Institutionen för Medicin

Structure and Function of Microsomal Prostaglandin E Synthase-1

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Abstract

The glutathione-dependent enzyme microsomal prostaglandin E synthase-1 (MPGES1) plays a pivotal role in inflammatory diseases. MPGES1 is up-regulated by pro-inflammatory cytokines in concert with cyclooxygenase (COX)-2, and the concerted action of both enzymes leads to the production of induced prostaglandin E2 (PGE2), a potent lipid mediator of inflammation, pain, and fever. Non-steroidal anti-inflammatory drugs (NSAIDs) as well as COX-2 specific inhibitors (COXIBs) are widely used analgesics that interfere with PGE2 production by inhibiting COX. However, use of these drugs is often connected with severe side effects such as gastrointestinal bleeding and cardiovascular events, respectively. This is because these drugs impair the levels of lipid mediators whose formation depends on COX but not on MPGES1. Therefore, specific inhibition of MPGES1 is regarded as a promising strategy in the treatment of inflammatory diseases. MPGES1 inhibitors are currently developed, and it is expected that these novel pharmaceuticals display less severe adverse drug effects while potently eliminating the pro-inflammatory effects of induced PGE2.

We have conducted studies on the structure and function of MPGES1 in order to understand how this enzyme and its inhibitors work on a molecular level, and the effects of MPGES1 inhibition have been investigated in several disease states. In paper I, the structure of the integral membrane protein MPGES1 was elucidated by electron crystallography. Heterologously expressed human MPGES1 was purified to apparent homogeneity and subjected to two-dimensional crystallisation in the presence of phospholipids. Elastic electron scattering induced by the protein crystals at various angles was used to calculate the three-dimensional structure at 3.5 Å, which was validated by site-directed mutagenesis of structurally and functionally important residues. MPGES1 shows a homotrimeric organisation. Reduced glutathione (GSH), an essential co-factor of MPGES1, binds between two adjacent subunits, but it is not directly accessible from the membrane. Therefore, it is probable that dynamic opening of the protein during the catalytic mechanism allows the substrate PGH2 to access the active site.

Some of the MPGES1 inhibitors potently block the activity of the human enzyme but do not show any effect on the rat orthologue. In paper II, we exploited this characteristic to investigate the inhibitor binding site of MPGES1. We could change the ability of rat and human MPGES1, respectively, to bind the inhibitor by creating chimeric enzymes. Mutation of single amino acids revealed that three residues, which are aligned at the entrance to the cleft between two adjacent subunits, have a gatekeeper function. The corresponding residues in rat MPGES1 restrict the access for competitive inhibitors to the active site. These results give direct evidence for the location of the active site and provide a model of how the substrate or competitive inhibitors of the enzyme enter the active site via the phospholipid bilayer of the membrane.

Animal models were used to investigate the effects of MPGES1 inhibition. For the studies in paper III, prostate and lung cancer cells with high constitutive or inducible expression of MPGES1, respectively, were used. Stable knock-down of MPGES1 was established in these cells, which resulted in markedly decreased enzyme activity and slower growth of xenograft tumours in nude mice. Increased apoptosis in response to genotoxic stress was observed, which could be attenuated by exogenous PGE2. This suggests a role of MPGES1 in tumour progression and beneficial use of specific MPGES1 inhibitors in cancer treatment. In paper IV, we investigated the consequences of MPGES1 deletion after myocardial infarction (MI), which was induced in MPGES1 knock-out mice and wt controls. No difference in infarction size was observed; however, MPGES1 knock-out mice showed worse left ventricular function and altered cardiac architecture 28 days after the event. In both groups prostanoid levels in the tissue were increased to a similar extend after MI, except for PGE2, which was found to be significantly lower in the knock-out mice. These results imply that MPGES1 derived PGE2 is important for cardiac tissue remodelling, and deletion of this enzyme results in worse cardiac function after MI. Therefore, use of MPGES1 inhibitors should be carefully considered for patients at cardiovascular risk.

Finally, we evaluated whether the urinary metabolite of PGE2, tetrnor-PGEM, can be used as a biomarker for inflammation. In paper V, we employed LC-MS/MS methodology to quantify tetrnor-PGEM without prior derivatisation in the urine from healthy and sick individuals. Levels of tetrnor-PGEM remained stable in healthy individuals before and after vaccination, a stimulus of local inflammation associated with mild general symptoms. In patients with fever and active disease, however, tetrnor-PGEM levels were elevated compared to healthy controls. The method is sensitive enough to detect baseline levels and will provide a helpful tool for the investigation of inflammatory diseases and the effects of MPGES1 inhibitors.

In conclusion, this thesis provides a deeper understanding of the enzyme MPGES1. The protein structure is presented, the location of the active site was identified, and a mechanism is suggested of how the substrate PGH2 or competitive MPGES1 inhibitors access the active site from the membrane. These inhibitors may be used as anti-inflammatory drugs, but also as anticancer treatment of certain types of prostate cancer. After MI, however, use of MPGES1 inhibitors might be harmful due to pivotal functions of MPGES1 derived PGE2 during heart tissue remodelling. In order to analyse depression of systemic levels of PGE2 caused by MPGES1 inhibitors an analytical method is presented for direct quantification of the urinary PGE2 metabolite tetrnor-PGEM.

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