Progress in the Molecular Characterization of Hepatobiliary Transporters

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Abstract
Over the last 25 years, our understanding of the driving forces for hepatobiliary elimination and knowledge of the molecular basis of uptake and efflux transport in hepatocytes have undergone fundamental changes. This refers to bile acids and many other endogenous substances as well as to drugs that are eliminated on the hepatobiliary route. In this development, not only molecular cloning, functional characterization, and localization of transporters were decisive, but also the discovery of hereditary mutations in genes encoding sinusoidal uptake transporters and canalicular export pumps in humans and rodents. Uptake by passive diffusion and elimination into bile driven by the electrochemical gradient are no longer considered relevant for hepatobiliary elimination in the intact organism. Furthermore, insights into the relative roles of uptake transporters and unidirectional ATP-driven efflux pumps were obtained when we established double-transfected polarized cell lines stably expressing, as an example, the hepatocellular uptake transporter OATP1B3 and the apical (canalicular) efflux pump multidrug resistance protein 2 (MRP2; ABCC2). ATP-dependent efflux transporters localized to the basolateral (sinusoidal) hepatocyte membrane, particularly MRP3 (ABCC3) and MRP4 (ABCC4), pump substances from hepatocytes into sinusoidal blood. Bile acids are substrates for human MRP4 in the presence of physiological concentrations of reduced glutathione, which undergoes co-transport. These efflux pumps have been recognized in recent years to play an important compensatory role in cholestasis and to contribute to the balance between uptake and efflux of bile acids and other organic anions during the vectorial transport from blood into bile. This sinusoidal efflux not only enables subsequent renal elimination but also facilitates the re-uptake of substances into neighboring hepatocytes located more centrally and downstream in the sinusoid.

Introduction

The driving forces and the specificity of the hepatobiliary elimination of organic anions, such as bile acids, bilirubin and its conjugates, glutathione conjugates, or leukotrienes, were poorly understood until the end of the 1980s. Discovery and cloning of uptake transporters in the human hepatocyte sinusoidal membrane and of ATP-dependent unidirectional efflux pumps in the canalicular and in the sinusoidal membrane have now enabled a molecular characterization of key components of hepatobiliary transport. In addition, many pathophysiological
conditions, such as cholestasis and some types of hyperbilirubinemia can now be interpreted on a molecular level. Importantly, the discovery of hereditary mutations leading to a deficiency of the respective transport protein in human liver has greatly supported, over the recent 20 years, the characterization of the role of certain transporters in human hepatobiliary elimination and its disorders [1–7].

The interaction of uptake transporters and ATP-dependent efflux pumps during the vectorial transport across hepatocytes has been studied by the stable expression of recombinant human hepatocyte transporters in polarized double-transfected or multiple-transfected cell lines [8, 9]. Mathematical modeling of transport in these polarized double-transfected cells has indicated that uptake as well as unidirectional efflux transporters are required for transcellular transport at a high rate [10].

The localization of multidrug resistance protein 3 (MRP3) [11] and MRP4 [12] in the hepatocyte sinusoidal membrane was initially controversial. In the meantime, these transporters provide a molecular basis for our understanding of the compensatory basolateral efflux of bile acids and many conjugates under cholestatic conditions [13]. Functionally, this basolateral efflux has been observed early and noninvasively by positron-emission tomography in rats using the physiological leukotriene 11C-N-acetyl-leukotriene E4 [14], in the isolated perfused rat liver using dibromosulphophthalein [15], and recently by positron-emission tomography using the bile acid analog 11C-cholylsarcosine in pigs [16] and humans [17].

The Uptake Transporters for Organic Anions in the Human Hepatocyte Sinusoidal (Basolateral) Membrane

Following the cloning of the sodium-dependent bile acid transporters (NTCPs) in rat [18] and human [19] liver by Peter Meier’s group in Zürich, 3 sodium-independent uptake transporters for organic anions were cloned and localized in human hepatocytes: OATP1B1 [20–22], OATP1B3 [23], and OATP2B1 [24]. These human OATPs differ markedly from rodent OATPs [25]. OATPs mediate the uptake into hepatocytes of a broad spectrum of organic anions, including anionic conjugates, leukotrienes, bile acids, and many anionic drugs such as statins [25, 26]. OATPs seem to mediate exclusively the uptake of organic anions into cells in an exchange for bicarbonate, which leaves the cell [27]. Although OATP1B1 and OATP1B3 are capable of mediating the uptake of bile acids into hepatocytes, the predominant route for bile acid uptake is via NTCP. This is convincingly demonstrated by the extremely elevated plasma levels of conjugated bile acids in human hereditary NTCP deficiency [7]. Whereas OATP1B1 and OATP1B3 are predominantly, if not exclusively, expressed in human hepatocytes, OATP2B1 is expressed, in addition to hepatocytes, in many other tissues [26]. This should be taken into account when certain drugs, such as fluvastatin, are transported by OATP2B1, in addition to OATP1B1 and OATP1B3 [28]. Another aspect to be noted is the lack of OATP1A2 in the human hepatocyte sinusoidal membrane [25]. However, OATP1A2 is strongly expressed in the luminal membrane of the blood-brain barrier [29] and may contribute to the uptake of drugs and several endogenous substances, such as unconjugated bilirubin [30], into the brain.

The ATP-Dependent Export Pumps for Organic Anions in the Human Hepatocyte Canalicular Membrane

The hepatocyte canalicular membrane contains a set of primary-active ATP-dependent export pumps responsible for the steep uphill transport from hepatocytes into bile of glucuronides, bile acids, glutathione conjugates, and other organic anions. The discovery of ATP-dependent transport of glutathione conjugates and glucuronides in the canalicular membrane [31, 32] was followed by the cloning and characterization of rat and human Mrp2/MRP2, ABCC2; (for review see [33]). Discovery of the ATP-dependent transport system for bile acids in rat canalicular membranes [34–36] preceded the cloning and characterization of the human bile salt export pump (BSEP; ABCB11; [4, 5, 37]). An additional efflux pump for organic anions, particularly for sulfoconjugates [38], has been localized to the human canalicular membrane [39] and is termed breast cancer resistance protein (BCRP, ABCG2; [40]). MDR1 P-glycoprotein (ABC1B), an efflux pump for organic cations that was localized already in 1987 in the human hepatocyte canalicular membrane [41], is a minor contributor to bile flow but serves in the elimination of several cationic drugs [42] and cationic natural products such as berberine [43]. For the elimination of many organic cations into bile, the multidrug and toxin extrusion transporter, MATE1 encoded by the SLC47A1 gene [44, 45], seems to be more important than MDR1 P-glycoprotein.

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The ATP-Dependent Export Pumps for Organic Anions in the Human Hepatocyte Sinusoidal (Basolateral) Membrane and Their Compensatory Role in Cholestasis

The molecular basis of the efflux of bile acids and other organic anions from hepatocytes into blood under normal and cholestatic conditions was only functionally understood until recently. We have localized 3 members of the multidrug resistance protein subfamily specifically to the basolateral membrane of human hepatocytes: MRP3 (ABCC3) [11], MRP4 (ABCC4) [12], and MRP6 (ABCC6) [46]. These basolateral MRPs are unidirectional pumps releasing organic anions into sinusoidal blood. The transport activity of human MRP3, contrary to rodent Mrp3, is rather low with bile acids [47], but human MRP3 is an efficient transporter for monoglucuronosyl and bisglucuronosyl bilirubin [48]. The substrate specificity of human MRP4 is broad [49] and includes the cotransport of bile acids with reduced glutathione [50] as well as leukotrienes [51]. MRP4 may be considered to be the first important basolateral efflux pump for bile acids in humans. MRP6 has been identified recently as a basolateral efflux pump for nucleotides, particularly for ATP, leading to extracellular pyrophosphate after hydrolysis by ectoenzymes [52].

A compensatory function of members of the MRP family in the basolateral hepatocyte membrane, particularly when the activity of canalicular efflux pumps is impaired, was first proposed in 1996 on the basis of immunodetection of MRP isoforms using an antibody with broad specificity [2]. This concept has been supported by the upregulation of the rodent Mrp3 protein in Mrp2-deficient and cholestatic livers [53, 54], and by the specific detection of basolateral MRP3 [11] and MRP4 [12] in human and rat hepatocytes. However, it should be em-
phesized that the rate of upregulation and the relative contribution of basolateral bile acid efflux is much higher in rat than in human hepatocytes [55]. A strong upregulation of human MRP4 was demonstrated in patients with hereditary deficiency of BSEP (ABCB11) [56]. This is in line with the function of MRP4 as a basolateral bile acid efflux pump [12, 50], and enables a better understanding of the enhanced urinary excretion of bile acid conjugates, which are formed in hepatocytes, under conditions of cholestasis [57–59].

The concept of the function of the basolateral efflux pumps of the hepatocyte as a compensatory mechanism in cholestasis also raises the question of a cycling or re-entry of bile acids and their conjugates into hepatocytes along the sinusoid [50]. Such a cycling or re-entry of bile acids may be important also under physiological conditions. Efflux into sinusoidal blood via MRP4, and possibly also via OSTA/β, is required when the rate of sinusoidal bile acid uptake into hepatocytes via NTCP, OATP1B1, and OATP1B3 exceeds the capacity of the BSEP in the canalicular membrane.

Insights from Polarized Cells Stably Expressing Recombinant Hepatocyte Transporters

Vectorial transport across polarized cells controls the half-life of many endogenous and xenobiotic substances in the mammalian organism and contributes to their detoxification and terminal elimination. We have constructed double-transfected polarized cells comprising a recombinant uptake transporter and an apically localized recombinant efflux pump [8]. Initially, we used stably expressed human hepatocyte transporters, which we had originally cloned and localized, namely, OATP1B3 (formerly OATP8) for uptake and MRP2 (ABCC2) for efflux, to investigate transcellular transport in polarized MDCKII cells [8]. Additional transporter combinations and substrates were subsequently published by Yuichi Sugiyama’s group in Tokyo [9, 60], many other laboratories [61–63], as well as by our own group in Heidelberg [28, 43, 64–66]. These double-transfected polarized cells have clearly established that both uptake and efflux transport proteins are necessary to obtain the manifold increase in the rate of transcellular transport comparable to the transporter interaction and transport efficiency observed in the intact liver [8, 10]. Moreover, these double-transfected polarized cells provide valuable tools for the identification of transport substrates, transported drugs, and transport inhibitors [8].

An overview of the current state of the transporters in the human hepatocyte plasma membrane is given in figure 1.

Disclosure Statement

The author declares no conflict of interest related to this review.

References


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