

Effect of mycophenolic acid in experimental, nontransplant glomerular diseases: new mechanisms beyond immune cells

Agnes Hackl¹ · Rasmus Ehren¹ · Lutz Thorsten Weber¹

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Abstract Mycophenolic acid (MPA) was introduced into clinical practice as immunosuppressive drug therapy to prevent allograft rejection. Since then, its clinical application has widened. Our goal was to review the lessons learned from experimental nontransplant glomerular disease models on the mechanisms of MPA therapy. T and B lymphocytes are preferentially dependent on de novo purine synthesis. By inhibiting the rate-limiting enzyme of de novo purine synthesis, MPA depletes the pool of deoxyguanosine triphosphate (dGTP) and inhibits proliferation of these immune cells. Furthermore, MPA can also induce apoptosis of immune cells and is known to inhibit synthesis of fucose- and mannose-containing membrane glycoproteins altering the surface expression and binding ability of adhesion molecules. However, MPA exerts a direct effect also on nonimmune cells. Mesangial cells are partially dependent on de novo purine biosynthesis and are thus susceptible to MPA treatment. Additionally, MPA can inhibit apoptosis in podocytes and seems to be beneficial in preserving the expression of nephrin and podocin, and by attenuation of urokinase receptor expression leads to decreased foot-process effacement. In summary, our manuscript sheds light on the molecular mechanisms underlying the antiproteinuric effect of MPA. Overall, MPA is an excellent treatment option in many immunologic glomerulopathies because it possesses immunosuppressive properties, has a remarkable effect on nonimmune cells and counteracts the proliferation of mesangial cells, expansion of mesangial

matrix, and foot-process effacement of podocytes combined with a low systemic toxicity.

Keywords Mycophenolate mofetil · Experimental · Nontransplant glomerular diseases · Nonimmune effect of mycophenolic acid · Mesangial cells · Podocytes

Introduction

Mycophenolate mofetil (MMF) was the product of a program of drug development led by Nelson, Allison, and Eugui of Roche Laboratories [1, 2]. This orally administered pro-drug is hydrolyzed by esterases in the intestine and blood to release mycophenolic acid (MPA), a potent, selective, noncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH).

MMF was introduced into clinical practice in 1993 as a complementary immunosuppressive drug to glucocorticoids, calcineurin inhibitors, and cell cycle inhibitors to prevent allograft rejection (Fig. 1). Since 1993, the clinical application of MMF has significantly widened and has become part of first-line therapy in lupus nephritis [3, 4]. MMF has proven not to be inferior to cyclophosphamide for both induction and maintenance therapy of class III, IV, or V lupus nephritis. Moreover, African American and Hispanic patients had a better response rate to MMF than to cyclophosphamide [5–7]. There is a vast body of literature in favor of MMF being an inherent part of treatment in complicated cases of pediatric idiopathic nephrotic syndrome. Kidney Disease: Improving Global Outcomes (KDIGO) guidelines suggests MMF as second-line medication in frequently relapsing nephrotic syndrome (FRNS) [8]. Gellerman et al. carried out a randomized, multicenter, open-label, crossover trial comparing MMF and cyclosporin A (CsA) therapy in 60 children with FRNS over a 2-year period [9]. MMF was associated with significantly more relapses per

✉ Agnes Hackl
agnes.hackl@uk-koeln.de

¹ Pediatric Nephrology, Children's and Adolescents' Hospital, University Hospital of Cologne, Kerpener Street 62, 50937 Cologne, Germany

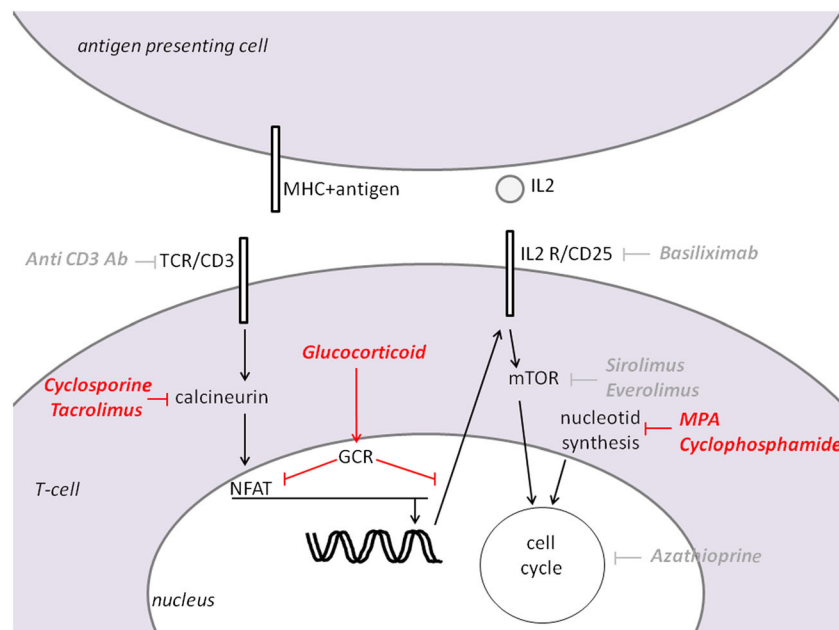


Fig. 1 Mechanism of action of different immunosuppressive drugs demonstrated in a T cell. In the treatment of glomerular diseases, the following immunosuppressive drugs can be administered (*red*): (1) Glucocorticoids act via nongenomic and genomic effects, e.g. by inhibiting transcription of genes that encode cytokines such as IL-2. (2) MPA is a noncompetitive inhibitor of the inosine monophosphate dehydrogenase, suppressing nucleotide synthesis. (3) Cyclophosphamide is an alkylating agent forming DNA crosslinks both between and within DNA strands, which leads to cell apoptosis. (4) Calcineurin inhibitors

(cyclosporin A and tacrolimus) bind to cyclophilin and in this complex inhibit the phosphatase calcineurin and the transcription of IL-2, accordingly. (5) The monoclonal anti-CD20 antibody (rituximab) influences the function of B cells, leading to inactivation and apoptosis of these cells (not shown). The other immunosuppressive drugs shown are applied in transplant immunology (*grey*). *MHC* major histocompatibility complex, *IL-2* interleukin-2, *IL-2 R* interleukin-2 receptor, *TCR* T-cell receptor, *GCR* glucocorticoid receptor, *NFAT* nuclear factor of activated T cells, *mTOR* mechanistic target of rapamycin, *MPA* mycophenolic acid

patient in the first year, but this effect was no longer seen in the second year of the trial, potentially due to a carryover effect from the first year. At the same time, the mean glomerular filtration rate was lower in patients receiving CsA and a post hoc analysis demonstrated exposure-dependent comparable efficacy of MMF and CsA. In line with that, other investigators have shown the efficacy of MMF not to be inferior compared with CsA [10, 11]. These encouraging results have been obtained mostly in minimal-change nephropathy in which MMF may help to reduce the use of steroids in patients who are often very young and are therefore more susceptible to steroid-associated side effects. The results obtained in patients with focal segmental glomerulonephritis (FSGN) and idiopathic membranous nephropathy were less promising [12–16]. The effectiveness of MMF in immunoglobulin (Ig)A nephritis as well as Henoch–Schönlein purpura nephritis (HSPN) again is auspicious. In HSPN, MMF can be considered as an option for maintenance therapy [17, 18]. In terms of IgA nephritis, conflicting results have been published, but in combination with renin-angiotensin system (RAAS) inhibition, MMF seems to be superior to monotherapy with an RAAS inhibitor [19, 20]. Albeit its general effectiveness in primary glomerulonephritis is still under debate [7, 12], MMF shows promising results especially in proteinuric renal diseases with an underlying immunological etiology via its active compound, MPA.

In the last two decades, a vast body of evidence has shown that this compound possesses effects not only on immune cells but also on different nonimmune cells, including glomerular cells, which makes MPA an attractive therapeutic choice in case of immune- as well as nonimmune-mediated glomerular diseases. The rationale for use of MPA in glomerular diseases was thoroughly summarized years ago by Badid et al. in 2001 and by Zatz et al. in 2002 [21, 22]. However, since then, substantial new publications have emerged. Therefore, our goal was to review the growing evidence for direct effects of MPA on glomerular cells, shedding light on the potential underlying mechanisms of the clinically established MPA therapy in glomerular diseases.

Immune-mediated effects of mycophenolic acid

Inhibited proliferation of immune cells

MPA has a selective antiproliferative effect on lymphocytes. By inhibiting IMPDH, the rate-limiting enzyme of the de novo purine synthesis, MPA depletes the pool of deoxyguanosine triphosphate (dGTP) required for DNA synthesis. IMPDH occurs in two isoforms: types I and II. The type II isoform is nearly five times more susceptible to inhibition by MPA than

the type I isoform. T and B lymphocytes possess only the type II isoform and are preferentially dependent on the de novo pathway of purine synthesis, making them an ideal target of the principal mechanism by which the immunosuppressive activity of MPA is exerted (Fig. 2). It is less well known, but important to note, that MPA significantly depletes GTP levels in monocytes also. This effect has been comprehensively summarized by Allison and Eugui [1, 2] and further confirmed by others in the last 20 years [23–25].

Facilitated apoptosis of immune cells

Although the principal mode of action of MPA on lymphocytes is cytostatic, it can also induce apoptosis of immune cells. Activated peripheral blood leukocytes showed 24 % apoptotic cells, while in the presence of 0.5 mM MPA, this was increased up to 67 %. A human T-lymphocytic cell line incubated with 1 mM MPA showed 82–98 % apoptotic cells as compared with 12 % in the absence of MPA [26]. Cohn et al. further examined the effects of MPA not only in a human T-lymphocytic cell line but also in two human monocytic precursor cell lines and observed MPA-induced apoptosis in each cell line. However, the incidence of apoptotic cells was lower in monocytic cell lines than in the lymphocytic line [27]. In concordance with these data, Andrikos et al. proved that therapeutic levels of MPA led to increased apoptosis rates of monocytic cells [28]. Moreover, the combination of CsA, steroid, and MMF increased the rate of apoptosis to as high as 95 %. Thus, immunosuppressive therapy can contribute to the high rate of apoptosis observed among mononuclear cells of

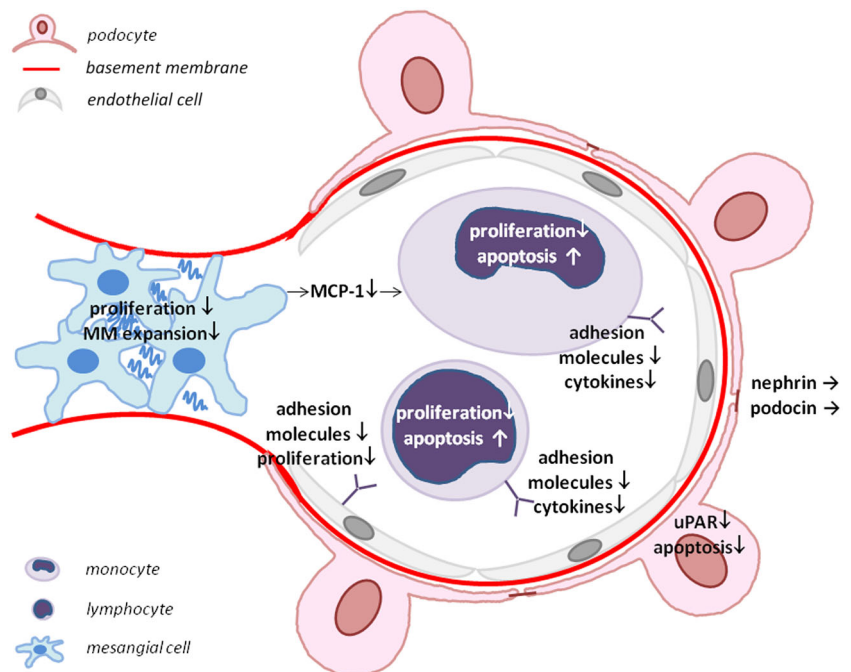
transplanted patients. Takahashi et al. demonstrated that MPA is more effective in potentiating T-cell apoptosis compared with rapamycin and CsA [29]. Additionally, the Fas ligand, which belongs to the tumor necrosis factor family and induces apoptosis, accumulated during MPA therapy [30]. Thus, MPA action may not have a “guanine starvation” mechanism only but may also function via an active feedback control mechanism of cell proliferation.

Decreased recruitment of immune cells

It is accepted that MPA depletes pools of dGTP in human lymphocytes and monocytes, thereby inhibiting not only cell proliferation but also synthesis of fucose- and mannose-containing saccharide components of membrane glycoproteins. These are recognized by the family of adhesion molecules, which are involved in the interaction between lymphocytes and either endothelial or antigen-presenting cells or target cells. However, other studies concluded that MPA has no effect on the surface expression of adhesion molecules but alters the binding ability of these molecules. Nevertheless, MPA leads to impairment of lymphocyte and monocyte recruitment and adhesion to and penetration via endothelial cell surface. Thus, activation of these cells is highly disturbed and cytokine production reduced.

Allison et al. first published data using flow cytometry and showed that MPA depletes the terminal mannose content of different membrane glycoproteins, thus altering cell adhesion in activated human peripheral blood lymphocytes [1]. In accordance with that, MPA was shown to reduce adhesion of

Fig. 2 Potential mechanisms of action of mycophenolic acid (MPA) in glomerulopathies. MPA has several mechanisms of action, all of which may contribute in different degrees to treat glomerular diseases. *MM* mesangial matrix, *MCP-1* monocyte chemoattractant protein 1, *uPAR* urokinase receptor



peripheral blood lymphocytes to human umbilical vein endothelial cells (HUVEC)-cultured monolayers by up to 70–80 % and T-lymphocyte penetration up to 50–60 % [31]. In another study, MPA prevented not only the accumulation of T-cell adhesion ligands, very late antigen 4 (VLA-4), and P-selectin glycoprotein ligand 1 (PSGL-1) receptor on the membranes of T-cell pseudopodia, but reduced the binding capacity of PSGL-1, VLA-4, and leukocyte-function-associated antigen 1 by 70 %, 50 %, and 30 %, respectively [32]. Beyond adhesion molecules, cytokines were also found to play a crucial role in the mechanism of action of MPA. Some authors demonstrated that the monocyte chemoattractant protein 1 (MCP-1), an important chemotactic factor for macrophages and lymphocytes, was strongly reduced by MPA treatment [33, 34]. Another molecule, osteopontin, which is involved in the accumulation of intrarenal macrophages, was also described as being highly down-regulated by MPA treatment [35]. Finally, because of suppression of macrophage activation, the production of various macrophage cytokines is also highly reduced [21, 24]. Accordingly, a great body of evidence shows that the infiltration of immune cells is prevented by MPA treatment in diabetic glomerulopathy [33, 36, 37], in the 5/6 ablation model [38–40], in spontaneously hypertensive rats [41], in a model of adriamycin-induced nephropathy followed by unilateral nephrectomy [42], in Heymann nephritis [43], and in rapidly progressive glomerulonephritis [35]. It can be concluded that MPA possesses potent infiltration-blocking properties.

Non-immune-mediated effects of mycophenolic acid

All results detailed above show that a major part of the clinically observed glomeruloprotective action of MPA is mediated through immune cells. However, there is data showing that MPA also exerts a direct effect on nonimmune cells, such as mesangial cells, endothelial cells, and podocytes.

Inhibited proliferation of mesangial cells

Activation and proliferation of mesangial cells is a key feature of many glomerular diseases caused by immunological or other mechanism of injury. It is also known that in mesangial cells, the salvage pathway for guanosine synthesis is less active than in other cell types in glomeruli such as, for example, endothelial cells. Nevertheless, mesangial cells are partially dependent on de novo purine biosynthesis and susceptible to MPA.

Cell proliferation is a highly regulated process that includes enhancing factors such as cyclins, cyclin-dependent kinases, and inhibitory proteins. Chiara et al. applied a model of anti-thy1-antigen nephritis and showed that MPA treatment during the early phase of mesangial cell proliferation causes a

decrease in cyclin gene expression (in both B and D groups) and a parallel increase in levels of cell-cycle inhibitor proteins, such as p27^{kip1} [44]. A direct effect of MPA on mesangial cell proliferation was further supported by experimental results. Ziswiler et al. and Hauser et al. demonstrated that in vitro, MPA can dose-dependently inhibit growth of cultured mesangial cells, leading to almost total arrest of cell proliferation. This effect seemed to be reversible, since administration of guanosine was able to prevent it [45, 46]. Furthermore, in an in vivo model of anti-thy1.1 nephritis, MPA decreased the bromodeoxyuridine-positive proliferating cells in the mesangium [45]. In summary, in vitro and in vivo results identify mesangial cells as being sensitive targets of MPA's antiproliferative action, which is mediated by guanosine depletion.

Inhibition of mesangial matrix expansion

The response of mesangial cells to different challenges includes an excessive accumulation of matrix proteins, leading to fibrosis. Some studies clearly show that MPA has intrinsic antifibrotic properties. Namely, MPA exerts a direct inhibitory effect on the secretion of osteopontin in a platelet-derived growth factor (PDGF)-treated rat mesangial cell line in vitro [47]. Furthermore, MPA was found to counteract the stimulatory effect of fetal calf serum and transforming growth factor (TGF)- β on the production of fibronectin and collagen type I, which may be mediated at least in part by dGTP depletion [48]. In agreement with these results, collagen type III deposition in the 5/6 ablation model was also significantly reduced by MPA treatment [49]. In addition, Ziswiler et al. demonstrated a decrease in the expression of α -smooth muscle actin and an attenuated accumulation of extracellular matrix (ECM) in a nephritic animal model in case of MPA treatment [45]. However, the authors found that in cultured mesangial cells, MPA did not affect the expression of three major matrix proteins: fibronectin, and collagen types I and IV. Therefore, the authors concluded that the attenuation of matrix deposition in vivo appeared to be most likely the consequence of the reduced cell number rather than of a specific effect of MPA on ECM protein synthesis. In summary in vivo data clearly show an antifibrotic effect of MPA, but to date, whether this reflects a direct inhibitory effect on production of cytokines and ECM proteins or rather an indirect response of the mesangium due to prevention of cell proliferation in the glomeruli has not been fully determined.

Maintenance of podocyte function

It is well known that nephrin, the podocyte transmembrane slit-diaphragm protein and podocin, the membrane-associated protein of band-7-stromatin family, are necessary for the proper function of the renal filtration barrier. Nakhoul

et al. investigated an adriamycin-induced nephrotic model accompanied by severe disruption of slit diaphragm structure and consequent rapid and profound loss of nephrin and podocin. MPA treatment seemed to be beneficial and preserved the expression of nephrin and podocin. However, this favorable response did not reach a significant level [50]. In line with that, in a diabetic model, it was revealed that MPA is able to protect against the loss of nephrin [51]. Furthermore, Wu et al. demonstrated that glomeruli of diabetic rat express significantly less nephrin and podocin, which can be restored by MPA treatment. Furthermore, they described that macrophage recruitment and expression of interleukin (IL)-1 and tumor necrosis factor (TNF)- α was also ameliorated [36]. To note, Takano et al. found that the macrophage-derived cytokines IL-1 and TNF- α markedly suppressed activity of the nephrin gene promoter [52], thus one can postulate that in this experimental setting, less infiltration of macrophages prevented the phenotypic changes of podocytes and the consequent loss of nephrin. As a further aspect, MPA was found to inhibit podocyte depletion in the early stage of streptozotocin-induced diabetes mellitus, partly by inhibiting apoptosis via decreasing cleaved caspase-3 and bax expression and increasing bcl-2 expression [51, 53]. Furthermore, urokinase receptor (uPAR) has been recently identified to have a down-stream signal pathway in podocytes, leading to foot-process effacement and urinary protein loss via a mechanism that includes lipid-dependent activation of α 5 β 3 integrin [54]. MPA appeared to decrease uPAR messenger RNA (mRNA) expression and protein levels, leading to decreased foot-process effacement and proteinuria in lupus nephritis [55].

Effects on endothelial cells

Data on how expression of endothelial cell adhesion molecules is influenced by MPA are rather contradictory. Olejarz et al. reported that MPA significantly inhibits TNF- α -induced intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) mRNA, and protein expression in human aortic endothelial cells [56]. Additionally, Huang et al. proved that not only did inhibition of TNF- α induce expression of ICAM-1 but also a multifactorial effect of MPA on endothelial cells showing decreased angiogenesis of endothelial cells in a three-dimensional collagen gel culture system, reduced migration of endothelial cells, and proliferation inhibition of these cells [57]. While others could not confirm the reduction of ICAM-1 by MPA treatment, they found a diminished E- and P-selectin expression on endothelial cell membranes in the presence of MPA [31, 32, 58, 59]. Nonetheless, there are reports showing no effect of MPA on adhesion molecule expression [60–62]. So, further studies are warranted to delineate the effect of MPA on endothelial cells.

Effects on tubular epithelial cells

The effect of MPA on tubular epithelial cells deserves attention in order to understand the complexity of MPA mechanism of action. In the pathomechanism of ischemia–reperfusion (I/R) renal failure, which is mainly characterized by tubular injury, endothelin-1 (ET-1) plays a pivotal deteriorating role. When cultured rabbit proximal tubular epithelial cells (PTEC) were treated with different immunosuppressives, a slight to moderate decrease in ET-1 was observed following administration of MPA, while treatment with CsA or tacrolimus (Tac) resulted in a significant increase in ET-1 [63]. Another mechanism of tubular injury is exposure to high levels of proteinuria, which increases MCP-1 expression in tubular epithelial cells, thus facilitating the infiltration of immune cells. Shui et al. showed that this effect was prevented by MPA, which was partly dependent on the p38—mitogen activated protein kinase (MAPK) pathway [64]. Furthermore, Copland et al. demonstrated that MPA even influences epithelial mesenchymal transformation (EMT). MPA not only prevented a transforming-growth-factor-induced transition of human PTEC into myofibroblasts, but it markedly inhibited proliferation of PTEC and returned myofibroblasts to a PTEC morphology [65].

The beneficial effect of MPA on tubular epithelial cells was also described *in vivo*. In diabetic nephropathy, MPA has a protective function via suppression of the EMT [66]. Additionally, Iturbe et al. showed that angiotensin-II-induced tubulointerstitial injury, including tubular dilation and sloughing, was significantly reduced by MPA treatment, as were tubulointerstitial proliferative activity and T-cell infiltration and activation [67]. Finally, several publications emphasize the capability of MPA to inhibit expression of adhesion molecules on the surface of inflammatory cells and ultimately prevent their infiltration into the kidney. Accordingly, a significantly better tubulointerstitial score was seen in Fisher 344→Lewis rat transplant model, in anti-thy1-induced chronic–progressive glomerulosclerosis, in subtotal ablation, in long-term I/R injury, and in diabetic nephropathy [33, 68–71].

Taken together, the beneficial effects of MPA therapy extend beyond glomeruli to preventing accompanying tubular injury via suppressing the proteinuria-induced infiltration of immune cells in the tubulointerstitium and via inhibiting EMT.

Conclusion

Overall, MPA seems to be an excellent treatment option in many immunologic glomerular diseases. First MPA possesses widely accepted immunosuppressive properties. Moreover, it has a remarkable effect on nonimmune cells and ability to counteract with proliferation of mesangial cells, expansion

of mesangial matrix, and foot-process effacement of podocytes, as discussed in this review (Fig. 2). In addition, it has a relatively low systemic toxicity. Therefore, future prospective randomized studies will need to examine the antiproteinuric value of MPA in glomerular diseases beyond established use in immunological renal disease, such as lupus nephritis.

Compliance with ethical standards

Conflict of interest LTW has received unrestricted scientific grants from Novartis Pharma GmbH and Roche Pharma AG.

AH and RE declare no conflict of interest.

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