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Mycophenolate mofetil: implications for the treatment of glomerular disease

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Introduction

Mycophenolate mofetil (MMF) is a specific inhibitor of inosine monophosphate dehydrogenase, which is involved in de novo purine synthesis. MMF is a suppressor of both T and B cell lymphocyte proliferation [1,2] and has been used successfully for the prevention of acute and chronic rejection of renal allografts [3,4]. MMF is more effective than azathioprine in preventing acute rejection. Interestingly, although MMF has been introduced as an immunosuppressive drug, it has also effects on non-immune cells. In particular it has an antiproliferative action on vascular smooth muscle cells [5,6] and this may be responsible, at least in part, for the beneficial effect of MMF on chronic graft dysfunction [7].

Only limited information is available with respect to the use of MMF for the treatment of autoimmune diseases. There are experimental studies documenting that MMF inhibits the development of experimental autoimmune uveoretinitis in rats [8] and the development of diabetes in BB rats. Furthermore, it ameliorates the renal lesions in several models of experimental glomerular disease [9]. These experimental studies provide a strong rationale to examine whether MMF is useful or not in preventing progressive renal failure in patients with glomerular diseases as well.

Unfortunately, the information in literature concerning its use in human glomerulopathies is limited. Only the results of some small preliminary trials in immune-mediated glomerular diseases are available [9], but these trials have shown some benefit of MMF, particularly when conventional treatment has failed. An advantage of MMF is that it provides immune suppression without causing major bone marrow suppression. It certainly represents a new and promising approach to the treatment of inflammatory glomerular disease.

Mechanisms of action and pharmacokinetics

Myophenolic acid (MPA), the active metabolite of MMF, is a reversible and non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme for the de novo pathway of purine synthesis [2]. As a result, it interferes with the cellular synthesis of guanosine and deoxyguanosine nucleotides. Proliferating lymphocytes are more dependent on the de novo pathway of purine synthesis than on the salvage pathway. Consequently, MPA inhibits preferentially lymphocyte proliferation [10]. When MMF is given per os, it is quickly absorbed. The bioavailability is 94% and the T_{max} 0.8 h. The MMF ester is converted into MPA. The latter is largely bound to plasma proteins (97%) and the free fraction is increased in patients with the nephrotic syndrome. MPA undergoes hepatic conjugation transforming it to an inactive phenolic glucuronide. Ninety per cent of this metabolite is eliminated via renal excretion. This metabolite

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cumulates in patients with renal insufficiency and may be responsible for the poor gastrointestinal tolerance of MMF in these patients, particularly chronically dialysed patients [11].

Action of mycophenolate on specific cell functions

Selective inhibition of lymphocyte functions

MMF has a selective antiproliferative effect on lymphocytes. *In vitro* lymphocyte proliferation in response to different mitogens (PHA, staphyloccal protein A) is inhibited in the presence of MPA at low concentrations (<100 nmol/l). *In vitro*, at a concentration of 100 nmol/l, MPA inhibits antibody production by B lymphocytes, which have been activated by staphylococcal protein A. Inhibition of the antibody response has also been shown in mice [1]. Similarly, the humoral immune response to antihaemophilus influenza vaccine was significantly reduced in renal transplant recipients treated with MMF [12]. Furthermore, MMF suppresses the formation of specific antibodies when transplant patients received polyclonal antithymocyte globulins [13].

Deoxyguanosine nucleotide depletion

When GTP in lymphocytes is depleted by MPA, the transfer of fucose and mannose to glycoproteins (including glycoprotein adhesion molecules) is inhibited [10]. This is important because adhesion molecules facilitate the attachment of leucocytes to endothelial cells and to target cells, thus increasing recruitment of inflammatory cells in damaged tissues. Expression of one group of adhesion molecules, the selectins, on the cell surface plays a major role in the initial interaction between leucoytes and endothelial cells [14]. Adhesion molecules also participate in the initiation and effector phase of the immune response. Finally, adhesion molecules are involved in the interaction between antigen presenting cells and lymphocytes as well as in the interaction between effector lymphocytes and target cells.

Effects on non-immune cells

At higher concentrations (10–100 mmol/l), which may be reached in the clinical setting, MPA has effects on cells not related to the immune system. Of note, this is particularly an antiproliferative effect on vascular smooth muscle cells [5]. *In vitro*, MPA inhibits vascular smooth muscle cell proliferation even when pro-proliferative stimuli (angiotensin II, β-FGF) are present. This effect is not shared by other immunosuppressing drugs, for example, cyclosporin or tacrolimus [5]. In the balloon injury model of the rat, intimal hypertrophy and smooth muscle cell hyperplasia are abrogated, at least partially [15]. This antiproliferative effect on vascular smooth muscle cells may be of relevance concerning the effect of MMF on chronic allograft dysfunction [7]. This action of MMF is unique and not shared by other immunosuppressive drugs. As some glomerulopathies are associated with vascular lesions, for example, onion skinning and microthrombus formation, which resemble vascular rejection, MMF might be of use in these types of glomerular disease as well.

Mycophenolate mofetil and experimental models of glomerular disease

MMF was effective in the prevention of progressive nephritis in murine models of SLE [16]. In the mouse MRL/lpr lupus model, Van Bruggen *et al*. [17] found significant reduction of albuminuria and of glomerular immunoglobulin and C3 deposition. In the same model, MMF improved survival, albumin excretion, haematuria antiDNA IgG levels, and glomerular C3 deposition. These effects are comparable with those of intraperitoneal cyclophosphamide treatment [18].

In the Heymann membranous glomerulonephritis model of the rat, early administration of MMF for 4 weeks prevented the appearance of proteinuria, glomerular IgG deposits as well as interstitial infiltration by inflammatory cells [19]. In contrast, administration of MMF late in the course of the disease did not prevent renal damage.

It is particularly remarkable that MMF was effective not only in models of primary glomerular inflammation, but also in the primarily non-inflammatory kidney remnant model, for instance, the rat with subtotal nephrectomy. The combination of MMF and angiotensin converting enzyme inhibitors provided superior renal protection [20]. MMF attenuated renal injury by interfering with the cumulativeness and proliferation of inflammatory cells. MMF limited the increase in blood pressure, glomerular hypertrophy and glomerular hyperfiltration. We have recently documented that MMF treatment significantly improves renal function and reduces renal injury after subtotal nephrectomy in rats [21]. In particular, MMF reduces compensatory hypertrophy of the remnant kidney and markedly decreases cell proliferation, myofibroblast formation and collagen III deposition [22]. The ability of MMF to suppress not only the immune response, but also smooth muscle cell proliferation makes the drug a candidate for preventing renal fibrosis, as myofibroblasts share many features with vascular smooth muscle cells. Further support for this novel therapeutic approach is provided by experiments where mesangial cell proliferation and differentiation into myofibroblasts was significantly inhibited by MPA in mesangioproliferative glomerulonephritis [23]. Proliferation of tubular cells is reduced by MMF as well, as shown by *in vivo* [22] and *in vitro* [24] experiments. This effect may contribute to the renoprotective action of MMF, as tubular cells play a role in the genesis of renal fibrosis [25]. Even when started late in the course of the disease, combined treatment with MMF and

angiotensin converting enzyme inhibitors stabilises established renal injury in the remnant kidney model [26]. It follows that MMF may have therapeutic potential, in the early stage by interfering with the inflammatory process, and in later stages by interfering with the development of fibrosis.

Mycophenolate mofetil in human glomerular disease

MMF and recurrent glomerulonephritis in allografts

Some preliminary data suggest that MMF reduces the rate of recurrence of IgA nephropathy in kidney allografts [27]. This is controversial, however, as other authors failed to observe prevention of recurrent IgA nephropathy or focal segmental glomerulosclerosis in patients treated with MMF even when treatment started at the time of engraftment [28,29]. An anecdotal observation showed clinical improvement of a relapse of ANCA-positive vasculitis in a cadaveric kidney transplant when the patient was treated with MMF. This observation is remarkable since the patient had received cyclophosphamide therapy [30].

Mycophenolate mofetil and primary glomerular diseases

The effect of MMF on glomerular diseases has been investigated in several small series. Although they comprise only a small number of patients, they are of particular interest, as usually the patients treated with MMF had been resistant to conventional treatment [31]. Several reports in various glomerular diseases showed improvement or stabilization of renal function in the majority of patients treated with MMF [32,33].

Mycophenolate mofetil and lupus nephritis

Remarkable improvement in serum creatinine and proteinuria was seen in 13 patients with relapsing or resistant lupus nephritis who had received MMF treatment for an average of 12.9 months [34]. A recent study evaluated efficacy and safety of MMF in combination with corticosteroid treatment in severe lupus nephritis. This was compared with a treatment schedule comprising first corticosteroids in combination with cyclophosphamide and subsequently in combination with azathioprine. In 42 patients with severe lupus nephritis, the rates of remission and the rates of relapse were similar in the two groups, but more side effects were seen with cyclophosphamide than with MMF [35]. A histological study in 15 patients with severe lupus nephritis showed that treatment with MMF for 6 months led to a more pronounced reduction of glomerular immune deposits, glomerular necrosis, microthrombus formation and vascular changes as compared with 12 patients who had received cyclophosphamide [36].

Mycophenolate mofetil and vasculitis

MMF might also be useful for the treatment of vasculitis [37]. Nowack *et al*. [38] reported on 11 patients with vasculitis (and with pauci-immune RPGN) (nine with Wegener's granulomatosis and two with microscopic polyarteritis. Following induction, therapy with cyclophosphamide MMF was administered at a dose of 2 g/day for 15 months as maintenance therapy in association with low dose steroids. In one patient, cyclophosphamide had been discontinued after 2 months because of haematological side effects and then MMF was introduced. Only one patient with Wegener's granulomatosis amongst the 11 patients relapsed on maintenance therapy; in the other patients, disease activity and proteinuria diminished.

Nephrotic syndrome

MMF caused improvement or stabilisation of renal function in seven patients with membranous glomerulonephritis [33]. Miller *et al*. [39] reported on 16 patients treated with MMF at a dose ranging from 0.5 to 2 g/day for a mean duration of 8 months. In these patients, other treatments had failed. Six of the 14 patients who completed the trial had more than 50% reduction of proteinuria with stable serum creatinine levels. MMF had to be discontinued in two patients because of adverse effects. A similar evolution was reported in a small series of children with steroid-dependent and steroid-resistant nephrotic syndrome [40,41]. The role of MMF in patients with FSGS is uncertain, because MMF failed to prevent recurrence of the nephrotic syndrome after transplantation [28,29]. Furthermore, in 20 patients with FSGS MMF failed to reduce proteinuria [42,43].

IgA nephropathy

Only anecdotal observations are available which reported a beneficial effect of MMF [33]. A marked decrease in proteinuria was seen in 18 patients with IgA nephropathy treated with MMF for 24 months compared to 18 patients treated with prednisone [44].

Conclusions

MMF may have therapeutic applications beyond immunosuppression in transplant recipients. This is particularly true for immune-mediated glomerular disease. Preliminary results suggest that MMF is effective in several types of glomerulonephritis after conventional therapy had failed. The toxicity of MMF is low compared with cyclophosphamide. MMF combines two actions: as an immunosuppressive agent it reduces the inflammatory process early on and subsequently it interferes with the genesis of fibrosis. Randomized controlled studies are obviously mandatory, (i) to provide definite evidence for the efficacy and safety and (ii) to define indications for, and treatment

modalities of, MMF. In view of the relatively good tolerance observed during pilot studies, it is rational to use MMF as a salvage therapy in patients who failed to respond to conventional treatment or in whom conventional treatment was discontinued because of intolerance or development of complications.

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