Expression and membrane localization of MCT isoforms along the length of the human intestine

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Gill, Ravinder K., Seema Saksena, Waddah A. Alrefai, Zaheer Sarwar, Jay L. Goldstein, Robert E. Carroll, Krishnamurthy Ramaswamy, and Pradeep K. Dudeja. Expression and membrane localization of MCT isoforms along the length of the human intestine. Am J Physiol Cell Physiol 289: C846–C852, 2005.—Recent studies from our laboratory and others have demonstrated the involvement of monocarboxylate transporter (MCT)1 in the luminal uptake of short-chain fatty acids (SCFAs) in the human intestine. Functional studies from our laboratory previously demonstrated kinetically distinct SCFA transporters on the apical and basolateral membranes of human colonocytes. Although apical SCFA uptake is mediated by the MCT1 isoform, the molecular identity of the basolateral membrane SCFA transporter(s) and whether this transporter is encoded by another MCT isoform is not known. The present studies were designed to assess the expression and membrane localization of different MCT isoforms in human small intestine and colon. Immunoblotting was performed with the purified apical and basolateral membranes from human intestinal mucosa obtained from organ donor intestine. Immunohistochemistry studies were done on paraffin-embedded sections of human colonic biopsy samples. Immunoblotting studies detected a protein band of ~39 kDa for MCT1, predominantly in the apical membranes. The relative abundance of MCT1 mRNA and protein increased along the length of the human intestine. MCT4 (54 kDa) and MCT5 (54 kDa) isoforms showed basolateral localization and were highly expressed in the distal colon. Immunohistochemical studies confirmed that human MCT1 antibody labeling was confined to the apical membranes, whereas MCT5 antibody staining was restricted to the basolateral membranes of the colonocytes. We speculate that distinct MCT isoforms may be involved in SCFA transport across the apical or basolateral membranes in polarized colonic epithelial cells.

monocarboxylate transporter; short-chain fatty acids; absorption; short-chain fatty acid transport; mammalian colon

SHORT-CHAIN FATTY ACIDS (SCFAs) acetate, propionate, and butyrate are monocarboxylates, which represent the most abundant anions in the colonic lumen. SCFAs are produced by anaerobic fermentation of undigested carbohydrates by enteric bacteria. SCFAs are rapidly absorbed in the colon and serve as the preferential source of energy for colonic enterocytes (22) and influence various cellular functions (22). For example, butyrate in particular has been shown to induce cell differentiation and regulate the growth and proliferation of colonic mucosa (5). Intraluminal instillation of SCFAs has proven beneficial in the amelioration of diversion and ulcerative colitis (4, 19) as well as colorectal carcinoma in experimental animals and humans (25). Other effects of SCFAs in the intestine include stimulation of sodium chloride absorption and maintenance of colonic epithelial integrity (9).

Several studies have attempted to investigate the mechanism(s) of absorption of SCFAs across the plasma membrane of the intestinal epithelial cells. In this regard, SCFA absorption across the intestinal mucosa has been shown to occur through the nonionic diffusion of protonated SCFAs and/or a carrier-mediated anion exchange (7, 9). We have clearly demonstrated (16, 17) the presence of HCO3−-dependent, carrier-mediated anion exchange for SCFA uptake in the apical membranes of the human ileum and colon. Recent studies from our laboratory (12) and others (28, 29) have demonstrated the involvement of monocarboxylate transporter (MCT)1 in the absorption of SCFAs across the apical membrane in the human intestine. It was shown previously that absorbed butyrate is not completely metabolized by colonocytes and thus could exit across the basolateral membrane into the bloodstream (30, 34). In this regard, previous functional studies from our laboratory and others have characterized a kinetically distinct basolateral SCFA/anion exchanger in the basolateral membrane of human (34) and rat colon (27). The basolateral membrane SCFA/anion exchangers exhibited Km for butyrate of 17.5 ± 4.5 mM compared with the luminal SCFA/anion exchangers (Km for butyrate 1.5 ± 0.2 mM) (34), indicating the involvement of possibly distinct molecular isoforms of the MCTs expressed in the colon. However, the molecular identity of the basolateral membrane SCFA transporter and whether it is encoded by a MCT isoform other than MCT1 are not known yet.

Fourteen MCT isoforms have been identified to date in mammals, each having a unique tissue distribution (13). Direct demonstration of the functional transport for mammalian MCT1–4 has been demonstrated (14). MCT1 is ubiquitously expressed but is especially prominent in heart and red muscle (24). MCT2 and MCT3 distribution has been shown to be more restricted (18, 21, 23, 35). MCT5 is primarily found in placenta (26), MCT6 in kidney and placenta (26), MCT7 in pancreas and brain, and MCT8 in liver, kidney, and myocardium (26). Our RT-PCR studies (26) showed that MCT1, -3, -4, -5, and -6 isoforms were expressed in the human colonic carcinoma cell line Caco-2. The possibility of multiple MCT isoforms expressed in the human intestine makes the establishment of the molecular identity of the apical and basolateral SCFA transporters complex. Therefore, detailed studies are needed to understand the membrane localization as well as the regional transport of individual SCFAs.
expression of MCT isoforms to clearly define their role in the transport of SCFAs, especially across the basolateral membrane of polarized human intestinal epithelial cells.

The current studies were designed to assess the expression and membrane localization of different MCT isoforms along the length of the human intestine with immunoblotting and immunohistochemical studies. Our findings demonstrate that the MCT1, -4, and -5 isoforms are the predominant isoforms expressed in the human colon, with MCT1 localized to the apical membrane and MCT4 and -5 isoforms localized to the basolateral membranes. MCT3 expression was found to be significantly low compared with other MCT isoforms, and it was found to be basolaterally localized.

**MATERIALS AND METHODS**

**RNAse protection assay.** Human tissue samples from different regions of the human intestine were obtained from pinch biopsies and immediately suspended in RNAzol solution for total RNA extraction. Collection of these specimens was performed according to protocols approved by the Institutional Review Board of the University of Illinois at Chicago and by the Human Investigation Committee of the Jesse Brown Veterans Affairs (VA) Medical Center. Total RNA was extracted from various tissue sources with RNAzol solution supplied by the manufacturer (Tel-Test, Friendswood, TX). For quantitation of mRNA level, the RNase protection assay was used because of its high sensitivity and feasibility with small quantities of RNA. The RNase protection assay was performed as previously described by us (12). Briefly, [32P]CTP-labeled antisense MCT transcript was synthesized from cDNA template corresponding to MCT1 cloned into pGEM-T.

**Materials.** Extracts from various tissue sources with RNAzol solution supplied by the manufacturer (Tel-Test, Von Hoff, TX) were used to synthesize labeled antisense RNA probes.

**Synthesis of peptide antigens.** Synthetic peptides corresponding to the COOH terminus of MCT1 [amino acids (aa) 482–500], MCT4 (aa 201–219), MCT5 (aa 488–505), MCT3 (aa 202–221), and MCT6 (aa 510–523) were designed and synthesized. A search in the BLAST database revealed no membrane proteins with significant homologies to the peptide sequences used for immunization: MCT1, ESQDQKDTEGGPKEEESPV; MCT4, KSENNSGKDKGSSLSAHG; MCT5, PKAVLQAKQTALGWNSPT; MCT3, VTAQPGSGPPRPSRLL; and MCT6, FLEMDLAKNHERHVQME.

**Immunoblotting.** For immunoblotting studies, briefly, 150 μg each of purified human small intestinal and colonic apical membranes were solubilized in Laemmli sample buffer (2% SDS, 100 mM dithiothreitol, 60 mM Tris, pH 6.8, 0.01% bromophenol blue) and separated on 12% Tris-glycine SDS-polyacrylamide gels. The separated proteins were electroblotted onto nitrocellulose membranes and stained with Ponceau S to ensure equal loading of the protein in each lane. The blot was then probed with MCT1, MCT4, or MCT5 (overnight, 4°C, diluted 1:2,000–1:5,000) or MCT3 (overnight, 4°C, diluted 1:50) antibody. The bands were visualized using enhanced chemiluminescence according to the manufacturer’s instructions (ECL; Amersham).

**Immunohistochemical analysis.** Formalin-fixed paraffin-embedded tissues of human colonic crypts were obtained from human pinch biopsy samples or tissues that had been surgically removed. Collection of these specimens was performed according to protocols approved by the Human Investigation Committee of the Jesse Brown VA Medical Center and the Institutional Review Board of the University of Illinois at Chicago. Immunohistochemical studies were carried out on 5- or 7-μm paraffin sections of colonic tissues mounted on (3-aminopropyl)triethoxysilane-treated slides. The slides were deparaffinized in xylene for 20 min to remove the embedding media and washed in absolute ethanol for 5 min. The slides were then gradually rehydrated gently in a series of alcohol washes, including 96%, 85%, and 50%, and placed in distilled water for 5 min each. The activity of endogenous peroxidase was blocked by incubating the slides for 1 h in 0.1% Triton X-100, the slides were then incubated for 1 h at room temperature (RT) in 20% normal goat serum in PBS containing 1% BSA to block nonspecific antibody binding. This was followed by incubation of the slides with rabbit polyclonal antibodies to human MCT isoform-specific antibodies, diluted 1:125 (MCT1, 4°C, overnight) or 1:100 (MCT5, RT, 1 h). After the slides were washed three times for 5 min with PBS, liquid DAB+chromogen (3,3′-diaminobenzidine solution, Dako) was applied (until the brown chromogen stain was detected). The reaction was stopped by immersing the slides in distilled water. The slides were counterstained with hematoxylin for 30 s to counterstain the cell nuclei. For control experiments, the sections were incubated with preimmune rabbit serum and with primary antibody omitted.

**Image acquisition.** The immunostained slides were examined with a Nikon Eclipse E400 microscope. Digital images were captured with a spot insight digital camera.

**Statistical analysis.** All experiments were performed with at least three or four freshly isolated membrane preparations from different organ donors.

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RESULTS

MCT1 is apically localized. Our previous functional studies (12) showed the involvement of the MCT1 isoform in the luminal uptake of SCFAs in Caco-2 cells. To further determine the expression of MCT1 and its membrane localization, we performed immunoblotting studies in purified apical and basolateral membranes isolated from different regions of human small and large intestine, using isoform-specific MCT1 antibodies. As shown in Fig. 1A, MCT1 antibody detected a protein band of ~39 kDa mainly in apical membranes isolated from human small and large intestine. The specificity of the MCT1 band was confirmed by peptide competition experiments in which the protein blot was incubated with MCT1 antibody pretreated with either 5-fold or 20-fold excess of the 19-residue competitive MCT1 peptide. The 39-kDa band was totally competed out in the presence of excess of peptide (20-fold) (Fig. 1B). Figure 1C shows the relative intensity of the MCT1 band in the apical membranes as assessed by densitometric analysis. These results showed that MCT1 protein band abundance increased along the length of the human intestine, with a predominant expression in the distal colonic membranes, followed by proximal colon, ileum, and jejunum.

In addition, immunohistochemical studies using MCT1 antibody were also carried out on paraffin sections of colonic crypts obtained from the human biopsy samples. In parallel with immunoblotting results, the immunohistochemical staining pattern suggested that expression of MCT1 is predominantly confined to the apical membranes (Fig. 2A). No staining was observed in colonic crypts stained with preimmune serum (Fig. 2B).

MCT1 mRNA expression in human gastrointestinal tract. In parallel to the immunoblotting studies, we also determined the MCT1 mRNA relative abundance across different regions of the human intestine by RNase protection assay. The [32P]cRNA probes for human MCT1 and GAPDH (internal standard) were hybridized to total RNA extracted from the available pinch biopsy samples. Subsequently, the RNase-digested bands with the predicted sizes were observed and quantified by measuring the ratio of their representative den-
sities to that of GAPDH. The results in Table 1 show MCT1 mRNA to be maximally expressed in colonic regions compared with the duodenum. These quantitative studies could not be performed in jejunum and ileum regions because of the lack of sufficient biopsy samples from these regions.

**MCT4 is basolaterally localized.** Previous functional studies (12, 28, 29, 34) suggested the presence of distinct SCFA transporters in human apical and basolateral colonic membranes. Therefore, experiments were performed using other MCT isoform-specific antibodies to assess their membrane localization and expression. Immunoblotting studies using MCT4 isoform-specific antibodies suggested that MCT4 is an ~54-kDa protein band expressed only in the basolateral membranes (Fig. 3A). No band was detected when MCT4 antibody was incubated with its competitive peptide (Fig. 3B). As shown in Fig. 3C, the abundance of MCT4 in the basolateral membranes was in the order distal colon >> proximal colon >> ileum. Jejunum showed no expression of MCT4. The MCT4 antibody, however, was not found to be suitable for the immunohistochemical analysis.

**Expression and membrane localization of MCT5.** Immunoblotting studies performed with MCT5 antibody detected a ~54-kDa band predominantly in the basolateral membranes. No expression of MCT5 was detected in apical membranes isolated from different regions of the human intestine (Fig. 4A). This band was specific, as shown by the absence of the band in the presence of the competitive peptide (Fig. 4B). Similar to MCT4, MCT5 was found to be abundantly present in the distal colon, with relatively less expression in the proximal colon and ileum (Fig. 4C).

Immunohistochemical staining revealed that staining of MCT5 antibody was restricted to the basolateral membranes of colonic cryptic cells (Fig. 5A). No staining was observed in negative controls, when the slides were incubated with the preimmune serum (Fig. 5B) or when the primary antibody was omitted (not shown).

**MCT3 is present in ileum.** Previous studies have suggested that MCT3 is primarily expressed in the retinal epithelium (23, 35). Our studies detected an ~52-kDa band in immunoblotting studies only when MCT3 antibody was used at very low dilutions (1:50) compared with the relatively higher dilutions used for other MCT isoforms (1:2,000–1:5,000). The MCT3 band was mainly expressed in basolateral membranes (Fig. 6A). Unlike MCT4 and MCT5, the relative expression of MCT3 was higher in the ileum compared with the colonic regions (Fig. 6B). MCT6 expression could not be detected in either apical or basolateral membranes isolated from human small intestinal and colonic regions, even at the very high concentrations of the antibody (1:20 dilution) used for these studies.

**DISCUSSION**

Using purified plasma membrane vesicles, we previously demonstrated (16, 17) the presence of apical membrane SCFA/HCO₃⁻ exchange activity in ileal and colonic regions of the
human intestine. We have also reported (34) the presence of kinetically distinct SCFA/HCO$_3^-$ exchange activity in the basolateral membranes of the human proximal colon. Ample evidence from our previous studies (12) and those of others (28, 29) utilizing various molecular approaches and membrane and cellular uptake experiments using substrate analogs and specific inhibitors suggested that the apical SCFA/anion exchange process in the human colonicocytes is mediated via MCT1, a prototype of the MCT gene family. For example, our data demonstrating the involvement of MCT1 in apical SCFA uptake included inhibition of $[^{14}$C]butyrate uptake by the MCT substrate lactate and inhibition by the typical MCT inhibitor hydroxycinnamate and by suppression of butyrate uptake by overexpression of antisense MCT1 in the human colonic Caco-2 cell line (12). However, whether the basolateral SCFA/HCO$_3^-$ exchanger represents yet another MCT isoform is not clear. In the present study, we demonstrate for the first time the expression of different MCT isoforms along the length of the human intestine and the membrane localization of their polypeptides in the intestinal epithelial cells.

MCT1 was the first isoform to be cloned from Chinese hamster ovary cells and has been shown to transport monocarboxylates such as lactate and pyruvate (14, 24). With respect to membrane localization studies of MCT1 in intestine, Garcia et al. (11), utilizing immunohistochemistry, previously demonstrated that MCT1 was localized to basolateral membranes in hamster cecal epithelial cells. In the rat jejunum, immunohistochemistry studies have shown the localization of MCT1 predominantly to the basolateral membranes of immature crypt cells, but in mature surface cells MCT1 was shown to shift to the luminal membranes (32). In contrast, studies conducted by Ritzhaupt et al. (28) using Western blotting have demonstrated localization of MCT1 to the pig and human colonic luminal membranes. Similarly, Buyse et al. (6), using immunofluorescence, have shown the apical localization of MCT1 in Caco-2 cells. MCT1 protein expression was shown to follow a gradient across the crypt-surface axis, with a peak in the apical membrane of the surface cells of human colon (20). Using two different but complementary techniques of immunoblotting and immunohistochemistry, we have now shown that MCT1 expression is restricted to the luminal membranes of human intestinal epithelial cells as well as in colonic crypts. Consistent with the protein expression, parallel studies to examine MCT1 mRNA relative abundance by RNase protection assay suggested higher MCT1 mRNA expression (relative to GAPDH as an internal control) in the human colon compared with duodenum. Our present study is the first to determine MCT1 expression (at both protein and mRNA levels), distribution, and localization in different regions of the human intestine. We have shown that the expression of MCT1 in-
creased along the length of the human intestine, with maximum expression in the distal colon. This observation is not surprising, because the colon represents the major site for SCFA production and absorption (9). Our immunoblot studies showing a size of ~39 kDa for the MCT1 band (which was competed by specific peptide) mainly in the apical membranes also detected faint bands in the basolateral membranes of the ileum and colon. This faint expression can be explained by a possible cross-contamination of basolateral membranes with apical membranes.

MCT4 has been shown to be the predominant isoform of skeletal muscles, where it is primarily responsible for lactate extrusion out of the muscle (14). With immunoblot, we have shown that MCT4 (~54 kDa, which was competed by its specific peptide) is expressed in ileum and colon, with more predominant expression in the distal colon. MCT4 expression was restricted to the basolateral membranes in the human intestine. Studies of MCT1 and MCT4 expressed in Xenopus oocytes have shown that kinetic characteristics of these two isoforms are substantially different ($K_m$ for lactate: MCT1 3.5 mM, MCT4 17–34 mM) (2). The affinity of MCT1 expressed in Xenopus oocytes for various monocarboxylates ranges from $K_m$ of 0.7 mM for pyruvate to 4–6 mM for acetate and 10–12 mM for β-hydroxybutyrate (13). On the other hand, MCT4 expressed in Xenopus oocytes exhibits much lower affinity for substrates compared with MCT1, with $K_m$ values ~5- to 10-fold higher (13). Our previous studies (34) in human proximal colonic basolateral membranes demonstrated the presence of a distinct SCFA/HCO$_3^-$ transporter with a $K_m$ for butyrate of 17.5 mM. Although these studies compared the affinity of the basolateral transporter for butyrate, but not lactate, it is likely that MCT4 represents the basolateral SCFA transporter in the human colon. Similar to MCT4, MCT5 expression was highest in distal colon, followed by proximal colon and ileum. Both immunoblot and immunohistochemical studies confirmed that MCT5 was also localized to the basolateral membranes. MCT5 has not yet been functionally characterized (14). Future studies on MCT5 isoform function are needed to determine whether it could also represent one of the potential candidates mediating SCFA transport in the basolateral membranes of human colonic epithelial cells.

MCT3 was found to be expressed at relatively low levels compared with other isoforms, because it was detected only at a primary antibody concentration ~20 times higher than that used for the detection of other isoforms. Studies have shown that MCT3 is exclusively localized to basolateral membranes of retinal pigment epithelium (RPE), where it plays an important role in lactate efflux from RPE into the choroidal blood supply (23, 35). Our studies showed that MCT3 was present in both the small intestine and colonic regions, localized to the basolateral membranes. However, unlike other basolaterally expressed isoforms (MCT4 and MCT5), MCT3 expression was in the order ileum >> proximal colon > distal colon > jejunum. Therefore, it could be speculated that MCT3 might not play an important role in SCFA transport in the human colon. Although our previous RT-PCR studies showed the expression of MCT1, -3, -4, -5, and -6 isoforms in the human colonic carcinoma cell line Caco-2 (26), the present studies showed that MCT6 protein expression was absent from the human intestine.

Fig. 7. Proposed model of expression of MCT isoform(s) in the human colon. SCFA, short-chain fatty acid.

Our findings showing that human colonic epithelial cells express three different MCT isoforms on the basolateral membrane appear intriguing, but not unusual. Such a phenomenon is also very common for Na$^+$- and Cl$^-$-absorbing isoforms in the human intestine. For example, Na$^+$/H$^+$ exchanger (NHE) isoforms NHE2 and NHE3 are both expressed on the apical surface of intestinal epithelial cells but are regulated differentially and even reciprocally under some conditions (8). NHE1, in contrast, is present on the basolateral surface (31). Similarly, anion exchanger (AE) isoforms AE2 and AE3 are both localized to the basolateral surface of human intestinal epithelial cells (1).

Therefore, the apparent question that remains to be answered is, What could be the possible reason for the coexpression of different MCT isoforms on the basolateral surface? It could be speculated that these different isoforms exhibit distinct kinetic properties for different SCFAs or, on the other hand, may be important in maintaining housekeeping functions. Also, it is possible that different MCT isoforms are differentially regulated under acute or chronic conditions and might play distinct roles in the pathophysiology of different inflammatory conditions where SCFAs are implicated.

In summary, a speculative model demonstrating the expression of potential transporters involved in SCFA absorption in the human intestinal epithelial cell is shown in Fig. 7. Our immunoblotting studies using purified apical and basolateral membrane fractions from organ donor intestines demonstrated that MCT4 and MCT5 proteins were expressed on the basolateral membrane domains, whereas MCT1 expression was predominantly on the apical membrane domains. It could be speculated that both MCT4 and MCT5, or either one of them, might represent the key basolateral SCFA transporter. The present studies have formed an important background for future investigations into further understanding of SCFA influx and efflux across the polarized membranes of human intestinal epithelial cells and establishing the function and identity of the basolateral SCFA transporter(s).
HUMAN INTESTINAL MCT ISOFORMS

REFERENCES


GRANTS

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