Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 3 pp. 353–361, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

REVIEW

MYCOPHENOLATE MOFETIL – A NEW ATHEROPREVENTIVE DRUG?

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Abstract: Atherosclerosis is a form of chronic inflammation in which endothelial cell dysfunction, fibroproliferative process, oxidative stress and inflammatory cell activation are linked to plaque development and destabilization. T-lymphocytes also play a key role in pathogenesis of atherosclerosis. As a consequence, the suggested concept that modulation of an immunological response could be an appropriate target in the prevention of cardiovascular disease, is an important focus of research. Mycophenolate mofetil (MMF) is an inhibitor of inosine monophosphate dehydrogenase (IMPDH), that exerts anti-proliferative and pro-apototic effects, particularly on activated T-lymphocytes. MMF has other anti-atherogenic effects at the level of endothelial cells, monocytes/macrophages, smooth muscle cells and dendritic cells. In addition, MMF exhibits anti-oxidative properties. The present review paper provides an overview about the mechanisms of anti-atherosclerotic properties of MMF.

Keywords: atherosclerosis, mycophenolate mofetil, endothelium, monocyte/macrophage, T-lymphocyte, adhesion molecules, oxidative stress

Atherosclerosis, which causes cardiovascular diseases, is a specific form of chronic inflammation. Atherosclerosis is modified by lipid disorders, oxidative stress and the fibroproliferative process. Endothelium dysfunction plays a key role in the initiation as well as in the progression of atherosclerosis. Moreover, of great importance is that atherosclerosis has both an innate and adaptive defensive mechanism. An innate response depends on macrophages – the main inflammatory cells – playing an important role in the pathogenesis of atherosclerosis. A mechanism of the adaptive response depends on lymphocytes T and B. These cells are able to modulate the atherosclerosis by a secretion of immunoregulatory cytokines and antibodies. Many suggestions present a view that the modulation of the immunological response could be an appropriate target in the prevention of cardiovascular diseases, therefore, mycophenolate mofetil seems to be a reasonable proposal as a drug. The scope of this study is to gather all knowledge about the mechanisms of the anti-atherosclerosis properties of this drug.

Mycophenolate mofetil – structure, metabolism and mechanisms

Mycophenolate mofetil (MMF) is the pro-drug of mycophenolic acid (MPA), which has been isolated from one kind of fungus – Penicillium fungus (1).

Taking a clearly chemical point of view, its structure is expressed as follows: (E)-6-(1,3-dihy-

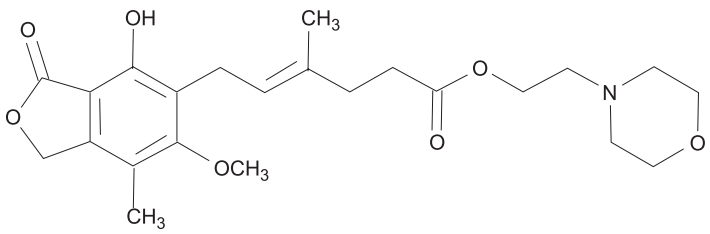

Figure 1. The structure of mycophenolate mofetil

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dro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenonian 2-morpholinoethyl (Fig. 1). After oral administration and absorption of MMF, the ester linkage is rapidly hydrolyzed by esterases to yield MPA, an active immunosuppressive agent. The bioavailability of oral MPA from MMF is 94% while the maximum plasma concentration occurs about 2 h after administration. MPA undergoes a hepatic glucuronidation through a glucoronozyl transferase to form a mycophenolic acid glucuronide (MPAG), an inactive metabolite, which is secreted into bile. Subsequently, it is converted back to MPA by bacterial glucuronidases and then re-absorbed, and recirculated. At least 90% of MMF is excreted in urine as MPAG (1).

A correct synthesis of purine is necessary to obtain nucleotides, particularly guanosine triphosphate (GTP) and deoxyguanosine triphosphate (dGTP), which are used to synthesize DNA and glycoprotein. The purine synthesis occurs *via* two major pathways: the *de novo* pathway and the salvage pathway (Fig. 2). In the *de novo* pathway, 5-phosphoribosyl-1-pyrophosphate (PRPP) is converted by PPPP synthetase to inosine monophosphate (IMP), which is further modified to guanosine monophosphate (GMP) by the rate limiting enzyme, inosine monophosphate dehydrogenase (IMPDH). IMPDH binds IMP and cofactor NAD, which receives hydrogen from IMP. $NADH_2$ is then released, forming xanthosine monophosphate (XMP), which is subsequently converted to GMP. In the salvage pathway, guanine obtained as a result of nucleotide acid degradation is converted to GMP.

In 1969, Franklin and Cook described the effect of MPA on IMPDH and its ability to inhibit the synthesis of nucleotide acids in eucariotic cells. MPA occupies the catalytic site in IMPDH, which is occupied by NAD and H_2O, thereby inhibiting IMPDH (2). Whereas most human cells types have the capacity to synthesize the guanosine nucleotides by the use of both pathways mentioned above, lymphocytes are almost completely IMPDH-dependent (1, 3). To date, two isoforms of IMPDH have been identified. Stimulated T lymphocytes strongly express isoform II, which has a 5-fold higher affinity to MPA compared to isoform I, of which is constitutively expressed in most cells. Hence, inhibition of IMPDH with MPA is followed by the depletion of the pool of GTP required for DNA synthesis, predominantly in stimulated T-lymphocytes. As a result, MPA inhibits the T-lymphocyte proliferation throughout the blocking cell cycle in the G1 phase. Furthermore, MPA inhibits a production of antibodies stimulated by miogens and antigens, but also induces apoptosis in activated T-lymphocytes. Features of MPA mentioned above are responsible for its immunosuppressive action.

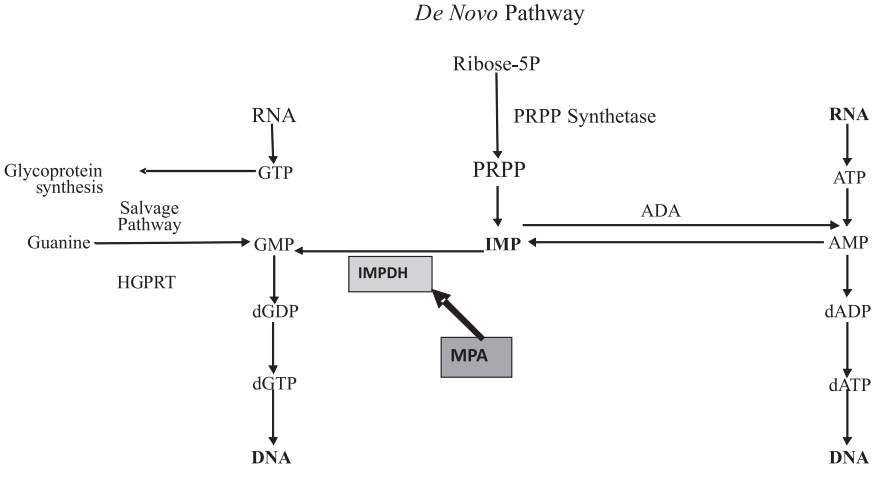
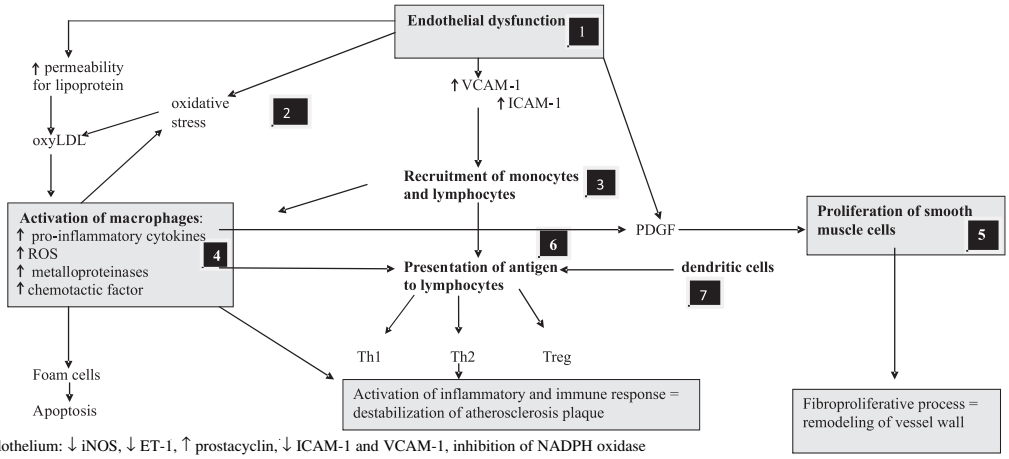


Figure 2. Purine biosynthesis pathways
ADA – adenosine deaminase, dADP – deoxyadenosine diphosphate, dATP – deoxyadenosine triphosphate, dGDP – deoxyguanosine diphosphate, dGTP – deoxyguanosine triphosphate, HGPRT – hypoxanthine-guanine phosphoribosyl transferase, IMP – inosine monophosphate, IMPDH – inosine monophosphate dehydrogenase, MPA – mycophenolic acid, PRPP – 5-phosphoribosyl-1-pyrophosphate



1. Endothelium: ↓ iNOS, ↓ ET-1, ↑ prostacyclin, ↓ ICAM-1 and VCAM-1, inhibition of NADPH oxidase
2. ↓Oxidative stress
3. ↓ Recruitment of monocytes and lymphocytes: decreased affinity of adhesion molecules for their ligands on endothelium
4. Macrophages: ↓ proliferation, ↑ apoptosis, ↓ pro-inflammatory cytokines, ↓ metalloproteinases, ↓ oxidative stress
5. Smooth muscle cells: ↓ proliferation
6. T Lymphocytes: ↓ proliferation, ↑ apoptosis ↓ INFγ, ↑ Treg
7. 6. Dendritic cells: ↓ maturation, ↓ IL-12, ↓ INFγ

Figure 3. Schematic representation of anti-atherogenic properties of MMF
ET-1 – endothelin-1. iNOS – inducible NO synthase, INF – interferon γ, ICAM-1 – intercellular adhesion molecule-1, oxyLDL – oxidized LDL, PDGF – platelet derived growth factor, ROS – reactive oxygen species, Treg.T – regulatory T-lymphocytes, VCAM-1 – vascular cell adhesion molecule-1

Effect of MPA on endothelial cells

Endothelial dysfunction plays a key role in the pathogenesis of atherosclerosis. A hallmark of endothelial dysfunction is the impaired bioavailability of NO, which is caused firstly by diminished synthesis and release, and secondly, by inactivation through increasing local reactive oxygen species (ROS) (4, 5). NO released by endothelial cells is responsible for relaxing smooth muscle cells, and in consequence, controlling intravascular pressure and blood tissue perfusion. Moreover, NO inhibits an aggregation of platelets, adhesion of leukocytes, proliferation of smooth muscles and inflammatory response. NO is synthesized from L-arginine by the constitutive endothelial NO synthase (eNOS; NOS3) in a small amount (6). Another type of NO synthase, so called "inducible NOS" (iNOS; NOS2), synthesizes a higher concentration of NO in activated macrophages or endothelial cells over a longer period.

The activated macrophages also produce ROS, which combine with NO to form peroxynitrites – highly reactive molecules that can react with protein, and in this way, change their function, and therefore, the function of endothelium. It is assumed that iNOS exerts pro-atherogenic activity by protein nitrosylation as well as by downregulation of eNOS activity (7).

It would be therapeutically desirable to inhibit iNOS activity, but not eNOS. Both NOS isoforms require tetrahydrobiopterin (BH_4), which is an essential cofactor for converting L-arginine into NO (6). However, whereas BH_4 is tightly bound to eNOS, the iNOS isoform requires continuous *de novo* synthesis of this co-factor. As it is well known, MPA decreases intracellular content of BH_4 by reducing the intracellular GTP, which is essential for synthesis of this co-factor (8). According to studies by Senda et al. (9), MPA inhibits interferon γ (INFγ) and tumor necrosis α (TNFα)-stimulated iNOS activity, whereas basal NO production, mediated by eNOS, remained unaffected.

Furthermore, endothelial dysfunction, leading to impairment of vasodilatation, is also due to impaired prostacyclin synthesis and up-regulated production of endothelin-1, which is a strong vasoconstrictor. As known from further studies, MPA increased the prostacyclin release and inhibited TNFα-stimulated synthesis of endothelin-1 (10, 11). Based on the data, it might be postulated that MPA exhibits beneficial effects on the vasodilatory function of endothelium.

Endothelial dysfunction is also manifested by shifts from anti-adhesive to pro-adhesive phenotype, which is essential for both progression of atherosclerosis, as well as for the inflammatory process

taking place in the vessel wall (12). This feature of endothelial dysfunction is associated with the appearance of adhesion molecules on their surface as mediators of interactions between cells of the vascular wall and leukocytes, thus, these interactions are essential for the adhesion and trans-endothelial migration of leukocytes. The family of endothelial adhesion molecules includes: selectins E and P, intercellular adhesion molecule-1 (ICAM-1), ICAM-2, vascular cell adhesion molecule-1 (VCAM-1), and platelet-endothelial adhesion molecule-1 (PECAM-1). So far, the best recognized and probably the most important for atherosclerosis are: VCAM-1 and ICAM-1 (13).

Many studies performed *in vitro* on HUVEC (human umbilical vein endothelial cells) stimulated with range of cytokines have shown various effects of MPA on the endothelial adhesion molecules and delivered numerous conflicting results. The study by Hauser et al. (14) reports that MPA did not change the ICAM-1 expression stimulated by TNFα, however, it increased TNFα-induced VCAM-1 and the selectin E expression. Some study results demonstrated that pre-treatment of HUVEC with MPA enhanced the induction of ICAM-1 by interleukin-1β (IL-1β) (15). Other studies demonstrated that MPA revealed to be a strong inhibitor of IL-1β-stimulated of VCAM-1 and the selectin E expression, but its inhibitory effect on the induced expression of ICAM-1 was much weaker (16). Similarly, Rabb et al. (17) demonstrated that MPA inhibited the TNFα-stimulated expression of VCAM-1 and ICAM-1.

An interaction between endothelial adhesion molecules and their ligands on the surface of leukocytes depend on their structure and affinity. As known, mannose and fucose are transferred to glycoproteins through guanosine nucleotides (1). Theoretically, a depletion of GTP by MPA should inhibit the transfer of mannose and fucose to surface receptors, along with the adhesion molecules.

Functional confirmative evidence of this observation was to demonstrate that MPA inhibits adhesion of monocytes to endothelial cells (18) and adhesion and penetration of lymphocytes (16).

The data mentioned above suggest that MMF treatment for medicinal purposes has the capacity to reduce infiltration of circulating monocytes and lymphocytes to the site of inflammation, including atherosclerosis plaque, as has been confirmed in animal studies (19).

MPA impact on monocytes / macrophages

The endothelial dysfunction, simultaneously with a local secretion of chemotactic factors, allows for local recruitment of monocytes inside the intima. Monocytes accumulating in the vascular wall undergo a series of changes typical for macrophages; then scavenger receptors appear on their surface, which participate in the internalization of foreign antigen, for example, modified LDL (20, 21). This leads to their activation and the formation of foam cells. Activated macrophages secrete many factors modulating the course of atherosclerosis. They are the source of many inflammatory cytokines, including IL-1, TNF and IL-6. They secrete many chemotactic factors for monocytes and lymphocytes. They produce an increased number of ROS, which are responsible for oxidative stress in the vascular wall and thus contribute to the formation of oxidized low density lipoprotein (oxyLDL), the main pathogenic factor of atherosclerosis. They are also a source for growth factors, including the platelet derived growth factor (PDGF). The activated macrophages produce metalloproteinases, i.e., enzymes that digest connective tissue matrix, therefore, they might contribute to the destabilization and rupture of atherosclerosis plaque, and by that means leading to acute clinical complications of atherosclerosis. Moreover, macrophages as cells presenting antigen, are able to present it to the lymphocytes $CD4^+$ with the use of major histocompatibility complex (MHC) class II and thus, become a kind of bridge between adaptive and innate responses of the organism.

MPA, by decreasing the level of GTP in monocytes / macrophages, may contribute to a decrease in monocytes recruitment to the intima, and thus to the impairment of inflammatory response of macrophages, which is responsible for atherosclerosis progression. In an earlier study, it was shown that MPA treatment of monocytes decreased a monosylation of glycoproteins and their attachment to endothelial cells and laminin (22). Further studies confirmed this observation showing a reduction of monocyte binding to HUVEC or selectin E after MMF treatment (18). Moreover, these studies demonstrated that MMF attenuated lipopolysacharide (LPS) or INFγ-stimulated expression of ICAM-1 and MHC of class II. Furthermore, MPA also revealed other properties, which may contribute to decreasing the number of monocytes / macrophages in atherosclerotic lesion. Studies performed by Cohn et al. (23) have demonstrated that MPA inhibited a monocyte proliferation as well as arrested their cell cycle in the S phase and increased their apoptosis. Interestingly, exogenous guanosine added before MPA treatment only partially reversed MPA-induced changes in these parameters, which suggests the existence of another type of mechanism, not only the impact of IMPDH itself.

These observations from *in vitro* studies were confirmed in results carried out *in vivo*. It has been demonstrated, by the use of animal experimental model for diabetes, that MMF inhibited glomerular infiltration of macrophages (24). A simultaneous MMF application to mice ApoE ($^{-/-}$), treated with a fat enriched diet, has led to a decrease of the number of monocytes in aorta sections (25). MMF therapy may also lead to attenuation of the inflammatory response. Studies performed by Weimer et al. (26) demonstrated that monocytes received from patients with long-term stable graft functions treated with MMF, secreted a diminished amount of IL-1 and TNF in response to LPS. MMF therapy in patients with a carotid artery stenosis was associated with the decreased expressions of metalloproteinases in sections of these vessels (27).

The above mentioned data suggest that MMF treatment may lead to a decrease in the recruitment of monocytes / macrophages and their activation, and thus to the impairment of inflammation in atherosclerotic plaque.

Effect of MPA on smooth muscle cells

Smooth muscle cells play a key role in the pathogenesis of atherosclerosis (28). Under the influence of locally secreted growth factors (mainly from activated macrophages) mostly PDGF, they migrate from the media to the intima, where they further proliferate and synthesize components of connective tissues. Thanks to the activity of the smooth muscle cells, present in the intima, it first comes forward to form fibrous cap, covering up the atherosclerotic lesion, and then it remodels the vessel wall and narrows it so that it finally leads to the impairment of blood tissues perfusion.

It has been demonstrated that MPA inhibited the PDGF-stimulated proliferation of rat smooth muscle cells and the synthesis of connective tissue components (29). MPA also inhibited a proliferation of the smooth muscle cells induced by endothelin-1 (30) or oleic acid (31). As mentioned earlier, MPA inhibited the secretion of inflammatory cytokines. It is known that IL-1 and TNF stimulate the endothelial and the smooth muscles cells to synthesize PDGF (32). Moreover, IL-6 is a strong stimulator of smooth muscle proliferation. The results of the studies *in vitro* were proven in the animal model for aorta transplantation, in which an application of MMF caused a decrease in appearance of the smooth muscle cells in the intima and an essential reduction of their replication rate (33). Another study, targeted for evaluating MPA impact on the formation of atherosclerosis in a transplanted aorta, showed that the application of this drug inhibited all essential histological features of atherosclerosis in the transplant by reducing smooth muscle cell replication (30).

The above mentioned data suggest that the application of MMF may lead to the impairment of smooth muscle cell proliferation and to the reduction of connective tissue component secretion, thus inhibiting the remodelling of the vessel wall.

MPA impact on lymphocytes

In the chronic inflammatory process, essential for atherosclerosis, there are not only monocytes / macrophages-dependent mechanisms of innate responses involved, but also these adaptive response mechanisms that remain under control of lymphocytes T (34). Lymphocytes T, present on atherosclerosis plaque, include $CD4^+$ (helper lymphocytes) and $CD8^+$ (cytotoxic lymphocytes), wherein $CD4^+$ are more dominative. Active lymphocytes $CD4^+$ support both a humoral and a cellular response. An effective response emerges from the point, in which naive lymphocytes T encounter cells presenting antigen. Here is where bonding of antigens comes forward, cut into small fragments, tied with MHC class II on the surface of cells presenting antigen, by the TCR receptors present on the surface of $CD4^+$.

Macrophages and dendritic cells are the main cells presenting antigen. In order to effectively activate lymphocytes T, except TCR bonding with antigen fragments tied with MHC class II, a co-stimulation is essential, in other words, a simultaneous bonding of proper proteins on lymphocytes and on the cells presenting antigen. As regards to atherosclerosis, the most known are interactions between CD40L on lymphocytes and CD40 on cells presenting antigen. Activation of lymphocytes T may lead to the creation of different types of lymphocytes T. Among $CD4^+$ lymphocytes that dominate in atherosclerotic plaque, the most abundant are lymphocytes Th1, responsible for the cellular response, secreting mainly INFγ and TNFα. Lymphocytes Th2 presence occurs much less, which is responsible for the humoral response and secretion of IL-4, IL-5 and IL-10. It has been suggested that Th1 lymphocytes promote the development and progression of atherosclerosis, whereas Th2 lymphocytes are anti-atherogenic. An essential role of lymphocytes T in the pathogenesis of atherosclerosis was proven in many study results obtained from genetically modified mice, which disclosed an inhibition of the response, dependent from these cells and truly decreased the progression of atherosclerosis, whereas entering $CD4^+$ for mice ApoE ($^{-/-}$) increased the progression of atherosclerosis (35).

In recent years, there were some suggestions that regulatory lymphocytes T (Treg) had a beneficial effect for atherosclerosis (36). This is in regards to a population of lymphocytes T that is responsible for the maintenance of tolerance and control of the immunological response. It has been suggested that the balance between an efector response (Th1 and Th2) and the regulatory immunological one, or lack of one, as refers to atherosclerosis, played an essential role in its development and progression. A transfer of lymphocytes Treg to mice ApoE $(^{-/-})$ led to the inhibition of both the Th1 and the Th2 response with a simultaneous increase of IL-10, the cytokine ranging from a variety spectrum of anti-atherosclerotic activity (37).

As previously mentioned, MPA has a 5-fold higher affinity to IMPDH type II, and the main form is expressed in stimulated lymphocytes (1). An inhibition of this dehydrogenase finally led to the depletion of a pool of GTP required for DNA synthesis, predominantly in activated lymphocytes T. As a consequence, MPA inhibits the lymphocytes T proliferation by blocking them in the G or S phase of the cell cycle. In addition, MPA induced the apoptosis in the activated lymphocytes T from 12 to 82% (23). During the studies performed on mice injected with the superantigen staphylococcal enterotoxin B (SEB), it was proven that MMF accelerated the elimination of SEB-reactive lymphocytes T through an induction of their apoptosis (38).

The depletion of the pool of GTP by MPA also inhibits transfer of mannose and fuctose into glycoproteins, being present on the surface of lymphocytes T, including the glycoproteins responsible for attaching to the endothelial cells (1). Thus, treatment of T-lymphocytes with MPA resulted in attenuation of their adhesion to endothelial cells, as was proven in many studies. The studies performed by Blaheta et. al. (39) revealed a strong inhibition of both the adhesion and the penetration rate of $CD4^+$ and $CD8^+$ T-lymphocytes suggesting that it occurs mainly as a result of reduced binding ability of two essential adhesion molecules for this process: very late antigen (VLA-4 – ligand for VCAM-1) and lymphocyte function-associated antigen (LFA-1 – ligand for ICAM-1).

As seen from the data mentioned above, MPA has the capacity to decrease the number of lymphocytes in the sites of inflammation, including the atherosclerosis plaque, throughout the inhibition of proliferation, an induction of apoptosis and a decreasing attachment to the endothelial cell. This has been confirmed in several *in vivo* studies. The studies performed on rats after cardiac transplantation demonstrated that MMF reduced an accumulation of LFA-1-positive leukocytes in some sections of vessels and the extent of a transplant vasculopathy (40).

Some functions of activated T-lymphocytes might also be impacted by MPA. *In vivo* studies demonstrated that MPA inhibited a phytohemagglutinin or CD3 antibody-stimulated production of INFγ (41). In the earlier cited studies performed on mice injected with superantigen SEB, it was demonstrated that MMF decreased serum levels of TNFα and INFγ was stimulated by this antigen (38). That fact is of much importance taking into consideration that INFγ produced by lymphocytes Th1 was a strong activator of macrophages. Moreover, it inhibited a synthesis of collagen and stimulated macrophages to produce metalloproteinases. Hereby it contributed to the impairment of the fibrous cup covering atherosclerotic plaque, and as a consequence, the formation of plaque prone to rupture. The recently published study results, as regards to patients with a carotid artery stenosis that received MMF for 2 weeks prior to undergoing carotid endarterectomy, were quite interesting (27). In most MMF treated patients, a decreased number of activated lymphocytes T with a simultaneous increase in the number of lymphocytes Treg in sections of carotids was observed.

MPA impact on dendrtic cells

Dendritic cells are major presenters of antigen to the naive T-lymphocytes, thereby initiating their proliferation and differentiation to Th1 or Th2 cells (36). For an efficient antigen presentation, a dentritic cell maturation, as well as up-regulation of MHC molecules and co-stimulatory molecules (CD40 and CD68), are required. As known from *in vitro* results, an oxyLDL, the main pathogenetic factor of atherosclerosis, may be this type of antigen that participates in the maturation and the activation of the dendritic cells (42). The potential role of the dendritic cells in atherosclerosis was underlined by a detection of matured dendritic cells in the plaque co-localized with the activated T lymphocytes (43).

A frequent presence of the dentritic cells, close to the T-lymphocytes in the rupture prone regions, suggests that these cells presenting antigen activate the T-lymphocytes, thereby promoting plaque progression and destabilization. It is widely considered that IL-12, produced by some dendritic cells, played a key role in differentiating lymphocytes T in Th1 as regards to atherosclerosis (36).

As seen from *in vitro* studies performed on murine dendritic cells, MMF impaired their ability

to stimulate allogenic T lymphocytes and reduced the expression of CD40 and CD68 (44). Furthermore, it inhibited the LPS-stimulated production of IL-12. A similar observation was made on human dendritic cells stimulated with LPS or TNFα, which led to evidence that MPA inhibited LPS or TNFα-induced dendritic cells maturation (45). Moreover, MPA inhibited IL-12 and INFγ synthesis induced by both mediators as well as the ability to stimulate allogenic lymphocytes T. An addition of exogenous guanosine reversed the above mentioned MPA impact.

Anti-oxidative properties of MPA

As seen from the basis of knowledge, oxidative stress plays a key role in the pathogenesis of atherosclerosis (46, 47). The increase of oxidative stress is the result of a balance disorder between the production of ROS and the activity of the mechanisms responsible for their removal. An increased ROS formation may, in many ways, contribute to the development and progression of atherosclerosis. First of all, they are responsible for the formation of oxyLDL, the main pathogenic factor of atherosclerosis. ROS may impair the vasoprotective function of the endothelial cells by a rapid inactivation of NO. ROS contribute to the intracellular signalling cascades associated with the inflammatory response. They may function as second messengers by activating some kinases and transcription factors, notably nuclear factor-κB (NF-κB). Moreover, they regulate an expression of many growth factors and pro-oncogenes like c-Myc, c-Fos and c-Jun, hitherto contributing to a migration and a proliferation of smooth muscles, and further to the remodelling of the vessel wall (48). An NADPH oxidase is the principal cause of the increase of oxidative stress in atherosclerosis and is the main source of ROS in endothelial cells (49). The classical NADPH oxidase complex is composed of two cell-membrane subunits: $p22^{phox}$ and $gp91^{phox}$, which form a flavocytochrome B558. The transfer of an electron from NADPH to the molecular oxygen – generating the superoxide radical – is catalyzed by the oxidase, as a result of its activation through a translocation of the cytoplasmic regulatory subunits $p47^{phox}$, $p67^{phox}$, $p40^{phox}$ and Rac, and the binding with cytochrome B558. Some drugs, which maintain the endothelial function and prevent cardiovascular disease progression (statins for example), partly exert their beneficial effects by inactivating endothelial NADPH oxidase (50).

The previous studies showed that MPA exhibits anti-oxidative properties. The studies performed on the endothelial cells showed that MPA inhibited NADPH oxidase activation by reducing the amount of membrane bound Rac1, as well as its activity (51). As it is known, Rac1 is a GTP-dependent protein. Thus, MPA induced inhibition of the endothelial ROS formation may be caused by the depletion of cellular GTP content, which is the likely cause of the subsequent attenuation of Rac1 and NAPH oxidase activity. Another study performed on smooth muscle cells showed that an inhibitory effect of MPA on PDGF-stimulated production of ROS was only partially restored by egzogenous guanosine (29). Going forward, the authors made a further study, which showed that MPA addition into a probe containing H_2O_2 resulted in rapidly reduced hydrogen peroxide concentration. It was assumed that carboxyl, methyl and hydroxyl portions of MPA are expected to react directly with H_2O_2, resulting in a decrease of its amount.

MMF impact on development and progression of atherosclerosis as observed in *in vivo* study

By the use of rabbits fed a high-cholesterol diet, resulting in pathology relevant to the general human population, Greenstein et al. (52) performed studies in order to evaluate the impact of MMF on atherogenesis. These dieted animals simultaneously received MMF (80 mg/kg) for 12 weeks. After a completion of the test, aortic tissue section was immunohistochemically evaluated. In the MMF treated group, plaque area was significantly decreased by 46% and a number of macrophages and smooth muscle cells in this group were reduced to a comparable level of the control group. Also, Romero et al. (53), using the same model of atherogenesis, found that MMF (30 mg/kg, 12 week) reduced atherosclerosis in the aorta by over 50%. Furthermore, the MMF reduced the smooth muscle cell proliferation, and intimal macrophages and foam cell infiltration, as well as the total aortic cholesterol content. Recently, study results were accessible on the other experimental atherosclerosis model by using ApoE $(^{-/-})$ mice, treated simultaneously with 30 mg/kg of MMF and high-fat diet for 3 to 12 weeks (25). In both MMF-treated groups, the macroscopic and the histologic aortic atherosclerosis lesions was significantly reduced in comparison with the control group. Furthermore, the MMF treatment decreased the T-lymphocyte proliferation and cell number, as well as the aortic content of macrophages and their proliferation.

Recent studies have demonstrated that a systemic lupus erythematosus (SLE) was associated

with an increased risk for cardiovascular disease. A study performed on mice LDL ($^{-/-}$) with SLE-susceptible gen, demonstrated that atorvastatin attenuated atherogenesis in mice without the transplanted gen, but failed to reduce the atherosclerotic lesion size in LDL ($^{-/-}$) SLE mice, in spite of a significant reduction in serum cholesterol levels (54). A treatment with MMF or MMF + atorvastatin attenuated atherogenesis in both groups of animals.

It is worth mentioning, although it was not the scope of this publication, that MMF had beneficial effects in cardiac transplant patients beyond the suppression of tissue rejection. MMF treatment resulted in diminished intimal thickening and cardiac allograf vasculopathy compared to those of other immunosupressive drugs. A wide discussion about the results of these studies was available in publication by Gibson et al. (55).

As can be deduced from the presented overview, MMF exerts a plethora of anti-inflammatory effects that could be hypothesized to attenuate pivotal processes in atherosclerosis and it could be a viable preventive agent in people exposed to a significant risk for cardiovascular disease. It seems quite advisable to perform further studies to test this hypothesis.

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Received: 29. 01. 2013