ORIGINAL ARTICLES—LIVER, PANCREAS AND BILIARY TRACT

Mycophenolate Mofetil in Autoimmune Hepatitis Patients Not Responsive or Intolerant to Standard Immunosuppressive Therapy

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Background & Aims: **The immunosuppressive treatment for autoimmune hepatitis (AIH) patients is prednisone and azathioprine. Ten percent to 20% of patients do not respond or are intolerant of standard treatment. The aim of this study was to assess the biochemical, histologic, and hematologic parameters during mycophenolate mofetil (MMF) treatment in AIH patients who did not respond to or were intolerant of prednisone and/or azathioprine.** ***Methods:*** **A retrospective study was performed of 15 AIH patients who received MMF either as monotherapy or in combination with prednisone after failure or intolerance of the initial regimen. Records were reviewed as to initial therapy, reasons why MMF was initiated, liver enzyme levels, histology on MMF, and complications.** ***Results:*** **The mean age was 60 ± 15 years. All patients were started on MMF at 1 gram twice a day, 3 on MMF monotherapy, and 12 on prednisone and MMF. The average MMF treatment duration was 41 months. Alanine aminotransferase levels decreased significantly from 91.73 ± 88.69 to 60.87 ± 71.2 ($P = .03$) on MMF treatment. Inflammatory scores (2.59 ± 0.97 to 1.14 ± 1.21, $P = .02$) and Ishak fibrosis scores (4.10 ± 1.37 to 2.5 ± 1.51, $P = .02$) also decreased. No significant hematologic complications were noted during MMF treatment.** ***Conclusions:*** **Administration of MMF, either as monotherapy or in combination with prednisone, results in biochemical and histologic improvement in AIH patients who are prednisone and/or azathioprine intolerant or resistant without the development of significant complications. MMF should be studied prospectively as an alternative agent in the treatment of autoimmune liver disease.**

Autoimmune hepatitis (AIH), first described in the 1950s, is a type of chronic hepatitis of unknown cause, with genetic and environmental components.[1] This form of chronic liver disease generally is progressive and often is associated with fluctuating levels of liver enzymes in the patient's serum. AIH occurs in both adults and children. Although the cause of AIH is unknown, the pathogenesis is believed to be based on a loss of tolerance to self-antigens in genetically susceptible persons who can be identified by serologic and immunogenetic markers. It is defined by the finding of specific pathologic changes, including plasma cell infiltrates in association with a pattern of autoantibodies. The classic form of AIH disease is classified into type 1 or type 2.[2] A number of other forms of autoimmune liver disease exist (ie, primary biliary cirrhosis, sclerosing cholangitis, among others). These diseases may overlap with the classic form of AIH described earlier.

The American Association for the Study of Liver Diseases has recommended prednisone in combination with azathioprine, or high doses of prednisone alone in patients with severe or progressive AIH as defined by liver histology.[3] Although prednisone abates inflammation inherent to this disease, azathioprine is used predominantly as a corticosteroid-sparing agent. Between 10% and 20% of the patient population, however, do not respond to or are intolerant of the current standard treatment.[4]

The goal of this study was to assess biochemical, hematologic, and histologic parameters in patients with AIH who did not respond to or were intolerant of prednisone and/or azathioprine and were treated with mycophenolate mofetil (MMF) (Roche Pharmaceutical, Nutley, NJ).

Patients and Methods

We made an electronic query of all patients with AIH who had been referred to the Division of Hepatology and Department of Transplantation for evaluation and management. We then reviewed all the charts of patients with AIH who had received MMF as part of their treatment. Institutional review board approval to review these records was obtained. We found 24 patients who had been treated with MMF. We excluded 9 patients with either co-existing hepatitis C by enzyme-linked immunosorbent assay or by polymerase chain reaction, or who were treated with tacrolimus (Prograf, Astellas Pharmaceuticals, Deerfield, IL). None of the patients received liver transplantation at the time of the study. Thus, 15 charts were reviewed for the patient's date of diagnosis, initial treatment medications, and to determine the reasons why the patients failed the initial course of prednisone and azathioprine and were changed to MMF. All patients had to be taking MMF for at least 1 year. Initial therapy was either prednisone (30 mg/day) or prednisone/azathioprine (30 mg/day prednisone and 50 mg/day azathioprine). Some patients were treated initially by local referring physicians. Thus, when first seen by our group, doses of prednisone were lower. MMF was added to prednisone or replaced azathioprine if patients developed unacceptable side effects from prednisone and/or azathioprine or had a suboptimal response, defined as progressively active disease as assessed

Abbreviations used in this paper: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; MMF, mycophenolate mofetil.

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Table 1. Reasons for Discontinuing Prednisone and/or Azathioprine

Patient	AIH score	No response	Prednisone side effects	Azathioprine side effects
1	14	No response (B)		Nausea
2	11	No response (B, H)	Hypertension	
3	14		Diabetes mellitus	Leukopenia
4	19			Rash
5	20	No response (B)	Cushingoid changes	
6	15	No response (H)	Cushingoid changes	Nausea
7	19	No response (H)		Leukopenia, thrombocytopenia
8	19	No response (B)	Diabetes mellitus, cushingoid changes	Leukopenia
9	17	No response (B)	Cataracts, glaucoma, osteopenia	
10	11	No response (B)	Multiple adverse events	
11	17			Nausea, vomiting
12	14		Cushingoid changes	
13	N/A	No response (B)		Leukopenia, neutropenia
14	N/A	No response (B)		Leukopenia
15	14	No response (B)		

NOTE. AIH pretreatment score: definite AIH, >15; probable AIH, 10–15.
B, biochemical nonresponse (ie, progressive increase or nonnormalization of transaminase levels); H, histologic nonresponse (ie, progression of fibrosis compared with baseline biopsy specimen).

by liver biopsy (including fibrosis), and/or a persistent increase of transaminase levels while on standard therapy. All the patients had been tested for antinuclear antibody and anti–smooth muscle antibody. All patients were hepatitis C virus RNA negative by quantitative assay. Selected patients had been tested for antimitochondrial antibody and anti–liver-kidney microsomal-1 antibody, liver-specific membrane protein, and liver cytosol antibody (Specialty Laboratories, Santa Monica, CA).

Liver biopsy specimens from patients taking MMF were compared with pre-MMF treatment biopsy specimens for changes in fibrosis and inflammation scores. Inflammatory activity was graded using the Scheuer[5] system, with grade 0 corresponding to none to minimal portal/lobular activity and grade 4 corresponding to severe inflammation with evidence of bridging necrosis. Fibrosis stage was scored according to the Ishak et al[5] scheme, and ranged from 0 to 6.

Efficacy and safety end points of the study were as follows: (1) histologic improvement in terms of inflammatory activity and degree of fibrosis using the Scheuer[5] and Ishak et al[6] scoring systems, (2) normalization or reduction in alanine aminotransferase (ALT) levels, and (3) changes in hematologic parameters.

Statistics

We used nonparametric tests (Wilcoxon rank-sum test) to compare pretreatment and on-treatment values. Statistics were performed with Statview software v1.05 (SAS Institute, Cary, NC).

Results

A total of 15 patients were included in the study. Eleven patients were women (73%), and the mean age was 60 ± 15 years. Most patients were Caucasian (80%), 2 were Hispanic, and 1 was Asian.

Thirteen patients had type 1 AIH, and 2 had overlap AIH and PBC. All 15 patients were either antinuclear antibody or anti–smooth muscle antibody positive; 10 patients were antinuclear antibody positive, 9 patients were anti–smooth muscle antibody positive, and 4 were positive for both. All of the AIH type 1 patients tested negative for anti–liver-kidney microsomal-1 antibody. The patients with AIH–PBC overlap syndrome were positive for antimitochondrial antibody, antinuclear antibody, and anti–smooth muscle antibody. The pretreatment AIH score was calculated for 13 patients and ranged from 11 to 20. A score of 10–15 was interpreted as probable for AIH (N = 7) whereas a score of more than 15 was interpreted as definitive for AIH (N = 6).

Twelve patients were on combination prednisone-azathioprine, and 3 patients were on prednisone monotherapy before commencement of MMF. Fourteen patients (95%) had significant adverse events to the prednisone-azathioprine treatment, and 11 patients (73%) had a suboptimal response to therapy. Five patients were switched to MMF after only 3, 4, 4, 12, and 14 months of standard treatment because of the development of significant side effects to azathioprine, mostly leukopenia. The mean duration of prednisone ± azathioprine therapy before MMF was 15.7 ± 14.6 months. The reasons for changing to MMF are summarized in Table 1.

On pre-MMF liver biopsy specimens, the inflammatory activity ranged from 1 to 4 with a mean grading score of 2.59 ± 0.97. Fibrosis stage was scored according to the Ishak[5] scheme, and ranged from 2 to 6 with a mean stage of 4.10 ± 1.37. The mean time interval between pre-MMF and on-MMF biopsy procedures was 31 months, whereas the mean time interval between MMF initiation and on-MMF biopsy procedures was 23 months. All patients had been started on MMF at a dose of 1 g twice a day.

Three patients were treated with MMF alone and 12 patients were given prednisone + MMF. The average duration of MMF treatment in this study was 41 months. Table 2 summarizes the biochemical and histologic parameters of patients while still on prednisone and/or azathioprine (pre-MMF) and after initiation of MMF (end-MMF).

There was a decrease in the level of serum transaminases in all patients after MMF administration although normal ALT levels were observed only in 11 (73%) patients. Serum bilirubin levels normalized in 14 (93%) patients. Changes in hematologic parameters were not clinically significant. Platelet counts remained greater than 100,000 in 12 (80%) patients. Although 2 patients had platelet counts in the 50,000 range several months after institution of MMF, no bleeding episodes were noted. The white blood cell count decreased to less than 5000 cells/mm^3 in 5 patients. One patient developed leukopenia at less than 3000 cells/mm^3, however, no infectious complications were noted

Table 2. Efficacy and Safety of MMF

	Pre-MMF	End MMF	*P* value
ALT level	91.73 ± 88.69	60.87 ± 71.20	.03
Bilirubin level	1.95 ± 3.21	0.82 ± 0.45	.22
γ-globulin level	3.78 ± 1.02	3.40 ± 0.75	.29
White blood cell count	6.86 ± 3.07	5.76 ± 2.04	.18
Platelet level	145.20 ± 70.72	172.93 ± 77.51	.37
Hemoglobin level	12.96 ± 1.84	13.00 ± 1.75	.86
Hepatic necroinflammatory score (Scheuer)[5]	2.59 ± 0.97	1.14 ± 1.21	.02
Fibrosis score (Ishak et al)[6]	4.10 ± 1.37	2.5 ± 1.51	.02

NOTE. Means ± SD shown.

and no growth factor support was required. Hemoglobin levels remained stable.

We found a statistically significant decrease in both the hepatic necroinflammatory and fibrosis scores with MMF treatment (Table 2). Patients on MMF alone had similar Ishak[5] fibrosis scores (2.33 ± 1.53) compared with those on prednisone + MMF (2.57 ± 1.62, $P = .83$).

Five patients were younger than 50 years of age, and 10 patients were older than age 50. However, we did not find any difference in the MMF response between the 2 age categories.

Seven patients had mild-to-moderate (stages 1–4) fibrosis and 8 had cirrhosis (stages 5–6 by Ishak[5]). The larger decreases in ALT levels occurred in patients with advanced fibrosis, although this trend did not reach statistical significance. Patients with advanced fibrosis did not have significantly different changes in hematologic parameters compared with those with lesser fibrosis scores. Of the 6 patients with stage 5 fibrosis, 5 were restaged and the median fibrosis score was 3 (fibrosis 1, 2, 3, 3, and 4) on MMF. The 2 stage 6 patients were unchanged.

Two patients had AIH–PBC syndrome. Although biochemical improvement was noted in both patients, the fibrosis stage remained the same before and after MMF treatment.

On follow-up evaluation, 10 patients remain on MMF: 1 patient is on 250 mg twice a day, 1 patient is on 500 mg twice a day, 5 patients are on 1.5 g twice a day, and 3 patients are on 1 g twice-daily dosing. MMF dosing was adjusted, according to chart notes, for changes in ALT levels, symptoms, and, if performed, evidence of active disease on liver biopsy examination. Four of these 10 patients are on MMF monotherapy, and 6 are taking MMF and prednisone (the usual prednisone dose was 5–10 mg/day). Five patients discontinued MMF. One patient stopped the MMF-prednisone regimen because of noncompliance, and eventually developed cirrhosis. Three patients were switched to tacrolimus, 2 of whom had further increases in liver enzyme levels despite several months of MMF treatment. One patient was lost to follow-up evaluation.

Throughout the MMF treatment, there were no infectious complications noted. One patient developed diarrhea with MMF 500 mg twice a day, but the diarrhea resolved with dose reduction of MMF to 250 mg twice a day. Another patient was taken off MMF because of sore gums and teeth loosening. All patients were maintained on a proton pump inhibitor while on MMF and there was no report of abdominal discomfort or pain.

Discussion

AIH is the first chronic liver disease to show a significant improvement in patient survival and reversal of cirrhosis with effective medical therapy. Disease remission is achieved in 80% of patients who are treated with prednisone and azathioprine.[7] Some patients, however, are intolerant to prednisone or azathioprine because of well-known side effects. In addition, patients may be resistant to this combination treatment, fail to normalize transaminase levels, and to improve histology, and may develop progressive liver disease.[8–12] For some patients who develop decompensated cirrhosis despite standard immunosuppression, liver transplantation may be the only therapeutic option.[13,14] Because biochemical and histologic remission need to be pursued in the treatment of AIH, additional or alternative therapeutic agents among nonresponders to standard immunosuppression have been considered. Immunosuppressive drugs such as cyclosporine A (GenGraf; Abbott, North Chicago, IL, or Neoral; Novartis, East Hanover, NJ), budesonide (Budenofalk; Dr. Falk Pharma UK Ltd., Buckinghamshire), and tacrolimus (Prograf, Astellas Pharmaceuticals) have been used successfully in resistant cases of AIH.[9–12] Cyclosporine A, a calcineurin inhibitor, was shown to induce remission in AIH in both children and adults.[11,12] Tacrolimus, another calcineurin inhibitor, was used in a study of 21 patients with chronic active AIH, with improvement in the biochemical profile after 3 months of treatment.[9] Similarly, 11 patients with steroid-refractory AIH treated with tacrolimus for a median of 25 months showed significant ALT and aspartate aminotransferase improvement at the end of the follow-up evaluation.[10] However, both cyclosporine and tacrolimus have substantial side effects including nephrotoxicity, neurotoxicity, hypertension, hyperlipidemia, and diabetes mellitus.[15]

MMF, discovered 50 years ago, is an ester of mycophenolic acid that was developed as an oral prodrug of mycophenolate for use in organ transplantation. Its method of action is interfering with DNA synthesis by inhibiting the enzyme inosine monophosphate dehydrogenase. Unlike the calcineurin inhibitors, it is devoid of neurotoxic and nephrotoxic effects.[16,17] The main toxicities of MMF are related to bone marrow suppression, opportunistic infections such as cytomegalovirus, and direct intestinal mucosal injury that can result in ulcerations. MMF has been approved by the Food and Drug Administration for the prevention of organ transplant rejection for both kidney and liver transplants. Although commonly used as an immunosuppressant for organ transplantation, it also is used to treat autoimmune diseases such as rheumatoid arthritis, psoriasis, and lupus nephritis. Table 3 summarizes the relevant literature concerning the use of MMF in AIH. Richardson et al[18] recently showed that 7 AIH patients who did not respond to azathioprine had an improvement in their ALT level, a decrease in histologic inflammation, and received a lower prednisone dose after treatment with MMF. Similarly, the Canadian Association for the Study of the Liver published a study of MMF among 16 AIH patients who had failed conventional therapy, and concluded that the drug was effective (7 of 11 patients, 64% sustained transaminase normalization) and well tolerated.[19] Two other studies suggested varying degrees of biochemical improvement (Table 3).[20,21]

We evaluated 15 patients with AIH who had failed prednisone and/or azathioprine treatment because of either progressive liver disease by histology or secondary to intolerance/side

Table 3. Summary of Clinical Experiences During MMF Treatment of AIH Patients

	Total patients, N	Dose MMF, mg/day	Biochemical response	Histologic response
Richardson et al[18]	7	2000	5/7 (71%)	Inflammatory activity: 7/7; fibrosis 0/7
Chatur et al[19]	16 (11/16 on MMF monotherapy)	500–2000	7/11 (64%)	Not assessed
Czaja and Carpenter[20]	8	500–3000 (median, 2000)	2/8 (25%) aspartate aminotransferase level improved	1 of 2, inflammation improvement
Devlin et al[21]	5	N/A	5/5 (100%) normal ALT level	1/5
Present study	15	500–3000	11/15 (73%) normal ALT level	Inflammatory activity: 11/15; fibrosis stage: 8/15

effects to therapy. We have provided data to suggest that MMF is a viable therapeutic option for patients with AIH who are not responsive to standard treatment. In our study, almost three quarters of patients normalized their liver enzyme levels, and the inflammatory and fibrosis scores decreased significantly on MMF treatment. Only 2 patients developed significant side effects from the use of MMF, 1 of whom had symptom resolution on dose adjustment. No significant bone marrow toxicity occurred in our patients and we had no instance of infectious complications associated with neutropenia. Importantly, patients with more advanced fibrosis did not have a significant worsening of hematologic parameters.

A potential drawback was the retrospective nature of this study. No controls were available to confirm the efficacy of MMF. However, biopsy specimens were reviewed by experienced pathologists. A re-review of all biopsy specimens was not possible because some biopsy procedures were performed and read in peripheral hospitals and therefore were not available. Because the improvement in the histology was in line with the improvement in the transaminase level, we postulate that reviewing a fraction of the histologic slides would not substantially alter the results. Another issue was the low prevalence of diarrhea: it is possible that this retrospective study resulted in an underestimation of the prevalence of gastrointestinal adverse events potentially caused by MMF.

We conclude that MMF was effective in selected AIH patients who had adverse events or did not respond adequately to conventional therapy. Although MMF is expensive ($16/day for a dose of 1 g twice a day), we believe that this drug merits further evaluation in prospective randomized controlled trials.

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