FUNCTIONAL REGULATION OF SLC XENOBIOTIC TRANSPORTERS BY PDZ ADAPTOR PROTEINS: ITS UTILITY IN DRUG SCREENING
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PDZ adaptor proteins have been suggested to directly interact with PDZ binding motif located at the C-terminus of several transporters. Some of PDZ adaptor proteins have been thought to regulate function of transporter(s) in terms of stabilization at the cell-surface, regulation of subcellular localization and/or direct modulation of transporting function. This may lead to development of novel transporter-expression system(s) which include the PDZ proteins functioning as appropriate regulatory systems for the transporters. In the present study, we aimed to investigate validity of Xenopus laevis oocytes that express both transporters and the PDZ proteins as a novel in vitro assay system for assessing transporter function. Double injection of cRNAs encoding SLC transporters (OCTN1, OCTN2, PEPT1, PEPT2, OATP-A) and the PDZ proteins (PDZK1, PDZK1NHERF1) significantly increased the uptake of substrate of each transporter by Xenopus laevis oocytes, compared with that after cRNA injection for the transporter alone. The cRNA injection for the PDZ proteins alone resulted in minimal increase in uptake of the substrate, compared with that after injection of water alone. Kinetic analysis for the transport function of OCTN1, PEPT2 and OATP-A showed that PDZK1 or PDZK1NHERF1 increased the V_max without changing K_m. According to biotinylation analysis, the increase in transport function as observed for OCTN1 was associated with the increase in cell-surface expression of the transporter. Thus, the present studies suggested promising utility of transporter/adaptor- coexpressing Xenopus laevis oocytes as a novel assay tool for assessing function of several transporters in vitro.

RELATIONSHIP BETWEEN GSH CONTENTS AND Mrp2 LOCALIZATION IN MULTIPLE ORGANS
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[Purpose] The multidrug resistance-associated protein 2 (Mrp2) is ATP-dependent export pump that mediates the transport of glutathione (GSH) and a variety of organic anions. It is mainly expressed on canalicular membrane of hepatocytes and also presented in the brush border membrane of renal tubular and intestinal epithelial cells. We have reported that Mrp2 was rapidly internalized by activation of Ca^{2+}- independent protein kinase C (novel PKC) under ethacrynic acid (EA)-induced oxidative stress condition in rat liver (Sekine et al, Free Rad. Biol. Med. 2006, 2166-2174). EA is conjugated with glutathione (GSH) and, thus, excess EA induces a lack of intracellular GSH, leading to an acute oxidative stress. Intestine serves as a major portal of compounds, including drugs and toxicants. Therefore, it is one of the most susceptible tissues to xenobiotics in the body, and easily affected by temporary oxidative stress. In this study, we examined the effect of oxidative stress on the localization of Mrp2 in rat intestine.

[Method] Rat everted intestinal sacs were incubated with or without 1 mM EA for 60 min to induce oxidative stress. Localization of Mrp2 and PKCs in intestine was analyzed. Intracellular GSH level was also measured.

[Results and Discussion] In contrast to liver, EA treatment did not change the localization of Mrp2 in intestine (109 ± 9.15%), although intracellular GSH was reduced to 52% of control and novel PKCs were selectively activated. The present results demonstrated the distinct effect of oxidative stress on the localization of Mrp2 in intestine and liver in spite of similar profile of PKC family activation. It might be possible that putative scaffolding protein(s) rather than Mrp2 itself was a target of novel PKCs.