was given at an initial dose of 0.1 mg/kg/day. The whole blood concentration of tacrolimus was monitored twice a week and the dosage of tacrolimus was adjusted to reach a concentration range between 10 and 15 ng/ml. Approximately 2 weeks after starting tacrolimus or 3 months after AZA, infliximab (5 mg/kg) was considered as an additional option for patients without complete fistula closure. In addition to these therapeutic protocols, corticosteroids or infliximab was administered to FCD patients with active luminal disease.

Definition of therapeutic efficacy

The clinical response to therapy for fistulas was classified as complete, partial, and no response. A complete response was defined as fistula closure with complete cessation of fistula drainage in cases with external fistula, and as disappearance of the fistula in patients with internal fistulas, as demonstrated by imaging studies such as radiographic or endoscopic examination, or magnetic resonance imaging. A partial response was defined as greater than 50% reduction in the size or number of fistulas. Clinical remission of CD was defined as a CDAI of less than150 points.

Statistics

Unless otherwise stated, all numerical data are expressed as the mean ± standard error. Differences in characteristics between patients with external and internal fistulas were analyzed using the Student's *t*-test or the Mann-Whitney *U*-test. Evaluation of the change in the CDAI score was analyzed using the Wilcoxon signed-rank test. Statistical analyses were performed using STATVIEW version 5.0 (SAS, Cary, NC). A p value of less than 0.05 was considered to be statistically significant.

Results

The overall mean observation period was 15.7 months (range, 6-25 months).

Baseline characteristics

The baseline characteristics of patients with fistulas are shown in Table 1. There were 2 women and 8 men, and the mean age was 30.2±2.9 years. One patient (patient 4) had colonic-type CD and the other 9 patients had ileocolonic-type. Six patients had external fistulas and 4 patients had internal fistulas. Mean disease duration in patients with external fistulas and in those with internal fistulas were 3.5±0.9 years and 9.7±3.5 years, respectively (p=0.072). Of 6 patients, 5 with external fistulas (83.3%) had perianal fistulas, 2 of whom had setons inserted before medical therapy. The remaining patient had an enterocutaneous fistula. The internal fistula types varied, as shown in Table 1, and all patients with internal fistulas had 2 or more fistulas. The CDAI scores prior to medical therapy were significantly higher in patients with internal fistulas than in patients with external fistulas (median: 267.0, range 200.5-319.0 vs 198.2, range 168.6-249.2; p=0.033). As for previous medical therapy, all patients except patient 5, who had already started AZA treatment in another hospital, had 5-ASA, and 5 patients (1 of 6 patients with external fistulas and all 4 patients with internal fistulas) used oral antibiotics. Only 1 patient (patient 8) was treated with infliximab for an enterovesical fistula prior to the present study, but had not responded to this agent.

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Table 1. Baseline Characteristics of 10 CD Patients with Fistula

Pt	Sex	Age (yr)	Type of CD	Duration of CD (yr)	Type/number of fistula	CDAI	Previous therapy	Surgical records
1	M	22	Ileocolonic	1	Perianal/1	168.6	5-ASA	
2	M	17	Ileocolonic	1	Perianal/1	188.6	5-ASA	
3	M	33	Ileocolonic	4	Perianal/1	203.5	5-ASA	Seton placement
4	M	26	Colonic	3	Perianal/2	187.0	5-ASA, MTZ	
5	M	28	Ileocolonic	5	Perianal/2	192.1	AZA	Seton placement
6	M	49	Ileocolonic	7	Enterocutaneous/1	249.2	5-ASA	Hemicolectomy, Small bowel resection
			Mean	3.5		198.2		
7	F	25	Ileocolonic	5	Enteroenteric/2	319.0	5-ASA, MTZ	
8	F	35	Ileocolonic	18	Duodenocolic/1, Enterovesical/1	284.8	5-ASA, MTZ, IFX	Hemicolectomy, Sigmoidectomy
9	M	29	Ileocolonic	13	Duodenocolic/2, Ileocolonic/2	263.8	5-ASA, CPFX	
10	M	38	Ileocolonic	3	Duodenocolic/1, Ileocolonic/1	200.5	5-ASA, MTZ	
			Mean	9.7		267.0		

All patients with external fistula, except for patient 5, were followed from the first day of the initial use of 5-ASA and antibiotics.
Patient 5 only had started with AZA treatment and seton placement.
The mean CDAI score in patients with internal fistula (267.0) was significantly higher than that in patients with external fistula (198.2).

Table 2. Clinical Outcome of Medical Therapy for FCD Patients

Pt	Period of observation (mo)	Clinical response of fistulas	Time of fistula closure (wks)	CDAI after therapy	Final outcome	Side effects
1	19	Complete response	12wks	119.6	Steroid stopped	-
2	15	Complete response	7wks	96	Steroid stopped	-
3	24	Complete response	10wks	139.6	Seton removed	-
4	19	Complete response	14wks	127.4		paresthesia
5	15	Complete response	4wks	84.7	Steroid stopped, Seton removed	-
6	6	Complete response	12wks	114.9		-
7	17	No response	-	85		-
8	25	Complete response	8wks	95.6		-
9	8	No response	-	252.2	Surgical treatment	-
10	9	No response	-	295.3	Surgical treatment	-

All patients with external fistula achieved complete closure of fistula with a combination of several medical therapies such as 5-ASA, antibiotics, immunomodulators, and infliximab. However, only one patient with internal fistula (patient 8) achieved complete closure of fistulas with these medical therapies.
Patient 7 with enteroenteric fistula achieved remission of luminal CD following treatment with tacrolimus, although the fistula did not close.

Clinical outcome

The clinical outcome of medical therapy for FCD is shown in Table 2. All patients with external fistulas achieved a complete response (100%), whereas only 1 of 4 patents with internal fistulas achieved a complete response (25%).

External fistulas

The therapeutic regimen for patients with external fistulas is shown in Fig. 1. Of 5 patients with perianal fistulas, 4 (patients 1-4; 80%) began treatment with 5-ASA plus antibiotics; for 3 patients (patients 1, 2, and 3), antibiotics were added to 5-ASA. None of these patients achieved a complete response. Two of these patients (patients 1 and 2) had active luminal disease and were treated with the addition of AZA plus prednisolone and achieved a complete response. One patient (patient 3) achieved a complete response after starting additional tacrolimus therapy. The remaining patient (patient 4) was refractory even to the addition of tacrolimus, and required additional treatment with infliximab to achieve a complete response. In patient 5, who had already been administered AZA at the initial visit, neither an increase in AZA dose nor the additional use of prednisolone was effective for fistula closure. Additional combined administration of 5-ASA plus CPFX, however, resulted in complete fistula closure. In patient 6, who had an enterocutaneous fistula, fistula closure was achieved with a combination of 5-ASA, CPFX, and tacrolimus. Four days after starting oral intake, however, the fistula recurred. Infliximab was then added, resulting in complete closure of the fistula. The mean time to a complete response in patients with external fistulas was 9.5 weeks (range, 4-12 weeks).

Internal fistulas

The therapeutic regimen for the 4 patients with internal fistulas is shown in Fig. 2. A complete response was achieved in only 1 patient (patient 8) that had both duodenocolic and enterovesical fistulas, and was refractory to infliximab, but responded to tacrolimus. One patient (patient 7) achieved remission of luminal CD (CDAI: 85 points), but the fistulas, which formed a short bypass, did not close even following treatment with tacrolimus. Patients 9 and 10, with long segmental and complicated fistulas, did not respond to medical therapy with antibiotics plus immunomodulators. Surgical treatment was eventually performed for these 2 patients due to intestinal obstruction during their clinical course.

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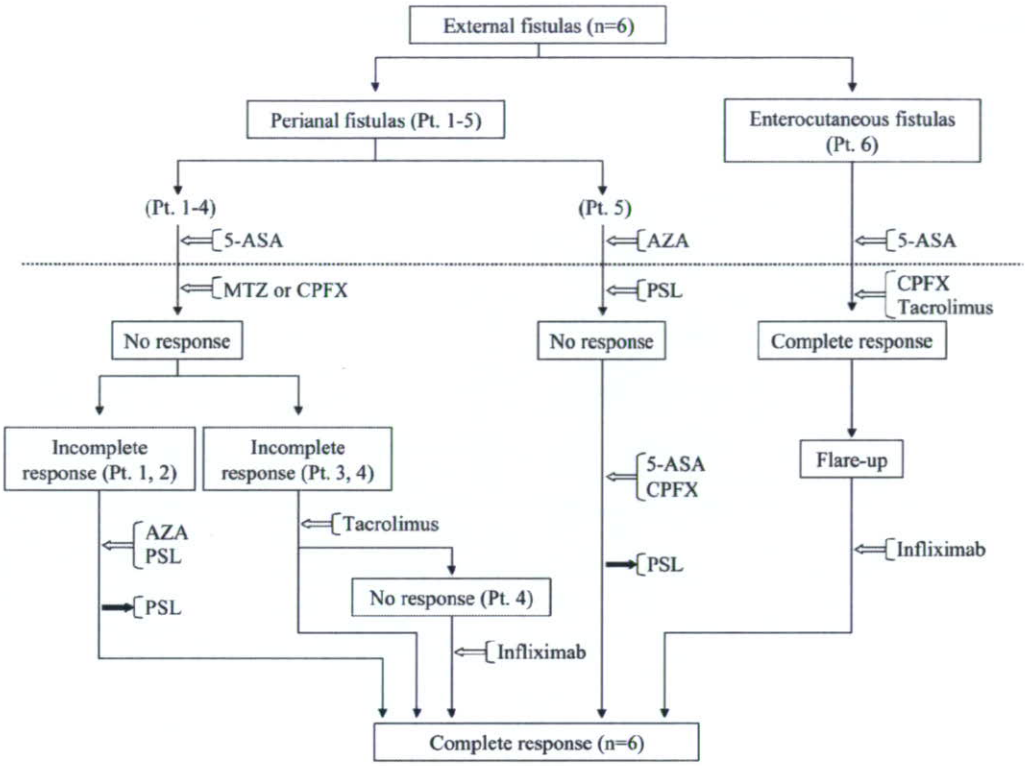

Figure 1. Therapeutic regimen for patients with external fistulas. All patients achieved a complete response to medical therapy. The dotted line indicates the time of the initial visit to our hospital. White and black arrows indicate the administration and discontinuation of drugs, respectively. †Patient 4 was treated with 5-ASA plus MTZ at the initial visit.

Change in CDAI after medical therapy

The CDAI score in patients with external fistulas decreased significantly from 198.2 (range 168.6-249.2) to 113.7 (range 84.7-139.6) after starting medical therapy for their fistulas (p=0.028), while there was no significant difference in the scores before and after medical therapy in patients with internal fistulas (median: 267.0, range 200.5-319.0 to 182.0, range 85.0-295.3; p=0.273; Fig. 3).

Withdrawal rate of corticosteroids and seton drainage

Corticosteroids used in 3 patients with perianal fistula were completely tapered off by using immunomodulators or antibiotics. Setons placed in 2 patients with perianal fistulas (patients 3 and 5) were also removed during follow-up after starting additional treatment with immunomodulator or antibiotics.

Side effects

There were no life-threatening side effects observed during the follow-up. Only one patient (patient 3) complained of paresthesia of the upper extremities following MTZ administration. This symptom was relieved by dose reduction.

Discussion

The present study revealed that among patients with CD, external fistulas were more responsive to medical therapies than internal fistulas. These results are consistent with previous data (9, 10). Indeed, all of our patients with external fistulas achieved closure of the fistulas as well as withdrawal from corticosteroids or removal of setons, whereas closure was achieved in only 1 of 4 patients with internal fistulas. All 6 patients with external fistulas were basically treated with antibiotics plus immunomodulators, and 4 of them never required infliximab. Interestingly, patient 5 who had perianal fistulas and had already taken AZA without antibiotics, obtained a complete response after starting additional antibiotic therapy. These results suggest that concomitant administration of antibiotics and immunomodulators is important for closure of external fistulas. Dejaco et al reported that antibiotic therapy is useful for inducing a short-term response in CD patients with perianal fistulas and for bridging the maintenance therapy with AZA (11). The early use of antibiotics appears essential for the treatment of perianal FCD because the control of sepsis is the first objective (12) and the immunosuppressive action of AZA or 6-mercaptopurine (6-MP) occurs slowly, possibly taking months to be effective (8, 12). Therefore, we consider that combination therapy with antibiotics and immunomodulators

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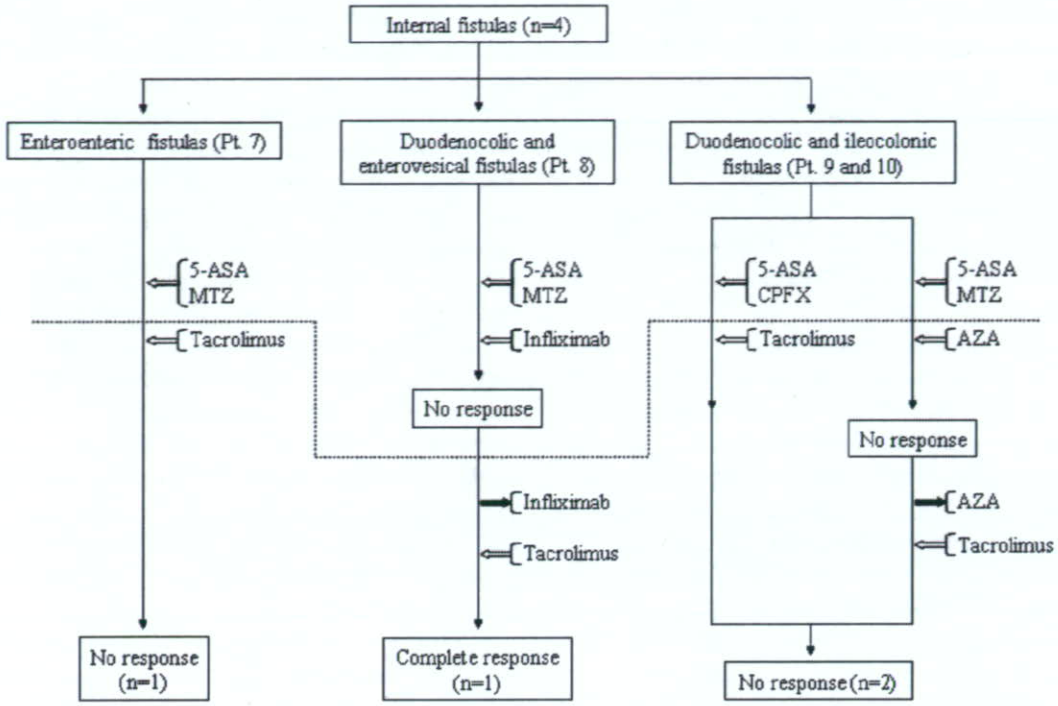

Figure 2. **Therapeutic regimen for patients with internal fistulas. Complete response to medical therapy was achieved in only 1 of 4 patients with an internal fistula (patient 8). The other patients were refractory to medical therapy; one of them (patient 7) achieved remission of luminal Crohn's disease without fistula closure, and the remaining patients (patients 9 and 10) required surgical treatment. The dotted line indicates the time of the initial visit to our hospital. White and black arrows indicate administration and discontinuation of drugs, respectively.**

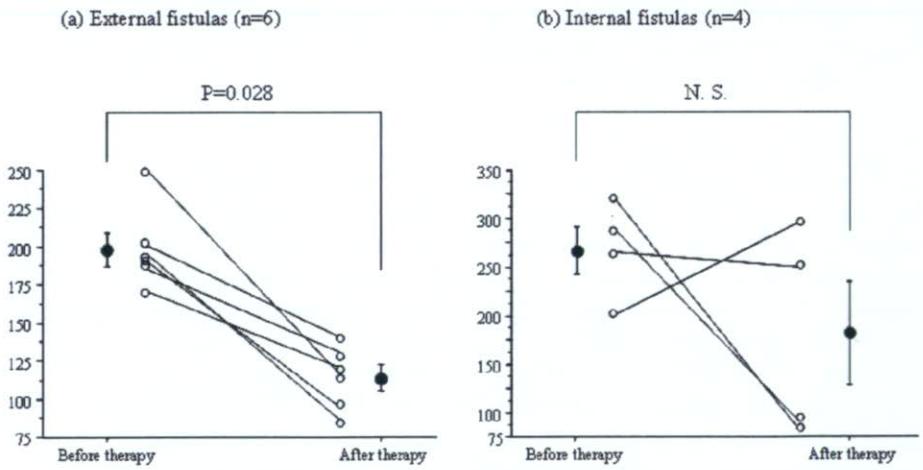

Figure 3. **Change in Crohn's disease activity index (CDAI) in patients with external fistulas (a) and in those with internal fistulas (b) after medical therapy. Closed circles represent the mean± standard error. N.S: not significant**

is suitable as an initial therapy for FCD.

It is generally accepted that long-term use of antibiotics is important for maintaining comfort in FCD patients and improves the quality of life for a long time (4). A subpopulation of patients with FCD quickly relapse after a dose reduction or discontinuation of antibiotics (4, 13-15), suggesting that the control of enteric bacteria is important for patients with FCD, particularly under immunosuppressive conditions. Thus, it appears reasonable to use antibiotics for both the induction and maintenance of remission in patients with FCD, as long as side effects do not occur.

AZA might be optimal as an initial immunomodulator for the treatment of FCD, because many reports indicate that AZA/6-MP is effective for both achieving and maintaining fistula closure, and long-term use of these drugs is well-tolerated and safe without any evidence of the development

of neoplasms or infection (4, 16-18). Moreover, tacrolimus, which was administered to some patients with FCD in this study, might be an alternative therapy because it is reported to be effective not only as a bridge therapy until the efficacy of AZA or 6-MP appears (19, 20), but also for long-term management of FCD (8, 21). Recently, two contradictory reports were published in which the efficacy of tacrolimus for FCD patients was evaluated (7, 8). A short-term, double-blind, placebo-controlled trial by Sandborn et al showed poor outcomes for external FCD patients treated with tacrolimus: tacrolimus was better than placebo for improving fistulas but not for inducing fistula remission, and nephrotoxicity was quite high (7). On the other hand, a prospective, uncontrolled, open-label study by Gonzalez-Lama et al demonstrated both short- and long-term efficacy and safety of oral tacrolimus therapy for perianal, enterocutaneous, and rectovaginal FCD patients refractory to conventional therapies, including infliximab, and only mild side effects were observed without nephrotoxicity (8). The discrepancy between the two studies might be due to the differences in the initial dose and blood concentration of tacrolimus. The initial dosage in the former study was 0.2 mg/kg per day, with adjustment of the whole blood concentration to 10 to 20 ng/ml, which is recommended for the early posttransplant period of organ transplantation and should not last for more than 2 to 3 months (22). In the latter study, however, tacrolimus was initiated at a dosage of 0.1 mg/kg per day, keeping the blood concentration under 15 ng/ml (8), which is similar to our recommended protocol for the treatment of patients with FCD. A higher initial dose of tacrolimus increases the risk of nephrotoxicity without a clinical response in FCD patients (23). Therefore, we consider that tacrolimus could be an alternative option for treating patients with FCD that is refractory to conventional therapy including infliximab, as in patient 8, as long as an appropriate initial dosage and adjustment of a whole-blood concentration are achieved.

Introduction of the anti-tumor necrosis factor-α antibody, infliximab, has greatly improved the quality of life in patients with FCD refractory to conventional therapy (5, 6, 12). In our study, combination therapy with antibiotics and immunomodulators was not always effective as revealed in patients 4 and 6 with perianal and enterocutaneous fistulas, respectively. In these cases, additional treatment with infliximab induced complete fistula closure. Recently, the early administration of infliximab, a so-called 'top-down' approach, was shown to be efficacious, however, some controversies still exist (24, 25). On the other hand, concomitant treatment with antibiotics or immunomodulators with infliximab had synergistic effects or reduced the magnitude of the immunogenic response to infliximab, as compared with infliximab alone (26-30). Therefore, whether infliximab administration should precede the administration of antibiotics or immunomodulators remains unclear. Based on the present data, however, the combination of antibiotics and immunomodulators can be used as an initial therapy, particularly for patients with external fistulas.

As for the treatment of internal fistulas, the efficacy of medical therapy has yet to be demonstrated in randomized controlled trials (9). In the present study, only 1 of 4 patients with internal fistulas achieved a complete response, indicating that internal fistulas are resistant to medical therapy. Patient 8 with the internal fistulas, who was reported previously (31), was refractory to infliximab, but alternatively responded to tacrolimus with complete closure. Patient 7 had enteroenteric fistulas with a very short bypass that did not warrant surgical intervention. Because these fistulas were associated with active inflammation, as reflected by the patient's CDAI score of 319, we treated this patient with tacrolimus. As a result, the abdominal symptoms subsided although the fistulas did not close. Perhaps a short bypass as in this case might not need to be closed because there was neither bacterial overgrowth nor disturbed absorption. Two other patients with internal fistulas were refractory to medical therapy and eventually required surgical treatment. Infliximab could not be used in these 2 patients because intestinal obstruction occurred within 2 weeks after the administration of tacrolimus. These two patients shared common features; their fistulas were long and complicated with intestinal stenosis. Thus, these radiologic findings suggest that such internal fistulas might be refractory to medical therapy and surgery should be selected. In this study, the longer mean disease duration and significantly higher mean CDAI in patients with internal fistula compared to those with external fistula might result in a poor response to medical therapies.

In conclusion, the present study focused on a medical treatment for Japanese patients with FCD. External fistulas were more responsive to medical therapies than internal fistulas, consistent with previous reports (9, 10). Combination therapies with antibiotics and immunomodulators might be considered as an initial therapy for external FCD. Moreover, infliximab can be administered as an additional option for cases refractory to combination therapy. In contrast, internal fistulas are refractory to these medical treatments. In the future, a prospective study with more patients is required to evaluate what kinds of treatments are optimal for patients with FCD.

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Abbreviation: IFX: Infliximab

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