200717011A

厚生労働科学研究費補助金

医療技術実用化総合研究事業

タクロリムスの難治性クローン病治療に向けての臨床試験実施計画に関する研究

平成19年度　総括研究報告書

主任研究者　千葉　勉

平成20(2008)年　4月

目　　次

厚生労働科学研究費補助金（医療技術実用化総合研究事業）
総括研究報告書

タクロリムスの難治性クローン病治療に向けての臨床試験実施計画に関する研究

主任研究者　千葉　勉　京都大学医学研究科消化器内科学講座・教授

研究要旨：

クローン病（CD）は若年者に発症する難治性の炎症性腸疾患で、現行の保険適応薬剤に対する抵抗例が少なからず存在する。そこで本研究では、本邦で開発された優れた免疫抑制薬であるタクロリムスの難治性CDに対する保険適応承認を最終目標として、その効果について質の高いエビデンスを得るための臨床試験を計画することを目的とした。その結果、タクロリムス高用量投与群において、投与後30日目ですでに優れた効果を認め、60日目には有効率100%、緩解率83.3%となった。また全例でステロイドを中止することが可能となった。さらに副作用について、軽微なもの以外特に認められなかった。以上、難治性CDに対してタクロリムス治療は極めて有効であり、今後保険適応承認に向けて、大規模臨床試験を行うことが必要であると考えられた。

【分担研究者】
西尾　彰功
京都大学医学研究科　准教授
仲瀬　裕志
京都大学医学研究科　講師

A. 研究目的

クローン病（CD）は難治性の炎症性腸疾患であり、わが国でも増加しつつあるが、主として若年者に発症し教育や就労が困難となる例が多い。CDの発症には大腸の常在細菌に対する異常な免疫反応が関与すると考えられているため、現在免疫抑制薬を中心とした治療が行われているが、現行の保険適応薬剤に抵抗性を示す難治例が少なからず存在し大きな臨床的課題となっている。特に瘻孔や狭窄例では長期食事摂取が困難なためQOLが大きく傷害され、その生活は悲惨であり新しい治療法の開発が待たれる。一方タクロリムスはわが国で開発された優れた免疫抑制剤で、すでに移植後

拒絶やGVHDの抑制、慢性関節リューマチなどに保険承認を受けて、優れた効果をあげている。またCDより患者数の多い潰瘍性大腸炎に対して現在臨床治験が進行中である。しかしながらCDについては、その効果が期待されながら患者数が少ないなどの理由から臨床治験の計画はないのが現状である。以上より本研究では、タクロリムスの難治性CDに対する保険適応承認を最終目標として、その効果について質の高いエビデンスを得るための臨床試験を計画することを目的とした。

B. 研究方法

（1）現行の内科治療（ステロイド、イムラン、レミケード等）で治療困難な難治性CD患者に対してタクロリムスを投与して、大規模臨床試験に向けて、投与量、投与期間、投与方法などを検討した。なお投与量の検討は血中タクロリムスのトラフ値を測定することにより行った。

（2）点滴または経口投与によって治療を開始し（経口摂取が不可能な症例に対して点滴施行）、点滴例は可能な限り2週間以内に経口投与へ移行した。投与量は目標血中濃度により3群（低用量　0-5、中用量　5-10、高用量　10-15ng/ml）に分けた。

（3）同時に瘻孔（内瘻、外瘻）、狭窄、痔瘻などの有無でその効果を比較し、適応対象の検討をおこなった。

（4）評価は、疾患活動性指数（CDAI）の変化でおこない、治療前より70点以上のCDAI減少を「有効」、150点以下となった例を「緩解」とした。また副次項目として、ステロイドの減量効果も検討した。

（5）タクロリムス投与期間中、血液検査、臨床症状などで、様々な副作用の出現についても検討を加えた。

C. 研究結果

（1）19例がエントリーされた。平均年齢は32歳（男性11例、女性8例）で、3群間で年齢に有意差はなかった。また男女比にも差はなかった。薬剤の投与量は、低トラフ群　7例、中トラフ群　6例、高トラフ群　6例であった。タクロリムス投与前のCDAIスコアは症例全体で368で、投与量の異なる3群間で差はなかった。

（2）タクロリムス投与30日後には、低、中、高トラフ群でCDAIはそれぞれ330、254、151とな

り、また 60 日後には、それぞれ 324、204、123 となった（添付図）。有効例は低、中、高トラフ群それぞれ 1/7 (14.3%)、4/6(66.7%)、6/6(100%)であり、また緩解例はそれぞれ 0/7(0%)、2/6(33.3%)、5/6(83.3%)となった。このうち中トラフ群にはタクロリムスを 90 日間投与したが、30 日目と比較してトラフ値は改善しなかった。

（3）タクロリムス投与によって、それぞれの群でステロイド減量効果を認めたが、その減量効果は低、中、高トラフ群の順に増強した。投与 30 日後には、高トラフ群では平均 22mg から 2.7mg、60 日目には投与例は 0 となった。このことからタクロリムスの特にステロイド減量効果における即効性が確認された。

（4）副作用として、2 例の患者（高トラフ群）に軽い血清クレアチニンの上昇を認めたが（1.8 および 2.0 mg/dl)、トラフ濃度を下げることによって正常に回復した。また一人の患者において一時的な Tremor が出現した（高トラフ群）が、これも減量により改善した。なおこれらはすべて高用量投与群であった。全経過をとおして全例重篤な感染症はみとめなかった。また全経過を通して中断例は認めなかった。

（5）タクロリムス投与により、4 例の瘻孔（内瘻）のうち 3 例で、また 2 例の外瘻の 2 例ともに閉鎖が確認された。

D. 考察

今回の臨床研究において、従来の保険適応薬剤治療に抵抗する難治性 CD に対してタクロリムス投与が極めて有効であることが確認された。すなわち特に高用量群において、投与 60 日目で有効 100%、また緩解 83.3%であり、さらにステロイド投与については全例が中止することが可能となった。さらに 4 例の内瘻症例中 3 例、2 例の外瘻症例中 2 例において閉鎖が確認された。一方、副作用については、やはり高用量群でのみ生じたいが、いずれも軽微なもので減量することによって消失した。

今回の臨床試験は、今後大規模な臨床試験を行うための投与方法（用量、投与期間など）を決定するためにおこなったものである。その結果投与量については、低中高の 3 群間の比較では、やはり高用量群でもっとも高い有効率、緩解率が得られ、

またステロイド減量効果も最も優れていた。これに対して、副作用については高用量投与群でのみ認められたが、いずれも軽微なものであった。以上より今後の臨床研究においては、やはり高用量を設定する必要があると考えられた。一方投与期間については、投与 30 日目よりは 60 日目のほうが CDAI はいずれの群でもより改善する傾向が認められた。しかしながら高用量群では、その改善は必ずしも著明なものではなく、投与 30 日目で、すでに十分な効果が得られた。さらに 90 日間投与した中用量群では、60 日目と比較して 90 日目に明らかな改善は認められなかった。以上より、タクロリムスの効果を判定する期間として、30、60 日のいずれかは議論する余地が残されているが、より短期で検討してもその効果は十分に明らかになるものと想定された。

今回副次効果項目として、ステロイド減量効果が検討されたが、特に高用量では、60 日目に全例がステロイドを中止することが可能であった。このように早期にステロイドを離脱できることは、ステロイド長期大量使用による、副作用などに悩む患者にとって大きな福音であり、タクロリムス治療が極めて優れた治療法であることを支持するものである。また瘻孔、外瘻についても優れた効果が得られたが、これも患者の QOL の改善に対して極めて大きな成果といえる。

副作用については、今回は高用量群は 60 日まで、中用量群では 90 日目までの投与をおこなったが、この間重症感染症も含めて、重篤な副作用は観察されなかった。したがってタクロリムスの高用量投与は、比較的短期間投与についてはほぼ安全であると考えられた。しかしながら、長期投与した場合の安全性はいまだ確認されておらず、この点については、今後の検討課題と言える。

以上より、今後大規模臨床試験を計画するにあたっては、短期、高用量投与が好ましいと考えられた。

E. 結論

（1）タクロリムスは難治性 CD に対する治療薬として、効果、副作用、コンプライアンスのすべての点で優れていた。

（2）またその特徴として、即効性、強いステロイド減量効果、瘻孔、外瘻への効果、などが認められた。

（3）なおこれらの効果は、特に高用量投与群で最も強く認められた。

（4）さらに上記の成績は、投与 30

日、60 日目ですでに明らかであった。

（5）以上より、今後難治性 CD 患者に対するタクロリムスによる大規模臨床試験の実施にあたっては、高用量による、比較的短期の投与試験が適切であると考えられた。

F. 健康危険情報

とくになし。

G. 研究発表

1）論文発表

1. Nakase H, Yoshino T, Ueno S, Uza N, Mikami S, Matsuura M, Chiba T: Importance of early detection of cytomegalovirus infection in refractory inflammatory bowel disease. Inflamm Bowel Dis 13:364:2007.
2. Nakase H, Mikami S, Matsuura M, Ueno S, Uza N, Inoue S, Kitamura H, Kasahara K, Yoshino T, Takeda Y, Chiba T: Rescue therapy with Tacrolimus for a patient with severe ulcerative colitis refractory to combination leukocytapehresis and high-dose of corticosteroid therapy. Int Med 46:717-720:2007.
3. Inoue S, Nakase H, Matsuura M, Ueno S, Uza N, Kitamura H, Mikami S, Tamaki H, Kasahara K, Chiba T: Open label trial of Clarithromycin therapy in Japanese patients with Crohn's disease. J Gastroenterol Hepatol 22:984-988:2007.
4. Mikami S, Nakase H, Ueno S, Matsuura M, Sakurai T, Chiba T: Involvement of cytomegalovirus infection in the ileal lesions of the patient with Behcet's disease. Inflamm Bowel Dis 13:802-803:2007.
5. Yoshino T, Nakase H, Ueno S, Uza N, Inoue S, Mikami S, Matsuura M, Ohmori K, Sakurai T, Nagayama S, Hasegawa S, Sakai Y, Chiba T: Usefulness of quantitative real-time PCR assay for early detection of cytomegalovirus infection in patients with ulcerative colitis refractory to immunosuppressive therapies. Inflamm Bowel Dis 13:1516-1521:2007.
6. Uza N, Nakase H, Ueno S, Inoue S, Mikami S, Tamaki H, Matsuura M, Chiba T: The

effect of medical treatment on patients with fistuklizing Crohn's disease: our experience with a retrospective study. Intern Med 2008 (in press)

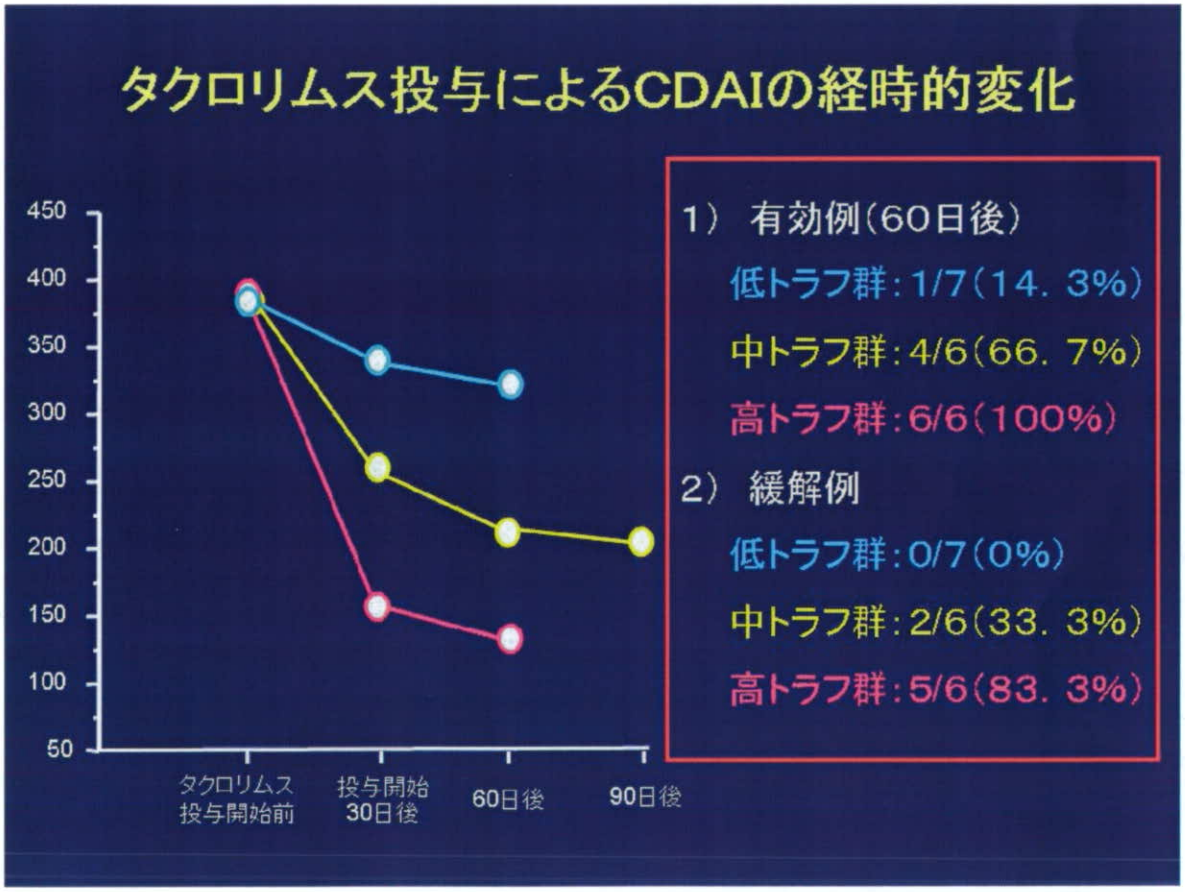
2）学会発表

1. Hiroshi Kitamura, Hiroshi Nakase, Yasuhiro Takeda, Takuya Yoshino, Katsuhiro Kasahara, Satoru Ueno, Norimitsu Uza, Satoko Inoue, Sakae Mikami, Minoru Matsuura, Yoshihiro Ishida, Kazuhiro Nagata, Tsutomu Chiba: The critical role of heat shock protein 47 in intestinal fibrosis associated with Inflammatory Bowel Diseases. Digestive Disease Week and the 108th Annual Meeting of the American Gastroenterological Association Institute・oral sessions, 2007.5.23, Washington DC.
2. Takuya Yoshino, Hiroshi Nakase, Satoko Inoue, Hiroshi Kitamura, Satoru Ueno, Norimitsu Uza, Sakae Mikami, Minoru Matsuura, Tsutomu Chiba: The usefulness of quantitative real-time PCR assay for early detection of cytomegalovirus in patients with UC refractory to immunosuppressive therapies. Digestive Disease Week and the 108th Annual Meeting of the American Gastroenterological Association Institute・poster sessions, 2007.5.21, Washington DC.
3. TANAKA Junya, WATANABE Norihiko, KIDO Masahiro, SAGA Kazuyuki, AKAMATSU Takuji, NISHIO Akiyoshi, CHIBA Tsutomu: Human TSLP enhances TLR-ligand-mediated IL-23 production by myeloid dendritic cells and has a potential to induce Th17 inflammatory responses. 2007 日本免疫学会総会・学術集会, 2007.11.20, Tokyo.
4. Tomohiro Watanabe, Naoki Asano, Peter J Murray, Keiko Ozato, Prafullakumar Tailor,Ivan J Fuss, Atsushi Kitani, Tsutomu Chiba, Warren Strober: Muramyl Dipeptide Activation of Nucleotide Binding Oligomerization Domain 2 Protects Mice from Experimental Colitis. The 3rd JSI-RCAI Immunology Workshop Regulation of the Mucosal Immune Response

-Crosstalk between Basic and Clinical Researches-, 2007.3.14, Tokyo.

H. 知的財産権の出願・登録状況
（予定を含む）

特許取得および実用新案登録など、該当なし。



別紙4　研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル	書籍全体の編集者	書籍名	出版社名	出版地	出版年	ページ
該当なし							

雑誌

発表氏名	論文タイトル名	発表誌名	巻(号)	ページ	出版年
Nakase H, Yoshino T, Ueno S, Uza N, Mikami S, Matsuura M, Chiba T	Importance of early detection of cytomegalovirus infection in refractory inflammatory bowel disease.	Inflamm Bowel Dis	13	364	2007
Nakase H, Mikami S, Matsuura M, Ueno S, Uza N, Inoue S, Kitamura H, Kasahara K, Yoshino T, Takeda Y, Chiba T	Rescue therapy with Tacrolimus for a patient with severe ulcerative colitis refractory to combination leukocytapehresis and high-dose of corticosteroid therapy.	Int Med	46	717-720	2007
Inoue S, Nakase H, Matsuura M, Ueno S, Uza N, Kitamura H, Mikami S, Tamaki H, Kasahara K, Chiba T	Open label trial of Clarithromycin therapy in Japanese patients with Crohn's disease.	J Gastroenterol Hepatol	22	984-988	2007
Mikami S, Nakase H, Ueno S, Matsuura M, Sakurai T, Chiba T	Involvement of cytomegalovirus infection in the ileal lesions of the patient with Behcet's disease.	Inflamm Bowel Dis	13	802-803	2007
Yoshino T, Nakase H, Ueno S, Uza N, Inoue S, Mikami S, Matsuura M, Ohmori K, Sakurai T, Nagayama S, Hasegawa S, Sakai Y, Chiba T	Usefulness of quantitative real-time PCR assay for early detection of cytomegalovirus infection in patients with ulcerative colitis refractory to immunosuppressive therapies.	Inflamm Bowel Dis	13	1516-1521	2007
Uza N, Nakase H, Ueno S, Inoue S, Mikami S, Tamaki H, Matsuura M, Chiba T	The effect of medical treatment on patients with fistuklizing Crohn's disease: our experience with a retrospective study.	Intern Med	47	193-199	2008

Importance of Early Detection of Cytomegalovirus Infection in Refractory Inflammatory Bowel Disease

To the Editor:

Cases of inflammatory bowel disease (IBD) associated with cytomegalovirus (CMV) infection is a very important issue for gastroenterologist.[1,2] In patients with IBD refractory to conventional therapy, we should always consider the possibility of CMV infection. We note with great interest the article by Dimitroulia et al.[3] Data for patients with IBD who were investigated in that study demonstrated that the CMV genome in intestinal tissue was detected by polymerase chain reaction (PCR) in 32.9% of total IBD patients, while the CMV genome in the blood was detected in 27.1% of these patients. A positive ratio of CMV genome in intestinal tissue is higher in patients with ulcerative colitis (UC) than in those with Crohn's disease. Moreover, 5.9% of IBD patients had detectable CMV genome in their intestinal samples but not in their blood. On the other hand, in the control group who had no inflammatory disease, 11.9% of individuals had a detectable CMV genome in their blood, but 2.2% in their intestine.

The authors suggested that a potential role of CMV in the mucosal immune disturbance of IBD might be suspected because CMV infection occurred in intestinal tissue more dominantly than in the blood. However, they described that a definitive causal role of CMV in exacerbating IBD is still unknown. The detection of the CMV genome in colonic tissue may not necessarily indicate active CMV infection because infection usually results from reactivation of latent virus.

Recently, we clearly demonstrated that the detected copy number of CMV DNA by our PCR method is higher in the inflamed colonic tissue than that in noninflamed colonic tissue in patients with UC refractory to immunosuppressive therapy.[4] Moreover, our data demonstrated that all three patients with UC were positive for the CMV genome in inflamed colonic tissue alone, although neither CMV antigenemia nor CMV inclusion bodies were detected. In addition, we investigated more patients with refractory UC. As a result, 18 (94.7%) of 19 patients with refractory UC who were positive for CMV DNA in inflamed colonic mucosa by our PCR method had neither CMV inclusion body nor CMV antigenemia. All patients were successfully treated with antiviral therapy (unpubl. data). These results strongly support that CMV infection was involved in the deterioration of patients with UC and the investigation of CMV DNA in inflamed colonic tissue by the PCR method is necessary for early detection of CMV infection. Thus, both Dimitroulia's and our data included important points in considering diagnosis and therapy for patients with moderate or severe IBD who might have concomitant CMV infection.

In summary, we strongly believe that CMV infection is involved in exacerbation of patients with IBD. Therefore, early detection of CMV infection in intestinal tissue is absolutely necessary for developing a therapeutic strategy. Quantitative real-time PCR assay in gastrointestinal tissue is a promising modality for the diagnosis of CMV infection.

Hiroshi Nakase MD, PhD
Takuya Yoshino, MD
Satoru Ueno, MD
Norimitsu Uza, MD
Sakae Mikami, MD
Minoru Matsuura, MD, PhD
Tsutomu Chiba, MD, PhD
Department of Gastroenterology & Hepatology
Graduate School of Medicine
Kyoto University
Kyoto, Japan

REFERENCES

1. Vega R, Bertran X, Menacho M, et al. Cytomegalovirus infection in patients with inflammatory bowel disease. *Am J Gastroenterol.* 1999;94:1053–1056.
2. Pfau P, Kochman ML, Fruth EE, et al. Cytomegalovirus colitis complicating ulcerative colitis in the steroid-naïve patient. *Am J Gastroenterol.* 2001;96:895–899.
3. Dimitroulia E, Spanakis N, Konstantinidou AE, et al. Frequent detection of cytomegalovirus in the intestine of patients with inflammatory bowel disease. *Inflamm Bowel Dis.* 2006;12:879–884.
4. Kou T, Nakase H, Tamaki H, et al. Cytomegalovirus infection in patients with ulcerative colitis diagnosed by quantitative real-time PCR analysis. *Dig Dis Sci.* 2006;51:1052–1055.

Supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Culture and Science of Japan (grant 18590677).

DOI 10.1002/ibd.20033
Published online 19 December 2006 in Wiley InterScience (www.interscience.wiley.com).

INTERNAL MEDICINE

□ CASE REPORT □

Rescue Therapy with Tacrolimus for a Patient with Severe Ulcerative Colitis Refractory to Combination Leukocytapheresis and High-Dose Corticosteroid Therapy

Hiroshi Nakase, Sakae Mikami, Minoru Matsuura, Satoru Ueno, Norimitsu Uza, Satoko Inoue, Hiroshi Kitamura, Katsuhiro Kasahara, Takuya Yoshino, Yasuhiro Takeda and Tsutomu Chiba

Abstract

A19-year-old man complaining of severe diarrhea and hematochezia was admitted to our hospital. Endoscopic findings and laboratory data revealed that he had ulcerative colitis (UC). Despite combination therapy with high-dose corticosteroids and intensive granulocytapheresis, his condition did not improve. Therefore, we initiated tacrolimus therapy. Intravenous administration of tacrolimus with a trough level of 10 to 15 ng/ml relieved his abdominal symptoms within 1 week. The patient experienced no UC relapse 1 year after treatment with oral tacrolimus. Tacrolimus is a promising therapy for patients with UC refractory to the combination of high-dose corticosteroids and leukocytapheresis.

Key words: ulcerative colitis, tacrolimus, leykocytapheresis

(DOI: 10.2169/internalmedicine.46.6219)

Introduction

Tacrolimus is a macrolide antibiotic isolated from *Streptomyces tsukubanesis* that has immunomodulatory properties; it is efficacious and widely used for the prevention of allograft rejection in patients undergoing liver transplantation (1). Although its action is similar to that of cyclosporin (CyA), the immunosuppressive effect is 10 to 20 times greater in vivo than that of CyA and its intestinal absorption is more reliable, even in the presence of gastrointestinal disease (2). Therefore, much attention has been directed to tacrolimus for patients with inflammatory bowel disease (IBD) that are refractory to conventional therapy. In several uncontrolled studies, tacrolimus improved fistulizing Crohn's disease (CD) and steroid-refractory ulcerative colitis (UC) (3, 4). A recent randomized control study demonstrated the efficacy and safety of oral tacrolimus for inducing remission of refractory UC (5). Here, we report a patient with severe UC which was refractory to combination high-dose corticosteroids and leukocytapheresis therapy and was successfully treated with tacrolimus.

Case Report

A 19-old-year man with no medical history was admitted to our hospital because of high fever and frequent bloody diarrhea. Physical examination revealed localized tenderness at the left lower abdomen and increased bowel sounds. His temperature was 38℃. The following pathologic laboratory findings were noted: white blood cell count 11600/mm^3, C-reactive protein (CRP) 14 mg/dl, and serum albumin 2.9 g/dl (Table 1). Repeated blood cultures and testing for parasitic and bacterial bowel pathogens were negative. Colonoscopy revealed dull colonic mucosa and an erythematous pattern with a granular texture and gross pitting, and a blurred vascular pattern. Computed tomography showed increased wall thickness throughout the entire colon and increased vascularization within the bowel walls. The patient was diagnosed with severe total type UC. Under this diagnosis, daily therapy with 50 mg of prednisolone (PSL), along with 3000 mg mesalamine was started. Despite this

Department of Gastroenterology & Hepatology, Graduate School of Medicine, Kyoto University, Kyoto
Received for publication September 2, 2006; Accepted for publication January 25, 2007
Correspondence to Dr. Hiroshi Nakase, hiropy@kuhp.kyoto-u.ac.jp

DOI: 10.2169/internalmedicine.46.6219

Table 1. Laboratory Data on Admission

Hematology			Normal range
WBC	11600/mm^3		3000-8500
Hb	11.8 g/dl		12.5-16.4
Plt	280000 /mm^3		167000-364000
Blood chemistry			
TP	6.7	g/dl	6.3-8.1
Alb	2.9	g/dl	3.9-5.1
GOT	17	IU/l	13-33
GPT	12	IU/l	8-42
ChE	157	IU/l	201-436
BUN	6	mg/dl	8-22
Cr	0.8	mg/dl	0.6-1.1
Na	137	mEq/L	136-144
K	3.5	mEq/L	3.6-4.8
CRP	14	mg/dl	0.2<

therapy, the patient's condition worsened. We then initiated treatment with 60 mg PSL and methylprednisolone pulse therapy (1000 mg for 3days). His condition improved 2 weeks after initiating this therapy. When the dose of PSL was tapered to 50 mg per day, however, his condition deteriorated again. He became increasingly anemic and hypoalbuminemic, and both blood in the stool and bowel movements increased. We thought that additional mesalamine enema might worsen the patient's symptoms and therefore did not perform it. Next, we started intensive granulocytapheresis (G-CAP) twice a week as additional therapy. Although we performed combination therapy with PSL and G-CAP six times, his condition did not improve. Colonoscopy revealed deep longitudinal ulcerations with mucosal erythema and edema at the sigmoid colon (Fig. 1). At this time, cytomegalovirus (CMV) antigenemia was negative. Histology and polymerase chain reaction method to detect CMV in a colonic biopsy specimen revealed no existence of concomitant CMV infection. We considered that it would be necessary to use immunosuppressive therapies with a strong and rapid onset of action for this patient because he was refractory to intensive G-CAP and high-dose corticosteroid therapy.

After informed consent was obtained from the patient and his family, tacrolimus was given by continuous intravenous infusion to adjust the serum trough levels of tacrolimus from 10 to 15 ng/ml. One week after initiating intravenous administration of tacrolimus, his abdominal pain disappeared and CRP became negative (Fig. 2). We then switched to oral administration of tacrolimus with the same trough level. Eight weeks after initiating the oral tacrolimus therapy, the administration of PSL was tapered off and the patient's condition was completely improved. Colonoscopy revealed regenerating epithelium at the sites of previous ulcerative lesions (Fig. 3). During this therapy, the patient did not experience any serious side effects other than tremor. We tapered the dose of tacrolimus to achieve a trough level of 5 to 10 ng/ml and he remained in remission 3 months later. One year later, the patient is still in remission without any side effects and the laboratory data show no signs of active inflammation.

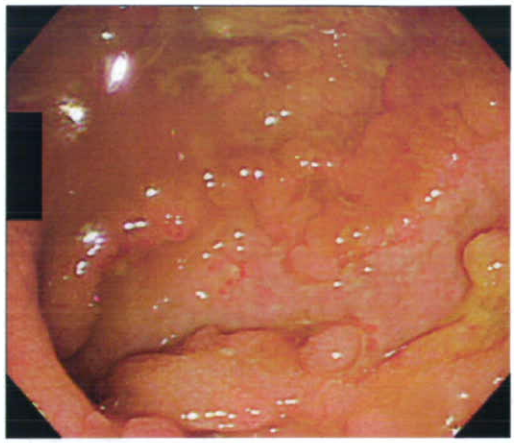


Figure 1. Colonoscopic view of the colonic ulcerations before administering tacrolimus.

Discussion

This is a case report of a 19-year-old Japanese patient with severe UC that was successfully treated by intravenous and oral administration of tacrolimus. The initial therapy for patients with moderate or severe UC is a combination of oral mesalamine and corticosteroids. Recently, the therapeutic effect of GCAP for active UC was reported in Japan. G-CAP is a new therapy in which granulocytes and monocytes are selectively absorbed by a G-1 Adacolumn. Naganuma et al reported that G-CAP therapy is a promising option for patients with moderate UC that are refractory to conventional therapy, with regard to reducing and avoiding PSL re-administration, but that five sessions of G-CAP is not very effective for patients with severe UC (6). In this case, we intensively performed G-CAP twice per week to reduce the colonic inflammation, because the patient did not respond to high-dose PSL administration. This combination therapy, however, was not effective for this patient. In addition, endoscopic findings revealed deep longitudinal ulcer, which was suggestive of impaired mucosal healing. We therefore started intravenous administration of tacrolimus, for expecting rapid onset of action and letting this patient go into remission as soon as possible, because azathioprine may be ineffective in this active phase for its delayed onset of action.

Generally, intravenous administration of CyA is effective as a rescue therapy for patients with severe UC. The rapid

DOI: 10.2169/internalmedicine.46.6219

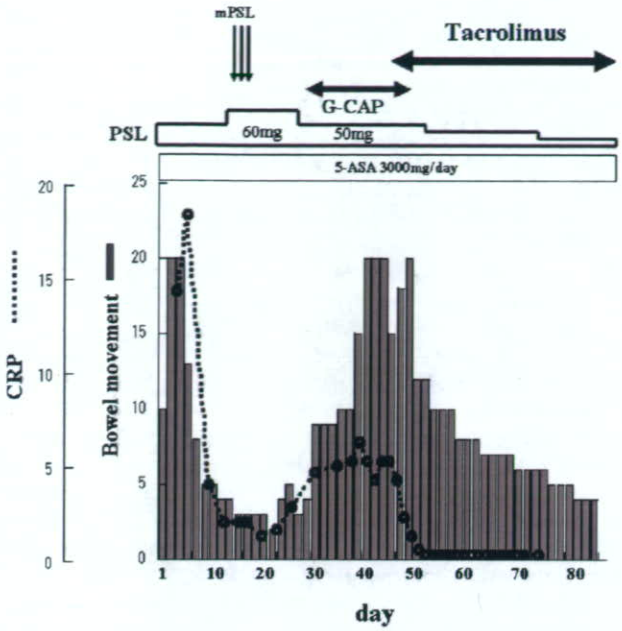


Figure 2. Clinical course of the patient successfully treated with tacrolimus.

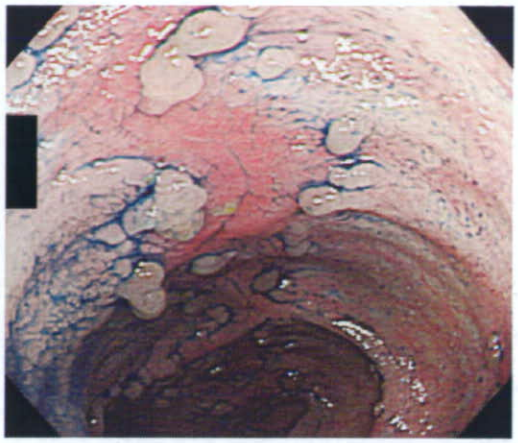


Figure 3. Colonoscopic view showing the appearance of regenerating epithelium at the previously ulcerated sites after tacrolimus administration.

efficacy of CyA to avoid emergency colectomy is approximately 50% to 80% in UC, but 35% to 67% of patients eventually undergo surgery (7-10). In addition, maintenance of remission with CyA requires high doses that are frequently associated with significant side effects such as gingival hyperplasia, hypertrichosis, hypertension, diabetes, nephrotoxicity, and neurotoxicity (11).

Another major disadvantage of CyA is the necessity to administer it intravenously to achieve sufficient, stable levels due to its variable intestinal absorption. The original oil-based oral formulation of CyA is characterized by high intra- and inter-patient's pharmacokinetic variability and poor bioavailability in patients with diarrhea, thereby preventing the stable blood levels of CyA (12).

Tacrolimus, however, is well-absorbed orally, compared to intravenous administration, even in severe colitis (4, 13). Moreover, recent evidence from transplant patients suggests that tacrolimus is superior to CyA with respect to immunosuppressive potency and has a lower incidence of side effects (14, 15). Based on these findings, we selected tacrolimus for this patient.

Tacrolimus has immunosuppressive properties similar to CyA, but is approximately 100 times more potent than CyA (16). Tacrolimus interacts with the calmodulin-dependent serine/threonine phosphatase calcineurin by binding of the immunophilin FKBP12. The main action is an abrogation of the translocation process of the nuclear factor of activated T-cells. This leads to a decrease in interleukin-2 levels, which in turn reduces the activation and proliferation of T cells (17). As for the therapeutic effects of tacrolimus in patients with IBD, some published case reports and uncontrolled studies suggest that tacrolimus therapy is beneficial in patients with steroid-resistant IBD or perianal fistulating CD (3, 4, 18, 19). A recent randomized study demonstrated dose-dependent efficacy and safety of oral tacrolimus for remission-induction therapy of refractory UC (5). Of refractory UC patients, that had been treated with a high trough concentration of tacrolimus (10～15 ng/ml), 68.4% had an improved disease activity index score with a reduction of more than four points within 2 weeks. Moreover, 20% of patients with refractory UC treated with such a high trough level of tacrolimus went into remission. These results suggest that the optimal treatment range, in terms of efficacy, for the induction of remission is 10 to 15 ng/ml. Therefore, we started tacrolimus therapy with aiming the trough level of 10～15 ng/ml, which resulted in the rapid improvement of the present patient's condition. In this case, although the patient has continued tacrolimus therapy for nearly 2 years, he has not experienced any serious side effects. He has been maintained in clinical remission with a trough level of tacrolimus of 5 to 10 ng/ml. There are currently only a few reports on the long-term efficacy and safety of tacrolimus as a maintenance treatment for patients with UC (18). In this regard, further clinical trials are needed to evaluate whether tacrolimus is an optimal drug for maintenance therapy for patients with UC (19, 20).

In conclusion, we report a patient with UC refractory to combination of corticosteroid and intensive G-CAP therapy, that was successfully treated with tacrolimus. Controlled trials comparing tacrolimus with CyA in patients with UC are not yet available, and it is therefore difficult at present to conclude which drug is superior. Tacrolimus, however, is an alternative option for patients with UC that are refractory to conventional therapy.

This work was supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Culture and Science of Japan (18590677).

DOI: 10.2169/internalmedicine.46.6219

References

1. The US Multicenter Fk506 Liver Study Group. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression in liver transplantation. N Engl J Med **331**: 1110-1115, 1994.
2. Kino T, Hatanaka H, Miyata S, et al. FK-506, a novel immunosuppressant isolated from a *Streptomyces*. II. Immunosuppressive effect of FK-506 in vitro. J Antibiot(Tokyo) **40**: 1256-1265, 1987.
3. Lowry PW, Weaver AL, Tremaine WJ, Sandborn WJ. Combination therapy with oral tacrolimus (FK506) and azathioprine or 6-mercaptopurine for treatment-refractory Crohn's disease perianal fistulae. Inflamm Bowel Dis **5**: 239-245, 1999.
4. Fellermann K, Herrlinger KR, Witthoeft T, Homann N, Ludwig D, Stange EF. Tacrolimus: a new immunosuppressant for steroid refractory inflammatory bowel disease. Transplant Proc **33**: 2247-2248, 2001.
5. Ogata H, Matsui T, Nakamura M, et al. A randomised dose finding study of oral tacrolimus (FK506) therapy in refractory ulcerative colitis. Gut **55**: 1255-1262, 2006.
6. Naganuma M, Funakoshi S, Sakuraba A, et al. Granulocytapheresis is useful as an alternative therapy in patients with steroid-refractory or -dependent ulcerative colitis. Inflamm Bowel Dis **10**: 251-257, 2004.
7. Lichtiger S, Present DH, Kornbluth A, et al. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. N Engl J Med **330**: 1841-1845, 1994.
8. Carbonnel F, Boruchowicz A, Duclos B, et al. Intravenous cyclosporine in attacks of ulcerative colitis: short-term and long-term responses. Dig Dis Sci **41**: 2471-2476, 1996.
9. Cohen RD, Stein R, Hanauer SB. Intravenous cyclosporin in ulcerative colitis: a five-year experience. Am J Gastroenterol **94**: 1587-1592, 1999.
10. Rowe FA, Walker JH, Karp LC, Vasiliauskas EA, Plevy SE, Targan SR. Factors predictive of response to cyclosporin treatment for severe, steroid-resistant ulcerative colitis. Am J Gastroenterol **95**: 2000-2008, 2000.
11. Kornbluth A, Present DH, Lichiger S, Hanauer S. Cyclosporin for severe ulcerative colitis: a user's guide. Am J Gastroenterol **92**: 1424-1428, 1997.
12. Armstrong VW, Oellerich M. New developments in the immunosuppressive drug monitoring of cyclosporine, tacrolimus, and azathioprine. Clin Biochem **34**: 9-16, 2001.
13. Peters DH, Fitton A, Plosker GI, Faulds D. Tacrolimus. A review of its pharmacology, and therapeutic potential in hepatic and renal transplantation. Drugs **46**: 746-794, 1993.
14. Vanrenterghem Y. Tacrolimus (FK506) in kidney transplantation. Transplant Proc **31**: 3298-3299, 1999.
15. van Hoff JP. Christiaans MHL Use of tacrolimus in renal transplantation Transplant Proc. **30**: 2171-2173, 1998.
16. Gummert JF, Ikonen T, Morris RE. Newer Immunosuppressive drugs: a review. J Am Soc Nephrol **10**: 1366-1380, 1999.
17. Bierer BE. Biology of cyclosporine A and FK506. Prog Clin Biol Res **390**: 203-223, 1994.
18. Baumgart DC, Pintoffl JP, Sturm A, Wiedenmann B, Dignass AU. Tacrolimus is safe and effective in patients with severe steroid-refractory or steroid-dependent inflammatory bowel disease—a long-term follow-up. Am J Gastroenterol **101**: 1048-1056, 2006.
19. Hogenauer C, Wenzl HH, Hinterleitner TA, Petritsch W. Effect of oral tacrolimus (FK506) on steroid-refractory moderate/severe ulcerative colitis. Aliment Pharmacol Ther **18**: 415-423, 2003.
20. Baurngart DC, Wiedenmann B, Dignass AU. Rescue therapy with tacrolimus is effective in patients with severe and refractory inflammatory bowel disease. Aliment Pharmacol Ther **17**: 1273-1281, 2003.

doi:10.1111/j.1440-1746.2006.04533.x

GASTROENTEROLOGY

Open label trial of clarithromycin therapy in Japanese patients with Crohn's disease

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

Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Key words
antibiotics, clarithromycin, Crohn's disease, immunomodulator, luminal bacteria.

Accepted for publication 10 April 2006.

Correspondence
Dr Hiroshi Nakase, Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University Hospital, Kawara-cho 54, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. Email: hiropy@kuhp.kyoto-u.ac.jp

Abstract

Background and Aim: The pathogenesis of Crohn's disease is unclear, but many studies suggest that luminal bacteria play an important role in chronic intestinal inflammation in patients with this condition. Clarithromycin is a macrolide antibiotic with immunomodulatory activity. The aim of this study was to evaluate the effect of clarithromycin therapy in Japanese patients with Crohn's disease.

Methods: Fourteen patients with active Crohn's disease (12 with ileocolonic, one with colonic, one with small bowel type) were treated with oral clarithromycin 200 mg twice daily for 4 weeks. Patients who showed a clinical response within 4 weeks continued the therapy for up to 24 weeks. Four patients also received azathioprine. Clinical activity was assessed with the Crohn's Disease Activity Index (CDAI) at entry and at 4, 12, and 24 weeks after starting clarithromycin.

Results: The mean CDAI score at entry was 343.5. Within 4 weeks, eight (57.1%) of the 14 patients showed clinical improvement, and five (35.7%) of the eight patients achieved remission. All of those eight patients continued clarithromycin therapy after 4 weeks, and six (42.9%) were in clinical remission at 12 weeks. Of the 14 total patients, four (28.6%) continued clarithromycin for more than 24 weeks, and have remained in remission. Patients who received azathioprine concomitantly had a better response to clarithromycin therapy. No severe side-effects were observed during the study period.

Conclusions: This open label study showed encouraging results of clarithromycin therapy in Japanese patients with active Crohn's disease.

Introduction

The etiology of Crohn's disease remains unclear, and both genetic and environmental factors seem to be involved in its pathogenesis. Recent data suggest that indigenous bacterial flora play an important role in the initiation and perpetuation of chronic intestinal inflammation in patients with Crohn's disease.[1] Therefore, antibiotics are considered as one of the basic therapies.[2] Generally, metronidazole and ciprofloxacin are the antibiotics most often used to treat mild to moderate luminal or fistulizing Crohn's disease.[3] In a double blind, placebo-controlled trial of metronidazole in Crohn's disease, there was a significant reduction in Crohn's Disease Activity Index (CDAI) scores and C-reactive protein levels in the metronidazole-treated group compared with placebo.[4] A controlled 6-month trial of ciprofloxacin in Crohn's disease demonstrated a significant reduction in CDAI scores in the ciprofloxacin-treated group.[5] In combination therapy with metronidazole and ciprofloxacin for 10 weeks, 55 of 72 patients (76%) showed a clinical response and 49 of 72 patients (68%) achieved clinical remission.[6] This combination therapy was also effective for Japanese patients with Crohn's disease.[7]

Clarithromycin is a broad-spectrum macrolide antibiotic. Macrolides, such as clarithromycin and azithromycin, bind to the 50S subunit of the 70S bacterial ribosome, and thereby inhibit bacterial protein synthesis. They accumulate in extremely high levels within macrophages and have prolonged intracellular half-lives.[8] These traits enhance their efficacy against intracellular organisms. Similar to other macrolides, clarithromycin acts not only as an antibiotic but also as an immunomodulator, which can enhance macrophage proliferation, phagocytosis, chemotaxis, and cytocidal activity.[9] Clarithromycin suppresses nuclear factor (NF)-κB activation in response to tumor necrosis factor (TNF)-α in monocytes and lymphocytes,[10] and thus represses production of cytokines such as interleukin (IL)-8, TNF-α, and IL-1.[11–13]

There are several reports of open label studies of treatment for Crohn's disease with clarithromycin,[14–20] although its therapeutic results are still inconclusive. The aim of our trial is to evaluate the

Journal of Gastroenterology and Hepatology 22 (2007) 984–988

effectiveness of clarithromycin in Japanese patients with active Crohn's disease.

Methods

Patients

Fourteen patients (10 male, 4 female) with active Crohn's disease (CDAI ≥ 150 for more than 3 weeks) were enrolled in this study. The diagnosis of Crohn's disease was confirmed by clinical, radiological, endoscopic, and histological findings. The study was approved by the ethics committee of Kyoto University Hospital. Informed consent was obtained from all patients. The baseline characteristics of the patients are shown in Tables 1 and 2. All the patients were older than 20 years of age (mean 33.6, range 26–48 years), with ileocolonic ($n = 12$), colonic ($n = 1$), or small bowel-type ($n = 1$) Crohn's disease. At entry, the mean CDAI score was 343.5 (range 164.9–529.2).

Four patients (28.6%) were receiving azathioprine. The dose of azathioprine had been unchanged for more than 3 months before starting clarithromycin. Only one patient (7.1%) was receiving corticosteroids (7.5 mg/day) for 6 weeks prior to clarithromycin therapy. Eleven patients (78.6%) were receiving 5-aminosalicylate or sulfasalazine, and 11 patients (78.6%) were receiving elemental diet therapy. These drugs and therapy were kept unchanged during this study except in the one patient receiving steroid therapy in whom the dose was reduced as he clinically improved.

Table 1 Baseline characteristics of 14 patients with Crohn's disease

Characteristic	*n* (%)
Sex	
Male	10 (71.4)
Female	4 (28.6)
Disease location	
Colonic	1 (7.1)
Ileocolonic	12 (85.7)
Small bowel only	1 (7.1)
Previous resection	4 (28.6)
Treatment	
Elemental diet	11 (78.6)
5-Aminosalicylate, SASP	11 (78.6)
Steroids	1 (7.1)
Azathioprine	4 (28.6)
Intestinal complication	
Stenosis	5 (35.7)
Abscess	2 (14.3)
Fistula	3 (21.4)

SASP, sulfasalazine.

Table 2 Mean characteristics at entry of 14 patients with Crohn's disease

Characteristic	Mean	Median	Range
Age (years)	33.6	32	26–48
Duration of disease (years)	12.5	11.5	1–25
Crohn's Disease Activity Index	343.5	361.9	164.9–529.2
C-reactive protein (mg/L)	27.2	16	1–94

Study design

Patients received clarithromycin 200 mg twice daily for at least 4 weeks while they continued baseline treatment. There have been several reports that long-term administration with 400 mg/day of clarithromycin was well tolerated, safe, and effective for Japanese patients with chronic bronchitis or sinusitis.[21] Therefore, we used the same dose in our clinical trial.

Four weeks after starting clarithromycin therapy, we evaluated the CDAI, and decided whether or not clarithromycin therapy should be continued. The responders continued clarithromycin therapy for 24 weeks. If patients showed no clinical response or relapsed, clarithromycin therapy was stopped and alternative treatment was started. In this study, four patients continued on clarithromycin therapy for a median of 55 weeks (range 36–76) to study the long-term outcomes and side-effects.

Clinical activity was assessed with the CDAI and C-reactive protein levels at entry, and at 4, 12, and 24 weeks after starting clarithromycin. Remission was defined as a CDAI score of 150 or less, and clinical improvement was defined as a decrease in the CDAI score of at least 100 points.[22]

Statistical analysis

Unless otherwise stated, all numerical data are expressed as the mean ± standard error. The differences of the characteristics between groups were analyzed by the Student's *t*-test, Mann–Whitney *U*-test or Fisher's exact probability test. The evaluation of change of the CDAI score was analyzed by the Wilcoxon signed-rank test.

Results

Clinical response and remission

At the 4-week evaluation, eight patients (57.1%) showed clinical improvement, and six patients (42.9%) showed no clinical response (Fig. 1). The mean CDAI score of all patients decreased significantly from 343.5 (range 164.9–529.2) to 199.8 (range 39.8–376.1) ($P < 0.01$).

At 4 weeks, five (35.7%) of the eight patients with clinical improvement had achieved remission. The mean CDAI score of the eight patients decreased from 349.5 (range 195.4–505.0) to 131.0 (range 39.8–262.2) at 4 weeks. The patient receiving corticosteroids also showed clinical improvement and was tapered off corticosteroids by 4 weeks.

Eight patients with clinical improvement within 4 weeks continued clarithromycin therapy, and six (42.9%) of the eight remained in clinical remission at 12 weeks. We classified the six patients as the responder group. The other two patients (14.3%) relapsed at approximately 6 weeks after starting clarithromycin therapy. In these patients, clarithromycin therapy was stopped, and alternative therapy was started.

Six patients (42.9%) without clinical response at 4 weeks stopped clarithromycin. The mean CDAI score of the six patients at entry and at 4 weeks was 335.6 (range 164.9–529.2) and 291.7

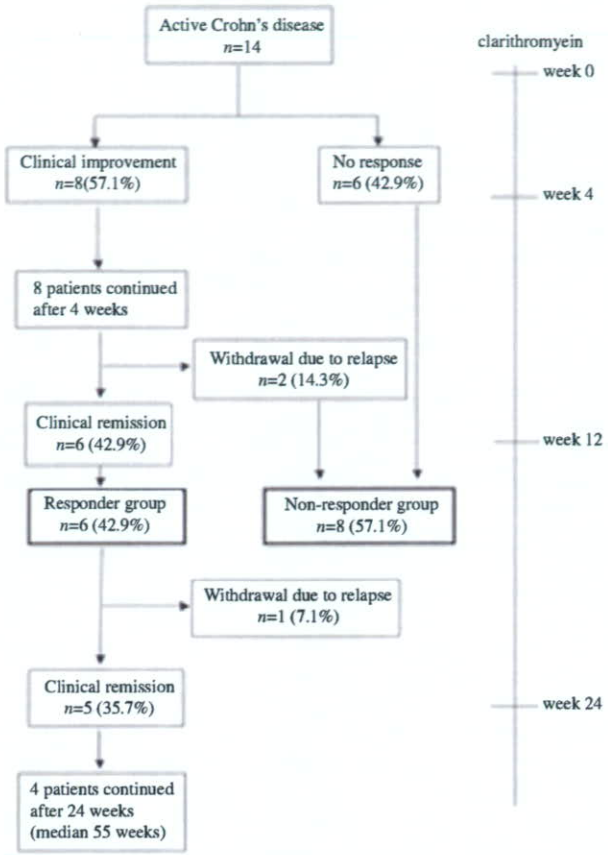


Figure 1 Clinical response in patients with active Crohn's disease receiving clarithromycin ($n = 14$). Clinical activity was evaluated by the Crohn's Disease Activity Index (CDAI) at entry, and at 4, 12, and 24 weeks after starting clarithromycin.

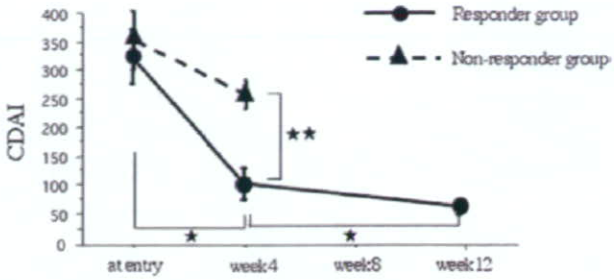


Figure 2 Change in the Crohn's Disease Activity Index (CDAI) score at entry, and at 4 and 12 weeks after starting clarithromycin in responder ($n = 6$) and non-responder ($n = 8$) groups. $^{*}P < 0.05$; $^{**}P < 0.01$.

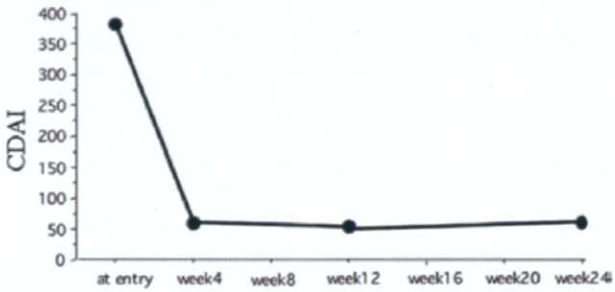


Figure 3 Change in the Crohn's Disease Activity Index (CDAI) score at entry, and at 4, 12, and 24 weeks after starting clarithromycin in one responder. In spite of treatment with azathioprine, his CDAI score was high at entry. After he started clarithromycin therapy, he achieved remission within 4 weeks, and stayed in remission for 24 weeks.

(range 232.4–376.1), respectively. We classified these six patients and the two patients who relapsed 6 weeks after starting clarithromycin as the non-responder group. The mean CDAI score of the non-responder group was 358.9 ± 47.2 (entry), and 272.2 ± 23.2 (4 weeks) (Fig. 2).

All of the responders continued clarithromycin therapy for more than 12 weeks. One of them relapsed at 20 weeks. He was treated with oral tacrolimus and infliximab therapy to achieve remission. The mean CDAI score of the responder group was 323.1 ± 46.9 at entry, 103.4 ± 26.9 at 4 weeks, and 71.2 ± 12.7 at 12 weeks (Fig. 2). Four patients (28.6%) continued clarithromycin therapy after 24 weeks, and all remained in remission.

An effective case

A 35-year-old man had had ileocolonic type Crohn's disease for 17 years. He had undergone intestinal resection twice due to vesico-rectal fistula and severe intestinal stenosis. He was started on azathioprine 16 months prior to our clarithromycin trial, but he often showed symptoms such as abdominal pain and distention. After he was started on clarithromycin therapy, his symptoms soon disappeared and he achieved remission within 4 weeks. His CDAI score during clarithromycin therapy was 384.1 at entry, 62.1 at 4 weeks, 52.5 at 12 weeks, and 62.1 at 24 weeks (Fig. 3). No side-effects were observed in this patient.

Concomitant therapy

In the responder group, three (50%) of six patients were receiving azathioprine, whereas only one of eight patients (12.5%) in the non-responder group was receiving azathioprine ($P = 0.24$) (Table 3). There were no differences in the numbers of patients who were receiving 5-aminosalicylate or sulfasalazine and elemental diet therapy between the responder and non-responder groups.

Relationship between responsiveness to clarithromycin and C-reactive protein levels or disease type

The mean serum C-reactive protein levels at entry of the non-responder group tended to be higher compared with the responder group (35.4 mg/L *vs* 16.3 mg/L, $P = 0.24$), although the difference was not statistically significant (Table 3). There was no difference in age, sex, duration of disease, disease location, intestinal complication, previous intestinal resection, or CDAI score at entry between the two groups (Table 3).

Table 3 Characteristics of responder and non-responder groups

Characteristic	Responder group ($n = 6$)	Non-responder group ($n = 8$)
Age (years)		
Mean	37.0 ± 3.5	31.1 ± 1.6
Range	26–48	27–38
Sex		
Male	5 (83.3)	5 (62.5)
Female	1 (16.7)	3 (37.5)
Duration of disease (years)		
Mean	13.2 ± 4.0	12.0 ± 2.3
Range	1–25	1–20
Disease location		
Colonic	1 (16.7)	0 (0)
Ileocolonic	5 (83.3)	7 (87.5)
Small bowel only	0 (0)	1 (12.5)
Intestinal complication		
Stenosis	2 (33.3)	3 (37.5)
Abscess	1 (16.7)	1 (12.5)
Fistula	2 (33.3)	1 (12.5)
Previous resection	1 (16.7)	3 (37.5)
Concomitant therapy		
Elemental diet	5 (83.3)	6 (75.0)
5-Aminosalicylate, SASP	4 (66.7)	7 (87.5)
Steroids	1 (16.7)	0 (0)
Azathioprine	3 (50)	1 (12.5)
CDAI score		
Entry	323.1 ± 46.9	358.9 ± 47.2
4 weeks	103.4 ± 26.9	272.2 ± 23.2
12 weeks	71.2 ± 12.7	-
C-reactive protein (mg/L)		
Entry	16.3 ± 3.0	35.4 ± 12.9
4 weeks	7.3 ± 2.9	18.5 ± 13.2
12 weeks	9.7 ± 3.8	-

Values shown as *n* (%) per group, or as mean ± SE. CDAI, Crohn's Disease Activity Index; SASP, sulfasalazine.

Side-effects

A minor side-effect was observed in only one patient (7.1%). He withdrew due to abdominal fullness, which improved after stopping clarithromycin. Patients on clarithromycin therapy for more than 24 weeks showed no side-effects.

Discussion

To our knowledge, this is the first demonstration of a therapeutic effect of clarithromycin in Japanese patients with Crohn's disease. A significant clinical response was observed in eight (57.1%) of 14 patients after 4 weeks, and remission was achieved in six patients (42.9%) at 12 weeks. Four patients (28.6%) continued the clarithromycin therapy for more than 24 weeks, with no serious side-effects. These results suggest that clarithromycin is effective for a subpopulation of patients with active Crohn's disease.

In a recently published open label study by Leiper *et al.*, 25 patients with active Crohn's disease received clarithromycin for 4 weeks continuing for up to 12 weeks.[20] Fifteen patients (60%) and nine patients (36%) were receiving corticosteroids and azathioprine, respectively. In that study, 16 patients (64%) showed clinical improvement, and 12 (48%) achieved remission within 4 weeks. At 12 weeks, 15 (60%) showed clinical improvement, and 11 (44%) remained in remission. This open label study demonstrated a good response to clarithromycin in patients with active Crohn's disease who had been resistant to other conventional therapy. Clarithromycin was also tested in patients with Crohn's disease in combination with ethambutol in a 3-month randomized, placebo-controlled study with a 1-year follow up.[17] Five of 15 treated patients (33%) had active Crohn's disease, and they were receiving corticosteroids, and none received immunosuppressive agents. The results of combination therapy with clarithromycin and ethambutol showed no apparent effect. Therefore, the effect of clarithromycin therapy on patients with Crohn's disease is inconclusive.

Large placebo-controlled studies of Crohn's disease have shown that within 3 months 26–42% of patients with active disease went into remission spontaneously.[23,24] However, after 1 year, only 15–18% of the patients were still in remission. Considering this natural history, the remission rate (42.9%) of our clinical trial at 12 weeks with clarithromycin therapy does not appear so high. However, about 80% of the patients remained in remission for 24 weeks after starting clarithromycin. These results suggested that 400 mg/day of clarithromycin therapy might be effective as maintenance rather than induction therapy of remission. In the future, to clarify this issue, we need to perform a dose escalation study with clarithromycin to investigate how much dose of clarithromycin is optimal for inducing remission of patients with active Crohn's disease.

There are several reports that antibiotic therapy is more effective in patients with colonic involvement than in those with small bowel disease alone.[4,25] In a study of combination therapy with rifabutin and clarithromycin or azithromycin, patients with involvement of both the small and large intestine achieved a better clinical response than patients with small bowel disease alone.[14] In contrast, combination therapy with clarithromycin, rifabutin, and clofazimine showed greater benefit in patients with small bowel disease alone. However, in the open label study of clarithromycin by Leiper *et al.*,[20] subgroup analysis by disease location revealed no significant difference between groups. In our study, we could not analyze the association between the effect of clarithromycin and disease location or phenotype, because of the limited number of patients. Therefore, further investigation in a greater number of patients is needed to clarify the clinical factors; such as disease location and phenotype, that influence effectiveness of clarithromycin therapy in patients with Crohn's disease.

This study revealed that the effectiveness of clarithromycin in patients with Crohn's disease was almost equal to that of single therapy with metronidazole or ciprofloxacin. Moreover, there were only a few minor side-effects of clarithromycin therapy unlike metronidazole and ciprofloxacin. Our data also showed that continuous clarithromycin therapy significantly reduced the mean CDAI score of responders at 12 weeks compared to that at 4 weeks. Thus, another advantage of clarithromycin might be the induction of immunomodulatory functions by its long-term administration, which are independent of its antibacterial activity. Clarithromycin is rapidly taken up by immune cells, which results

in a higher concentration in macrophages and polymorphonuclear cells with levels more than 30-fold the plasma concentration. Clarithromycin suppresses TNF-α, IL-1α, IL-1β, and granulocyte-macrophage colony-stimulating factor, while it increases the synthesis of IL-10 in macrophages.[12] In addition, it also enhances phagocytic function of macrophages.[9] Interestingly, in our study three (50%) of six patients in the responder group were receiving azathioprine, while only one (12.5%) of eight in the non-responder group was receiving azathioprine. The reason why combination therapy with clarithromycin and azathioprine tended to be more effective than azathioprine alone might be that clarithromycin modulates macrophage function, which is impaired in patients with Crohn's disease, in addition to azathioprine-induced lymphocyte suppression.

In conclusion, our uncontrolled trial of clarithromycin therapy in Japanese patients with Crohn's disease shows promising results. This study suggests that the administration of clarithromycin is a therapeutic option for Japanese patients with active Crohn's disease.

Acknowledgments

This work was supported by a Grant-in-aid for Scientific Research 16590590 from the Ministry of Culture Science of Japan, and by research funds JFE-2004 from the Japanese Foundation for Research and Promotion of Endoscopy, and the Japanese Society of Gastroenterology Research Grant to Hiroshi Nakase.

References

1 Bamias G, Marini M, Moskaluk CA *et al.* Down-regulation of intestinal lymphocyte activation and Th1 cytokine production by antibiotic therapy in a murine model of Crohn's disease. *J. Immunol.* 2002; **169**: 5308–14.
2 Greenberg GR. Antibiotics should be used as first-line therapy for Crohn's disease. *Inflamm. Bowel Dis.* 2004; **10**: 318–20.
3 Wild GE. The role of antibiotics in the management of Crohn's disease. *Inflamm. Bowel Dis.* 2004; **10**: 321–3.
4 Sutherland L, Singleton J, Sessions J *et al.* Double blind, placebo controlled trial of metronidazole in Crohn's disease. *Gut* 1991; **32**: 1071–5.
5 Arnold GL, Beaves MR, Pryjdun VO, Mook WJ. Preliminary study of ciprofloxacin in active Crohn's disease. *Inflamm. Bowel Dis.* 2002; **8**: 10–15.
6 Greenbloom SL, Steinhart AH, Greenberg GR. Combination ciprofloxacin and metronidazole for active Crohn's disease. *Can. J. Gastroenterol.* 1998; **12**: 53–6.
7 Ishikawa T, Okamura S, Oshimoto H, Kobayashi R, Mori M. Metronidazole plus ciprofloxacin therapy for active Crohn's disease. *Intern. Med.* 2003; **42**: 318–21.
8 Ives TJ, Schwab UE, Ward ES, Butts JD, Hall IH. Disposition and functions of clarithromycin in human THP-1 monocytes during stimulated and unstimulated conditions. *Res. Commun. Mol. Pathol. Pharmacol.* 2001; **110**: 183–208.
9 Xu G, Fujita J, Negayama K *et al.* Effect of macrolide antibiotics on macrophage functions. *Microbiol. Immunol.* 1996; **40**: 473–9.
10 Ichiyama T, Nishikawa M, Yoshitomi T *et al.* Clarithromycin inhibits NF-κB activation in human peripheral blood mononuclear cells and pulmonary epithelial cells. *Antimicrob. Agents Chemother.* 2001; **45**: 44–7.
11 Kikuchi T, Hagiwara K, Honda Y *et al.* Clarithromycin suppresses lipopolysaccharide-induced interleukin-8 production by human monocytes through AP-1 and NF-kappaB transcription factors. *J. Antimicrob. Chemother.* 2002; **49**: 745–55.
12 Takeshita K, Yamagishi I, Harada M, Otomo S, Nakagawa T, Mizushima Y. Immunological and anti-inflammatory effects of clarithromycin: inhibition of interleukin 1 production of murine peritoneal macrophages. *Drugs Exp. Clin. Res.* 1989; **15**: 527–33.
13 Morikawa K, Watabe H, Araake M, Morikawa S. Modulatory effect of antibiotics on cytokine production by human monocytes *in vitro*. *Antimicrob. Agents Chemother.* 1996; **40**: 1366–70.
14 Gui GP, Thomas PR, Tizard ML, Lake J, Sanderson JD, Hermon-Taylor J. Two-year outcomes analysis of Crohn's disease treated with rifabutin and macrolide antibiotics. *J. Antimicrob. Chemother.* 1997; **39**: 393–400.
15 Borody TJ, Leis S, Warren EF, Surace R. Treatment of severe Crohn's disease using antimycobacterial triple therapy: approaching a cure? *Dig. Liver Dis.* 2002; **34**: 29–38.
16 Shafran I, Kugler L, El-Zaatari FA, Naser SA, Sandoval J. Open clinical trial of rifabutin and clarithromycin therapy in Crohn's disease. *Dig. Liver Dis.* 2002; **34**: 22–8.
17 Goodgame RW, Kimball K, Akram S *et al.* Randomized controlled trial of clarithromycin and ethambutol in the treatment of Crohn's disease. *Aliment. Pharmacol. Ther.* 2001; **15**: 1861–6.
18 Rubin PH, Chapman ML, Scherl E, Sachar DB, Stamaty C, Present DH. Clarithromycin in active Crohn's disease: preliminary results of open label pilot study. *Gastroenterology* 1996; **110**: A1005.
19 Douglass A, Cann PA, Bramble MG. An open pilot study of antimicrobial therapy in patients with unresponsive Crohn's disease. *Gut* 2000; **46** (Suppl. II): A11.
20 Leiper K, Morris AI, Rhodes JM. Open label trial of oral clarithromycin in active Crohn's disease. *Aliment. Pharmacol. Ther.* 2000; **14**: 801–6.
21 Hashiba M, Baba S. Efficacy of long-term administration of clarithromycin in the treatment of intractable chronic sinusitis. *Acta Otolaryngol. Suppl.* 1996; **525**: 73–8.
22 Mannon PJ, Fuss IJ, Mayer L *et al.* Anti-interleukin-12 antibody for active Crohn's disease. *N. Engl. J. Med.* 2004; **351**: 2069–79.
23 Summers RW, Switz DM, Sessions JT Jr *et al.* National cooperative Crohn's disease study: results of drug treatment. *Gastroenterology* 1979; **77**: 847–69.
24 Malchow H, Ewe K, Brandes JW *et al.* European Cooperative Crohn's Disease Study (ECCDS): results of drug treatment. *Gastroenterology* 1984; **86**: 249–66.
25 Steinhart AH, Faegan BG, Wong CJ *et al.* Combined budesonide and antibiotic therapy for active Crohn's disease. *Gastroenterology* 2002; **123**: 33–40.

