Transpl Int (2004) 17: 463–467
DOI 10.1007/s00147-004-0745-0

CASE REPORT

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Villous atrophy induced by mycophenolate mofetil in renal-transplant patients

Received: 20 May 2003
Revised: 14 November 2003
Accepted: 10 March 2004
Published online: 21 August 2004

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Abstract Leucopenia and diarrhoea are the main side effects observed after the use of mycophenolate mofetil (MMF) in renal-transplant patients. The mechanism of diarrhoea remains unknown. We report on four cases presenting with severe diarrhoea, which appeared, respectively, at 4, 10, 24, and 66 months after MMF therapy had been started. All patients presented with weight loss and biological signs of malabsorption syndrome. Oesophago-gastroduodenoscopy revealed duodenal villous atrophy, which was confirmed by pathology examination. Anti-endomysium antibodies were negative. In all patients, diarrhoea disappeared within 1 month of MMF withdrawal without a gluten-free diet. A control oesophago-gastroduodenoscopy was performed in one patient 6 months later and was considered normal. None of the patients showed evidence of cytomegalovirus in enterocytes or cytomegalovirus-positive viraemia. In conclusion, villous atrophy induced by MMF might be one of the mechanisms of diarrhoea. It is mandatory to differentiate coeliac disease from MMF-induced villous atrophy because, in the latter case, a gluten-free diet is not required.

Keywords Mycophenolate mofetil · Diarrhoea · Duodenal villous atrophy · Malabsorption syndrome · Coeliac disease.

Introduction

To date, mycophenolate mofetil (MMF) has been commonly used after renal transplantation. MMF inhibits the de novo pathway of purine synthesis, which is mandatory for the proliferation and function of T and B lymphocytes. Several studies have reported on the beneficial effect of MMF, compared to azathioprine, in the prevention of acute rejection in renal-transplant (RT) patients [1, 2, 3]. The incidence of diarrhoea in RT patients receiving MMF ranged from 12% to 40% [1, 2, 3]. The mechanism of diarrhoea is still unknown. We describe four cases of severe diarrhoea that revealed duodenal villous atrophy in renal-transplant patients treated by MMF (Table 1).

Case reports

Patient 1

A 42-year-old RT patient on azathioprine/steroids was switched from azathioprine to MMF (1 g b.i.d.), 8.5 years after transplantation, because of a chronic allograft nephropathy. Twenty-four months later he was admitted for abdominal pain, chronic diarrhoea (six to eight watery stools per day), weakness and weight loss (6 kg). Standard stool culture and parasitological examination were negative. *Clostridium difficile* was not found. Biological parameters revealed severe normocytic anaemia, hypocalcaemia, hypo-albuminaemia and hypocholesterolaemia. Oesophago-gastroduodenoscopy revealed villous

Table 1 Patients' characteristics and biological parameters at diagnosis of villous atrophy (*HLA* human leukocyte antigen, *NA* not available)

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	42	48	26	52
Initial nephropathy	Chronic glomerulonephritis	Nephrocalcinosis	Alport's syndrome	Polycystic kidney disease
Time since renal transplantation (months)	126	32	100	100
Time since starting mycophenolate mofetil therapy (months)	24	16	51	66
Mycophenolate mofetil daily dose (g)	2	1.5	2	2
HLA DQ	2/7	2/7	1/2	2/5
Number of stools/day	6–8	5–6	5–6	10–15
Weight loss (kg)	6	12	8	10–15
MPA (mg/l)	NA	5.8	6.2	NA
Cytomegalovirus viraemia	–	–	–	–
Baseline creatinine (μmol/l)	150–170	250–300	120–140	140
Haemoglobin (g/dl)	7.1	8.5	11	11.9
VGM (μ^3)	88	87	92	109
White blood cells/neutrophil cells (/mm^3)	7000/3600	6800/2980	9000/5400	7600/3200
Vitamin B12 (pg/ml) ($210 < N < 920$)	630	918	220	204
Folate (ng/ml) ($2 < N < 4$)	6.7	2.18	2	2.7
Ferritin (μg/l) ($10 < N < 350$)	300	858	50	56
Calcium (mmol/l) ($2.25 < N < 2.55$)	1.96	1.23	2.5	2.28
Phosphorus (mmol/l) ($0.8 < N < 1.3$)	1.34	0.59	1.2	0.96
Cholesterol (mmol/l) ($3.8 < N < 6.2$)	2.65	4.25	2.69	1.9
Albumin (g/l)	30	30	40	40
IgA (g/l) ($0.85 < N < 2.72$)	1.74	NA	0.8	4.81
IgA anti-gliadin antibodies				
Before transplantation	NA	–	–	NA
At diagnosis of villous atrophy	–	–	+	+
IgA anti-endomysium antibodies				
Before transplantation	NA	-	-	NA
At diagnosis of villous atrophy	–	–	–	–
Anti-transglutaminase antibodies				
Before transplantation	NA	–	–	NA
At diagnosis of villous atrophy	–	–	–	+

atrophy (grade IV), confirmed by the histopathology examination (Figs. 1 and 2). Immunohistochemical study did not reveal cytomegalovirus (CMV) in the duodenal enterocytes. Colonoscopy was considered normal. Anti-gliadin, anti-endomysium and anti-transglutaminase antibodies were negative. MMF was replaced by azathioprine. Fifteen days later, the diarrhoea stopped, and the patient gained weight thereafter.

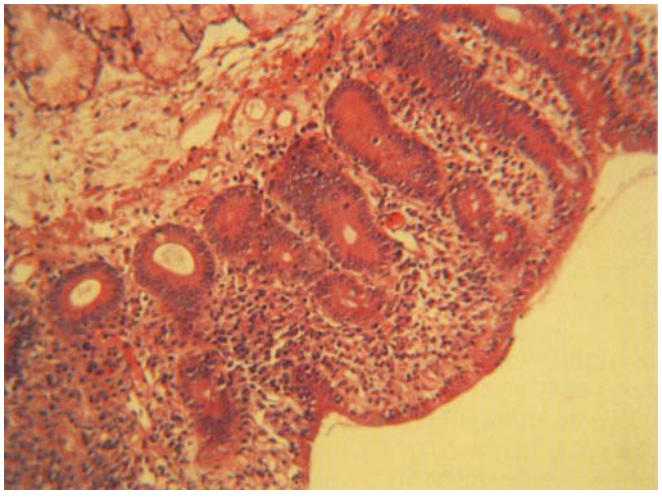

Fig. 1 Duodenal biopsy demonstrates severe villous atrophy. The lamina propria contains a moderately increased infiltrate of mononuclear cells and plasma cells (HES, ×160)

Patient 2

Sixteen months after a kidney transplantation, a 48-year-old woman presenting with renal allograft dysfunction was switched from cyclosporin A and azathioprine to tacrolimus and MMF (1.5 g/day). Ten months later, she presented with watery diarrhoea (up to five stools per day), and the weight loss reached 12 kg within 6 months. Standard stool culture and parasitological examination, as well as *Clostridium difficile*

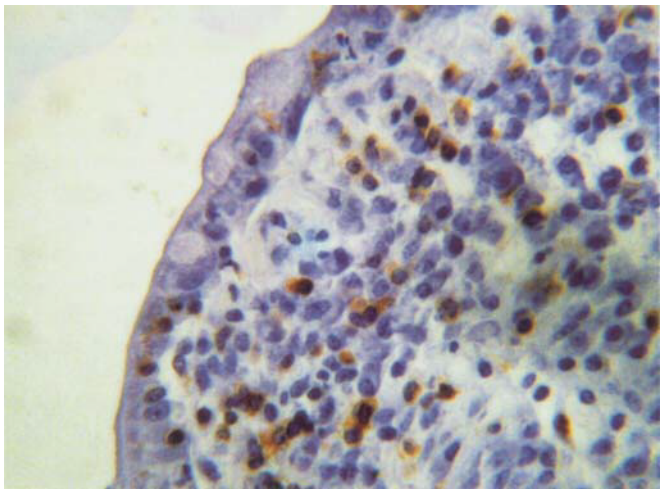

Fig. 2 The intra-epithelial lymphocytosis is not significantly increased (less than 30%). Immunohistochemical reaction for CD3, T lymphocyte marker

investigation, were negative. Serum creatinine level was 329 μmol/l. Biological parameters revealed normocytic anaemia, hypocalcaemia, hypophosphoraemia and hypo-albuminaemia. Oesophago-gastroduodenoscopy revealed duodenal villous atrophy (grade II). There were no CMV inclusion bodies in the duodenal cells, and immunohistochemical study found no cytomegalovirus. Anti-gliadin, anti-endomysium and anti-transglutaminase antibodies were absent before transplantation and at diagnosis of villous atrophy. MMF was subsequently stopped. The diarrhoea disappeared a month later, and the patient gained weight. An upper-tract endoscopy and duodenal biopsies were performed 7 months later. Duodenal histology was considered normal.

Patient 3

A 26-year-old man underwent renal transplantation in 1993. Initial immunosuppressive treatment was based on cyclosporin A, azathioprine and steroids. Four years later, azathioprine was replaced by MMF (1 g b.i.d.) because of chronic allograft nephropathy. Four months later, the patient complained of chronic diarrhoea (five to six watery stools per day), weakness and weight loss (8 kg). Repeated stool culture, parasitological examination and *Clostridium difficile* investigation were negative. Serum creatinine level was 170 μmol/l. Biological parameters revealed: moderate normocytic anaemia, hypocholesterolaemia and low vitamin E level. Serum folate and vitamin B12 levels were low. Colonoscopy revealed the presence of two erosions of the rectum. Oesophago-gastroduodenoscopy revealed duodenal villous atrophy (grade III), which was confirmed by pathology examination.

Cytomegalovirus was not found in the duodenal cells after immunohistochemical study. Colonoscopy and ileoscopy revealed only two ulcerations in the rectum. There were no signs of enterocolitis. Only immunoglobulin (Ig) A and IgG anti-gliadin antibodies were found to be positive. MMF was replaced by azathioprine. A month later, the diarrhoea stopped, and the patient gained weight. Fourteen months later, MMF was once again introduced at the daily dose of 1.5 g. Three months later he complained of chronic diarrhoea, weakness and weight loss (4 kg). Clinical and biological parameters remained negative. The patient declined an upper-tract endoscopy. MMF was stopped. The diarrhoea stopped a month later. A year later, anti-gliadin, anti-endomysium and anti-transglutaminase antibodies were found to be negative.

Patient 4

Twenty-six months after transplantation, a 52-year-old women presented an acute rejection, and, subsequently, azathioprine was replaced by MMF (1 g b.i.d.). Tacrolimus and corticosteroid doses remained unchanged. Sixty-six months later, she complained of diarrhoea (Ten to 15 watery stools per day), weight loss (4 kg), and weakness. When she was admitted to the hospital, her serum creatinine level was 140 μmol/l. Standard stool culture, parasitological examination, and *Clostridium difficile* investigation were negative. Biological parameters revealed macrocytic red cells without anaemia, low B12 vitamin level and hypocholesterolaemia. Oesophago-gastroduodenoscopy and pathology examination showed duodenal villous atrophy (grade IV) without CMV inclusion bodies. Immunohistochemical studies did not reveal the presence of cytomegalovirus in the duodenal cells. Both IgA and IgG anti-gliadin antibodies, as well as anti-transglutaminase antibodies, were positive. By contrast, anti-endomysium antibodies were absent. MMF was subsequently stopped. The diarrhoea disappeared a month later, and the patient gained weight.

Discussion

Leucopenia and gastrointestinal disorders, especially diarrhoea, are the main side effects observed in renal-transplant patients treated by MMF. Nevertheless, the mechanism of diarrhoea is still unknown. At our centre, almost 500 renal-transplant recipients receive MMF therapy. Of these, about 30% complain of occasional diarrhoea. However, few patients present with malabsorption syndrome requiring upper tract endoscopy. We report on four cases of severe diarrhoea that revealed duodenal villous atrophy. These abnormalities

might be one of the pathogenic mechanisms of diarrhoea induced by MMF.

After oral administration, MMF is absorbed in the stomach and is rapidly hydrolysed to mycophenolic acid, the active metabolite. Mycophenolic acid (MPA) inhibits inosine monophosphate dehydrogenase (IMPDH). There are two pathways for purine synthesis: the de novo purine-synthesis pathway, which depends on IMPDH, and the salvage pathway, which depends on hypoxanthine-guanine phosphoribosyltransferase. Lymphocytes rely quasi-exclusively on the de novo pathway. The anti-proliferative effect of MMF is also effective on cells other than lymphocytes, e.g. the rapidly dividing cells in the gut tract. Enterocytes depend approximately 50% on IMPDH. Subsequently, the intestinal epithelium is a target for the anti-proliferative effect of MMF. High doses of MMF inhibit the proliferation of basal epithelium cells of the small intestine in mice [4].

Pally et al. reported cases of jejunal villous atrophy in Lewis rats given MMF, at either 10–20 mg/kg or 20–30 mg/kg, irrespective of co-administration of cyclosporin A micro-emulsion [5]. In humans, Ducloux et al. had previously reported the first case of duodenal villous atrophy induced by MMF [6]. Berribi et al. suggested that MMF might have induced apoptosis in normal duodenal villi in an 8-year-old renal-transplant patient complaining of diarrhoea, weakness and weight loss [7]. It was also suggested that CMV infection was involved in MMF-induced diarrhoea [8]. None of our patients showed evidence of cytomegalovirus in their enterocytes or positive cytomegalovirus viraemia. Nevertheless, we did not look for other viruses by electronic microscopy.

Previous reports showed that the occurrence of side effects, i.e. diarrhoea and leucopenia, depends on the MMF dose. Most adverse effects were observed in patients receiving a daily dose of 3 g. In our report, the MMF dose of the four patients ranged from 1.5 to 2 g per day. None of these patients had leucopenia. However, in patients 2 and 4, who were receiving tacrolimus, the daily dose of MMF was high (1.5 g/day and 2 g/day, respectively). In addition, when assessed, MPA trough levels were relatively high (patients 2 and 3). Even though we did not assess MPA concentration in epithelial cells, we hypothesised that villous atrophy might have been triggered by a high concentration of MPA in epithelial cells.

All four patients presented severe chronic diarrhoea, weakness, significant weight loss, anaemia and malabsorption syndrome. Diarrhoea occurred a few months after the initiation of MMF therapy in one patient (patient 3); according to previous reports [9] MPA seems to be at a high concentration in the epithelial cells of gastrointestinal tract at that time [9]. By contrast, in patients 1, 2 and 4, diarrhoea occurred later, respectively, 18, 10 and 66 months after MMF had been initiated. Colonoscopy did not reveal signs of enterocolitis, as recently reported by Maes et al. [10]. Oesophagogastroduodenoscopy revealed histological duodenal villous atrophy in all patients. Other causes of duodenal villous atrophy were ruled out. None of these patients had coeliac disease or an infectious disease such as giardiasis. Patient 3 had anti-gliadin antibodies and patient 4 had anti-gliadin and anti-transglutaminase antibodies when villous atrophy was found. Despite the absence of a gluten-free diet, diarrhoea disappeared in all patients when MMF had been stopped. A year after MMF was stopped, anti-gliadin antibodies had disappeared in patient 3.

Coeliac disease is the other main cause of diarrhoea in the presence of duodenal villous atrophy. Over 95% of patients with coeliac sprue express the HLA DQ2 antigen [11]. In this report, even if all patients were of HLA DQ2 phenotype, this cause had been ruled out since none of the patients had presented with gastrointestinal symptoms and chronic diarrhoea before MMF therapy. Pathology examination did not reveal hyperplasia of crypts or an increase in intra-epithelial lymphocyte infiltration as described in coeliac disease.

The presence of anti-gliadin antibodies can be observed in many gastro-intestinal disorders. Only the presence of anti-endomysium IgA antibodies has been shown to have a specificity of 93% to 100% in coeliac disease [11]. In our report, when the patients were assessed (patients 1, 3 and 4) we did find an IgA deficiency. Finally, diarrhoea disappeared without a gluten-free diet. It is mandatory to make the difference between coeliac disease and MMF-induced villous atrophy, since, in this latter case, patients do not need a gluten-free diet.

In conclusion, in patients with severe diarrhoea and malabsorption syndrome, an upper-tract endoscopy and biopsies must be performed in order to search for villous atrophy. MMF inhibits the proliferation of epithelial cells and, possibly, intestinal IMPDH. This mechanism might be triggered by over-immunosuppression in patients with predisposing genetic factors, e.g. HLA DQ2. Villous atrophy might be one of the mechanisms of diarrhoea induced by MMF.

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