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Expression of Monocarboxylate Transporter 1 (MCT1) in the Dog Intestine

Yumiko SHIMOYAMA[1)], Doaa KIRAT[2)], Yuko AKIHARA[1)], Kazufumi KAWASAKO[1)], Misa KOMINE[1)], Kazuko HIRAYAMA[1)], Kazuya MATSUDA[1)], Minoru OKAMOTO[1)], Hidetomo IWANO[3)], Seiyu KATO[2)] and Hiroyuki TANIYAMA[1)]*

[1)]*Departments of Veterinary Pathology,* [2)]*Physiology and* [3)]*Biochemistry, School of Veterinary Medicine, Rakuno Gakuen University, 582–1 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069–8501, Japan*

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ABSTRACT. In this study, the expression and distribution of monocarboxyolate transporter 1 (MCT1) along the intestines (duodenum, jejunum, ileum, cecum, colon and rectum) of dogs were investigated at both the mRNA and protein levels. The expression of MCT1 protein and its distribution were confirmed by Western blotting and immunohistochemical staining using the antibody for MCT1. We identified mRNA coding for MCT1 and a 43-kDa band of MCT1 protein in all regions from the duodenum to the rectum. Immunoreactive staining for MCT1 was also observed in epithelial cells throughout the intestines. MCT1 immunoreactivity was greater in the large intestine than in the small intestine. MCT1 protein was predominantly expressed on the basolateral membranes along intestinal epithelial cells, suggesting that MCT1 may play an important role in lactate efflux and transport of short-chain fatty acids (SCFAs) to the bloodstream across the basolateral membranes of the dog intestine.

KEY WORDS: canine, intestine, monocarboxylate transporter 1.

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Monocarboxylic acid transporter 1 (MCT1) facilitates transport of monocarboxylic acids, such as lactic acid, pyruvic acid, short-chain fatty acids (SCFAs), and ketone bodies, across biological membranes [10]. It is an obligatory symporter that carries a dissociated proton-monocarboxylate pair with each transport cycle [9]. Based on sequence homology, MCT1 has been characterized as a member of the monocarboxylic acid transporter family of solute carriers (SLC16 family), which includes up to 14 subtypes (MCT1-14), although monocarboxylate substrate specificity has only been demonstrated for MCT1-MCT4 [9]. MCT1 is the best-characterized isoform and is found in the majority of tissues; in many cases, it is found in specific locations within the tissue.

The vertebrate gastrointestinal tract is populated by bacteria that can produce large amounts of fermentation metabolites, including SCFA and lactic acid. Studies using rat small intestinal basolateral membrane vesicles have demonstrated that lactic acid absorbed from the intestinal lumen or generated within the epithelium crosses the basolateral membrane of the enterocyte and enters the bloodstream via a facilitated diffusion [27] or anion exchange mechanism [4]. Garcia *et al.* [6,7] used immunohistochemistry to show that MCT1 protein is localized on the basolateral surface of epithelial cells throughout the gastrointestinal tract of the hamster. Subsquently, the study of Orsenigo *et al.* [20] showed that MCT1 is a major route of lactate efflux across the basolateral membrane of the rat jejunum. Furthermore, Ritzhaupt *et al.* [24] indicated that MCT1 protein is present on the luminal membrane of the human and pig colon and is involved in the transport of L-lactate and butyrate across the colonic luminal membrane.

There is data available that may provide some clues concerning the tissue distribution of the MCT1 protein in many animal species, including the hamster gastrointestinal tract [6, 7], rabbit small intestine [29], rat gastrointestinal tract [28], pig colon [24], sheep rumen [19], bovine gastrointestinal tract [14], ovine gastrointestinal tract [15], reindeer small intestine [16], and human colon [24] and small intestine [8]. The distribution is somewhat different between ruminants and monogastric animals. A study of ruminants showed that MCT1 is highly expressed in the forestomach [14], while in humans, the maximum expression is in the distal colon [8]. The cellular location of MCT1 also differs among species. Apical expression implies the contribution of SCFA influx from the lumen, and basolateral expression implies the contribution of SCFA efflux to the bloodstream [8, 20, 24].

Although the amount of SCFA production in the dog gastrointestinal tract is less than that of herbivores and omnivores[3], study of microflora in the intestines of dogs has shown that anaerobic bacteria produce SCFAs and lactate [30]. Other canine studies have shown that the highest SCFA concentrations are in the colon [3, 11]. Since SCFA is known to not only serve as a source of energy for the body but is also known to influence various cellular functions of intestinal epithelial cells [1, 3], it is important to know the transporter of these substrates. However, there is currently no published data on the expression of MCT1 in the dog intestine. The aim of the present study was to investigate the distribution and cellular localization of MCT1 along the intestines of the dog.

* CORRESPONDENCE TO: Prof. Dr. TANIYAMA, H., Department of Veterinary Pathology, School of Veterinary Medicine, Rakuno Gakuen University, 582–1 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069–8501, Japan.

MATERIALS AND METHODS

Animals: Four healthy beagle dogs (7–9 years old) were used in this study. The animals were euthanized by exsanguination under anesthesia with sodium pentobarbital. All the animals were treated according to the Laboratory Animal Control Guidelines of Rakuno Gakuen University, which basically conform with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, U.S.A. (NIH publication No. 86–23, revised 1985).

Sample collection: Tissue samples from the small (duodenum, jejunum, and ileum) and large intestines (cecum, colon, and rectum) were collected from the animals immediately after euthanasia and washed in ice-cold saline (0.9% NaCl). The epithelium of each region was scraped off using glass slides on ice. All the collected samples were immediately frozen in liquid N_2 and subsequently stored at –80°C until use for RT-PCR and Western blotting analyses. For light microscopy and immunohistochemical studies, tissue samples were immediately fixed in 4% paraformaldehyde for 24 hr. After fixation, the tissues were dehydrated through a series of graded concentrations of ethanol and xylene, embedded in paraffin, sectioned serially at 4 μm, and mounted on poly-L-lysine coated slides.

Reverse transcription-polymerase chain reaction (RT-PCR): Total RNA was extracted from the dog intestinal tissue using an RNeasy Mini Kit (Qiagen Sciences, Germantown, MD, U.S.A.) according to the manufacturer's instructions. One μg of total RNA was reverse transcribed into cDNA in a 20 μL reaction using Superscript II and oligo-d$(T)_{12-18}$ (Invitrogen, Carlsbad, CA, U.S.A.). PCR amplification was conducted on synthesized cDNAs using *Taq* DNA Polymerase (Takara, Bio, Inc., Otsu, Japan). The MCT1-specific PCR primers derived from the canine MCT1 cDNA sequence (GenBank Accession no. XM533065) were 5'- gtggatgcttgtcaggctgtgg-3' (sense primer) and 5'-ccagctacacagcagtttagtagg-3' (antisense primer; Hokkaido System Science Co., Ltd., Japan). The PCR conditions used were 94°C for 2 min; 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min; and a final extension at 72°C for 2 min using a thermocycler (iCycler, Bio-Rad, Hercules, CA, U.S.A.). In addition, to provide an appropriate internal PCR control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was amplified. PCR products were separated by agarose gel electrophoresis (1% agarose) and visualized with UV light following ethidium bromide staining. A reaction mixture with H_2O substituted for the template DNA was used for negative controls. The RT-PCR product was cloned in pSTBlue-AccepTor™ Vector (Novagen, EMD Biosciences, Inc., Merck KagaA, Darmstadt, Germany) using DNA ligase (DNA Ligation Kit, Version 1, Takara Bio, Inc., Otsu, Japan). The nucleic acid sequences of inserts were determined using an automated DNA sequencer (310 Genetic Anaylzer, Applied Biosystems Inc., Tokyo, Japan).

Western blot analysis: Tissues were homogenized in hypotonic buffer (20 mM Tris-HCl, 40 mM NaCl, and 1 mM dithiothreitol, pH 7.4) containing a complete Protease Inhibitor Cocktail (Nacalai Tesque, Inc., Kyoto, Japan). Homogenates were centrifuged at 200 × g for 10 min at 4°C to remove debris. The supernatants were then centrifuged at 200,000 × g for 30 min at 4°C. The membrane pellets were then resuspended in 62.5 mM Tris-HCl (pH 6.8), and protein concentrations were determined using the Lowry method [18]. Sixty μg of protein was separated by 10% SDS-PAGE [17] and transferred into nitrocellulose membranes. The membranes were then blocked with 5% (w/v) nonfat dry milk in PBS-T (0.1% Tween 20 in phosphate buffered saline) for one hr at room temperature and probed with the primary antibody at a dilution of 1:500 in PBS overnight at 4°C. The primary antibody used was chicken polyclonal anti-rat monocarboxylate transporter (MCT) 1 (Chemicon International, Inc., Temecula, CA, U.S.A.). After washing in PBS-T, the membranes were incubated with biotinylated goat anti-chicken IgY (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) diluted to 1:1,000 in PBS for 30 min at room temperature and with 0.2% avidin-biotin-peroxidase complex (ABC) reagent (Vectastain Elite ABC Kit; Vector Laboratories, Inc., Burlingame, CA, U.S.A.) for 30 min. Immunodetection was performed by chemiluminescence (ECL, Amersham International, UK) according to the manufacture's instructions. Rat pancreas, which has previously been the subject of MCT1 study, was used as the positive control [31]. A mouse monoclonal beta-actin antibody (Abcam, Cambridge, UK) was used as the internal standard. Negative control blots were probed with only the secondary antibody.

Light microscopy and immunohistochemical analysis: All sections were stained with hematoxylin and eosin (HE) by routine techniques. Immunohistochemical staining of the tissue sections was performed using the avidin-biotin-peroxidase method. After deparaffinization with xylene, the sections were subjected to antigen retrieval by heating them in a microwave oven in the presence of 0.01 M sodium citrate buffer (pH 6.0) for 15 min. They were then incubated in 3% (v/v) H_2O_2 in methanol at room temperature for 10 min to quench endogenous peroxidase activity, washed three times for 5 min in PBS, and incubated with Block Ace (Snow Brand Co., Ltd., Tokyo, Japan) at 37°C for 30 min to prevent nonspecific reactions. Subsequently, the sections were incubated overnight at 4°C in a humidified chamber with the primary antibody diluted to 1:300 in PBS. After washing with PBS, the sections were further incubated with biotinylated goat anti-chicken IgY at a dilution of 1:200 for 30 min. The sections were then treated with 2% avidin-biotin-peroxidase complex (ABC) reagent for 30 min. Finally, they were reacted with 0.5% (w/v) 3,3-diaminobenzidine tetrachloride (Kanto Chemical Co., Inc., Tokyo, Japan) in PBS containing 0.01% H_2O_2 for visualization of the bound antibody, and then they were counterstained with Meyer's hematoxylin. The primary antibody was replaced with antibody dilutent for negative control slides.

RESULTS

RT-PCR: Using the specific primers derived from the canine MCT1 predicted cDNA sequence, a 300 bp cDNA fragment was obtained by RT-PCR amplification of total RNA from different regions of the small and large intestines (Fig. 1). Product specificity was assessed by sequencing the amplified MCT1 cDNA fragments. The nucleotide sequence of the cDNA fragments showed 92%, 90%, 89%, and 89% homology with the analogous regions of human, hamster, rat, and bovine MCT1, respectively (GenBank accession numbers NM-003051, L25842, D63834, and BC104598, respectively).

Western blot analysis: The MCT1 antibody recognized a migrating protein with an apparent molecular mass of approximately 43-kDa in all regions of the small and large intestines (Fig. 2). The bands were the same size as the one obtained from the rat pancreas. This band was not observed in the negative control blots (data not shown).

Immunohistochemistry: The cellular location of MCT1 in jejunum and rectum are shown in Figs. 3 and 4, although immunoreactive staining for MCT1 was observed in epithelial cells throughout the intestines of the dogs. In the small intestine, immunoreactivity was observed over all mucosal epithelial cells, with decreasing intensity from the crypts to the tip of villi. In the absorptive cells of the most villous region, including the relatively immature cells of the villus bases, the staining was localized in the cell membrane, with greater intensity on the basolateral membrane. In contrast, in the immature epithelial cells of crypts, the reactivity was localized mostly in the lateral membrane. In the large intestine, immunoreactivity was observed over all mucosal epithelial cells, with decreasing intensity from the surface to the base of the crypts. The staining was localized primarily in the basolateral membrane throughout the crypt epithelium; however, the basal staining of epithelial cells was much weaker in the base of the crypts than on their surface. Intensity was stronger in the large intestine than in the small intestine. No specific staining was detected in the negative control slides.

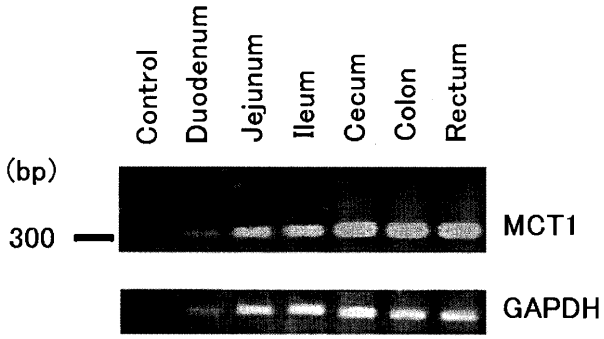

Fig. 1. RT-PCR analysis of MCT1 in the entire length of the dog intestine. Total RNA was collected from all regions of dog intestine. PCR was performed using MCT1-specific primers derived from the dog MCT1 cDNA sequence (GenBank Accession No. XM533065). The product was separated on 1% agarose gel. RT-PCR yielded an amplified band of the expected molecular size of 300 bp in all the indicated tissues. No band was observed in the negative control study. The lower panel shows the corresponding levels of GAPDH mRNA.

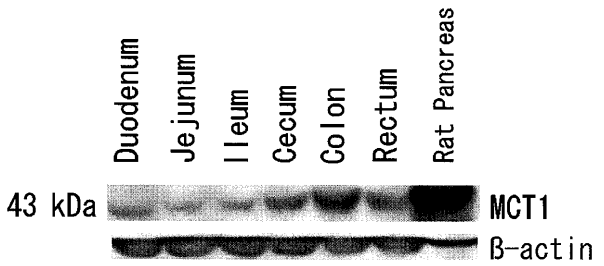

Fig. 2. Western blot analysis for MCT1 protein in the entire length of the dog intestine. Plasma membrane proteins (60 μg) from the indicated tissues were separated on 10% SDS-polyacrylamide gel, transferred to nitrocellulose, probed with MCT1 antibody, and visualized using the chemiluminescence technique. The lower panel shows β-actin as the internal standard.

DISCUSSION

The current study demonstrated the presence of MCT1 along the entire length of the dog intestine. The presence of MCT1 in the dog intestine was confirmed at the mRNA level by RT-PCR. The high sequence homology between the amplified dog MCT1 cDNA fragment and the analogous regions in various animal species indicates the presence of mRNA coding for MCT1 in the entire length of the dog intestine. Moreover, MCT1 protein was recognized as a 43-kDa band in all regions from the duodenum to the rectum, and this was in agreement with the predicted molecular size of the MCT1 protein (40–50 kDa) [21, 22, 23]. Additionally, immunohistochemical analysis revealed the presence of MCT1 protein throughout the intestine. In the immunopositive cells, staining was primarily localized in the basolateral membrane. The immunohistochemical data correlated quite well with the results obtained from Western blot study and showed greater intensity of epithelial MCT1 immunoreactivity in the large intestine than in the small intestine. Gill *et al.* [8] showed that the expression of MCT1 increases along the length of the human intestine, with the maximum expression in the distal colon. The pattern of MCT1 distribution in the dog intestine was comparably similar to those of monogastric animals, including humans, and correlates with the production site of SCFA and lactate in the dog gastrointestinal tract [2, 3, 11].

The microflora in the intestines of dogs are capable of fermenting starch substrates and producing SCFAs and lactate. SCFAs are largely produced in non-ruminants from colonic or cecal bacterial fermentation of non-digestible carbohydrates, with acetate, propionate, and butyrate representing about 70, 15, and 15% of the SCFAs produced, respectively [3]. It has been shown that the amount of butyrate absorbed is not completely metabolized by colonocytes [1, 5, 26] and

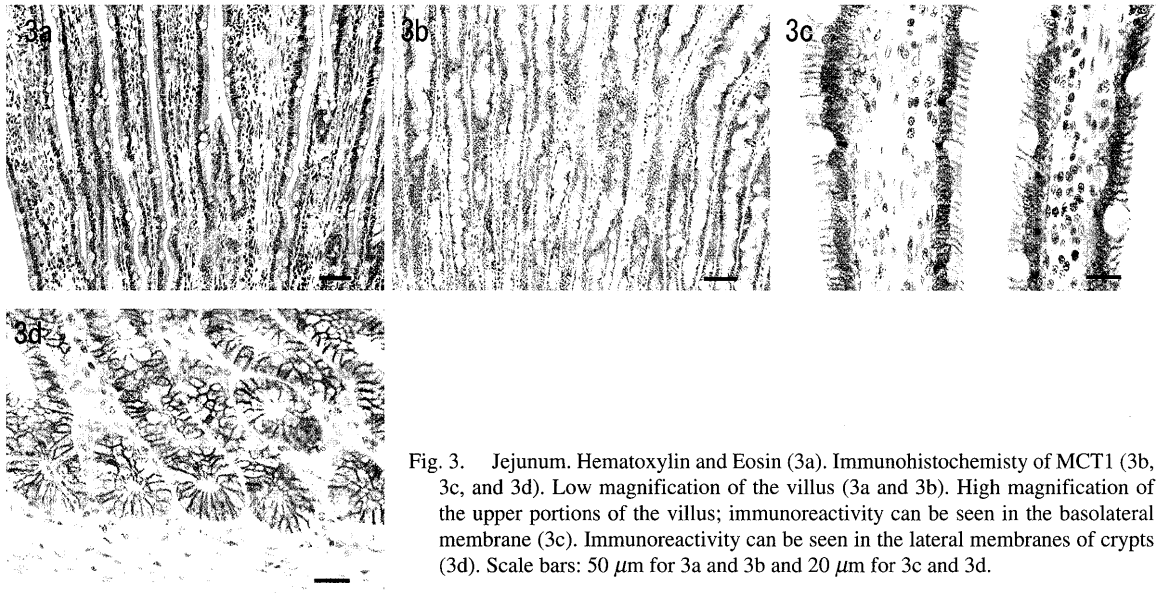

Fig. 3. Jejunum. Hematoxylin and Eosin (3a). Immunohistochemisty of MCT1 (3b, 3c, and 3d). Low magnification of the villus (3a and 3b). High magnification of the upper portions of the villus; immunoreactivity can be seen in the basolateral membrane (3c). Immunoreactivity can be seen in the lateral membranes of crypts (3d). Scale bars: 50 μm for 3a and 3b and 20 μm for 3c and 3d.

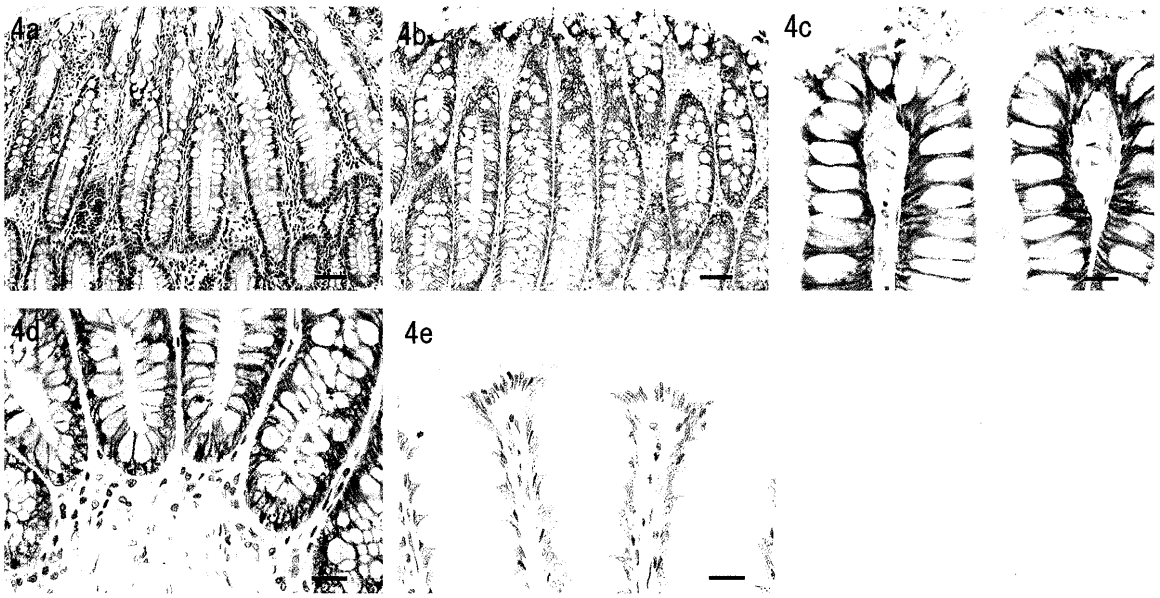

Fig. 4. Rectum. Hematoxylin and Eosin (4a). Immunohistochemistry of MCT1 (4b, 4c, 4d, and 4e). Low magnification of the crypt epithelium (4a and 4b). High magnification of the surface of crypts (4c) and base of crypts (4d); basolateral staining of the epithelium is stronger in the surface of crypts (4c) than the base of crypts (4d). Negative control (4e). No immunoreaction can be seen without the primary antibody. Scale bars: 50 μm for 4a and 4b and 20 μm for 4c, 4d, and 4e.

that it can be further transported across colonic basolateral membranes into the bloodstream. Moreover, studies [3, 13] have shown extensive metabolic use of SCFAs by the liver and other peripheral tissues. The gut is the primary non-hepatic organ exhibiting net lactate release in dogs. The intestine is believed to convert large amounts of glucose to lactate after a carbohydrate-rich meal. Lactate is thought to be a major substrate for gluconeogenesis in the livers of monogastric animals [12, 25]. Consequently, a specific transport mechanism seems to be required for export of lactate and SCFAs across the basolateral membranes of epithelia cells.

The immunohistochemical results of this study showed immunoreactivity on the basolateral membranes in most of the villus regions, including the relatively immature cells of the villus bases of the small intestine; on the other hand, the immunoreactivity of the immature cells of the villus crypts was mostly localized on the lateral membrane. In the small intestine, morphological changes are achieved during migration from the stem cells in the crypts to the crypt-villus

junction. When these cells reach the crypt-villus junction, their differentiation is complete. Although the reason for the decreasing intensity from the crypts to the tip of villi is uncertain, the alternation of localization of MCT1 in the small intestine may reflect the crypt-villus axis differentiation of epithelial cells. In the large intestine, immunoreactivity was observed mostly in the basolateral membrane throughout the crypt epithelium with decreasing intensity from the surface to the base of the crypts, and the basal staining of epithelial cells was much weaker in the base of crypts than in their surface. Since stem cells and proliferating cells in the large intestine reside at the base of the crypts and their differentiation is toward the lumen, the results of immunohistochemistry of the large intestine also seem to reflect the differentiation of epithelial cells. This suggests that MCT1 may contribute to efflux of SCFAs or lactate to the bloodstream in the mature epithelial cells of the dog intestine. Although the cellular localization of MCT1 was different, similar observations have been made in the rat jejunum; MCT1 was localized predominantly on the basolateral membranes of immature crypt cells, but immunohistochemistry showed that MCT1 shifted to the luminal membranes in mature surface cells [28]. Moreover, a study of the human intestine reported that MCT1 was localized in the apical membrane and that other isoforms (MCT4 and MCT5) were localized on the basolateral membrane [8]. Further functional studies and detection of other MCT isoforms are needed to clarify the implications of MCT1 expression in the dog intestine.

In conclusion, MCT1 protein is predominantly expressed in the basolateral membranes along the intestinal epithelial cells of dogs, suggesting that MCT1 may play an important role in lactate efflux and transport of SCFAs to the bloodstream across the basolateral membrane of the dog intestine. To our knowledge, this is the first study reporting the existence and tissue distribution of MCT1 in the dog intestine.

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