

Review Article

Themed Issue: Monocarboxylate Transporters in Drug Disposition
Guest Editor: Marilyn Morris

Overview of the Proton-coupled MCT (SLC16A) Family of Transporters: Characterization, Function and Role in the Transport of the Drug of Abuse γ -Hydroxybutyric Acid

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Received 4 March 2008; accepted 1 April 2008; published online 4 June 2008

Abstract. The transport of monocarboxylates, such as lactate and pyruvate, is mediated by the SLC16A family of proton-linked membrane transport proteins known as monocarboxylate transporters (MCTs). Fourteen MCT-related genes have been identified in mammals and of these seven MCTs have been functionally characterized. Despite their sequence homology, only MCT1–4 have been demonstrated to be proton-dependent transporters of monocarboxylic acids. MCT6, MCT8 and MCT10 have been demonstrated to transport diuretics, thyroid hormones and aromatic amino acids, respectively. MCT1–4 vary in their regulation, tissue distribution and substrate/inhibitor specificity with MCT1 being the most extensively characterized isoform. Emerging evidence suggests that in addition to endogenous substrates, MCTs are involved in the transport of pharmaceutical agents, including γ -hydroxybutyrate (GHB), 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors (statins), salicylic acid, and bumetanide. MCTs are expressed in a wide range of tissues including the liver, intestine, kidney and brain, and as such they have the potential to impact a number of processes contributing to the disposition of xenobiotic substrates. GHB has been extensively studied as a pharmaceutical substrate of MCTs; the renal clearance of GHB is dose-dependent with saturation of MCT-mediated reabsorption at high doses. Concomitant administration of GHB and L-lactate to rats results in an approximately two-fold increase in GHB renal clearance suggesting that inhibition of MCT1-mediated reabsorption of GHB may be an effective strategy for increasing renal and total GHB elimination in overdose situations. Further studies are required to more clearly define the role of MCTs on drug disposition and the potential for MCT-mediated detoxification strategies in GHB overdose.

KEY WORDS: butyrate; gamma-hydroxybutyrate; lactate; monocarboxylate transporters; SLC16A.

INTRODUCTION

Monocarboxylic acids play a major physiological role in that they represent an energy source for all cells in the body. Of these compounds, lactate is critically important as it is the end product of glycolysis and intracellular accumulation of lactate results in the inhibition of glycolysis. Furthermore, lactate can be oxidized in the brain and red skeletal muscle to fuel cellular respiration. As such, the transport of lactate and other monocarboxylic acids both into and out of cells is vital for cellular function.

Two transporter families have been identified that facilitate this need: the proton-coupled monocarboxylate transporters (MCTs) and the sodium-coupled monocarboxylate transporters (SMCTs). MCTs (SLC16A) were first identified in the mid-nineties and to date 14 members of this

family have been identified through sequence homology (1,2). Currently, seven isoforms have been functionally characterized and it has been demonstrated that not all members function as proton-coupled transporters and that a wide variety of endogenous and exogenous compounds are substrates, including lactate, pyruvate, butyrate, γ -hydroxybutyrate, bumetanide, and simvastatin acid (3–6). In contrast, the SMCT family contains only two members, SLC5A8 and SLC5A12, which were identified within the past 5 years (7–9). SMCTs have strikingly similar substrate specificities transporting short-chain monocarboxylates and sodium ions with ratios between 4:1 and 2:1 (Na:substrate) (9). These two distinct transporter families are further differentiated by their respective tissue distributions: SMCTs demonstrate a more restricted distribution (primarily kidney and intestine) while MCTs show a more ubiquitous distribution (4,9).

In addition, unlike SMCTs, some members of the MCT family have been demonstrated to transport exogenous compounds including drugs. The impact of MCT substrate/inhibitor specificity and tissue distribution needs to be further examined with respect to drug substrates, and the overall influence of MCTs on drug disposition. The present review

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focuses on the proton-coupled MCTs and aims to summarize our current understanding of their structure, function and regulation as well as their role in drug disposition using γ -hydroxybutyrate (GHB; a known MCT substrate) (10–12) as a specific example.

STRUCTURE, FUNCTION AND REGULATION OF MONOCARBOXYLATE TRANSPORTERS

The uptake of monocarboxylates was first demonstrated to be transporter-mediated in erythrocytes (13,14). Subsequently, the existence of a family of monocarboxylate transporters was proposed following the characterization of lactate transport in a variety of cell types (13,15,16). To date, 14 members of the MCT family have been identified through screening of genomic and expressed sequence tag (EST) databases (4). Hydropathy plots have predicted that MCTs have 12 transmembrane domains with the N- and C-termini located in the cytoplasm (2,4). The transmembrane domains (TMDs) are highly conserved between isoforms with the greatest sequence variations observed in the C-terminus and the large intracellular loop between TMDs 6 and 7, which has a range of 29–105 amino acid residues (2). This observed variability is common to transporters with 12 TMDs and it is thought that these sequence variations are related to substrate specificity or regulation of transport activity (2,17). Human tissue distribution of all currently identified isoforms has been investigated and is summarized in Table I. A number of recent reviews and articles have examined the tissue specific localization and physiological functions of MCT isoforms in both humans and rodents (18–25). Regulation of MCTs has been demonstrated to occur via transcriptional, translational and post-transcriptional mechanisms (26–28). These regulatory pathways appear to be age- and tissue-dependent, which further complicates the understanding of these pathways (27,28). Some MCTs require an ancillary protein (see Table I) which can be involved in cellular localization (29) or protein–protein interactions (30); however, the role of these accessory proteins in overall transporter function is not yet completely understood (29).

Functional characterization of MCT isoforms has been extended to seven isoforms (MCT1–4, 6, 8, 10) with the seven remaining MCT family members being classified as orphan MCTs (MCT5, 7, 9, 11–14). Table II provides a summary of currently identified substrates and inhibitors of functionally characterized MCT isoforms from humans and rats. Our current understanding indicates that the transport mechanism varies between MCT isoforms and that not all MCT isoforms transport monocarboxylates (e.g. MCT8). The following sections aim to provide an overview of our current understanding of individual MCT isoforms with respect to unique structural features, substrate/inhibitor specificity and regulation.

MCT1

MCT1 was first identified in Chinese hamster ovary cells when altered mevalonate transport resulting from a single point mutation was detected (15). Subsequently, human, rat and mouse homologues were cloned and functionally characterized (16,31–34). Tissue distribution of MCT1 is ubiquitous

For example, in the retinal pigment epithelium (RPE), expression is restricted to the apical membrane (2,17). Transport kinetics have been thoroughly explored using lactate for this isoform and have demonstrated that it functions as a proton-dependent cotransporter/exchanger (13,35). Transport occurs by ordered sequential binding with association of a proton followed by lactate binding. The complex is translocated across the membrane and the lactate and proton are released sequentially. Since the transporter functions as an exchanger, transport can occur bidirectionally; however, it is primarily responsible for the uptake of substrates (17).

While initial studies focused on the transport of lactate by MCT1, subsequent studies revealed that the substrate specificity of MCT1 was much less specific than initially thought (2,4,35). Substrate and inhibitor affinities are detailed in Table II. Transport of lactate was shown to be stereoselective with MCT1 having a greater affinity for L-lactate than D-lactate (35). Uptake of butyrate by intestinal epithelia cells is highly dependent on MCT1 expression; alterations in MCT1 levels results in altered uptake of butyrate which is the primary energy source for these cells (36,37). Interestingly, XP13512 (a gabapentin prodrug) was specifically designed to be a substrate for MCT1 in the intestine to improve the bioavailability of gabapentin (38,39). In addition to the transport of short-chain monocarboxylic acids, MCT1 was demonstrated to transport branched oxo-acids with a greater affinity than lactate (35). The higher affinity of these acids for MCT1 supports previous studies demonstrating their inhibitory potential towards lactate transport. Inhibitors of MCT1 fall into three broad categories: (1) bulky or aromatic monocarboxylates which act as competitive inhibitors (e.g. phenyl-pyruvate and α -cyano-4-hydroxycinnamate (CHC)); (2) amphiphilic compounds with divergent structures (e.g. quercetin and phloretin); and (3) some 4,4'-substituted stilbene-2,2'-disulphonates (e.g. DIDS) (4). Other isoforms can be distinguished from MCT1 based on the inhibitory potential of these compounds (Table II).

Relatively few studies have been conducted to assess the regulation of MCTs. Studies have indicated that altered physiological conditions and the presence of xenobiotics may alter the regulation of MCTs, in addition to altered expression at different developmental stages (40–42). MCT1 expression undergoes transcriptional, post-transcriptional and post-translational regulation and appears to be regulated in a tissue-specific manner (26–28). In colonic epithelium, exposure to butyrate resulted in a concentration- and time-dependent increase in MCT1 mRNA, protein expression and a corresponding increase in butyrate transport (43). These data suggest the possibility of altered transcriptional regulation; however, the authors further demonstrated increased transcript stability indicating additional post-transcriptional regulation mechanisms (43). High concentrations of lactate have also been demonstrated to increase MCT1 mRNA and protein levels in L6 cells (44). In contrast, treatment with testosterone resulted in increased skeletal muscle MCT1 protein expression and lactate transport in the absence of mRNA changes suggesting the importance of post-transcriptional regulation (27). These results indicate that careful experimental design is required to assess the

Table 1. The Human SLC16A Transporter Family

CT	UniGene name	Alternate (*former) Name	Sequence accession ID	Human gene locus	Tissue distribution	Subcellular location	Accessory protein	Transport mechanism	Ref.
CT1	SLC16A1		NM_003051	1p13.2	Ubiquitous	Apical and basolateral membranes	CD147	H ⁺ cotransporter exchanger	(4,19)
CT2	SLC16A7		NM_004731	12q14.1	Testis, liver, kidney, skeletal muscle, heart, brain, spleen, pancreas	Basolateral membrane	EMBIGIN	H ⁺ cotransporter	(4,29,51,53)
CT3	SLC16A8	REMP	NM_013356	22q13.1	Retinal pigment epithelium (RPE), choroids plexus, aorta, placenta, kidney	Basolateral membrane (RPE)	CD147	H ⁺ cotransporter	(4,19,60-62, 66)
CT4	SLC16A3	(*MCT3)	NM_004207	17q25.3	White muscle, white blood cells, tumors, RPE, brain kidney, placenta, small intestine, lung, heart	Basolateral membrane	CD147	H ⁺ cotransporter	(4,19,62,103,104)
CT5	SLC16A4	(*MCT4)	NM_004696	1p13.3	Placenta, intestine, colon			Orphan	(4,19)
CT6	SLC16A5	(*MCT5)	NM_004695	17q25.1	Kidney, muscle, placenta, intestine, brain, heart, pancreas, prostate, lung			Facilitated diffusion	(3,4)
CT7	SLC16A6	(*MCT6)	NM_004694	17q24.2	Pancreas, brain, muscle			Orphan	(4)
CT8	SLC16A2	XPCT (*MCT7)	NM_006517	Xq13.2	Liver, brain, kidney, heart, placenta			Orphan	(4,105)
CT9	SLC16A9		BN000144	10q21.2	Endometrium, testis, ovary, breast, brain, kidney, adrenal, retina			Orphan	(4)
CT10	SLC16A10	TAT1	NM_018593	6q21-q22	Intestine, kidney, skeletal muscle, heart, liver, placenta	Basolateral membrane		Facilitated diffusion/exchanger	(4,70)
CT11	SLC16A11		NM_153357	17p13.2	Skin, lung, ovary, breast, pancreas, RPE, choroid plexus			Orphan	(4)
CT12	SLC16A12		ENSG00000152779	10q23.3	Kidney			Orphan	(4)
CT13	SLC16A13		BN000145	17p13.1	Breast, bone marrow			Orphan	(4)
CT14	SLC16A14		BN000146	2q36.3	Brain, heart, ovary, breast, lung, pancreas, RPE, choroid plexus			Orphan	(4)

Table II. Comparison of Substrates and Inhibitors for Various MCT Isoforms in Humans and Rats

Species	Isoform	Expression System	Substrate	Km (mM)	Inhibitor	Ki ^a or IC50 ^b (μM)	References				
Human	MCT1	<i>Xenopus</i> oocytes	Lactate	3.5–6	Phloretin	28 ^a	(17,35,38,43,53)				
			Pyruvate	1.8–2.5	Quercetin	n.a.					
			Acetoacetate	5.5	CHC	425 ^a					
			α-Ketoisovalerate	1.3	pCMBS	n.a.					
			α-oxoisohexanoate	0.67	XP13512	0.620 ^b					
			α-oxoisovalerate	1.25							
			Butyrate	9							
	MCT2	<i>Xenopus</i> oocytes	Pyruvate		0.025	CHC	n.a.	(53)			
						L-Lactate	n.a.				
						GHB	n.a.				
	MCT3	ARPE-19 cells	Lactate	n.a.			(21)				
	MCT4	<i>Xenopus</i> oocytes	L-lactate	L-lactate	28	pCMBS	21 ^a	(64,65)			
				D-lactate	519	CHC	991 ^a				
				Pyruvate	153	Phloretin	41 ^a				
				D-β-hydroxybutyrate	130	NPPB	240 ^a				
				Acetoacetate	216	Fluvastatin	32 ^b				
				α-ketobutyrate	57	Atorvastatin	32 ^b				
				α-ketoisocaproate	95	Lovastatin	44 ^b				
				α-ketoisovalerate	113	Simvastatin	79 ^b				
				MCT6	<i>Xenopus</i> oocytes	Bumetanide	Bumetanide		0.084	Furosemide	46 ^b
Nateglinide							n.a.		Azosemide	21 ^b	
Prostaglandin F ₂ α	n.a.										
MCT8	COS1 and JEG3 cells	T ₃	T ₃	n.a.		(72)					
			T ₄	n.a.							
Rat	MCT1	<i>Xenopus</i> oocytes	Lactate	3.5	Phloretin	28 ^b	(12,35,58)				
			GHB	4.6	Quercetin	14 ^b					
					Benzbromaron	22 ^b					
	MCT2	MDA-MB231 <i>Xenopus</i> oocytes	γ-hydroxybutyrate	γ-hydroxybutyrate	4.6			(12)			
				Lactate	0.74	Phloretin	14 ^b		(58)		
				Pyruvate	n.a.	Quercetin	5 ^b				
	MCT4	<i>Xenopus</i> oocytes	L-lactate	L-lactate	34	CHC	24 ^b	(104)			
				Pyruvate	36.3	CHC	350 ^b				
				2-oxoisohexanoate	13	pCMBS	n.a.				
				Acetoacetate	31						
				β-hydroxybutyrate	65						
	MCT8	<i>Xenopus</i> oocytes	T ₃	T ₃	n.a.	N-bromoacetyl-T ₃	n.a.	(71)			
				T ₄	n.a.	Bromosulphothalein	n.a.				
	MCT10	<i>Xenopus</i> oocytes	L-Tryptophan	L-Tryptophan	3.8			(70)			
				L-Tyrosine	2.6						
L-Phenylalanine				7.0							
L-DOPA				6.4							

CHC α-Cyano-4-hydroxycinnamate, NPPB 5-nitro-2-(3-phenylpropylamino)benzoate, pCMBS p-chloromercuribenzenesulphonic acid, n.a. transporter kinetic parameters were not determined

The superscripts are used with the data in the same column of the table to indicate if the values are IC50 or Ki values

MCT1 and multiple regulation pathways appear to be involved in its regulation. The MCT1 5'-flanking and 3' untranslated regions were recently cloned and a variety of transcription factor binding sites were identified (26). In addition, increased MCT1 expression and activity have been reported in human neuroblastoma and melanoma cell lines resulting from low extracellular pH (41,45). Inhibition and silencing of MCT1 in neuroblastoma and glioma cell lines resulted in increased cellular pH leading to apoptotic cell

therapeutic target (41,46,47). Additional studies need to address the potential for varied physiological states and xenobiotics to alter MCT1 (or other isoforms) regulation, as this may impact the disposition of both endogenous and exogenous MCT substrates.

MCT1 is further regulated by its association with the cell surface glycoprotein CD147, which has a single transmembrane domain with the C-terminus located in the cytosol (48,49). Topology studies suggest that one MCT1 molecule

dimerization with another MCT1/CD147 pair (49). The initial association of CD147 and MCT1 is required for the translocation of MCT1 to the plasma membrane (48). Furthermore, studies indicate that covalent modification of CD147 results in inhibition of lactate transport as is seen with pCMBS-mediated inhibition of transport (48,50). In addition to MCT1, CD147 functions as an ancillary protein for MCT4 but not MCT2 (48).

MCT2

MCT2 was initially isolated and functionally characterized from a Syrian hamster liver library (51) with subsequent identification of homologues in rat (52) and human (53). In humans, expression of MCT2 is more restricted than MCT1 (Table I), with the greatest expression observed in the testis (53). In addition, species differences have been observed in the tissue distribution of MCT2. For example, rodents express higher levels of MCT2 in the liver, while MCT2 protein expression is not detectable in human liver (53). Brain MCT2 expression and cellular localization also appears to be highly species dependent (53–55). This variability in tissue expression may be a result of species differences in gene regulation. In both rodents and humans, MCT2 splice variants have been detected in a species and tissue-dependent manner suggesting that transcriptional and post-transcriptional regulation pathways play an important role in the tissue specificity of this isoform (52,53,55,56). Similar to MCT1, MCT2 requires an accessory protein for translocation to the plasma membrane. However, MCT2 requires gp70 (EMBIGIN), not CD147 (29). In addition, tissue specific post-translational regulation of MCT2 has recently been demonstrated in the mouse brain with the association of MCT2 and the scaffolding protein Delphinin which results in colocalization of MCT2 with δ -glutamate receptors (30,57). Further studies on the species- and tissue-specific regulation are required to identify the complex pathways involved in MCT2 regulation.

MCT2 has remarkably similar substrate specificity to MCT1. However, in contrast to the observed substrate affinities of MCT1, MCT2 was demonstrated to be a high affinity pyruvate transporter in humans ($K_m=25 \mu\text{M}$) which concurs with results obtained using hamster and rat MCT2 (Table II) (51,58). Furthermore, MCT2 is inhibited by phloretin and CHC, but not by the organomercurial thiol reagent pCMBS, which distinguishes it from MCT1 (4). It is thought that this difference in inhibitor sensitivity results from the requirement of MCT1 and MCT2 for different accessory proteins (4).

MCT3

MCT3 is believed to have the most restricted distribution of any MCT with expression in the basolateral membrane of the RPE and the choroid plexus in humans, rodents and chickens (21,59,60). However, recent studies demonstrated MCT3 expression in vascular smooth muscle cell lines (61), human aorta (61), human kidney (62) and human intestinal Caco-2 cells (unpublished), suggesting that MCT3 mRNA may be more widely distributed than originally thought. Furthermore, decreased MCT3 mRNA and protein expres-

which concurs with changes in smooth muscle cells characteristic of this disease state (61). The authors further demonstrated that DNA methylation of the MCT3 gene likely contributed to the observed expression changes (61).

Chicken MCT3 has been demonstrated to transport lactate in a yeast expression system ($K_m=6 \text{ mM}$) and demonstrates a profound resistance to prototypical MCT inhibitors (60). Additional information on human MCT3 substrates or inhibitors is not present in the literature nor is there detailed information regarding the regulation of MCT3.

MCT4

MCT4 demonstrates remarkable similarities to MCT1 with respect to tissue distribution, regulation and substrate/inhibitor specificity (Tables I and II). The principal difference between these isoforms lies in their tissue specific localization and substrate affinities. In contrast to MCT1, MCT4 is predominantly expressed in highly glycolytic cells such as white muscle and white blood cells suggesting that its physiological function is lactate efflux (17,63). MCT4 and CD147 expression were induced in MDA-MB231 cells (a highly invasive breast cancer cell line) supporting the metabolic switch to highly glycolytic cells in metastasis and the corresponding increase in lactate efflux (42). MCT4 localization at the plasma membrane was dependent on CD147 expression, which is consistent with results obtained for MCT1 (42). The role of MCT4 in lactate efflux is further supported by its high expression in the placenta where it is involved in the transfer of lactate into the maternal circulation (4). While there is a great degree of overlap in the substrate specificity of MCT1 and MCT4, these two isoforms differ in their substrate affinities with MCT4 having lower affinities for a range of monocarboxylates (64). In contrast to other MCTs, lactate transport via MCT4 is inhibited by a range of statin drugs which may play a role in cytotoxicities observed with statin administration (65).

MCT6

MCT6 was first identified by Price *et al.* in 1998 (66) through genomic and EST database screening. Northern blot analysis was used to determine the tissue distribution of MCT6 (Table I) with expression being predominantly in the kidneys (66).

In contrast to other members of the MCT family, MCT6 does not transport short-chain monocarboxylates or amino acids; rather, all substrates identified to date are pharmaceutical agents (Table II) (3). Murakami *et al.* (3) demonstrated that bumetanide uptake is mediated by MCT6 in a pH- and membrane potential-, but not proton-dependent manner suggesting that it may be net charge dependent. Furthermore, uptake of bumetanide was inhibited by probenecid and several thiazides, but not inhibited by lactate or succinic acid (3). This suggests that a carboxylic moiety is not essential for MCT6 affinity, as was anticipated based on results obtained with other MCT isoforms (3). MCT6 mRNA expression has been demonstrated along the entire length of the human intestine with the highest expression levels observed in the stomach (66,67). This expression pattern suggests that MCT6

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