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Changes in protein and mRNA expression levels of claudin family after mucosal lesion by intestinal ischemia/reperfusion

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ABSTRACT

Ischemia/reperfusion (I/R) injury of the intestine is the leading cause of organ dysfunction after restoration of blood flow after diverse events, including shock and intestinal transplantation. I/R injury must be overcome for successful small intestinal transplantation. Tight junctions (TJ) are the most apical component of the intercellular junctional complex in epithelial cells; they establish cell polarity and functioning as major determinants of epithelial barrier function. Among the proteins that comprise TJ, the claudin family is thought to play a crucial role in homeostasis in multicellular organisms. Therefore, the aim of this study was to examine the changes in function of TJ and behavior of the claudin family during intestinal I/R. Wistar/ST rats underwent intestinal ischemia by using the spring scale and surgical suture for 1 h, followed by 24 h of reperfusion. We examined the changes in area under the blood concentration curve (AUC) after oral administration of FD-4, which is a paracellular marker, and claudin-1, -2, -4, and -7 mRNA and protein expression levels in ileum. The structure of ileal mucosa was partly damaged and its function was diminished by intestinal I/R until 3 h after reperfusion, but were almost recovered 24 h after reperfusion. However, a time difference was shown between the recoveries of mucosal structure and function. Furthermore, a difference in the expression among various kinds of claudin was found. It was suggested that claudin-4 and multi-PDZ domain protein, which is a scaffolding protein, regulate intestinal paracellular permeability during intestinal I/R. Moreover, the changes in the expression level of claudin-2 were unique.

1. Introduction

Tight junctions (TJ) are the most apical component of the intercellular junctional complex in epithelial cells; they establish cell polarity and functioning as major determinants of epithelial barrier function (Anderson and Van Itallie, 1995). Dynamic regulation of TJ function is fundamental to many physiological processes, and disruption of TJ drastically alters paracellular permeability and is a source of many pathological states (Madara, 1998).

Several transmembrane proteins located in TJ strands have already been described, including occludin (Furuse et al., 1993), tricellulin (Ikenouchi et al., 2005), junctional adhesion molecule (Bazzoni, 2003), and the claudin protein family (Furuse et al., 1998b; Morita et al., 1999). Among the proteins that comprise TJ, the claudin family is thought to play a crucial role in homeostasis in multicellular organisms. The claudin family members have molecular masses of approximately 23 kDa, possess 4 transmembrane domains, and comprise a multigene family consisting of at least 27 members in mice/humans (Furuse et al., 1998a; Mineta et al., 2011; Tsukita et al., 2001). The claudin family confers barrier function as constituents of TJ strands and directly participates in the transport of materials across epithelia through the paracellular pathway by adjusting the tightness and the selectivity of TJ strands; however, the biological role of each claudin remains incompletely understood. Various cells, organs, and tissue compartments have unique distributions of the various claudins and, according to recent reports, claudin-1, -2, -3, -4, -5, and -7 are strongly expressed in rat intestine (Kinugasa et al., 2000; Rahner et al., 2001; Fujita et al., 2006).

In grafting of small intestine, intestinal mucosal barrier dysfunction causes bacterial translocation (Kubes et al., 1992), which often results in fatality. Ischemia/reperfusion (I/R) injury of the intestine is the leading cause of organ dysfunction after restoration of blood flow after diverse events, including shock and intestinal transplantation (Haglund, 1994; Schneider et al., 1994). Therefore, I/R injury must be overcome for successful small intestinal transplantation.

At present, details of the roles of the claudin family in recovery from disintegration of mucosal structure and barrier dysfunction by intestinal I/R injury have not been clarified. Furthermore, the identity of the claudin that is necessary for the acquisition of barrier function has not been clarified.

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We previously reported on an *in vivo* intestinal I/R model made by using the spring scale and surgical sutures (Tomita et al., 2008, 2009; Takizawa et al., 2011); the influence on the tissue surrounding the blood vessel in this model is less than that in the method of intercepting the blood flow with a clip for surgery. Therefore, we can examine the influence of intestinal I/R injury in detail using this model. In the present study, we examined the changes in intestinal mucosal structure that depended on intestinal I/R, that is, the changes in protein expression of the claudin family and permeation of paracellular marker. Moreover, we examined the late phase as well as the early phase of intestinal I/R.

2. Materials and methods

2.1. Materials

Fluorescein isothiocyanate-labeled dextran 4000, 10,000, 20,000, 40,000, and 70,000 (FD-4, FD-10, FD-20, FD-40, and FD-70) were purchased from Sigma–Aldrich Co., Ltd. All other reagents were of analytical grade or better.

2.2. Animals and experimental design

Male Wistar/ST rats (8 weeks old) were purchased from Japan SLC Ltd. (Shizuoka, Japan). All animal experiments were performed according to the guidelines of Tokyo University of Pharmacy and Life Sciences. The animals were fasted for 18 h before starting the experiment. Water was freely available during fasting. We used an *in vivo* intestinal I/R model made using the spring scale and surgical sutures as previously reported (Tomita et al., 2008, 2009; Takizawa et al., 2011). Briefly, the superior mesenteric artery and vein in rats were occluded by hanging using surgical sutures (Shirakawa, Tokyo, Japan) connected with the spring balance for 60 min (ischemia condition), followed by reperfusion based on cutting of sutures (reperfusion condition). The hanging force of a blood vessel during ischemia was 50 g (50 g load group) or 100 g (100 g load group). In CTRL, same surgical operation was treated but the ischemia process was deleted (sham operation). 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.

2.3. Determination of total protein level and phospholipid

An *in situ* ileum loop (60 cm length) was isolated in each rat 1, 3, 6, 12, and 24 h after reperfusion. The ileal luminal contents were thoroughly washed out with a sufficient amount of ice-cold saline and the ileum was then divided into several parts of the same length. The mucosal surface of the ileum was scraped off with a slide glass. The collected mucosa was homogenized in a buffer containing 0.05 mg/mL phenylmethylsulfonylfluoride, 300 mM mannitol, 12 mM Tris, and 5 mM EGTA (pH 7.1) with a tissue homogenizer. The amount of protein in homogenized sample was determined using Micro BCA Protein Assay Reagent Kit (Thermo, Rockford, USA).

The phospholipid that dropped out from ileal mucosa into phosphate-buffered saline of ileal loop (10 cm length) during 1 h was determined. We defined the data of 0–1 h after reperfusion as R = 1, 3–4 h after reperfusion as R = 4, 6–7 h after reperfusion as R = 7, 12–13 h after reperfusion as R = 13, and 24–25 h after reperfusion as R = 25. The concentration of phospholipid in the sample was measured using Phospholipid-C-Test Wako Kit (choline oxidase-DAOS method), according to the manufacturer's protocol (Wako, Osaka, Japan). The concentration of phospholipid in the sample was calculated by comparison with standard solutions of choline chloride and phospholipid.

2.4. Absorption experiments using the in situ closed loop method

Intestinal absorption was evaluated by improved versions of the methods described in our previous report (Iida et al., 2006). Anesthesia was induced using Somnopentyl® (pentobarbital sodium, 50 mg/kg). The animals were placed on a heating pad to maintain body temperature at 37 °C. The abdomen was opened by a midline longitudinal incision and a 7 cm ileal segment was isolated and cannulated at both ends with plastic tubing. The segment was rinsed with phosphate-buffered saline, pH 7.4. A sufficient amount of saline to avoid loss of fluid was dropped onto the surgical area, and then covered with a paper sheet to avoid loss of fluid. Sixty min after starting ischemia using surgical sutures connected with the spring balance, 3 mL of Krebs Henselite Bicarbonate Buffer (KHBB, pH 7.4) solution containing FDs (15 mg/kg) was administered into the loop. Sampling was performed from the portal vein at 0, 3, 5, 10, 15, 20, 30, and 60 min after intravenous administration of FDs. The concentration of FDs in plasma was measured using a fluorescent spectrophotometer (HITACHI FP-6500, Tokyo, Japan). The AUC of FDs was calculated by the trapezoidal method. We defined the data of 0–1 h after reperfusion as R = 1, 3–4 h after reperfusion as R = 4, 6–7 h after reperfusion as R = 7, 12–13 h after reperfusion as R = 13, and 24–25 h after reperfusion as R = 25, respectively.

2.5. Preparation of RNA and cDNA synthesis

Total RNA was isolated from ileal specimens using TRIzol reagents (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, dTTP), 10 mM Tris–HCL (pH 8.4), 25 mM KCl, 5 mM $MgCl_2$, 10 mM DTT, and 10 units of RNaseOUT recombinant ribonuclease inhibitor.

2.6. Analysis of gene expression level in intestine by quantitative reverse transcription-polymerase chain reaction (RT-PCR)

To perform the real-time RT-PCR, 96-well reaction plates with optical adhesive covers and ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) were used. Assay-on-Demand Gene Expression Products were purchased for the claudin family and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Table 1) (Sigma–Aldrich, Tokyo, Japan). Reverse transcription was performed for 1 μg 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 SDS 7000 system from Applied Biosystems using a Universal MasterMix (Applied Biosystems). 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) predesigned TaqMan Gene Expression Assays from Applied Biosystems (Table 1).

2.7. Western blotting

An *in situ* ileal loop (60 cm length) was isolated in each rat, the ileal luminal contents were thoroughly washed out with a sufficient amount of ice-cold saline, and the ileum was then divided into several parts of the same length. The ileum's mucosal surface was scraped off with a slide glass. The collected mucosa was homogenized in a buffer containing 0.05 mg/mL phenylmethylsulfonylfluoride, 300 mM mannitol, 12 mM Tris, and 5 mM EGTA (pH 7.1) with a tissue homogenizer. Protein level was determined by a method using Micro BCA Protein Assay Reagent Kit (PIERCE),

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

Gene		Sequence (5′–3′)	Amplicon size	GenBank ID
Claudin-1	Forward	TGTCCACCATTGGCATGAAG	118 bp	NM-031699
	Reverse	GCCACTAATGTCGCCAGACC		
Claudin-2	Forward	ACAGCACTGGCATCACCCA	108 bp	NM-001106846
	Reverse	GCGAGGACATTGCACTGGAT		
Claudin-3	Forward	CGTACAAGATGAGACGGCCAA	119 bp	NM-031700
	Reverse	AATCCCGGATGATGGTGTTG		
Claudin-4	Forward	AAGGCCAAGGTCATGATCACAG	102 bp	NM-001012022
	Reverse	GAAGTCGCGGATGACGTTGT		
Claudin-5	Forward	GTGAAGTAGCCACCAAACTGCC	106 bp	NM-031701
	Reverse	CCAGCTGCCCTTTCAGGTTAG		
Claudin-7	Forward	TGCTCCTGGATTGGTCATCAG	104 bp	NM-031702
	Reverse	CCTGCCCAGCCGATAAAGA		
GAPDH	Forward	TGAGGTGACCGCATCTTCTTG	102 bp	NM-017008
	Reverse	TGGTAACCAGGCGTCCGATA		

and that of each sample was prepared for 4 μg/μL. The protein expression levels of claudins in homogenate of ileal membrane were evaluated by Western blotting. Western blotting using monoclonal antibody (Zymed Laboratories Inc.) for claudin-1, -2, -4, and -7 was performed as reported previously (Sugibayashi et al., 2009).

2.8. Statistical analysis

All the results are expressed as mean value ± standard error (Mean ± S.E.). Statistical significance between groups was analyzed using Tukey's test and *P* value less than 0.05 was considered to be significant.

3. Results

3.1. Changes in total protein level and leaked phospholipid by intestinal I/R

The total protein level in the ileal mucosa 1 h after reperfusion was decreased in a hanging-force-dependent manner during ischemia when compared with that in the control condition. Moreover, a significant reduction of total protein level in mucosa 3 h after reperfusion was still observed, but a tendency for recovery was shown when compared with the data 1 h after reperfusion. Therefore, the protein level in ileal mucosa showed recovery as the time proceeded. Twelve and 24 h after reperfusion, no significant change was observed in both the 50 g load group and the 100 g load group (Fig. 1a).

In addition, 1 and 4 h after reperfusion, the amount of leaked phospholipid from ileal mucosa to ileal loop also increased in a hanging-force-dependent manner during ischemia, especially in the 100 g load group, when compared with that in the control condition. However, 13 and 25 h after reperfusion, no significant change was observed in either the 50 g or the 100 g I/R group (Fig. 1b).

3.2. Estimation of paracellular permeability from ileal lumen to hepatic vein with or without intestinal I/R

AUC of FD-4 1 h after reperfusion was increased in a hanging-force-dependent manner during ischemia when compared with that in the control condition. Moreover, in the 100 g load group, a significant increase of AUC of FD-4 was still observed 3 h after reperfusion, but the degree of increase of AUC tended to decrease when compared with that 1 h after reperfusion. Thereafter to 7, 13, and 25 h after reperfusion, AUC of FD-4 of both the 50 g load group and the 100 g load group did not show significant changes compared with that of the control condition (Fig. 2a).

AUC of 0–1 h after reperfusion (AUC_{0-1}) values of 5 kinds of FD as paracellular markers with different molecular weights were examined. They clearly decreased with increased molecular weight (Fig. 2b).

3.3. Effect of intestinal I/R on mRNA expression level of claudin-1, -2, -3, -4, -5, and -7 in ileum

Claudin-1 mRNA expression level decreased from the start of reperfusion, and recovered to the control level by 24 h (Fig. 3a). Claudin-2 mRNA expression level significantly decreased from the start of reperfusion, and 24 h after reperfusion, the level was still

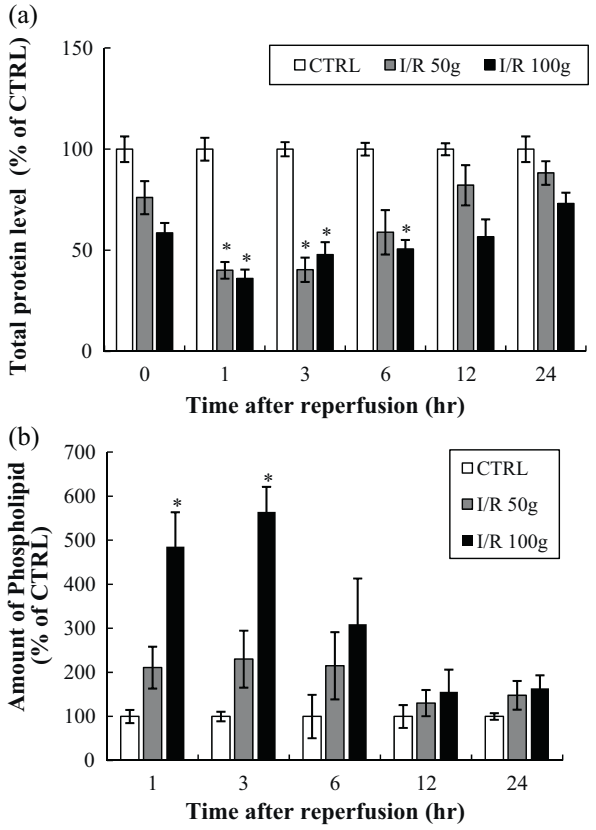

Fig. 1. Effect of intestinal I/R on total protein level in ileum (a) and leaked phospholipid from ileal mucosa (b). Results represent means and S.E. ($n = 11$ (R = 0, 3, 6, 12 and 24) and $n = 4$ (without R = 1) for each condition). The absolute value of total protein and phospholipid of CTRL were 3 mg/cm and 0.4 mg/cm, respectively. *$P < 0.05$ compared with control condition.

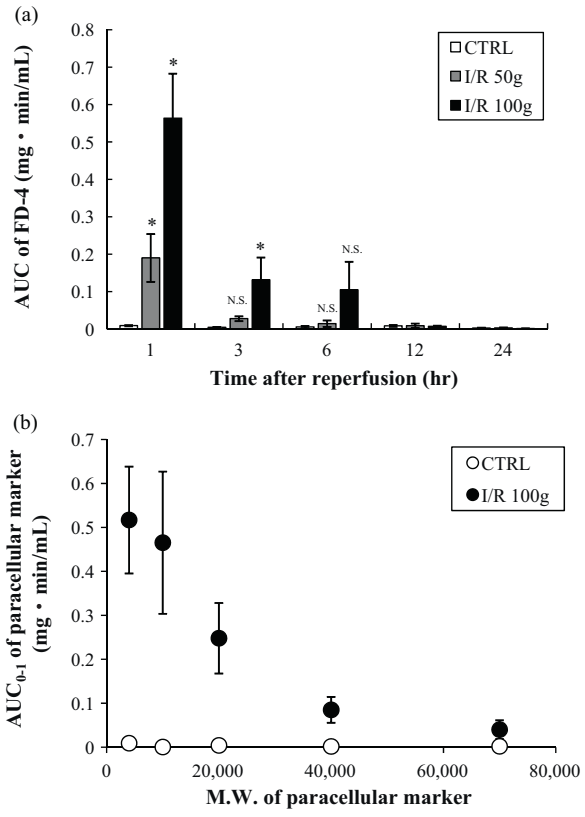

Fig. 2. Effect of intestinal I/R on AUC of FD-4 from ileal lumen (a) and AUC of five kinds of paracellular marker 0–1 h after reperfusion with or without 100 g of load (b). Data represent means and S.E. (n = 11 (R = 0, 3, 6, 12 and 24) and n = 4 (without R = 1) for each condition). *P < 0.05 compared with control condition.

decreased compared with that of the control condition (Fig. 3b). In contrast, claudin-4 mRNA expression level significantly increased from the start of reperfusion, and the maximum value was shown 1 h after reperfusion, and converged to the control level by 24 h (Fig. 3c). Claudin-7 mRNA expression level significantly decreased until 12 h after reperfusion, and 24 h after reperfusion, the level converged to the control level (Fig. 3d). For claudin-3 and -5 mRNA, no time- or lesion-dependent changes were found (data not shown).

3.4. Correlation between AUC of FD-4 and mRNA expression level of claudin family

No significant correlation was observed between AUC of FD-4 and the mRNA expression level of claudin-1 (R^2 = 0.2169), -2 (R^2 = 0.2041), or -7 (R^2 = 0.0704) (Fig. 4a, b and d). However, for claudin-4, a strong correlation was obtained (R^2 = 0.8576, P < 0.01) (Fig. 4c).

3.5. Effect of intestinal I/R on relative protein expression level of claudin-1, -2, -4, and -7 per total protein amount in ileum

The protein expression level of claudin was corrected by total protein level in ileal homogenate, and is shown as relative protein expression level in Fig. 6. Relative protein expression levels of claudin-1, -2, -4, and -7 were significantly decreased when compared with those in the control condition until 1 h after reperfusion (Fig. 5). Claudin-1 and -4 converged to the control level 6 h after reperfusion (Fig. 5a and c). Claudin-2 recovered to the control level 3 h after reperfusion, and converged to the control level 24 h after reperfusion (Fig. 5b). Claudin-7 was significantly decreased until 3 h after reperfusion, but it recovered to the control level 24 h after reperfusion (Fig. 5d).

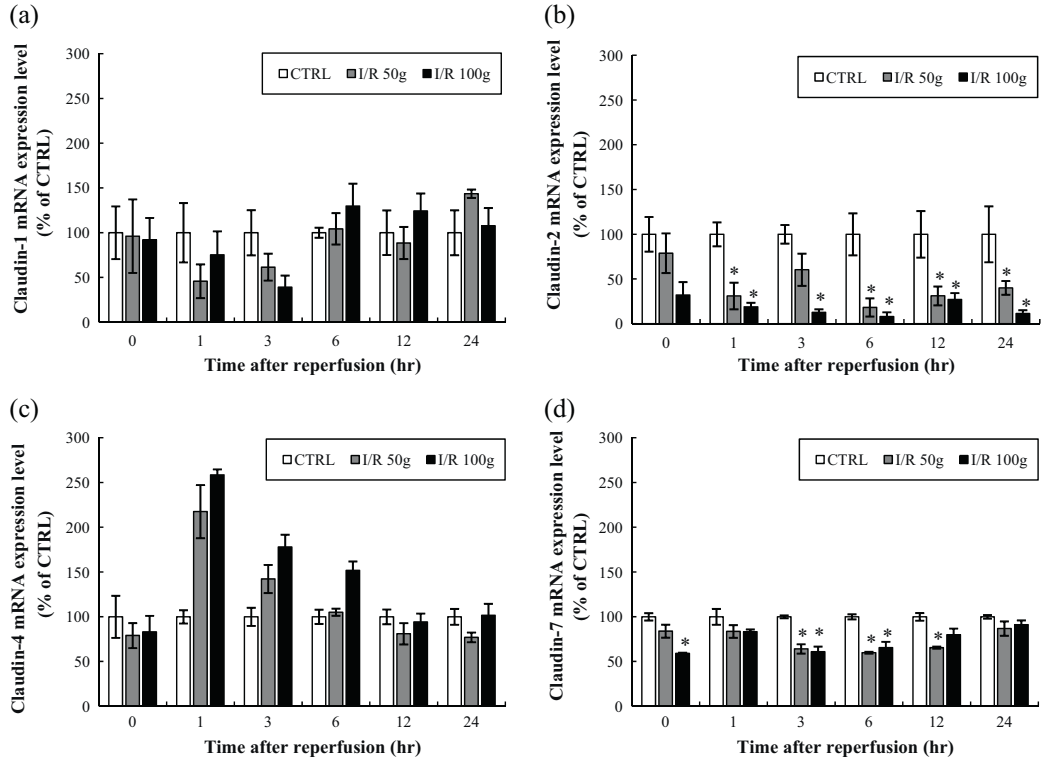

Fig. 3. Effect of intestinal I/R on mRNA expression level of claudin-1 (a), -2 (b), -4 (c), and -7 (d) in ileum. Data represent means and S.E. (n = 6 for each condition). *P < 0.05 compared with control condition.

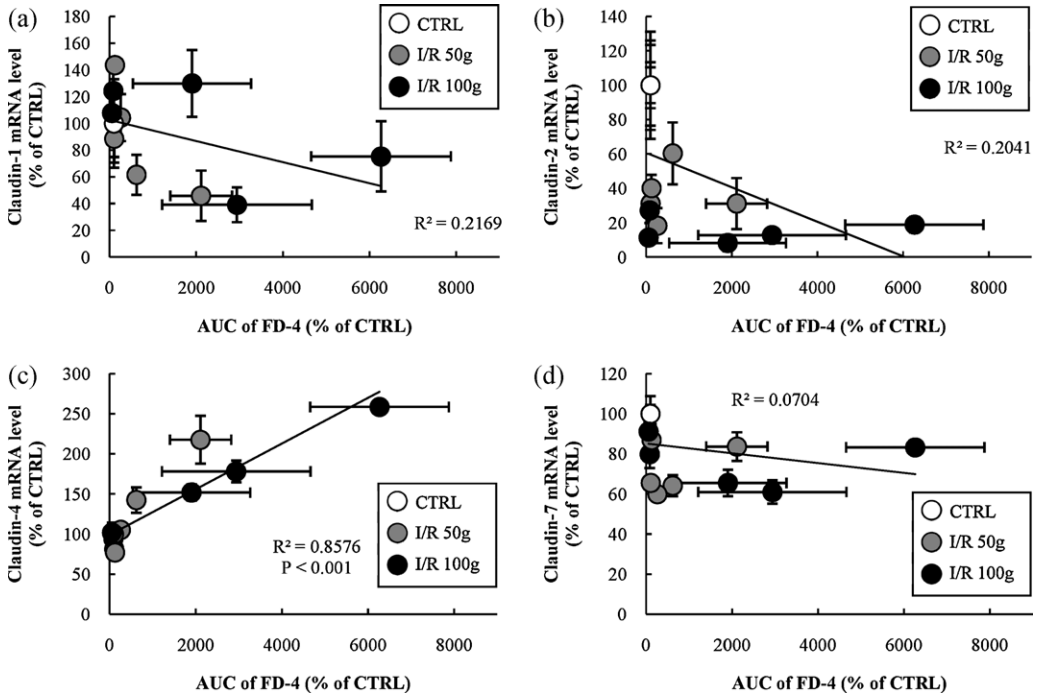

Fig. 4. Correlation between AUC of FD-4 and mRNA expression level of claudin-1 (a), -2 (b), -4 (c), and -7 (d) in ileum. Open circle: CTRL, Gray circle: I/R 50 g, Closed circle: I/R 100 g. Data represent means and S.E. (n = 4–11 for each condition).

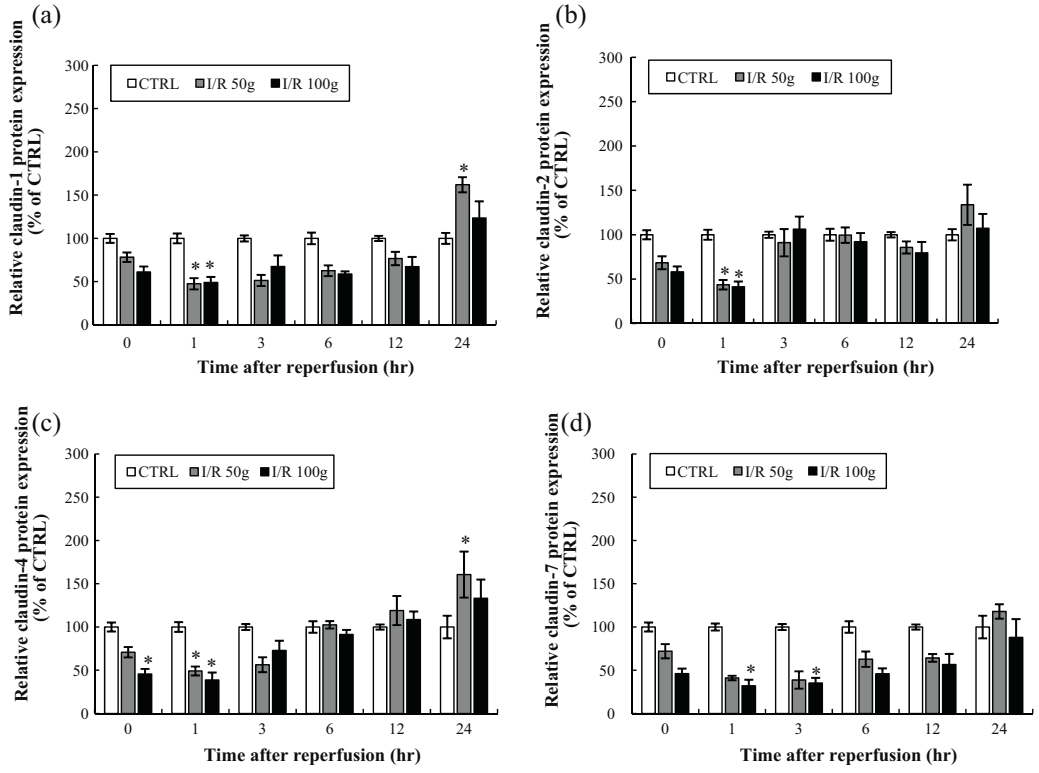

Fig. 5. Effect of intestinal I/R on relative protein expression level of claudin-1 (a), -2 (b), -4 (c), and -7 (d) in ileum. Data represent means and S.E. ($n = 4$ for each condition). $^{*}P < 0.05$ compared with control condition.

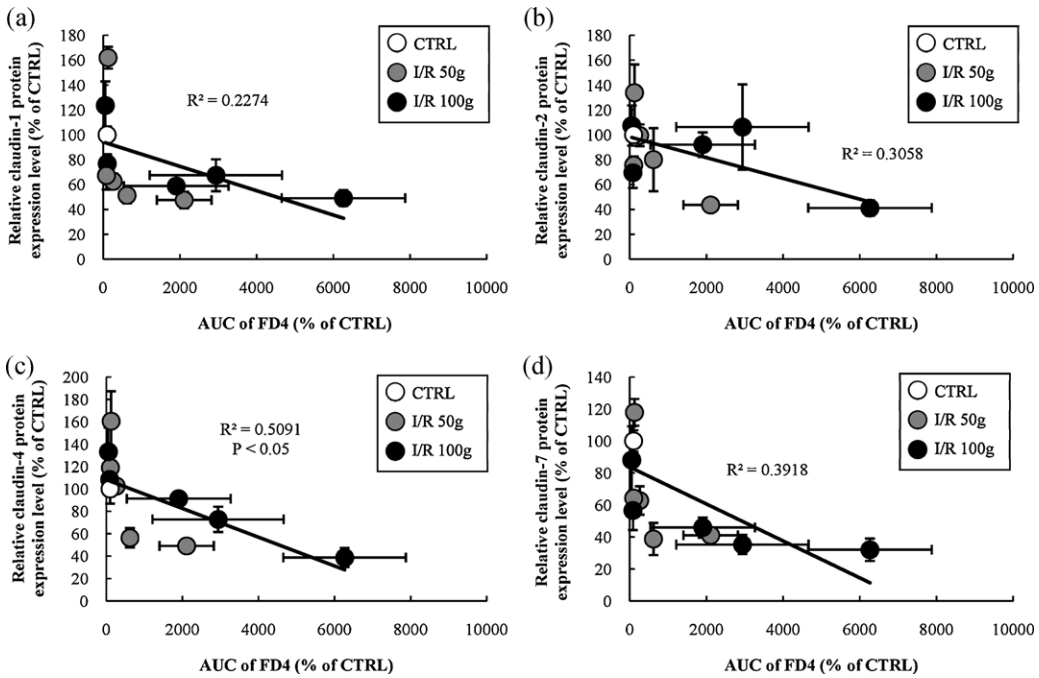

Fig. 6. Correlation between AUC of FD-4 and relative protein expression level of claudin-1 (a), -2 (b), -4 (c), or -7 (d) in ileum. Open circle: CTRL, Gray circle: I/R 50 g, Closed circle: I/R 100 g. Data represent means and S.E. (n = 4–11 for each condition).

3.6. Correlation between AUC of FD-4 and relative protein expression level of claudin family

No significant correlation was observed between AUC of FD-4 and claudin-1 (R^2 = 0.2274), -2 (R^2 = 0.3058), or -7 (R^2 = 0.3918) relative protein expression level (Fig. 6a, b, and d). However, for claudin-4, a significant correlation was obtained (R^2 = 0.5091, P < 0.05) (Fig. 6c).

4. Discussion

Intestinal I/R is directly and clinically associated with small bowel transplantation, circulatory system disease, and intestinal obstruction. In the present study, we examined the changes in structure and function of ileal mucosa and expression of claudin family until 24 h after reperfusion. Identifying molecular species of claudin family which strongly participates in a change of the membrane transport, not only leads to the prediction of drug disposition induced by the changes in the claudin species patients, but also to get information on the target factor of drug delivery system (DDS). It has been reported that claudin-4 strongly participated membrane permeation *via* paracellular pathway in the normal condition (Oshima et al., 2007). Even in the disease condition, it is considered that claudin-4 strongly participates to regulate the membrane permeation. Moreover the more claudin species than claudin-4 may participate in regulation of the membrane permeability in the disease condition. Therefore, we tried identification of claudin family which strongly participate in membrane permeation by evaluating the correlation between membrane permeation and the expression level of claudin family.

First, we examined the changes in the gastrointestinal mucous membrane by intestinal I/R. At 1, 3, and 6 h after reperfusion, total protein level in ileal lumen was significantly decreased in a hanging-force-dependent manner when compared with that in the control condition. In contrast, the amount of phospholipid that leaked from ileal lumen was significantly increased in a hanging-force-dependent manner. However, these changes were not observed 24 h after reperfusion (Fig. 1). In addition, we previously reported histological changes in ileal mucosa 1 h after reperfusion by intestinal I/R (Tomita et al., 2008). The progression of histological changes was observed 3 h after reperfusion, but the change was hardly observed 24 h after reperfusion (Tomita et al., 2009). These results suggested that mucosal lesion by intestinal I/R was induced at the same time as the start of reperfusion. However, the lesions were almost recovered by 24 h after reperfusion, although this depended on the severity of the lesion.

Moreover, we evaluated the changes in paracellular permeability during intestinal I/R by examining absorption of FD-4. It was shown that FD-4 follows polarized transport (Tomita et al., 2000), and the permeation may occur *via* the transcellular pathway as well as the paracellular pathway in this model. In Fig. 2b, AUC_{0-1} of FDs can be clearly seen to decrease with increased molecular weight (Fig. 2b), showing that these FD compounds permeate *via* the paracellular route. AUC of FD-4 was significantly increased by intestinal I/R 1 and 4 h after reperfusion, but recovered to the control level by 7 h after reperfusion (Fig. 2a). These results indicated that TJ opens by intestinal I/R but closes as time passes. This showed that the changes in structure and function of ileal mucosa by intestinal I/R were almost recovered by 25 h after reperfusion. Because a time difference was shown in the recoveries of mucosal structure and function, we examined the expression level of the claudin family.

According to recent reports, claudin-1, -2, -3, -4, -5, and -7 are strongly expressed in rat intestine (Kinugasa et al., 2000; Rahner et al., 2001; Fujita et al., 2006); therefore, we examined the expression level of these claudins in ileum. In Figs. 3b and 5b, a negative correlation cannot be observed for claudin-2 mRNA and protein expression levels. Generally, the protein expression level increases after induction of mRNA. However, claudin-2 did not show such behavior. Although the reason for this behavior has not been clarified, Telgenhoff et al. (2008) and Fujita et al. (2008) reported that claudin-2 protein forms a cation-selective channel, and the permeation of cationic material is promoted. The increase of claudin-2 induces the invasion of foreign substances such as bacteria; therefore, the mRNA expression level of claudin-2 was not increased during intestinal I/R. On the other hand, it was

reported that, in colorectal cancer tissues and active ulcerative colitis, the expression of claudin-2 was up-regulated compared with that in normal tissues (Kinugasa et al., 2007; Oshima et al., 2008). Therefore, it is clear that homeostasis of claudin-2 plays a key role, and it is suggested that claudin-2 is an extremely important factor for TJ construction and its remodeling. However, more detailed examination of claudin-2 is necessary.

On the other hand, a marked correlation of expression level of claudin-4 and AUC of paracellular markers, such as FD-4, was shown (Figs. 4c and 6c). These results suggested that claudin-4 regulates paracellular permeability, and supported the findings of Oshima et al. (2007) and Michikawa et al. (2008). Moreover, it was shown that claudin-4 may possibly become as a target factor of DDS also in the disease condition.

The claudin family confers barrier function as constituents of TJ strands and directly participates in the transport of materials across epithelia through the paracellular pathway by adjusting the tightness and the selectivity of TJ strands (Van Itallie et al., 2001; Furuse et al., 2001). Therefore, it is very important that we integrate findings for two or more kinds of claudin and scaffolding protein of TJ when discussing paracellular permeability.

Numerous reports about the claudin family show that it is important to clarify what kinds of claudin regulate paracellular permeability. In terms of the regulation of administration of drugs, changes in transepithelial electrical resistance as an index of the opening of TJ or changes in permeation amount of paracellular marker have been examined by knockdown of certain kinds of claudin by gene manipulation (Tamura et al., 2008) or regulation of claudin expression level by administration of chemical substance (Amasheh et al., 2008). On the other hand, to carry out appropriate drug therapy for a disease or in transplantation, it is extremely important to clarify which claudin influences restructuring of intestinal mucosa and regulation of paracellular permeability. In this study, we examined the function of the claudin family in detail and obtained extremely important information for clinical aspects and drug development. However, it has been reported that the claudin family has numerous functions related to different kinds of disease (Fujita et al., 2008; Kinugasa et al., 2007; Weber et al., 2008; Kim et al., 2008); thus, more detailed examination during intestinal I/R is necessary.

As described above, there are many reports about the changes in the expression level of claudin family in a cancer cell (Kinugasa et al., 2007; Oshima et al., 2008), but there are very few reports about the changes in the expression level of claudin family during gastrointestinal disease. The increase of claudin-1 and claudin-2 expression level in the patients of inflammatory bowel disease (IBD) (Weber et al., 2008) and Crohn (Prasad et al., 2005) were reported, but there are few reports about intestinal I/R injury. In addition, the increase of expression level of claudin-1 (Poritz et al., 2011) and -2 (Suzuki et al., 2011) were reported in various inflammatory model rats. However, because the molecular species of claudin family differ in all research, it is very difficult to compare those results since the disease and experimental conditions are different. Therefore, it is extremely important to accumulate many date about the changes in the expression level of claudin family in gastrointestinal disease. Further, it is necessary to cross talk about the information of the changes in the expression level of claudin family in each gastrointestinal disease, leading to development of pharmacotherapy in the gastrointestinal disease and DDS.

5. Conclusions

This is a first report of a study in which the expression levels of claudin family mRNA and protein were quantitatively evaluated during intestinal I/R, and in which associated paracellular permeability was discussed. It was shown that the damage of intestinal mucosa by intestinal I/R injury occurred with the start of reperfusion in a manner dependent on the hanging force during ischemia, and recovered by almost 24 h. In addition, the existence of an important relationship between restoration of paracellular permeability and remodeling of mucosal structure was shown by the correlation between FD-4 absorption and mRNA and protein expression levels of claudin-4. Furthermore, a time difference between paracellular permeability and claudin-4 expression level was found. Our results suggested that claudin-4 regulate paracellular permeability in intestine during intestinal I/R. Moreover, the behavior of the expression level of claudin-2 was unique during intestinal I/R.

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