Mycophenolic Acid Is a New Nox2 Inhibitor

Bernard Lassègue, Kathy K. Griendling

Organ transplantation owes its spectacular development in recent decades to the introduction of immunosuppressive drugs capable of preventing allograft rejection. However, vasculopathy remains a major cause of long-term graft failure; thus, a better understanding of its mechanisms is required to improve tolerance. Because endothelial dysfunction is an early manifestation of vascular disorders, Krötz et al.¹ in this issue of Hypertension, hypothesized that endothelial cells (ECs) may be directly affected by immunosuppressive treatment. Therefore, they tested 3 major classes of these drugs with distinct mechanisms of action and effects on the vasculature.

The first group includes cyclosporine A (CsA) and FK506 (tacrolimus). These are inhibitors of calcineurin, a ubiquitous calcium-dependent serine and threonine phosphatase (or PP2B). Calcineurin activates transcription factors of the nuclear factor of activated T cells family responsible for expression of surface receptors, cytokines, and chemokines in lymphocytes.² Calcineurin inhibitors thus induce immunosuppression by blocking lymphocyte proliferation. However, adverse effects include nephrotoxicity, hyperlipidemia, endothelial dysfunction, and hypertension. In the endothelium, calcineurin inhibitors can increase endothelin-1 release,² prevent dephosphorylation and activation of endothelial nitric oxide synthase (eNOS),³ and increase superoxide production.⁴

The second type of drug used by Krötz et al¹ is mycophenolic acid (MPA), an inhibitor of inosine monophosphate dehydrogenase, the rate-limiting enzyme in de novo guanosine synthesis. Lymphocytes depend on this enzyme, in contrast to most other cells, including ECs, which can also use an alternative salvage pathway for guanosine synthesis. Furthermore, whereas resting lymphocytes and other cell types express one type of inosine monophosphate dehydrogenase, activated lymphocytes express another isoform with >5 times greater sensitivity to MPA. Therefore, MPA depletes GTP preferentially in activated lymphocytes, leading to inhibition of DNA synthesis and proliferation, increased apoptosis of lymphocytes and monocytes, and immunosuppression.⁵ MPA is not nephrotoxic and does not affect lipidemia and blood pressure. It inhibits proliferation of vascular smooth muscle, experimental atherosclerosis, and graft vasculopathy. In the endothelium, favorable effects of MPA include inhibition of endothelin-1 formation, reduced expression of adhesion molecules, and enhanced prostaglandin I₂ release. However, because GTP is also required for de novo synthesis of tetrahydrobiopterin, an essential NOS cofactor, MPA also reduces NO formation. Although this effect may seem detrimental to endothelial function, it seems to preferentially affect inducible, rather than endothelial, NOS.⁵

The last immunosuppressor used by Krötz et al¹ is rapamycin (sirolimus), an inhibitor of mammalian target of rapamycin, a serine and threonine protein kinase effector of phosphatidylinositol 3-kinase involved in protein synthesis and cell cycle progression. Rapamycin blocks interleukin-mediated proliferation signals in lymphocytes, leading to immunosuppression, and also has antineoplastic activity. Rapamycin induces hyperlipidemia, but is not nephrotoxic per se, although it can increase CsA toxicity by reducing its degradation. In the vasculature, rapamycin may inhibit restenosis after angioplasty and allograft vasculopathy. However, it can lead to endothelial dysfunction by blocking vascular reendothelialization.⁶

It is apparent from this brief summary of their pharmacology that some immunosuppressors may protect, whereas others impair endothelial function. To understand the mechanisms by which these drugs exert their differential effects, Krötz et al¹ focused on the well-known ability of reactive oxygen species to inhibit endothelium-dependent relaxation by decreasing NO availability. Although eNOS itself may become uncoupled and produce superoxide, another major source of superoxide frequently upregulated in vascular disease is the reduced nicotinamide-adenine dinucleotide phosphate oxidase (Nox) family. These enzymes are composed in ECs of catalytic subunits (Nox1, Nox2, or Nox4) and regulatory subunits p22phox, p47phox, p67phox, and Rac1.⁷ In their study, Krötz et al¹ hypothesized that immunosuppressors directly affect expression and activity of some of these subunits and subsequent superoxide production in ECs.

These authors demonstrated that although rapamycin has no effect on superoxide, treatment of ECs for 6 to 24 hours with the calcineurin inhibitors CsA or FK506 is sufficient to double superoxide generation. This response was abolished by preincubation with the specific Nox inhibitor gp91ds-tat, indicating that it is entirely mediated by the oxidase. As suggested by the authors, one can speculate that calcineurin normally dephosphorylates and therefore inhibits p47phox, the subunit responsible for activation of Nox2 (and Nox1, which was not expressed here), but this remains to be determined. This result is important, because it suggests that targeting endothelial Nox2 or its activators may attenuate the major drawback of long-term treatment with calcineurin inhibitors.
In contrast, MPA significantly decreased superoxide in ECs and even reduced formation of the superoxide metabolite, hydrogen peroxide, after stimulation with a phorbol ester, a potent protein kinase C activator that stimulates Nox2 by phosphorylating p47phox. This suggests that MPA prevents Nox2 activation in ECs. Similarly, MPA was able to reduce phorbol ester–induced Nox2-mediated superoxide production in neutrophils, although in these cells this response is 10-fold higher than in ECs. The effect of MPA in ECs is not due to regulation of Nox2 or p47phox mRNA expression, but is inhibited by addition of guanosine, suggesting that it results from GTP depletion. This observation prompted the authors to further study Rac1, because this small G protein needs GTP to activate Nox2. They found that MPA inhibited both Rac1 translocation to the membrane and its activity. This inhibitory effect of MPA on EC Nox may help to explain its reported beneficial vascular activity in allografts, and because few oxidase inhibitors are currently known, it makes MPA an especially interesting experimental tool. Besides these important results, the study of Krötz et al. raises a few questions.

The authors suggest that MPA inhibited Nox in EC without reducing its expression. We agree that the mRNAs of catalytic subunits appeared unchanged. However, quantitative assays for p22phox and p67phox messages, as well as for all of the subunit proteins, would have to be carried out to definitely rule out an effect of MPA on Nox expression.

As noted earlier, the immunosuppressive effect of MPA is thought to result from preferential depletion of GTP in lymphocytes, but the present study suggests that inhibition of Nox in ECs was also due to GTP depletion. Therefore, it seems likely that the concentration of MPA used here was much higher than what occurs in vivo. Additional studies will be required to determine whether the IC50 of Nox inhibition by MPA in ECs is compatible with its plasma concentration during treatment.

The authors provide strong evidence that MPA inhibits superoxide production. However, its effect may be overestimated, because the cytochrome c assay includes a NOS inhibitor, and uncoupled eNOS is known to generate superoxide in ECs. Furthermore, MPA may also aggravate eNOS uncoupling by depleting tetrahydriobioterin in this system, although this effect was not observed in a previous study.

Inhibition of Rac1 undeniably suggests that MPA inhibits Nox, but it does not exclude other possible mechanisms of action. In fact, MPA reduced basal superoxide production in ECs twice as much as the other Nox inhibitors tested here. In the future, it may be possible to show that MPA directly inhibits Nox activity in EC homogenates and that Nox2 knockout is as effective as MPA.

In conclusion, Krötz et al. showed that the propensity of immunosuppressors to induce allograft vasculopathy seems to be correlated with their induction of Nox-mediated superoxide production in ECs, suggesting that Nox inhibitors could be used to correct this serious adverse effect of calcineurin inhibitors. MPA, in contrast to calcineurin inhibitors, reduced reactive oxygen species generation by inhibition of Rac1 and subsequent Nox2 activation in ECs. Future studies will undoubtedly determine whether this novel mechanism of action accounts for the protective effect of MPA in the vasculature.

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