Specific inhibition of MRP4 (ABCC4) function in platelets and effect of anti-inflammatory drugs on the transporter expression

Sophie Grammbauer, Andreas Böhm, Bernhard H. Rauch, Gabriele Jedlitschky
Department of Pharmacology, Center of Drug Absorption and Transport (C_DAT),
University Medicine Greifswald, Germany

Introduction and Objectives: MRP4 (ABCC4) has been identified as an important transporter for signaling molecules including especially cyclic AMP but also some pro-inflammatory lipid mediators in platelets and may thus represent a novel target to interfere with platelet function. This study had two objectives: to evaluate the role of MRP4 in platelet aggregation using a new selective MRP4 inhibitor and to characterize the modulation of the transporter expression by several anti-inflammatory and analgesic drugs.

Material and Methods: We determined the effect of Ceefourin 1, which was identified as a highly selective MRP4 inhibitor, on the aggregation of isolated platelets induced by various stimuli. Furthermore, we characterized the induction of the transporter by several anti-inflammatory and analgesic drugs in a megakaryoblastic cell line (M07e) on mRNA and protein level using quantitative Real-Time PCR and immunoblotting.

Results: Ceefourin 1 significantly reduced *ex vivo* platelet aggregation induced by collagen and ADP. A minor effect was observed when a thrombin receptor-activating peptide (AP1), arachidonic acid or the thromboxane receptor agonist U46619 was used as aggregating agent. In addition, the effect of several drugs on MRP4 expression was analyzed in M07e cells, which were used as model for hematopoietic cells. A significant increase of the MRP4 mRNA was observed after treatment of the cells for 48h with the classical NSAIDs (50 µM) acetylsalicylic acid (1.5 ± 0.1-fold), salicylic acid (1.7 ± 0.1-fold), ibuprofen (1.8 ± 0.2-fold), indomethacin (1.7 ± 0.3-fold) and diclofenac (1.7 ± 0.2-fold). The smallest effect was observed for naproxen (1.2 ± 0.2-fold). In addition, an induction of MRP4 was also observed for the COX-2 inhibitor celecoxib (2.0 ± 0.4-fold) and for paracetamol (1.5 ± 0.1-fold) (50 µM each) as well as for dexamethasone (1.8 ± 0.1-fold; 1 µM) (mean values ± SEM).

Conclusions: The selective MRP4 inhibitor Ceefourin 1 was able to significantly modulate platelet aggregation. Furthermore, several anti-inflammatory and analgesic drugs can induce MRP4 expression. This effect could contribute to the increased cardiovascular risk observed during treatment with these drugs.

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