Reduction of proteinuria with mycophenolate mofetil in predominantly membranous lupus nephropathy

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Introduction. Lupus membranous nephropathy (LMN) presents a difficult clinical problem as no particular treatment has been proven to be effective. Studies have shown good results with mycophenolate mofetil (MMF) in proliferative lupus nephropathy (LN) (WHO class III and IV disease).

Objectives. To study whether MMF treatment was effective in membranous predominant LN in patients resistant to or intolerant of other immunosuppressive agents.

Patients and methods. We retrospectively studied 10 patients with systemic lupus erythematosus who had biopsy-proven predominant LMN (six Vc patients and four Va or Vb patients). Previous treatments included cyclophosphamide, azathioprine, ciclosporin and corticosteroids. The following parameters were recorded at baseline and follow-up: blood pressure, ECLAM, proteinuria, serum albumin and creatinine, routine haematology and immunology.

Results. The study included eight women and two men, mean age 38.4 ± 7.1 yr (range 30–49 yr). The racial distribution was as follows: five Caucasian, and five Black patients. The mean treatment time with MMF was 18.8 ± 15.4 months (range 3–52 months). Twenty-four-hour urinary protein excretion was reduced from median 2.26 g (range 0–7.92 g) to median 0.66 g (range 0.08–3.85 g) at follow-up (P = 0.0039). Serum albumin increased significantly after treatment from median 29.5 g/l (range 14.0–42.0 g/l) to 33.5 g/l (range 23.0–40.0 g/l) at follow-up (P = 0.04). There were no significant changes in serum creatinine (P = 0.55).

Conclusion. MMF is a potentially useful immunosuppressive agent in reducing the proteinuria associated with membranous predominant LN.

Key words: Lupus membranous nephropathy, Systemic lupus erythematosus, Mycophenolate mofetil.

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease, associated with the presence of multiple autoantibodies, notably to nuclear components. The kidney is amongst the major organ systems affected, and lupus nephritis (LN) is a major cause of morbidity and mortality in SLE [1]. Several different and sometimes overlapping histopathological manifestations are recognized, and these have been classified by the World Health Organization (WHO) in a classification which has evolved since 1982, with the latest modification in 1995 [2, 3]. Lupus membranous nephropathy (LMN) is classified as class V LN and can occur in isolation, or more commonly together with proliferative lesions.

Although LMN accounts for a minority of LN patients, it also presents a difficult clinical problem as no particular treatment has been proven to be effective in this group, as clearly described in the review of Kolasinski et al. [4]. Mycophenolate mofetil (MMF) is an immunosuppressive agent which first found clinical application in transplantation where it showed superior efficacy when compared with azathioprine-containing regimens in the prevention of acute transplant rejection [5]. Following its success in transplantation, MMF has also been used in autoimmune disease in a variety of conditions including SLE, systemic vasculitis, myasthenia gravis and scleroderma [6]. Studies in SLE have mostly been open to date, and have in general shown good efficacy in LN. There is a published randomized controlled trial in proliferative LN (class IV), which showed similar efficacy in inducing remission of proliferative LN by treatment with MMF compared with oral cyclophosphamide [7]. Given the growing evidence base for the efficacy of MMF in LN, and the paucity of proven immunosuppressive treatment for LMN, we sought to consider whether MMF could be a potential treatment for this group of patients.

Patients and methods

Patients with LMN, who received therapy with MMF for 3 months or longer, were included in this retrospective study. Thirteen patients were identified from clinical records and biopsy information. Ten patients with predominantly membranous or pure membranous biopsy appearances were included, whilst three with extensive proliferative lesions were excluded.

All patients studied were under the care of the Lupus Unit at St Thomas’ Hospital or the Renal Unit at Guy’s Hospital. Patients were seen during the last 6 yr, and were under regular out-patient review. Nine patients fulfilled at least four of the revised American College of Rheumatology classification criteria for SLE [8, 9]. The remaining patient had features strongly suggestive of LN on

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biopsy, with positive ‘full house’ immunohistology, and strongly positive antinuclear antibody (ANA).

Patients underwent renal biopsy as part of their routine clinical care, and these biopsies were examined by a single renal histopathologist. The biopsies were routinely processed to paraffin and examined by light microscopy including immunohistology. Electron microscopy was performed on eight biopsies. The biopsies were routinely processed to paraffin and examined by a single renal histopathologist. The biopsies were classified according to the modified 1982 WHO classification. Regardless of the activity scores, the predominant membranous pattern with subepithelial immune deposits was the shared feature in all the biopsies. The membranous lesion was seen in silver methenamine stain, involving at least 75% of the capillary walls in over 50% of the glomeruli. This corresponded to a predominantly peripheral (capillary wall) positivity for immune deposits and was supported by electron microscopy with the presence of prominent subepithelial deposits, often accompanied by some mesangial and insignificant subendothelial deposits.

Data were collected from examination of patient records. These included standard renal parameters: serum albumin, urea and creatinine; 24-h urine protein collection; routine haematological measurements of full blood count (FBC) and erythrocyte sedimentation rate (ESR) were available. Immunological testing included measurement of complement components C3 and C4 by immunofluorescence, and measurement of anti-double-stranded DNA (dsDNA) antibodies by C. lucciæ immunofluorescence and/or radioimmunoassay.

Disease activity was assessed by the ECLAM (European Consensus Lupus Activity Measurement Index), which has been validated for retrospective use by Mosca et al. [11], and concomitant oral corticosteroid dose.

Data on cholesterol and triglyceride levels pre- and post-MMF treatment were analysed in seven patients. The patients were treated with MMF at a starting dose of 0.5 g/day, with maximum doses varying from 1–2.5 g. Most patients received MMF therapy because of continuing proteinuria despite other immunosuppressive therapy. One patient received MMF as initial therapy, and one patient who had already entered clinical remission with cyclosporin A and received MMF as maintenance therapy after cyclosporin A was stopped due to reduction in renal function.

All patients received concomitant corticosteroid therapy, and most patients had previously received treatment with one or more other immunosuppressive agents. Concomitant therapy included antihypertensive drugs [including ACE inhibitors (ACE-I) or angiotensin-II-receptor blockers (AIIRB) in nine patients]. In four patients, the dose of ACE-I or AIIRB was unchanged, in three it was reduced, while in two it was increased.

### Statistical analysis

A non-parametric test (Wilcoxon) was used for comparison between baseline values and post-treatment values of the different variables. A P value < 0.05 was considered statistically significant.

### Results

#### Baseline clinical parameters

There were eight women and two men, mean age 38.4 ± 7.1 yr (range 30–49 yr). Racial distribution was as follows: five Caucasian and five Black patients. The mean treatment time with MMF was 18.8 ± 15.4 months (range 3–52 months). Previous immunosuppressive therapy included azathioprine (eight patients—five discontinued for treatment failure and three due to adverse events), cyclophosphamide (two patients—one intolerance, one lack of efficacy) and cyclosporin A (two patients—one intolerance, one lack of efficacy). Previous treatment failure was because of lack of response to treatment (six patients), adverse effects (cytopenia two, reduction in glomerular filtration rate one, diarrhoea/dizziness one). Baseline clinical and antihypertensive treatment details are summarized in Table 1.

#### Renal disease activity

Details of renal histology, including activity and chronicity scores, are given in Table 2. Parameters of renal function at baseline and last visit can be found in Table 3. Twenty-four-hour urinary protein excretion was reduced significantly from median 2.26 g (range 0–7.92 g) to median 0.66 g (range 0.08–3.85 g) at follow-up (P = 0.0039). The patient commenced de novo on MMF was also commenced on prednisolone 60 mg as part of induction therapy, and excluded from analysis of protein excretion. Changes in urinary protein excretion are illustrated in Fig. 1. Serum albumin was increased significantly after treatment from median 29.5 g/l (range 14.0–42.0 g/l) to 33.5 g/l (range 23.0–40.0 g/l) at follow-up.
C3 showed a significant reduction from median 0.57 g/l (0.28–1.23) to median 0.66 g/l (range 0.32–1.46), \( P = 0.039 \). There were no significant changes in serum creatinine (\( P = 0.55 \)) or dsDNA antibodies (\( P = 0.12 \)).

Lupus disease activity

Measurements of ESR, prednisolone dose and ECLAM score are listed in Table 4. No significant change was seen in ESR and oral prednisolone dose (\( P = 0.054 \)). The patient commenced de novo on MMF was also commenced on prednisolone 60 mg as part of induction therapy, and excluded from analysis of steroid dose.

Lipid levels

There were no significant changes in serum cholesterol (\( P = 0.81 \)) or serum triglycerides (\( P = 0.56 \)) after MMF treatment.

Adverse events

During the follow-up period, two patients developed infectious complications—one patient had herpes zoster (requiring temporary dose reduction) and one patient had repeated episodes of ear infection. Five patients developed gastrointestinal symptoms (diarrhoea, nausea and vomiting), one patient needed discontinuation of MMF and two patients required dose reduction secondary to the gastrointestinal adverse events.

Discussion

Over the past 6 yr, MMF has successfully been introduced as a potential treatment for SLE, particularly LN. This followed its initial introduction in transplantation and a demonstration of its efficacy in mouse models of lupus. MMF has shown good efficacy in the treatment of patients with LN, and the largest group studied to date has been those with class IV LN. There have been open studies [12, 13], but the randomized controlled trials have compared MMF as induction therapy for LN with cyclophosphamide. In 2000, Chan et al. [7] reported similar efficacy in class IV LN between 21 patients treated with MMF compared with 21 patients treated with oral cyclophosphamide, while Ginzler et al. [14] recently reported (in abstract form) a superior overall remission rate in the MMF group as compared with pulsed intravenous cyclophosphamide (administered according to the National Institutes of Health protocol) in patients with class III, IV, and V LN. Subset analysis of LMN patients in this study has not been published.

MMF is metabolized to mycophenolic acid (MPA), the active moiety. MPA is an inhibitor of inosine monophosphate dehydrogenase (IMP-DH), which catalyses a rate-limiting step in the de novo synthesis pathway of purine nucleotides, on which lymphocytes are reliant. The type II isoform of IMP-DH, expressed in stimulated rather than resting lymphocytes, is inhibited more strongly by MMF. MMF inhibits both B- and T-lymphocyte proliferation, and decreases antibody production, as well as affecting glycosylation and expression of adhesion molecules [15].

However, MMF has effects beyond simple immunosuppression, reducing progression of renal disease in the rat remnant kidney.
model and inhibiting mesangial cell proliferation in the anti-Thy-1 animal model. It reduced proteinuria in the Heymann nephritis model of membranous nephritis [15].

LMN represents a difficult treatment dilemma for clinicians. Options might include a more conservative approach in patients with normal renal function and low-grade proteinuria, to a powerful immunosuppressive regime in patients who have impaired renal function and high-level proteinuria. A non-randomized prospective trial with 10 patients showed some efficacy for cyclosporin, though no patient had complete resolution of proteinuria [16]. There is also experience with other immunosuppressive agents (azathioprine, chlorambucil, cyclophosphamide), usually together with corticosteroids, though no clearcut optimal treatment has emerged [4].

The current lack of a definitive treatment strategy in LMN, and the toxicities of many of the currently used drugs: cyclosporin A (nephrotoxicity), cyclophosphamide (ovarian failure, bone marrow suppression) and chlorambucil (bone marrow suppression), suggests a possible remit for assessing the effect of MMF in LMN.

Ginzler et al. [14] reported lower incidence of severe pyogenic infections and mucocutaneous herpes in the MMF group compared with the cyclophosphamide-treated patients. This lower toxicity of MMF compared with cyclophosphamide may be an important advantage in LMN, as class V LN may be more benign in terms of progression to renal failure compared with class IV lesions [17]. Hence, the risk:benefit ratio for aggressive immunosuppressive therapy may differ between these groups.

Maximum dosage of MMF in our study varied from 1–2.5 g with median dose 1.5 g. This is comparable to that used by Chan et al. (1 g b.d., halving at 6 months) [7]. In the study by Contreras et al. [18] patients received a median MMF dose of 1.5 g during the first 12 months and it was subsequently decreased, and in renal transplantation (Tricontinental study) [19], patients were divided into two groups receiving 2 and 3 g of MMF, respectively. However, Ginzler et al. [14] used 3 g in the induction study.

The data in the literature in relation to MMF and idiopathic membranous nephropathy (IMN) are scant. Miller et al. [20] studied 16 patients with difficult-to-treat IMN. Six out of 16 patients halved their proteinuria, after a mean of 6 months’ treatment. Two patients experienced partial remissions (at least 50% decrease in proteinuria, total proteinuria <3 g/24 h), though no patient achieved complete remission (<0.3 g/24 h). Experience with MMF in LMN is also lacking. Buratti et al. [21] reported normalization of renal function and reduction in proteinuria in four out of four paediatric patients with LMN, though they found little effect in children with class IV disease. Denton et al. [22] reported sustained reduction in proteinuria, and stable graft function in a SLE patient who developed LMN in his renal transplant.

Subgroups of LMN were recognized by the division into Va and Vb (Va, pure membranous; Vb, associated with mesangial lesions) Vc and Vd (Vc, associated with focal proliferative change; Vd, associated with diffuse proliferative change) in the 1982 WHO classification [2]. However, the most recent WHO modification (1995) proposed that membranous change in association with proliferative features should be assigned to either class III or IV [3]. A recent proposal (published in 2004) from the International Society of Nephrology and the Renal Pathology Society suggests that cases should only be classified as membranous (class V) where membranous lesions involve over 50% of the tufts in more than 50% of the glomeruli; all our cases satisfied these criteria. Furthermore this proposal recommends that where membranous and proliferative changes co-exist, both should be reported [23].

In our study, we felt it was appropriate to include patients with predominant membranous lesions with associated minor proliferative changes (Vc, Vd in the previous classification) as well as pure membranous changes (Va, Vb). We did not include patients with predominant and extensive proliferative lesions, even if they had associated membranous change to minimize the potential contribution of response of proliferative change to MMF. Such an effect cannot be definitively excluded in the study group, as a number of patients with falls in proteinuria did have associated proliferative change. Repeat renal biopsies were not performed in our patients unless clinically indicated, though this could theoretically have provided direct information on the effect of MMF on membranous changes.

In this study we have shown that MMF can be a useful treatment for some patients with LMN. As with much of the MMF experience in the literature, this group included patients who had previously failed other immunosuppressive therapies and had on-going significant proteinuria prior to commencing MMF. Three patients with nephrotic-range proteinuria responded (two Vc, one Vb), with levels of proteinuria decreasing below 1 g/24 h. Two other patients with proteinuria over 2 g (both Vc) decreased to less than 1 g/24 h. In other patients, the initial proteinuria was less marked than might be expected in LMN, possibly related to prior immunosuppressive and ACE-I therapy.

In some of our patients the administration of concomitant ACE-I or AIIRB might also be relevant, though it is difficult to dissect the relative effect of these drugs in relation to MMF. In four patients, the dose of ACE-I or AIIRB was unchanged, in three it was reduced, while in two it was increased. It is of note that in the nephrotic patients with the largest falls in proteinuria there was no major influence of ACE inhibition. In the absence of contraindications, patients should receive ACE-I or AIIRB therapy in addition to their immunosuppressive treatment. There were no significant changes, as would be expected after a significant reduction in 24-h proteinuria levels, in the serum lipid plasma concentration. It is important to point out that the pre-treatment levels were only elevated in two patients, and both were taking lipid-lowering drugs.

Although MMF has shown potential in this and other small studies, it is clear that to establish it in the forefront in LMN alongside its emerging reputation in proliferative LN, larger groups of patients will need to be studied in a controlled prospective, long-term study; preferably patients with pure LMN. Furthermore, it may also be useful to examine its use in patients who have not received any prior immunosuppressive therapy so that its true effect can be measured, rather than restricting its use to resistant patients.

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