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BMCL Digest

Transporter-mediated tissue targeting of therapeutic molecules in drug discovery

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ABSTRACT

Tissue concentrations of endogenous chemicals and nutrients are in large part regulated by membrane transporters through their substrate specificity and differential tissue distributions. These transporters also play a key role in the disposition of therapeutic agents thus affecting their efficacy and safety profile. A transporter-mediated tissue targeting strategy, where the structural features recognized by the transporters are incorporated into the therapeutic molecule, is emerging as an effective approach in drug discovery. In this digest, we review this phenomenon and highlight recent cases in the design of liver and kidney targeted drug molecules.

The efficacy and safety profile of drugs are dictated by their target selectivity, pharmacokinetics, tissue distribution and free drug concentrations in targeting organs. Many efficacious compounds fail in development due to adverse responses derived from exposure in non-targeted organs.[1] Tissue-selective delivery of pharmacological agents is emerging as an effective approach to enhance the efficacy and improve the therapeutic window in drug discovery. Delivery of active molecules to targeted organ by tissue-targeted carrier including antibody, peptide, aptamer, micelles or nanoparticles, has been explored over the years.[2–6] The subsequent diffusion of the released active molecule into systemic circulation has limited the application of these platforms. In addition, the identification of carriers with appropriate ADME properties remains a major hurdle in the field.

Tissue concentrations of endogenous chemicals and nutrients are in large part regulated through their structure-dependent interactions with the networks of influx and efflux membrane transporters. Local disposition of chemicals is also directly affected by protein binding, passive partitioning into lipid and membrane matrix, and sequestration by lysosomes in their acidic pH environment. Over 400 membrane transporters are involved in the formation of the traffic networks through their differential distribution throughout the body. The ATP-binding cassette transporters (ABC-transporters) use the energy from ATP hydrolysis to direct their substrates movement. Most solute carrier protein (SLC) transporters mediate the influx of their substrates either by facilitated diffusion as a channel, or by active transport against concentration gradient with coupled ion exchange as driving force. Over the years, knowledge of the identity, substrate specificity, and the distribution profile of these influx and efflux transporters across species has increased considerably.[7,8] A transporter-mediated tissue-targeting strategy, where the structural features recognized by the transporters are incorporated into the therapeutic molecule design, is emerging as an effective approach in drug discovery. In this digest, we review this phenomenon and highlight recent cases in the design of liver and kidney targeted drug molecules.

Liver targeting: Orally ingested drugs reach the liver after absorption in the intestine mainly via the portal blood before their distribution into the systemic circulation. A high blood flow (~1 L/min in human) carries nutrients and other chemicals into the liver for metabolic processing and/or re-distribution to other organs. A number of organic cation and anion transporters are highly expressed on intestinal enterocytes and hepatocytes and mediate the uptake of these nutrients through their selective and overlapping substrate specificity. Organic cation transporter 1 (OCT1), organic anion transporter 2 (OAT2), sodium taurocholate co-transporting polypeptide (NTCP), and organic anion transporting polypeptide protein 1B1 (OATP1B1) and 1B3 (OATP1B3) are highly expressed in liver. The existence of these abundant and tissue specific transporters provides opportunities to explore liver-targeted drug design, utilizing the core structure recognition elements on their substrates. Expression analysis of key drug transporters from

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Table 1
Major OATP transporter distribution in human[8,9]

Transporter	Tissue distribution
OATP1A2	Brain and retina
OATP2B1	Liver, kidney, intestine, lung, placenta
OATP1B1	Liver
OATP1B3	Liver
OATP4C1	Liver, kidney

Table 2
Transporter specificity of some statins

Compounds	Transporter specificity	Refs.
Atorvastatin (**1**)	OATP1B1	15
Pravastatin (**2**)	OATP1B1, OATP1B2	16
Rosuvastatin (**3**)	OATP1B1, 1B3, 2B1, NTCP	17
Pitavastatin (**4**)	OATP1B1, OATP1B3	18

mouse, rat, dog, monkey and human has revealed the presence of both overlapping and species-specific distribution patterns (Table 1). Therefore the capacity of these common transporters needs to be further evaluated to facilitate the design of tissue-targeted molecules with well understood cross species difference and low potential risk of drug-drug interaction.[8,9]

Statins: Statins are a class of competitive inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme-A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis highly expressed in liver. Earlier systemically distributed HMG-CoA reductase inhibitors reduced cholesterol but also caused a number of dose-limiting adverse responses in animal studies. Drug leakage into the muscle is thought to be associated with the incidence of myopathy.[10,11] Statins used in the clinic are liver-targeted molecules, with the restricted tissue disposition feature essential for their clinic tolerability. Statins are substrates of OATPs that are highly expressed in the liver and intestines. Most OATP substrates are anionic amphipathic molecules as exemplified by bile acids. The beta-hydroxy heptanoic acid moiety, shared among many statins (Fig. 1), is the core structural element for the reversible binding in the active site of HMG-CoA reductase. It also serves as the homing moiety for liver-targeting through its interaction with the OATP transporters. With a similar unbound free fraction of 4% in plasma and liver, the liver concentrations of atorvastatin (**1**) in rats reached 50–60 and 160–420-fold higher over that in plasma and muscle respectively.[12] Altered plasma drug exposure has been observed for some statins in subjects with OATP1B1 polymorphism or upon the co-administration of other OATP1B1 inhibitors, implicating its dominant contribution in regulating their dispositions.[13,14] These observations also reveal the importance of utilizing multiple transporter networks in the design of tissue-targeted molecules to mitigate the risk of drug–drug interaction.

In addition to OATP1B1, other OATPs with an overlapping substrate recognition feature also contribute to the uptake and enrichment of many statins in liver (Table 2). After initial absorption in intestine and uptake by liver via the portal blood, pravastatin (**2**) and pitavastatin (**4**) are excreted into bile and reabsorbed in the gut, forming an entero-hepatic recirculation circuit. This recycling process, on top of the transporter-mediated direct uptake in hepatocytes, contributes significantly to their enhanced liver exposure.[19] Studies of the lactone (prodrug) and acid forms of statins (**5** and **6**, Fig. 2) demonstrated the interconversion between the two forms influences their transporter interaction and contributes to their pharmacokinetic difference.[20]

Glucokinase activators: Glucokinase (GK) converts cellular glucose to glucose-6-phosphate for subsequent metabolism and serves as a key regulator of glucose homeostasis. Small molecule-based allosteric activators of GK in liver promote hepatic glucose uptake, reduce hyperglycemia, and they may represent promising opportunity in the treatment of type 2 diabetes (T2D).[21] However, earlier systemically distributed GK-activators suffered from a dose-limited hypoglycemia response, from excessive GK activation in pancreatic β-cells leading to insulin overproduction.[22,23] To mitigate the risk of the excessive GK activation in pancreas, liver-targeted carboxylic acid-containing GK-activators were designed with a preferential activation of the enzyme in hepatocytes over that in pancreatic β-cells. Besides a low passive permeability to minimize distribution into extra-hepatic tissues, these molecules were optimized as OATP substrates for enhanced liver uptake. These efforts resulted in the discovery of hepato-selective GK-activators.[24] Replacing the methyl with the carboxylic acid in the systemically distributed **7** (Fig. 3) led to the liver-selective compound **8**, a potent GK-activator with a >50-fold liver-to-pancreas ratio of tissue distribution in rodent and non-rodent species. In contrast to compound **7**, compound **8** reduced fasting and postprandial glucose with no hypoglycemia in diabetic models, leading to its selection as a development candidate for treating diabetes.[25]

Stearoyl-CoA desaturase-1(SCD1) inhibitors: Another recent example of liver-targeted molecules is the design of SCD1 inhibitors by scientists at Merck Frosst Laboratories. SCD1 is a long chain fatty acyl-CoA desaturase highly expressed in liver, and mainly responsible for the de novo production of oleic acid. Elevated SCD1 activity is associated with obesity and several forms of cancers. SCD1 inhibition reduces de novo lipogenesis and improves insulin sensitivity in obese diabetic models. SCD1 inhibitors represent promising agents in the treatment of several disorders including type 2 diabetes, nonalcoholic steatohepatitis (NASH) and cancers. However, systemic SCD1 inhibition also causes dose-limiting adverse responses of dry skin and hair loss in association with excessive local lipid depletion.[26] To mitigate these adverse events while maintaining therapeutic effects, liver-selective SCD1 inhibitors were designed using a set of complementary assays to guide the optimization of structure–activity-relationship (SAR). First, compounds with enhanced potency in hepatocytes over HepG2 cells were selected in a direct target-engagement based cell assay,

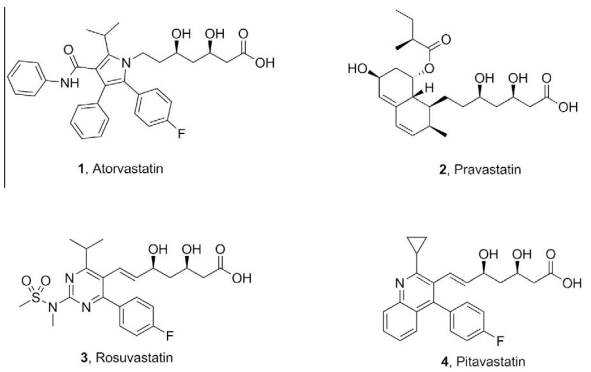

Figure 1. Statins.

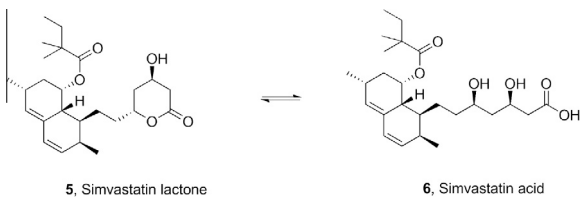

Figure 2. Simvastatin lactone and simvastatin acid.

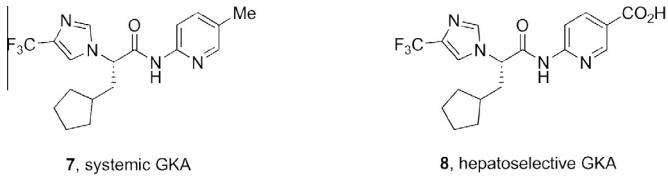

Figure 3. Glucokinase activators from Pfizer.

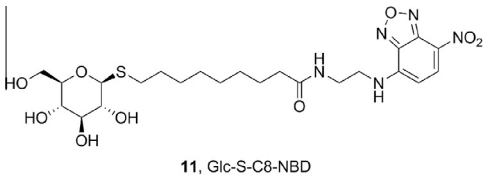

Figure 5. Renal selective alkylglucoside.

leveraging the high preservation of drug transporters in fresh hepatocytes. Concurrently, compounds with low passive cell permeability were selected to minimize diffusion-mediated non-selective tissue disposition. In addition, a tracer-based direct target-engagement assay in liver was used to guide the SAR optimization in vivo. This effort led to the identification of MK-8245 (Fig. 4 and **9**), a tetrazole acetic acid-based liver-targeted SCD1 inhibitor. Compound **9** is a substrate of OATP1B1 and OATP1B3, with a liver-to-plasma distribution ratio of >10-fold and a liver-to-skin ratio of >30-fold across a number of species including mouse, rat, dog and monkey. In contrast to the systemically distributed compound **10** which caused severe hair loss, skin and eye abnormalities within 7 days of oral treatment in DIO mice, compound **9** did not elicit any of these adverse responses after 4-weeks of daily dosing at efficacious doses, leading to its selection as a clinic candidate for the treatment of diabetes and dyslipidemia.[27,28]

In summary, both liver-targeted enzyme inhibitors and allosteric activators have been successfully designed by embedding the core structure recognition elements of OATP substrates. This approach has emerged as a practical strategy to enhance liver exposure over other organs for enhanced efficacy or improved therapeutic window.

Kidney targeting: Kidney is the key organ involved in the balanced regulation of nutrients, water and electrolytes in the body through filtration, secretion and reabsorption. When blood enters the afferent arteriole, approximately 20% of the volume is filtered by the glomerulus into the nephron. The rest returns to the peritubular capillaries and back to the trunk blood. In a healthy adult, the glomerular filtration rate (GFR) is about 180 L/day. Substances with low plasma protein binding are filtered into the renal tubule including salts, glucose, amino acids, urea, and other metabolites. Most electrolytes, amino acids, glucose and peptides are reabsorbed during their transit in the tubule space through active transporters or osmotic gradient, with less than 1% of the total fluid passing through kidney is excreted as urine. Tubular secretion, the process of transferring chemicals from peritubular capillaries to the tubular lumen involves multiple transporters including OCT, OAT, OATP, sodium phosphate transporter (NPT) and ABC-transporters.[29] Despite an increasing knowledge of their expression and substrate specificity, little has been published on how to leverage these transporters for targeted delivery of molecules to kidney.

During earlier searches for a kidney-targeting platform, alkyl glucosides were found to be effective vectors with an optimal chain length from 7 to 11 carbon units, with the fluorescent analog of Glc-S-C8-NBD (Fig. 5 and **11**) being 200-times more concentrated in kidney over plasma.[30,31] The origin of this chain length dependency, which may implicate the involvement of a transporter-mediated process, remains unresolved. Interestingly, these alkyl glucosides also inhibited a sodium glucose co-transporter (SGLT)-like activity in kidney brush border membrane vesicle (BBMV).[32] As far as renal targeting using a sugar moiety is concerned, its discovery may trace back to Phlorizin (Fig. 6 and **14**) which contains an O-glucoside moiety. It was first isolated in 1835 from the bark of apple trees and only recently identified as a dual SGLT1 and SGLT2 inhibitor.

Among the multiple glucose transporters in kidney, SGLT2 is a high capacity/low affinity (K_m ~2 mM) transporter highly

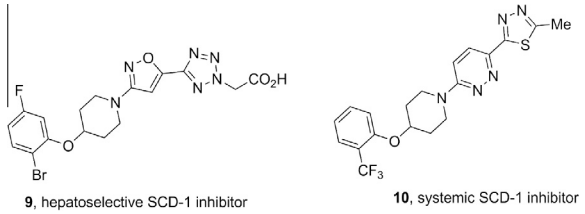

Figure 4. SCD-1 inhibitors.

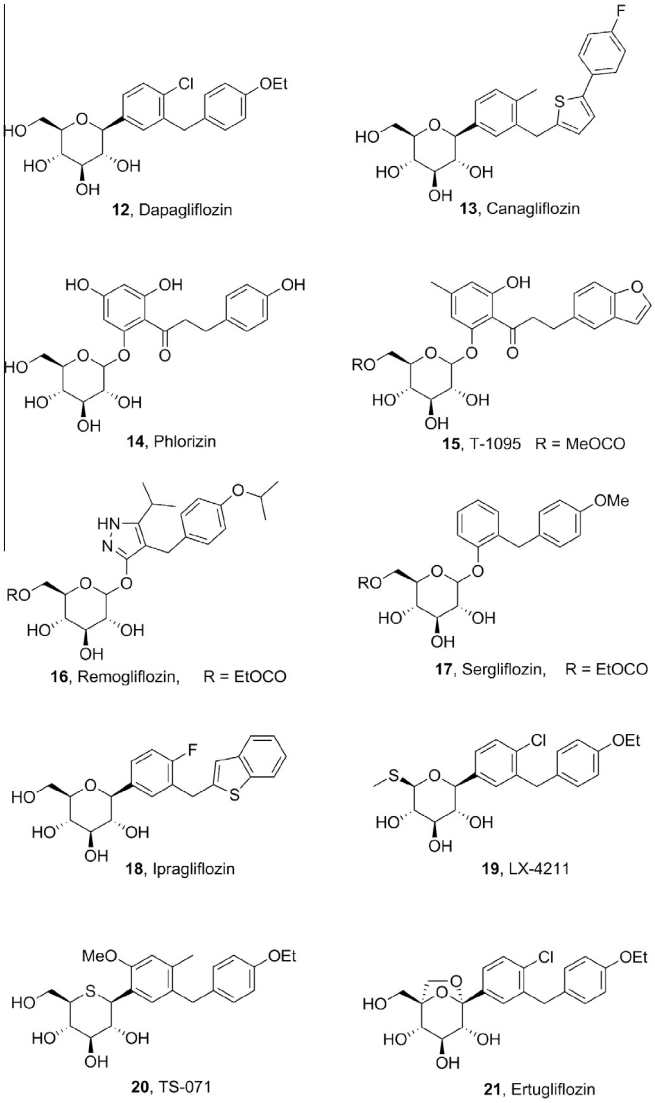

Figure 6. SGLT2 inhibitors.

expressed in the apical membrane of the renal proximal tubule (S1 segment).[33,34] Approximately 90% of glucose post filtration in rat is reabsorbed through SGLT2 and the rest of glucose is reabsorbed via the high affinity SGLT1 glucose transporter in the distal tubule segment. Genetic deletion of SGLT2 leads to daily loss of up to 150 g glucose without apparent side effects, supporting the development of SGLT2 inhibitors for treating diabetes.[35] By suppressing glucose reabsorption in the proximal tubules and enhancing glucose loss in the urine, inhibition of SGLT2 lowers blood glucose and also reduces overall caloric load. Dapagliflozin (**12**), Canagliflozin (**13**) and Ipragliflozin (**18**) are potent competitive SGLT2 selective inhibitors with IC_{50} of 5 nM, 2 nM and 7 nM, respectively.[36,37] The glucoside moiety, shared among SGLT2 inhibitors, has been recognized as the core structural element in eliciting the high affinity interaction with SGLT2 transporter. The prodrug T-1095 (**15**), Remogliflozin (**16**) and Sergliflozin (**17**) share the same O-glucoside motif as in Phlorizin (**14**). Modifying the aryl region of these O-glucosides can improve selectivity over other glucose transporters (SGLT1, SGLT3, SGLT6, GLUT1, GLUT12) as exemplified by Remogliflozin and Sergliflozin.[38–42] The O-glucoside linkage can be hydrolytically labile in vivo via a potential β-glucosidase-mediated cleavage. Replacing it with the C-glucoside linkage overcomes this potential liability which leads to the discovery of *C*-glucosyl SGLT2 inhibitors, Dapagliflozin (**12**) and Canagliflozin (**13**). These two drug molecules reduce HbA1c and cause weight loss in obese diabetic patients. Since their mode of action in lowering glucose is independent on the degree of insulin deficiency or insulin resistance, SGLT2 inhibitors are complementary to other anti-diabetic medications. Other C-glucosides, LX-4211 (**19**), TS-071 (**20**) and Ertugliflozin (**21**), have also been nominated for clinical evaluation.[43–46]

In spite of an extensive SAR knowledge on SGLT2 inhibitors, there is little literature on their renal disposition in relationship to the local target engagement. Some SGLT2 inhibitors were reported to maintain high drug concentrations in kidney. For example, TS-071 (**20**) has a kidney-to-plasma ratio of 24–35 folds with its main elimination via liver metabolism in rat.[47] However, the underlining mechanism that induces enhanced renal disposition for this type of molecules remains unknown. High expression level of SGLT2 in renal proximal tubule surface is one of the factors, inferring from the mouse kidney membrane proteins containing ~15 pmole of high affinity ^{3}H-dapagliflozin binding site.[48] In addition to the renal enrichment profile, several SGLT2 inhibitors share a low renal clearance rate across species.[49,50] Despite of low renal clearance, effective urinary glucose excretion can be achieved after a single dose of these SGLT2 inhibitors which reflects substantial local target engagement. These phenomena are suggestive of extensive retention or recirculation of the drug molecules during the tubule transition.

In view of these evidences, it is tempting to propose the presence of a transporter-mediated drug enrichment process for the glucosyl-containing molecules in the renal tubular space of kidney as illustrated by the Scheme in Figure 7. In this scheme, transporter [**A**] represents different uptake transporters of glucose (SGLT2, SGLT1, etc) that are highly expressed on the renal tubule epithelium. Inhibitors from glomerular filtration directly bind to transporter [**A**] with high affinity to block transcellular glucose flux. These drug molecules passing down the tubule could be re-absorbed by putative transporter [**B**] into the peri-tubular capillaries and possibly recirculate back to tubule fluid after systemic circulation. This transporter mediated reabsorption in couple with recirculation process may contribute significantly to the high drug level in kidney. The glucoside structure of the kidney-targeting molecules might be a common structural motif for transporter recognition. It would be interesting to test if the chain length dependency of the renal enriched alkyl glucosides (i.e., compound **11**) is partially coupled to this process.

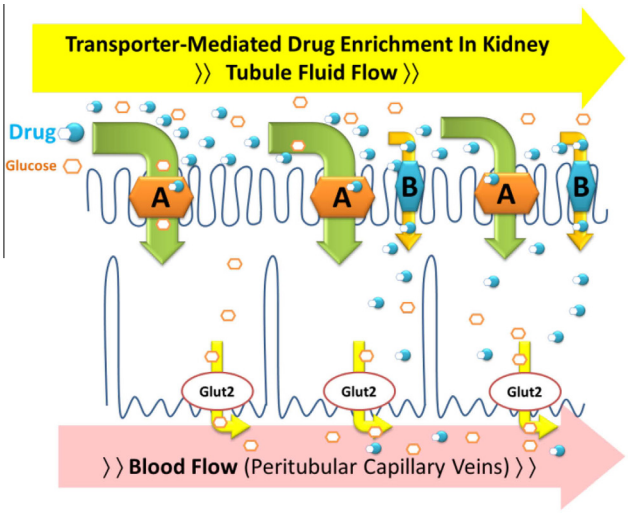

Figure 7. Proposed scheme illustrating the key components in transporter-mediated drug enrichment in kidney. The different uptake transporters of glucose on the tubule epithelium (SGLT-2, SGLT-1, etc) are represented as [**A**]. The putative transporters to take up glucoside-like structure are represented as [**B**]. In post-glomerular tubule fluid, the filtered drugs (with high affinity to glucose-transporters) will accumulate in tubular lumen through binding to highly expressed transporter [**A**]. Meanwhile active uptake of drug molecules along the tubule by transporter [**B**] enables local enrichment of drug concentration.

Prospective and summary: In summary, selective tissue targeting as a systematic approach to improve efficacy and reduce adverse effects has been widely recognized. Some principles, based on the accumulating knowledge during the development of liver-targeted compounds, are being actively explored for the design of compounds with preferred tissue distribution. Different classes of liver-targeted drugs have been developed to improve therapeutic window through specific substructure recognition by the liver-enriched high capacity transporters. Analysis of recent literature suggests that the kidney disposition of glucosyl-containing SGLT2 inhibitors may be impacted by the active transport via renal SGLT-like transporters, despite the lack of direct evidence. Here we propose that a transporter-mediated local enrichment process might occur in the renal tubular space for some transporter-substrate analogs, leading to an enhanced renal disposition. To date, there is a limited literature on selectively targeting tissues beyond liver and kidney. We would like to encourage the exploration of transporter-mediated tissue-targeting strategy more proactively. It is therefore of importance to gain further knowledge on the expression pattern and their substrate recognition feature of these transporters for the future design of tissue-targeted molecules.

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