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The 2nd Croatian Symposium on Membrane Transporters (2. hrvatski simpozij o transporterima): Membrane Transporters in Toxicological and Pharmacological Research

Ivan Sabolić[1] and Tvrtko Smital[2]

Unit of Molecular Toxicology, Institute for Medical Research and Occupational Health[1], Laboratory for Molecular Ecotoxicology, Division for Marine and Environmental Research, Rudjer Boskovic Institute[2], Zagreb, Croatia

Numerous studies in the past 25 years have shown that most compounds, generated in metabolic processes (metabolic intermediates and products; endogenous compounds) or introduced orally or parenterally (nutritive compounds, medicaments, environmental toxins and other xenobiotics), do not enter or exit cells freely, by simple diffusion. Rather, their transmembrane movement is mediated by various membrane-bound proteins (transporters) localized in the cell membrane. Most membrane transporters belong to the SLC (SoLute Carriers) superfamily, which in humans encompass ~400 members grouped in 52 families (1). The functional properties and roles in cell physiology have been described for most of them, but about 40% of all SLCs are still orphans, without known substrates and biological characterization (1-5). The well-characterized members act as secondary- or tertiary-active exchangers, coupled transporters or electrochemically-driven facilitators in mediating the transport of amino acids, sugars, cholesterol, fatty acids, vitamins, inorganic ions, essential and some toxic metals, and various organic anions and cations, including drugs used in human and veterinary medicine. Depending on the electrochemical gradients of their substrates, these transporters can operate in influx or efflux mode, and are especially important in the intestine, liver and kidneys for handling the (re)absorptive and secretory processes. Due to their roles in the physiology of various organs, pathophysiology in various human diseases, and pharmacology (drug therapy, drug transport, drug-drug interactions, drug toxicity, drug development), the most characterized are the organic anion (OATs), cation (OCTs) and zwitterion transporters (family SLC22) (2-7), multidrug and toxins extruders (MATE/SLC47) (4, 8), and sodium-independent (GLUTs/SLC2) and sodium-dependent (SGLTs/SLC5) glucose transporters (9-11). Functional defects or malfunctions of the specific SLC transporters due to either inactive gene, diminished gene expression, or single nucleotide polymorphisms (SNPs)-dependent gene variants have been implicated in various human conditions and diseases, such as primary carnithine deficiency, rheumatoid arthritis, inflammatory bowel disease, cisplatin-induced toxicity, urolithiasis, mood-related disorders, diabetes, diminished renal secretion of organic anions and cations, variations in liver handling and renal secretion of organic cations, variations in drug pharmacokinetics and therapeutic efficiency, occurrence of drug-drug interactions and drug-induced organ toxicity, etc. (2, 4-6, 8).

Another important superfamily of membrane transporters comprises several families of the primary-active ABC (ATP Binding Cassette) members that operate as the ATP-driven efflux pumps (12). Most renown of these transporters are multidrug resistance protein 1/P-glycoprotein (MDR1/P-gp/ABCB1), various MRPs (multidrug resistance associated proteins/ABCC family), and breast cancer

Correspondence to: Dr Ivan Sabolić, Unit of Molecular Toxicology, Institute for Medical Research and Occupational Health, Ksaverska c. 2, Zagreb, HR-10000. E-mail: *sabolic@imi.hr*

resistance protein (BCRP/ABCG2). Their main physiological role is elimination from the cells and body various cationic and some anionic metabolic products which are too large to be substrates for the SLC transporters. In humans, P-gp is located in the intestinal and renal proximal tubule cells (apical membrane), hepatocytes (canalicular membrane), brain capillaries (endothelial cells, being an active component of blood brain barrier), and placental syncytiotrophoblast (apical membrane). As an efflux pump, P-gp exports various xenobiotics from these tissues, thus protecting them from drug toxicity but also causing resistance to various therapeutic drugs. MRPs are more ubiquitous, and also mediate multiple drug resistance to cancer therapy, whereas BCRP is particularly responsible for poor effects of breast cancer chemotherapy. In addition, variable effects of drug therapy, drug-drug interactions and drug-induced organ toxicity in different individuals, as well as some specific genetic diseases may be related to the gene polymorphism of these transporters (12-15). Recent studies have indicated that some transporters of the ABC families (and also from the SLC families) can be therapeutic or diagnostic targets in specific diseases.

In vitro studies on identification and detailed molecular characterization of various membrane transporters, followed or complemented by the *in vivo* evaluation of its physiological and/or defence role in established animal research models, represents an important and well-established research area in pharmacology and toxicology. However, although the orthologs of the above-mentioned human membrane transporters have been detected and extensively studied in rodents and other experimental animals, in many aspects their translational relevance to human transporters appear to be questionable due to species differences in their characteristics and expression (16).

From the viewpoint of environmental toxicology, however, membrane transporters are important as integral elements of the cellular defence mechanisms involved in processing of endo- and xenobiotics in other animals. For example, aquatic organisms are continuously exposed to a variety of environmental chemicals originating from various anthropogenic sources, and evolutionary conserved cellular defence mechanisms that mediate the overall bioavailability and potential toxicity of xenobiotics have been shown to be similar to those described in mammals. They include the regulation of absorption by uptake (SLC) transporters, the well-documented biotransformation by phase I and phase II enzymes (17), and finally the more recently demonstrated active efflux of xenobiotics by the ABC proteins (18). Furthermore, recent ecotoxicological studies have shown that the expression levels of some membrane transporters in specific organs of wild animals may be relevant indicators of the environmental pollution (17, 19). Finally, it has been demonstrated that the transport activity of ABC transporters can be sensitive to the presence of environmental chemicals which can act as specific inhibitors (18, 20, 21). These chemicals have the potential to block the ABC transporters' mediated active efflux of xenobiotics, causing a significant increase in their intracellular accumulation. From an ecotoxicological viewpoint, the main consequence of this type of inhibition is an increase in sensitivity of aquatic organisms toward the many xenobiotics typically present in aquatic environments.

Studies on the role of membrane transporters in ecotoxicological context are rare in comparison to efforts in mainstream toxicology, especially regarding research on SLC transporters. Despite their physiological importance and well documented role in the cellular detoxification in mammals, none of these transporters have been characterized in non-mammalian organisms before recent studies done on zebrafish (22-24), and the knowledge about polyspecific uptake transporters in non-mammalian species remains modest. Consequently, apart from new insights relevant for our fundamental understanding of the evolution, biological role and (eco)toxicological significance of polyspecific membrane transporters, this type of research is a necessary prerequisite for a better understanding and ultimately prediction of toxicity, and should enable a more reliable risk assessment in the context of both human and environmental toxicology.

All these findings in the fast-growing field of membrane transporters led us to organize the 1st Croatian symposium on membrane transporters in 2013, which largely dealt with the physiological aspects of transporters in various experimental models performed by Croatian scientists in the Croatian research institutions. The presentations were held in Croatian. The present symposium was designed to be more international, with contributions from a few renown scientists from abroad, with all presentations in English and focused on a few selected transporters with a view toward the increasingly important pharmacological and (eco) toxicological impacts.

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