

Effects of Intestinal Ischemia/Reperfusion on Organic Cation Transporter mRNA Expression Level in the Rat Small Intestine

Yusuke Takizawa^{1,2*}, Yoshifusa Tobe², Nasa Sakamoto², Masahiro Hayashi³¹Division of Clinical Pharmaceutics, Department of Pharmaceutical Sciences, Nihon Pharmaceutical University, 10281, Komuro, Ina-machi, Kitaadachi-gun, Saitama, 362-0806, Japan²Department of Drug Absorption and Pharmacokinetics, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1, Horinouchi, Hachioji, Tokyo, 192-0392, Japan³Laboratory of Molecular Pharmaceutics and Technology, Faculty of Pharmacy, Takasaki University of Health and Welfare, 60, Nakaorui-machi, Takasaki, Gunma, 370-0033, Japan*Corresponding author: Yusuke Takizawa
DOI: 10.21276/sajp.2019.8.2.3

| Received: 12.02.2019 | Accepted: 22.02.2019 | Published: 28.02.2019

Abstract

Original Research Article

The SLC22 transporter family, which belongs to the secondary active transporters, consists of many members that are expressed in the kidney, liver, small intestine and other tissues. Among them, organic cation transporter (OCT) 1 and OCT2, which transport many important substances, are physiologically important transporters. On the other hand, after organ transplantation, the transplanted organs (tissue) are exposed to ischemia/reperfusion (I/R) injury. Moreover, patients must undergo immunosuppressive therapy and are exposed to many drugs in many cases. Herein, we examined changes in mRNA expression levels of OCT1 and OCT2 in the rat small intestine during intestinal I/R. An intestinal I/R model was made by occluding the superior mesenteric artery and vein in rats using surgical-sutures and spring balance for 60 min, followed by reperfusion. In both the jejunum and ileum, a significant change in OCT1 and OCT2 mRNA expression was observed, and the changes in the ileum continued for at least 24 hr after reperfusion. The changes in expression level of mRNA differed between OCT1 and OCT2, as well as between the jejunum and ileum. Data on the changes in expression level of the transporters during intestinal I/R is very important from the viewpoint of drug therapy.

Keywords: intestinal ischemia/reperfusion, OCT1, OCT2, jejunum, ileum, scaffold protein.**Copyright © 2019:** This is an open-access article distributed under the terms of the Creative Commons Attribution license which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use (NonCommercial, or CC-BY-NC) provided the original author and source are credited.

INTRODUCTION

Membrane-bound transporter proteins translocate molecules over cellular membranes. Most transporters belong to three major groups [1]; "channels, facilitated transporter" that move solutes down their electrochemical gradient, "primary active transporters" that use ATP to generate an ion/solute gradient to establish transportation, and "secondary active transporters" that utilize existing energy to transport materials [2].

The SLC22 transporter family, which belongs to the secondary active transporters, consists of many members that are expressed in the kidneys, liver, small intestine and other tissues. A recent evolutionary analysis found that SLC22 transporters fall into at least six subfamilies: OAT (organic anion transporter), OAT-like, OAT-related, OCT (organic cation transporter), OCTN (organic cation/carnitine transporter), or OCT/OCTN-related [3]. These transporters, especially

OAT1, OAT3, OCT1 and OCT2, are actively studied by many researchers.

OCT1 and OCT2 transport tetraalkylammoniums (tetramethylammonium, tetrabutylammonium), neurotoxins and monoamines (histamine, serotonin, dopamine, adrenaline), endogenous cations (thymine, choline, guanidine), and cationic drugs (imipramine, amiloride, cimetidine, chlorpheniramine, diphenhydramine) [4]. Moreover, OCTs transport some anticancer drugs such as imatinib and cisplatin [5, 6]. Thus, OCT1 and OCT2, which transport many important substances, are considered to be physiologically important transporters.

After organ transplantation, patients must undergo immunosuppressive therapy. Moreover, as patients have decreased immunity, they are more likely to develop complications. Therefore, patients are exposed to many drugs in many cases. In particular, after small bowel transplantation, organ (tissue) dysfunction due to ischemia/reperfusion (I/R) often

occurs [7]; therefore, it is important to elucidate the changes in expression of transporters, including the OCT family. In this study, we evaluated the changes in mRNA expression of OCT1 and OCT2 in the rat small intestine during intestinal I/R.

MATERIALS AND METHODS

Materials

All reagents were of analytical grade or higher.

Animals

Eight-week-old Male Wistar rats were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan). The animals were housed in a clean room maintained at $23 \pm 2^\circ\text{C}$, with a relative humidity of $55 \pm 10\%$ and a 12 hr light-dark cycle. The animals were fasted for 16-18 hr before starting the experiment. The animals had free access to water during fasting. All animal experiments were performed according to the guidelines of Tokyo University of Pharmacy and Life Sciences.

We previously reported the *in vivo* intestinal I/R model established using a spring balance and surgical sutures [8, 9]. Briefly, the superior mesenteric artery (SMA) and vein (SMV) in rats were occluded by hanging using surgical-sutures (Shirakawa, Tokyo, Japan) connected to a spring balance for 60 min (ischemia condition), followed by reperfusion by cutting the sutures (reperfusion condition). The hanging force on the SMA and SMV during ischemia was 50 g

(50-g load group; I/R 50 g) or 100 g (100-g load group; I/R 100 g). All experiments were performed under anesthesia with Somnopentyl® (pentobarbital sodium, 50 mg/kg), and the body temperature was maintained at 37°C with a heat lamp.

Preparation of total RNA and cDNA synthesis

Total RNA was isolated from small intestinal specimens using TRIzol® reagent (Invitrogen Co. Ltd., Paisley, UK) according to the manufacturer's instructions. Complementary DNA (cDNA) was prepared from total RNA using GeneAmp9600 (Applied Biosystems, Foster City, CA, USA) for real-time PCR according to the manufacturer's instructions. The two-step reaction mixture contained 2 µg of RNA, 100 ng of random hexamers, 0.5 mM dNTP mix (dATP, dCTP, dGTP, and dTTP), 10 mM Tris-HCl (pH 8.4), 25 mM KCl, 5 mM MgCl₂, 10 mM DTT, and 10 units of RNaseOUT recombinant ribonuclease inhibitor.

Analysis of the gene expression level in the intestine by quantitative real-time reverse transcription polymerase chain reaction (RT-PCR)

To perform real-time RT-PCR, 96-well reaction plates with optical adhesive covers and the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) were used. Assay-on-Demand Gene Expression Products were purchased for OCT1, OCT2, ezrin, radixin, moesin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Table-1).

Table-1: Sequences of primers used for real-time RT-PCR

Gene		Sequence (5'-3')	Amplicon Size	GenBank ID
OCT1	Forward	CTC CTG CTG ACC TGA AGA TGC T	104 bp	NM-012697
	Reverse	GGT GTG CTT CCT CAG GTT GG		
OCT2	Forward	TTC CAG CCG CCT TCA TCA	101 bp	NM-031584
	Reverse	AGA GGC TAG ACA GGC TGC TCC T		
Ezrin	Forward	CAG TTG CCG AAT CAC TTC CC	118 bp	NM-019357
	Reverse	AAA GTA TGG CAC CCG TGT GTG		
Radixin	Forward	CCC AGA AGA ACG AAC GCG T	117 bp	NM-001005889
	Reverse	CCT TGA CGT TCT CAG CAT GGA		
Moesin	Forward	GTT CTA TGC TCC TCG GCT TCG	103 bp	NM-030863
	Reverse	CAC CTC AAT GGT GTC AGG CTT T		
GAPDH	Forward	TGA GGT GAC CGC ATC TTC TTG	102 bp	NM-017008
	Reverse	TGG TAA CCA GGC GTC CGA TA		

Reverse transcription was performed for 1 μ g of RNA using a cDNA High Capacity Archive kit (Applied Biosystems, Foster City, CA, USA) and random hexamers as primers. Quantitative PCR was performed on an ABI PRISM 7000 system from Applied Biosystems using SYBR[®] qPCR Mix. The PCR conditions were 10 min at 90°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. All assays were RNA-specific (spanning exon-exon junctions) pre-designed SYBR green Gene Expression Assays from Applied Biosystems.

Statistical analysis

All results are expressed as the mean \pm standard error (S.E). Significance between groups was analyzed using the Student's t-test; $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Confirmation of Regional Difference in the Expression Levels of OCT1 and OCT2 in the Rat Small Intestine

As there was no information on the regional difference in OCT expression level in the small intestine of Wistar rats, we confirmed a regional difference in the mRNA expression of OCT1 and OCT2 (Fig-1). All parts of the small intestine were removed and divided into 10 segments (Upper: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10: Lower). The mRNA expression level of OCT1 in the upper region of the small intestine was higher than that in the lower region. In contrast, the mRNA expression level of OCT2 in the lower region of the small intestine was higher than that in the upper region. For both OCT1 and OCT2, the lowest expression level was observed in the middle segment.

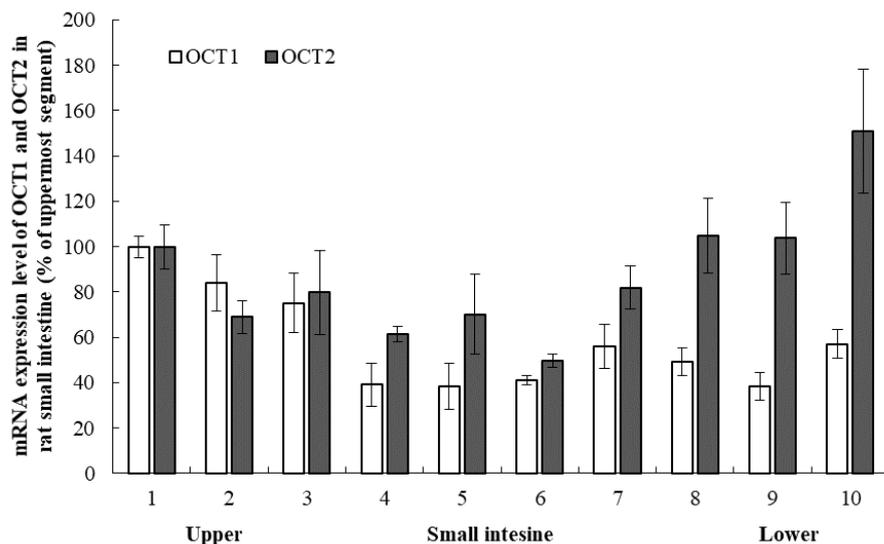


Fig-1: mRNA expression level of OCT1 and OCT2 in the rat small intestine. Results are presented as the mean \pm S.E. (n = 6)

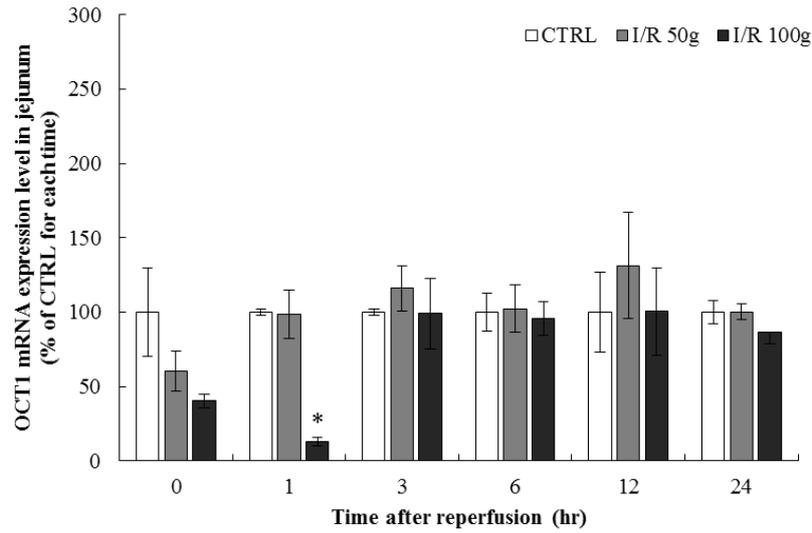
In the human small intestine, the mRNA expression levels of OCTs are very low compared with those in the colon, kidney, and liver [10, 11]. As the mRNA expression level of OCTs is very low in normal conditions, the change in expression due to disease may have a greater influence.

Changes in the mRNA Expression Level of OCT1 and OCT2 by Intestinal I/R

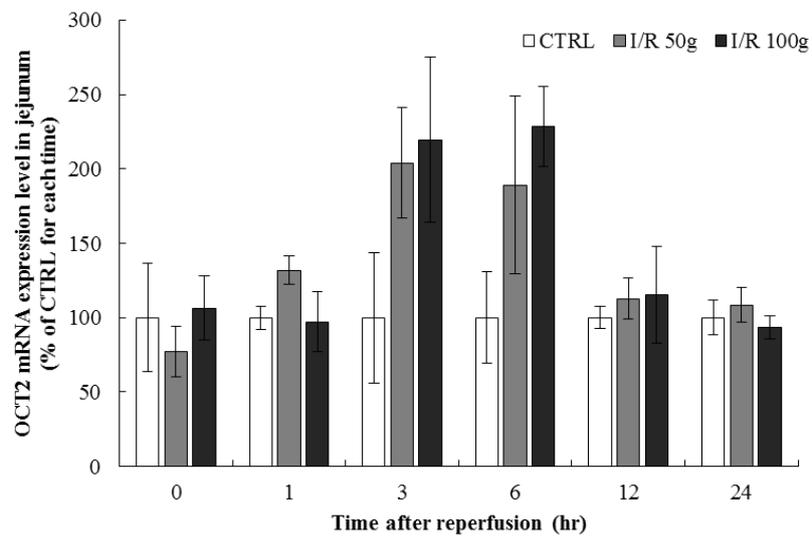
Next, we examined changes in the mRNA expression level of OCT1 and OCT2 by intestinal I/R (Fig-2). In the jejunum (the segment of No.1), the OCT1 mRNA level was decreased in a load-force-dependent manner during intestinal ischemia. This

decrease was observed until 1 hr after reperfusion and it returned to the control level by 3 hr after reperfusion (Fig-2a). In contrast, the changes in the mRNA expression level of OCT2 were not observed until at least 1 hr after reperfusion, and it increased at 3 to 6 hr after reperfusion (Fig-2b). OCT 1 and OCT 2 exhibited opposing changes.

On the other hand, ileal (the segment of No. 10) OCT1 and OCT2 mRNA expression was increased at the early stage of intestinal I/R, and decreased at the late stage (Fig-3a, 3b). Thus, similar behaviors were observed in the ileum.

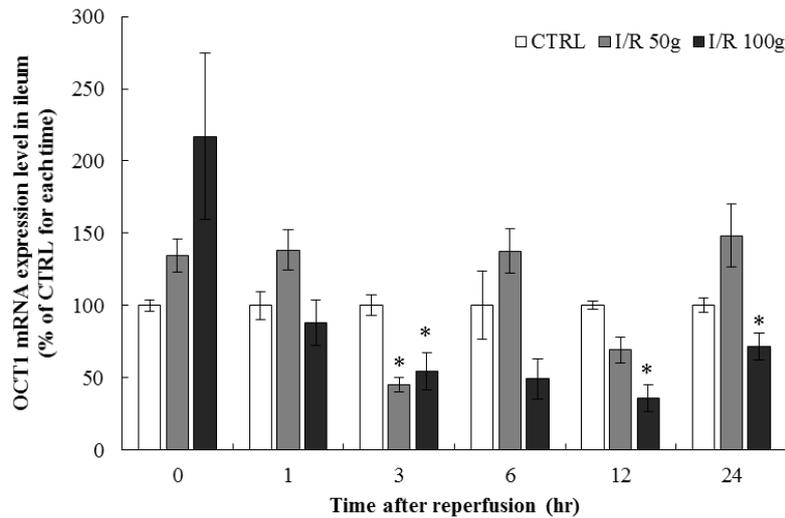


(a)

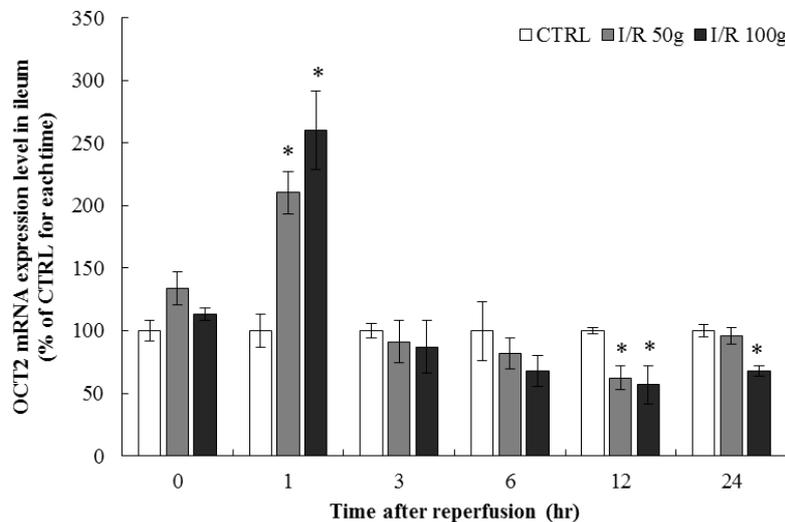


(b)

Fig-2: mRNA expression level of OCT1 (a) and OCT2 (b) in the rat jejunum during intestinal I/R. Results are presented as the mean \pm S.E. (n = 6). *P < 0.05 vs. each control condition (CTRL)



(a)



(b)

Fig-3: mRNA expression level of OCT1 (a) and OCT2 (b) in the rat ileum during intestinal I/R. Results are presented as the mean \pm S.E. (n = 6). *P < 0.05 vs. each control condition (CTRL)

As our intestinal I/R model was made by occlusion of the SMA and SMV near the duodenum, the influence of I/R likely differs between the upper part of the small intestine and the lower part. The OCT1 and OCT2 mRNA expression levels were markedly different between the jejunum and ileum, and further investigation is needed to clarify the detailed regulatory mechanism of OCT mRNA during intestinal I/R. Furthermore, we only examined mRNA, and examination of changes in the OCT protein amount is necessary.

Our research group previously reported changes in the mRNA and protein expression levels of P-glycoprotein (P-gp) and multidrug resistance-associated protein 2 (MRP2), which are typical and important ABC transporters during intestinal I/R [12, 13]. Even if these results are considered together, as the expression levels of these transporters are not similar,

independent transcription regulation pathways may exist for each transporter. Moreover, these transporters may thus be affected by intestinal I/R via different pathways.

Changes in the mRNA Expression Levels of Ezrin, Radixin, and Moesin by Intestinal I/R

Many factors, including nuclear receptors, such as PXR, have been reported to be involved in the regulation of transporter expression [14, 15]. Among them, it was reported that ezrin, radixin, and moesin (ERM) scaffold proteins have very important roles in cell adhesion and signal transduction, as well as in regulating transporters such as P-gp and MRP2 [16-18].

Lastly, we examined the changes in the mRNA expression levels of ERM proteins during intestinal I/R (Fig-4). In the 100-g ischemia group, the jejunal radixin mRNA expression level was significantly increased

during intestinal I/R (Fig-4c), but no significant change in the other mRNA expression levels was observed in the jejunum or ileum (Fig-4a, 4b, 4d, 4e, 4f). From these results, as the ERM mRNA expression level was not dependent on intestinal I/R, the effects of intestinal I/R on the mRNA expression level of ERM proteins are

unclear. Moreover, the relationship between OCT and ERM proteins during intestinal I/R was not clarified. Further investigation on regulation mechanisms of transporters during some disease conditions is necessary for safer drug therapy.

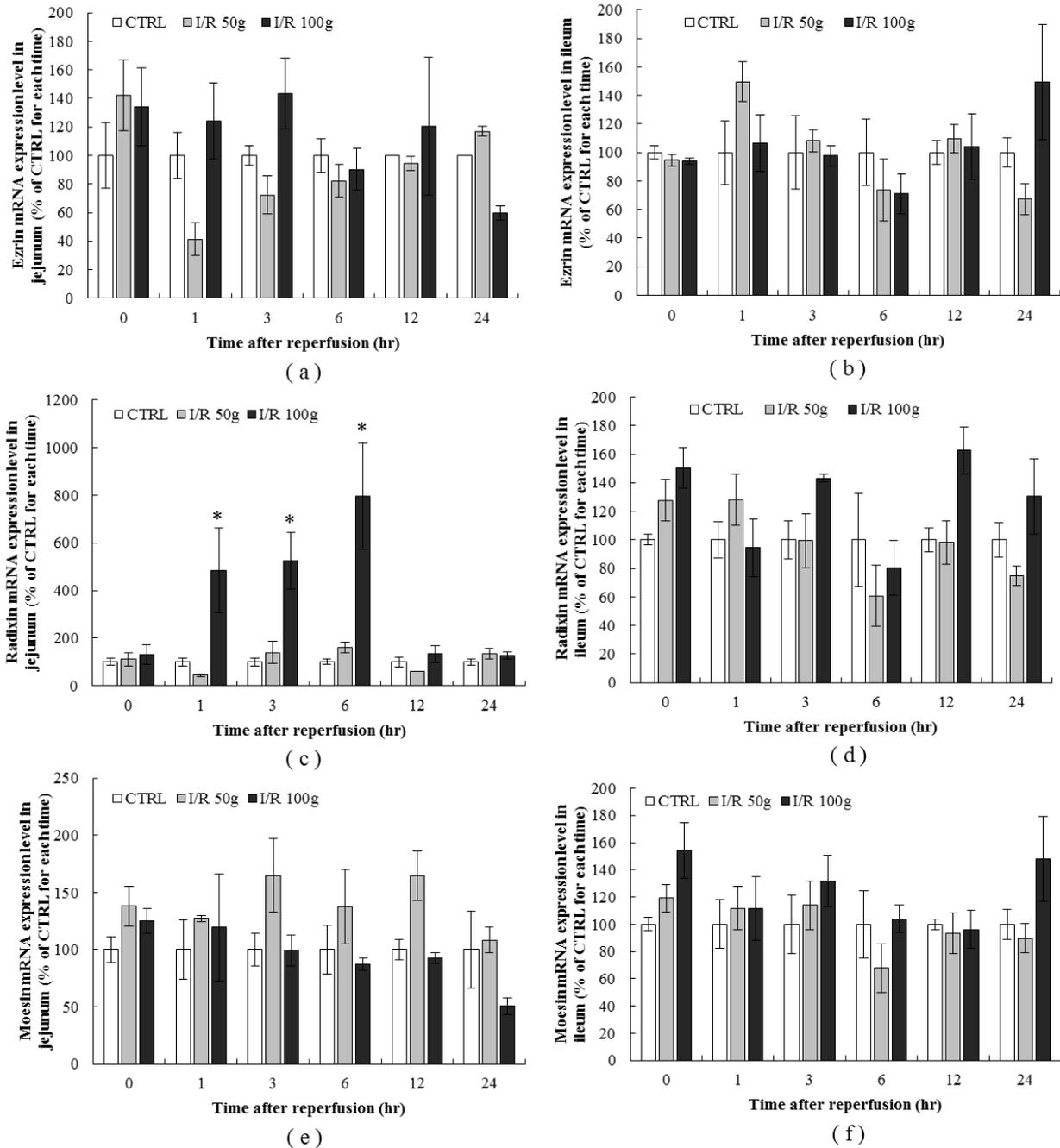


Fig-4: mRNA expression level of ezrin (a, b), radixin (c, d), and moesin (e, f) in the rat jejunum (a, c, e) and ileum (b, d, f) during intestinal I/R. Results are presented as the mean \pm S.E. (n = 6). *P < 0.05 vs. each control condition (CTRL)

CONCLUSION

This is the first report of changes in OCT1 and OCT2 mRNA expression levels in the rat small intestine during intestinal I/R. The changes in mRNA

expression differed between OCT1 and OCT2, and between the jejunum and ileum. Data on the changes in expression levels of the transporters during disease conditions is very important from the viewpoint of drug

therapy; therefore, further studies are required in the future.

Acknowledgements

The authors thank Miss Minami Nakagawa, Dr. Takahito Furuya, Mr. Yuki Aizawa, and Miss Nozomi Goto for technical assistance.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

- Almén MS, Nordström KJ, Fredriksson R, Schiöth HB. Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin. *BMC biology*. 2009 Dec;7(1):50.
- Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, Bruford EA. The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. *Pflügers Archiv*. 2004 Feb 1;447(5):465-8.
- Zhu C, Nigam KB, Date RC, Bush KT, Springer SA, Saier Jr MH, Wu W, Nigam SK. Evolutionary analysis and classification of OATs, OCTs, OCTNs, and other SLC22 transporters: structure-function implications and analysis of sequence motifs. *PLoS One*. 2015 Nov 4;10(11):e0140569.
- Urakami Y. Molecular diversity of organic cation transporter (OCT) mediating renal excretion of drugs. *Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan*. 2002 Nov;122(11):957-65.
- White DL, Saunders VA, Dang P, Engler J, Venables A, Zrim S, Zannettino A, Lynch K, Manley PW, Hughes T. Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: higher doses of imatinib may overcome the negative impact of low OCT-1 activity. *Blood*. 2007 Dec 1;110(12):4064-72.
- Filipski KK, Mathijssen RH, Mikkelsen TS, Schinkel AH, Sparreboom A. Contribution of organic cation transporter 2 (OCT2) to cisplatin-induced nephrotoxicity. *Clin Pharmacol Ther*. 2009; 86(4): 396-402.
- Zhi-Yong S, Dong YL, Wang XH; Bacterial Translocation and Multiple System Organ Failure in Bowel Ischemia and Reperfusion. *J Trauma*, 1992; 32: 148-53.
- Tomita M, Takizawa Y, Kishimoto H, Hayashi M. Assessment of ileal epithelial P-glycoprotein dysfunction induced by ischemia/reperfusion using in vivo animal model. *Drug metabolism and pharmacokinetics*. 2008;23(5):356-63.
- Takizawa Y, Kishimoto H, Kitazato T, Tomita M, Hayashi M. Changes in protein and mRNA expression levels of claudin family after mucosal lesion by intestinal ischemia/reperfusion. *International journal of pharmaceuticals*. 2012 Apr 15;426(1-2):82-9.
- Englund G, Rorsman F, Rönnblom A, Karlsson U, Lazorova L, Gråsjö J, Kindmark A, Artursson P. Regional levels of drug transporters along the human intestinal tract: co-expression of ABC and SLC transporters and comparison with Caco-2 cells. *European Journal of Pharmaceutical Sciences*. 2006 Nov 1;29(3-4):269-77.
- Meier Y, Eloranta JJ, Darimont J, Ismail MG, Hiller C, Fried M, Kullak-Ublick GA, Vavricka SR; Regional distribution of solute carrier mRNA expression along the human intestinal tract. *Drug Metab Dispos*. 2007; 35(4): 590-594.
- Takizawa Y, Kishimoto H, Kitazato T, Tomita M, Hayashi M. Changes in the localization of ileal P-glycoprotein induced by intestinal ischemia/reperfusion. *Biological and Pharmaceutical Bulletin*. 2011 Mar 1;34(3):408-14.
- Takizawa Y, Sakamoto N, Tobe Y, Hayashi M. Changes in function and expression level of multidrug resistance-associated protein during intestinal ischemia/reperfusion. *Sch Acad J Pharm*, 2018; 7(2): 60-66.
- Austin G, Holcroft A, Rinne N, Wang L, Clark RE. Evidence that the pregnane X and retinoid receptors PXR, RAR and RXR may regulate transcription of the transporter h OCT 1 in chronic myeloid leukaemia cells. *European journal of haematology*. 2015 Jan;94(1):74-8.
- Hyrsova L, Smutny T, Trejtnar F, Pavek P. Expression of organic cation transporter 1 (OCT1): unique patterns of indirect regulation by nuclear receptors and hepatospecific gene regulation. *Drug metabolism reviews*. 2016 Apr 2;48(2):139-58.
- Tsukita S, Yonemura S, Tsukita S. ERM (ezrin/radixin/moesin) family: from cytoskeleton to signal transduction. *Current opinion in cell biology*. 1997 Jan 1;9(1):70-5.
- Yang Q, Onuki R, Nakai C, Sugiyama Y. Ezrin and radixin both regulate the apical membrane localization of ABCC2 (MRP2) in human intestinal epithelial Caco-2 cells. *Experimental cell research*. 2007 Oct 1;313(16):3517-25.
- Luciani F, Molinari A, Lozupone F, Calcabrini A, Lugini L, Stringaro A, Puddu P, Arancia G, Cianfriglia M, Fais S. P-glycoprotein-actin association through ERM family proteins: a role in P-glycoprotein function in human cells of lymphoid origin. *Blood*. 2002 Jan 15;99(2):641-8.