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Functional Characterization of Recombinant Human Multidrug Resistance Protein 4 (MRP4/ABCC4)

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Multidrug resistance, intrinsic or acquired, presents a major complication to the successful treatment of human cancers. In the past years, many studies have been performed to identify the cellular mechanisms of multidrug resistance. One of these mechanisms is the reduction of intracellular drug accumulation, leading to the reduction of intracellular drug concentration that can reach a target. Several ATP-dependent efflux pumps have been identified to play potential roles in this mechanism of multidrug resistance. Multidrug resistance protein 4 (MRP4), a member of the ABCC subfamily, mediates multidrug resistance against several drugs used in antiretroviral and cancer therapy.

At the beginning of the present study, little was known about the physiological function and substrate specificity of MRP4. Therefore, the focus of this study was the functional characterization of human MRP4 and its localization in human tissues.

We cloned the *MRP4* cDNA, which was subsequently used to stably transfect two different mammalian cell lines. In both clonal cell lines MRP4 was localized in the plasma membrane. In addition, two polyclonal antibodies were generated, which specifically recognized human, mouse, and rat MRP4.

Using these antibodies, we localized MRP4 in the basolateral plasma membrane of human, mouse, and rat hepatocytes, and human hepatoma HepG2 cells. Recombinant human MRP4, expressed in V79 hamster fibroblasts and studied in plasma membrane vesicles, mediated ATP-dependent cotransport of reduced glutathione (GSH) or *S*-methyl-glutathione (Me-SG) together with cholyltaurine, cholylglycine, or cholate. Several monoanionic bile salts and the

quinoline derivative MK571 were potent inhibitors of this unidirectional transport. The K_m values were 2.7 mM for GSH and 1.2 mM for the non-reducing *S*-methyl-glutathione in the presence of 5 μM cholyltaurine, and 3.8 and 7.7 μM for cholyltaurine in the presence of 5 mM *S*-methyl-glutathione or GSH, respectively. Transport of bile salts by MRP4 was negligible in the absence of ATP or without *S*-methyl-glutathione or GSH. These findings identify a novel pathway for the efflux of GSH across the basolateral hepatocyte membrane into blood where it may serve as an antioxidant and as a source of cysteine for other organs. Moreover, MRP4-mediated bile salt transport across the basolateral membrane may function as an overflow pathway during impaired bile salt secretion across the canalicular membrane into bile. In conclusion, MRP4 mediates the efflux of GSH from hepatocytes into blood by cotransport with monoanionic bile salts; the latter can reenter hepatocytes via basolateral bile salt uptake transporters.

Further, MRP4 was co-expressed together with the cyclooxygenase COX-1 isozyme in human bladder, ureter, corpus cavernosum, prostate, and seminal vesicles. In contrast, MRP4 was co-expressed together with the COX-2 isozyme only in human seminal vesicles. Studies in inside-out membrane vesicles from MRP4-transfected cells demonstrated that MRP4 mediates ATP-dependent transport of the prostanoids PGE_2, $PGF_{2\alpha}$, and TXB_2. The transport of each of these prostanoids was stimulated in the presence of GSH, *S*-methyl-glutathione, or ophthalmate. The K_m values were 3.5 μM for PGE_2 in the absence of GSH and 11.9 μM in the presence of 5 mM GSH. In addition, several nonsteroidal anti-inflammatory drugs (NSAIDs) were potent inhibitors of the MRP4-mediated PGE_2 transport. However, most of the NSAIDs did not produce any inhibition of the transport when 5 mM GSH was present, with the exception of the indoleacetic acid derivatives, indomethacin and sulindac sulfide. GSH is present in most mammalian cells at millimolar concentrations and thus, under these physiological conditions, the K_m value for PGE_2 is most likely 11.9 μM and only indoleacetic acid derivatives are able to inhibit the MRP4-mediated PGE_2 transport. In conclusion, MRP4 is an export pump for prostanoids, and GSH is able to stimulate their transport, resulting in a high efficiency transport. Under these conditions, only indoleacetic acid derivatives, here exemplified by indomethacin and sulindac sulfide, show, in addition to inhibition of COX isozymes, a novel site of action by inhibiting MRP4-mediated PGE_2 transport.