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Dissecting Oxidative Stress following Ischemic Reperfusion

Numerous studies have shown that production of reactive oxygen and nitrogen species (ROS and RNS) during reperfusion causes tissue damage due in part to inactivation of mitochondrial electron transport chain proteins. The damage is paradoxical as the vasodilator, nitric oxide (NO), produced from sheer-stressed endothelial cells is a protective species that should increase blood flow. Likewise, superoxide dismutase (SOD) can convert the superoxide to hydrogen peroxide that should then also trigger vasodilation via activation of protein kinase G. It has been hypothesized that, instead, NO and superoxide react with each other to give peroxynitrite. The peroxynitrite is a potent oxidant that is known to inactivate mitochondrial proteins. It has been difficult to validate this chain of events in vivo due to the short half-lives and limited detection modalities for ROS and RNS. In this issue, the article by Xu et al. provides compelling support for this mechanistic scenario via the clever use of endothelial $NOS^{-/-}$ mice, blood flow measurements, SOD mimetics, and in vivo electron paramagnetic resonance oximetry. In the untreated control mice, there is considerable tissue damage, loss of contractile function, and damage to mitochondrial proteins. The SOD mimetic agents efficiently trap the burst phase superoxide via rapid conversion to hydrogen peroxide to produce increased blood flow, improved contractile functional recovery, and suppressed inactivation of mitochondrial proteins. Much of the protection is lost when the mice are also treated with glibenclamide, suggesting that the primary vasodilatory effect of NO and hydrogen peroxide is via activation of sarcolemmal ATP-sensitive potassium channels. Collectively, these results confirm the proposed mechanisms that underlie oxidative stress following ischemic reperfusion and suggest therapeutic options that can be used to ameliorate the subsequent tissue damage.

See article at *J Pharmacol Exp Ther* 2008, **327:**402-410.

An Unexpected Role for Peripheral Serotonin 5-HT_{2A} Receptors

The neurotransmitter serotonin, 5-hydroxytryptamine (5-HT), is a pleiotropic agent in the brain that has important roles in cognition. An important serotonin receptor in the brain, 5-HT_{2A}, is also expressed in peripheral tissues; however, functional roles for serotonin signaling outside of the central nervous system are not well defined. In this issue, the article by Yu et al. shows that functionally selective activation of the 5-HT_{2A} receptors in smooth muscle produces a profound suppression of tumor necrosis factor α (TNF-α)-mediated inflammatory responses. The authors examine the effect of a highly selective 5-HT_{2A} agonist, (*R*)-DOI [(*R*)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane], in a well established model system for the study of inflammatory responses, primary cultures of rat aortic smooth muscles. It was found that (*R*)-DOI potently suppressed TNF-α-triggered expression of inflammatory markers, such as intracellular adhesion molecule 1 (*ICAM-1*), vascular adhesion molecule 1 (*VCAM-1*), interleukin-6 expression, nitric-oxide synthase activity, and nuclear factor-κB (NF-κB) nuclear translocation. The authors made two surprising discoveries. First, (*R*)-DOI blocks TNF-α effects with an IC_{50} of 10 to 20 pM. Second, it can do so when administered hours after TNF-α treatment. Such potency is unprecedented, and the observation of efficacy, even when administered after initiation of an inflammatory response, opens the door to possible treatments of numerous pathologies, such as atherosclerosis, rheumatoid arthritis, or Alzheimer's disease. The super potency of (*R*)-DOI is also an excellent example of selective, functional receptor activation. Although other agents with similar agonist affinity for 5-HT_{2A} suppress TNF-α effects, they do not exhibit the potency of (*R*)-DOI. It is known that (*R*)-DOI preferentially activates the phospholipase-C_β protein kinase C (PKC) axis as opposed to activation of phospholipase-A_2, although the results also implicate participation of a nontraditional PKC. Thus, it remains to be seen whether the potent inflammatory effects seen here also involve nontraditional effector coupling of the G-protein-coupled 5-HT_{2A}. Regardless, the study provides important insights into 5-HT_{2A} function in the periphery while also opening the door to a new and exciting avenue for development of novel anti-inflammatory agents.

See article at *J Pharmacol Exp Ther* 2008, **327:**316-323.

T-Cell Migrations during Inflammatory Bowel Disease

Although the etiology of inflammatory bowel disease (IBD) is complex, it bears a number of similarities to other inflammatory diseases that are partly mediated by rogue T cells, such as rheumatoid arthritis and pulmonary fibrosis. In some of these other pathologies, it has become clear that the CXCL12 chemokine and its receptor, CXCR4, are important for targeting of both regulatory and effector T cells to the sites of inflammation. Interestingly, because CXCR4 is also a co-receptor for HIV entry into $CD4^+$ T cells, there have been a number of efforts to develop receptor antagonists. These efforts also have spawned new research efforts in the use of CXCR4 antagonists for treatment of other immunologic pathologies. In this issue, the article by Mikami et al. describes a study in IBD patients and in a murine colitis model that sought to determine whether the CXCL12/CXCR4 axis has a role in IBD. It was found that CXCR4 expression in peripheral T cells was increased in patients with ulcerative colitis and that the increases were correlated with disease severity. Increased CXCL12 mRNA levels were also found in colonic mucosal biopsies from IBD patients with active disease. These finding were also seen in the murine colitis model, and selective expression of knocked-in green fluorescent protein-CXCL12 was observed in submucosal lesions. Furthermore, the severity of the pathology in the murine model was ameliorated with a CXCR4 antagonist. In the mouse, it was also found that the antagonist inhibited migration of regulatory T cells into the lesions and proximal tissues. Although a number of interesting questions about the balance of Th1 versus Th2 T cell participation in mediating the inflammatory response remain, these results firmly place the CXCL12/CXCR4 axis in the etiology of IBD and that observation immediately suggests possible therapeutic interventions.

See article at *J Pharmacol Exp Ther* 2008, **327:**383-392.