Temperature Dependency of Bidirectional Flux in the Rat Intestine Subjected to Graded Ischemia

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This study examined the effect of temperature and ischemia on permeation of fluorescently-labeled dextran (M.W. = 4 kDa; FD4) across rat intestinal mucosa. Permeability was evaluated ex vivo using an everted gut sac technique in both the mucosal-to-serosal (M→S) and serosal-to-mucosal (S→M) directions. At baseline (B), 30-min of ischemia (I-30) and 60-min of ischemia (I-60), intestinal segments were prepared and incubated at 37℃, 15℃ and 4℃ for 30 min. Clearance (nl/min/cm^2) was calculated based on the accumulated amount of FD4 at 30 min. Both M→S and S→M fluxes increased with increasing temperature at B, I-30 and I-60. Ischemic gut (I-30 and I-60) had about a three-fold higher (M→S)/(S→M) flux ratio than that of normal gut ($p < 0.001$). At 4℃, neither M→S nor S→M flux was different between B and I-30, but both M→S and S→M fluxes significantly increased at I-60, suggesting an increase in permeation via a passive mechanism. Increased bidirectional fluxes at 37℃ were obtained in the I-30 and I-60 gut sacs when compared to B. We conclude that FD4 is actively transported across the intestinal mucosa in the S→M direction and that ischemic injury increases passive diffusion of the probe across the gut wall.

Keywords: *Permeability, Gut ischemia, Temperature dependency mechanism*

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The intestinal mucosal barrier is of importance in the limitation of systemic contamination by intraluminal microbe and their products. There has been a growing evidence of physiologic and morphologic studies that the epithelial barrier selectively restricts the movement of solutes with a large molecular radius (*i.e.* albumin, 36 micron) rather than that of smaller radius (as small as 3 micron). These findings could be explained by larger number of population of small pores (4-7 micron in radius) than that of large pores (65 micron in radius) which perforate through the mucosal layer[1]. Recently, as results of several studies, there are two possible routes for passive permeation of epithelium by hydrophilic molecules and ions. One is the transcellular pathway , and the other is the paracellular pathway. Theoretically, more than 85% of passive movement of hydrophilic solutes is thought to be permeated through the paracellular pathway[2]. The tight junction between adjoining epithelial cells act as a limiting barrier, controlling permeation via the paracellular pathway, and serving as "selective permeability" the normal intestinal epithelium[3]. Under physiologic conditions, intracellular signals are transmitted to the tight junction and translated into constriction or dilation of the apical "pore", contributing to the mucosal permeability[4].

Several techniques have been developed for *in vivo* assessment of epithelial barrier integrity in clinical and experimental animals. In this regard, two main approaches are used to assess intestinal permeability. One is loading a hydrophilic permeability probe and subsequent monitoring the appearance of the molecule in urine[5] or plasma[6]. The other more popular one is measurement of plasma-to-lumen clearance by infusion of an appropriate hydrophilic probe while the lumen of an isolated segment of intestine is perfused with a buffer solution[7,8]. Although useful, the latter technique, which accounts for opposite to the direction of translocation, provides uninterpretable results during ischemia and abruptly

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high permeability during reperfusion[9]. In addition to endothelial flux, this method employs serosal-to-mucosal permeation of solutes by which unclear mechanisms account for both physiologic and pathologic conditions[10].

We hypothesized that bidirectional flux of intestinal barrier would be adenosine triphosphate (ATP) dependent mechanism of hydrophilic solutes. The aims of this present study is: 1) to determine whether alterations in temperature influence the transportation of hydrophilic solutes in the mucosal-to-serosal direction and vise versa, 2) to evaluate the bidirectional flux of small intestine subjected to graded ischemia.

Material and Method

Animals

Sprague-Dawley rats (250-350 g) were used in this study. The animals were maintained at 22°C and 12 h light-dark cycle, with free access to standard laboratory chow and water *ad libitum*. Before operation, the rats were fast overnight but allowed water *ad libitum*. The research protocol was executed in accordance with the guidelines of the Institute Animal Use and Care Committee of Beth Israel Deaconess Medical Center and complied with regulations regarding animal care as published by the American Physiological Society and the National Institute of Health.

Mesenteric ischemia model

The rats were then anesthetized with intraperitoneal injections of sodium pentobarbitol (30 mg/kg) and ketamine (20 mg/kg) and maintained under anesthesia with intermittent intramuscular injection of ketamin (10 mg/kg) as needed. The animals were kept on a temperature-controlled surgical board (37°C) and allowed to breathe spontaneously. Via a midline laparotomy incision, the small bowel from upper jejunum to terminal ileum was gently irrigated with warm normal saline. During the operation, the eviscerated bowel was kept moist by overlaying with saline saturated gauze. One hundred and two segments (4-5 segments/rat) of 12-cm small intestine were systematically randomized selected to be subjected to normal, 30-min and 60-min ischemia. Ischemia was induced by ligating both mesenteric arteries and veins with silk 4/0. The intestinal segment was divided into 3 pieces; 8 cm for an assessment of intestinal permeability, 3 cm was frozen in liquid nitrogen and stored at -70°C for adenosine triphosphate (ATP) measurement and 1 cm was fixed in 10% buffered formalin histological examination.

Intestinal permeability measurement: Everted gut sac technique

Mucosal-to-serosal flux. Intestinal permeability was measured as previously described by Wattanasirichaigoon et al[9]. Briefly, the preparation of everted gut sacs was performed in ice-cold modified Krebs-Henseleit bicarbonate buffer (KHBB, pH 7.4). One end of the gut segment was ligated with 4-0 silk. The gut sac was then everted onto a thin plastic rod, and then the gut sac was secured with 4-0 silk to the grooved tip of a 5 mL plastic syringe containing 1.5 mL of KHBB. The everted gut was gently distended by injecting the 1.5 mL of KHBB. The sac was then suspended in a 100 mL beaker containing 80 mL of KHBB. Fluorescein-isothiocyanate dextran (MW 4,000 Da; FD4) was added to a final concentration of 20 microgram/mL. The beaker was incubated in either 4°C (ice cooling), 15°C or 37°C (water bath), and the bathing solution was continuously bubbled with 95% O_2 and 5% CO_2.

A 1.0 mL sample was taken from the beaker at the beginning of the experiment to determine the initial mucosal FD4 concentration. Following a 30 min incubation, the length and diameter of gut sac were measured and serosal fluid within the gut sac was then aspirated back to the syringe. A 1.0 mL serosal sample was taken after removing the gut sac from the syringe. The samples were cleared by centrifugation for 10 min at 1,000 x *g* and 4°C. The supernatant (300 micro liter) was diluted with phosphate buffered saline (3.0 mL), and fluorescence was measured using a Perkin-Elmer LS-50 fluorescence spectrophotometer (Palo Alto, CA) at an excitation wavelength of 492 nm (slit width = 10.0 nm), and an emission wavelength of 515 nm (slit width = 10.0 nm). These numbers were used with the following equations to determine the permeation rate and the apparent clearance rate of FD4 from the segment of small intestine.

Serosal-to-mucosal flux. The everted gut was gently distended by injecting the 1.5 mL of FD4 (5 mg/mL). The sac was then suspended in a 100 mL beaker containing 80 mL of KHBB. The beaker (mucosal chamber) was incubated for 30 min in either 4°C (ice cooling), 15°C (temperature-controlled) or 37°C (water bath), and the bathing solution was continuously bubbled with 95% O_2 and 5% CO_2. After the start of incubation, 100 ml samples of mucosal chamber (KHBB) were obtained to be used for measurement of the mucosal concentrations of the FD4 every 5 min. Concentrations of FD4 were determined as described above. From the length (L)

and diameter (D) of the sac, the serosal surface area (A_{ser}) was calculated according to equation 1.

$$A_{ser} = \pi L D_{ser} \quad (1)$$

The internal diameter (D_{ser}) was calculated by volume (V) distending the serosal compartment as equation 2

$$D_{ser} = \sqrt{\frac{4V}{\pi L}} \quad (2)$$

The following equations were used to calculate the permeation rate (PR) in mg/min and (serosal-to-mucosal) clearance (C*sm*) in $nl.min^{-1}.cm^{-2}$ of FD4:

$$M_{muc} = ([FD4]_{muc}) \times 80 \quad (3)$$

$$PR = M_{muc} / \text{incubation time} \quad (4)$$

$$Csm = 1{,}000\,(PR / [FD4]_{ser}) / A_{ser} \quad (5)$$

where M is the mass of FD4 in the mucosal chamber at every 5 min of incubation period, $[FD4]_{ser}$ is the FD4 concentration of 5 mg/mL in the serosal fluid. To calculate the clearance of expected mucosa function (C_{calc}) after ischemia was induced, the following formula was used:

$$(C_{normal})^{-1} = (C_{calc})^{-1} + (C_{isc})^{-1} \quad (6)$$

where C_{normal} is the clearance of normal intestinal mucosal, C_{isc} is the clearance of ischemic gut. The back-calculated clearance of expected ischemic mucosal function (C_{calc}) demonstrated that the contribution of intact mucosal architecture to the total barrier of the intestinal wall (%CMB), expressed as the following:

$$\%CMB = 100 - [100 \times (C_{isc} - C_{normal})/C_{isc}]$$

Measurement of ATP

Tissue ATP levels were determined using the luciferin/luciferase method. A piece of small intestine (approximately 0.2 g) was homogenized in 2.0 mL of an aqueous solution of 2% (w/v) trichloroacetic acid / 2 mM EDTA using a glass homogenizer (10 stokes). The homogenate was centrifuged at 10,000 *g* for 5 min at 4°C. The supernatant was diluted 1:200 in Tris-EDTA buffer (0.10 M Tris/2 mM EDTA, pH 7.75). The diluted supernatant (50 micro liter) was then added to 150 micro liter of Tris-EDTA buffer, followed by the addition of 50 ml of the luciferin/luciferase reagent (BioOrbit, Turku, Finland). Bioluminescence measurements were subsequently performed with the use of a LKB model 1250 luminometer (Turku, Finland). Following this measurement, a known quantity of an ATP standard was added to the samples as an internal control, and the corresponding bioluminescence was measured. The remaining pellets were digested overnight in 1 M NaOH at 4°C and assayed for protein concentration as described above. ATP levels are expressed as nanomoles ATP/mg tissue protein.

Histopathologic examination

A portion of the small bowel was resected as a normal, at the end of 30 and 60 min of ischemia and following 60 min of reperfusion All specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Mucosal lesions were blindly graded on a scale of 0 to 5 with the following criteria as described[10]. Grade 0, normal mucosa; Grade 1, subepithelial space formation; Grade 2, moderate epithelial lifting confined to the tip of the villi; Grade 3, extensive epithelial lifting, a few tips are denuded; Grade 4, denuded villi, dilated exposed capillaries, increased cellularity in the lamina propria; Grade 5, hemorrhagic ulceration. Ten individual readings were obtained from each slide, and the mean measurements were reported.

Statistical analyses

Data were analyzed with a statistical software package (SigmaStat for Windows, Jandel Scientific, Erkrath, Germany) and expressed as mean $\pm$ standard error of the mean (SEM). Statistical significance in differences between means within groups or between different groups was determined using one-way ANOVA and unpaired Student's t-test, respectively. A p-value of less than 0.05 was considered significant.

Results

Normal serosal permeability to FD4 under incubation with different temperature over the 30-min duration is shown in Fig. 1. We did not find any significant differences of bidirectional clearance in terms of regional differences (data not shown). There was a lag period during 0-10 min period, followed by a steady-state permeation during 10-20 min period, with a permeation rate (slope) of 0.09 ± 0.03, 0.66 ± 0.18, and 1.45 ± 0.14 micro g/min/cm^2 under 4°C, 15°C and 37°C, respectively. In I-30 gut sacs, the permeation rate calculated from slope of each curve in Fig. 2 were 0.14 ± 0.02, 1.3 ± 0.31, and 3.05 ± 0.56 micro g/min/cm^2 under 4°C, 15°C and 37°C, respectively. With similar calculations, the permeation rates of the I-60 gut sacs were 0.97 ± 0.06, 1.73 ± 0.29, and 4.78 ± 1.05 mg/min/cm^2 under 4°C, 15°C and 37°C, respectively (Fig. 3). The S-to-M cumulative permeability of FD4 incubated *ex vivo* at 4°C using the everted gut sac remained unchanged over the 30-min period in normal and I-30 gut sacs, whereas a significantly increased permeability was observed in I-60 gut sac. The cumulative permeability of these three conditions, as shown in Fig. 1-3, was increased with an increase in temperature. The

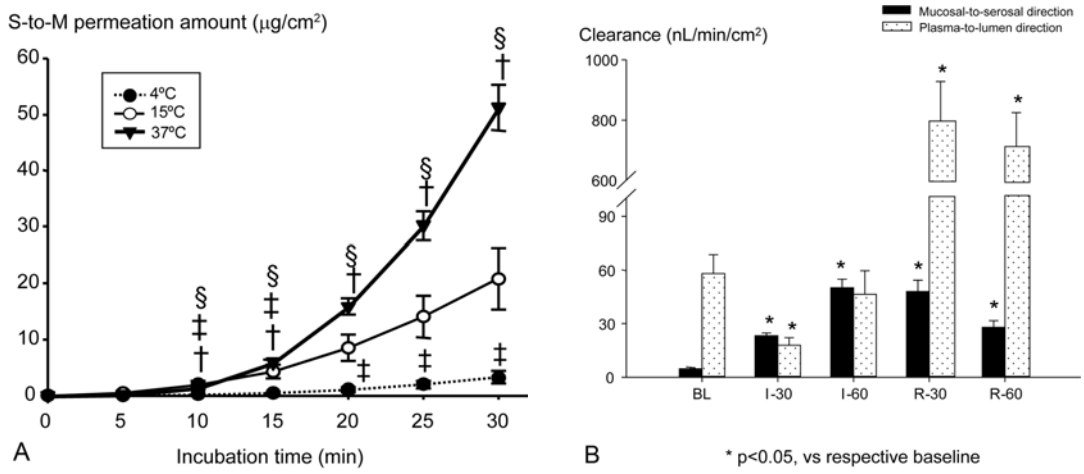

Fig. 1 Serosal-to-mucosal permeation amount was calculated by cumulative trans-serosal permeation of FD4 (A) in the everted gut sac preparations of rat normal small intestine during 30-min incubation under 4°C, 15°C and 37°C conditions (n = 5-6 specimens/each from 4 rats). Values were expressed as mean ± SEM. Cumulative permeation showed the relatively steady state (slope = permeation rate; r^2 = 0.97-99, $p < 0.001$) during 5-15 min, followed by a pronounced increase in permeability (B) thereafter. † $p < 0.05$, 15°C vs. 37°C, ‡ $p < 0.05$, 4°C vs. 15°C, § $p < 0.05$, 37°C vs. 4°C using unpaired t-test

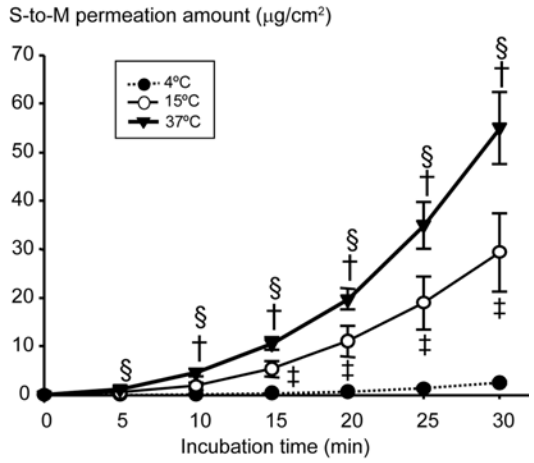

Fig. 2 Serosal-to-mucosal permeation amount was calculated by cumulative trans-serosal permeation of FD4 in the everted gut sac preparations of rat small intestine subjected to 30-min ischemia, under 4°C, 15°C and 37°C of temperature (n = 5-6 specimens/each from 4 rats). Vertical lines indicate SEM. Cumulative permeation showed the relatively steady state (slope = permeation rate; r^2 = 0.97-99, $p < 0.001$) during 5-15 min, followed by a pronounced increase in permeability thereafter. Closed circles represent cumulative permeability under 4°C, open circles represent 15°C, and closed triangles represent 37°C. † $p < 0.05$, 15°C vs. 37°C, ‡ $p < 0.05$, 4°C vs. 15°C, § $p < 0.05$, 37°C vs. 4°C using unpaired t-test

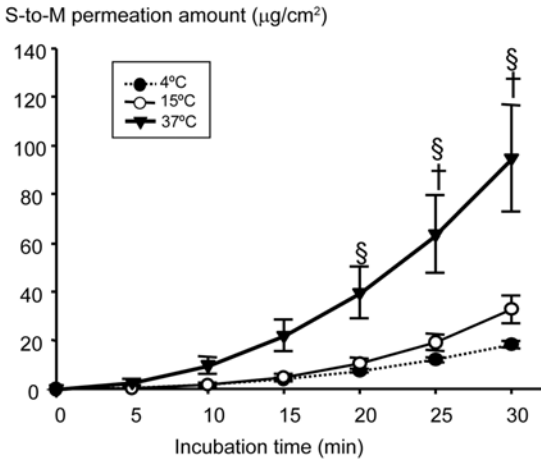

Fig. 3 Serosal-to-mucosal permeation amount was calculated by cumulative transerosal permeation of FD4 in the everted gut sac preparations of rat small intestine subjected to 60-min ischemia, which was incubated for 30 min under 4°C, 15°C and 37°C of temperature (n = 5-6 specimens/each from 4 rats). Vertical lines indicate SEM. Cumulative permeation showed the relatively steady state (slope = permeation rate; r^2 = 0.97-99, $p < 0.001$) during 5-15 min, followed by a pronounced increase in permeability thereafter. Closed circles represent cumulative permeability under 4°C, open circles represent 15°C, and closed triangles represent 37°C. † $p < 0.05$, 15°C vs. 37°C, § $p < 0.05$, 37°C vs. 4°C using unpaired t-test

15°C's permeability curve of each condition was in the mid-way between those of 4°C and 37°C. In addition, the permeation rate (slope) of each condition (normal, I-30 and I-60) was significantly increased as an increase in temperature ($p < 0.05$).

The M-to-S clearance of each condition under three different temperature of incubation was depicted in Fig. 4A. The M-to-S clearance of I-60 and I-30 gut sacs were significantly higher than that of normal gut sacs at any temperature ($p < 0.05$). Mucosal permeability of I-60 gut sac was higher than I-30 when incubated at only 4°C and 15°C, but not significant at 37°C. The results of S-to-M clearance were depicted in Fig. 4B. Under 4°C-incubation, significantly higher S-to-M clearance in I-60 was observed when compared to those of normal and I-30 ($p < 0.05$). The S-to-M clearance of I-60 gut sacs with 15°C and 37°C incubation did not show any significantly higher than that of I-30. There was no difference of S-to-M clearance between normal and I-30 gut sacs, but I-60 showed significantly higher than normal at any temperature (Fig. 4A).

Considering the physiologic condition of 37°C incubation, Fig. 5A demonstrated that much higher S-to-M flux than the opposite direction ($p < 0.001$) was found (n = 4-6 specimens from 6-8 rats). Percent changed from baseline of flux ratio (M-to-S : S-to-M) in ischemic guts were not significant difference at 4°C, but significantly difference were reached at both 15°C and 37°C (Fig. 5B). In this regard, however, ischemic gut (I-30 and I-60) had about a three-fold higher (MS)/(S→M) flux ratio than that of normal gut ($p < 0.001$).

The back-calculated clearance of ischemic mucosa (C_{calc}) showed that the contribution of normal

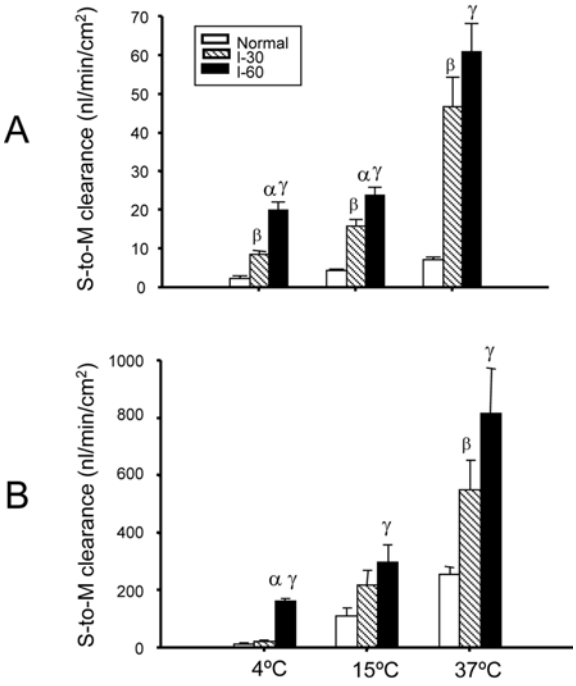

Fig. 4 Intestinal permeability of FD4 was assessed by both mucosal-to-serosal (A) and serosal-to-mucosal (B) directions. Mucosal-to-serosal clearance of normal, I-30 and I-60 small intestine was calculated from permeation amount of FD4 in the serosal side after 30-min incubation. Serosal-to-mucosal clearance of normal, I-30 and I-60 small intestine was calculated from permeation amount of FD4 in the mucosal side after 30-min incubation. The clearance was calculated as shown in the Methods and Materials. $^{\alpha}$ $p < 0.05$, I-30 vs. I-60; $^{\beta}$ $p < 0.05$, normal vs. I-30 and $^{\gamma}$ $p < 0.05$, normal vs. I-60 using unpaired t-test

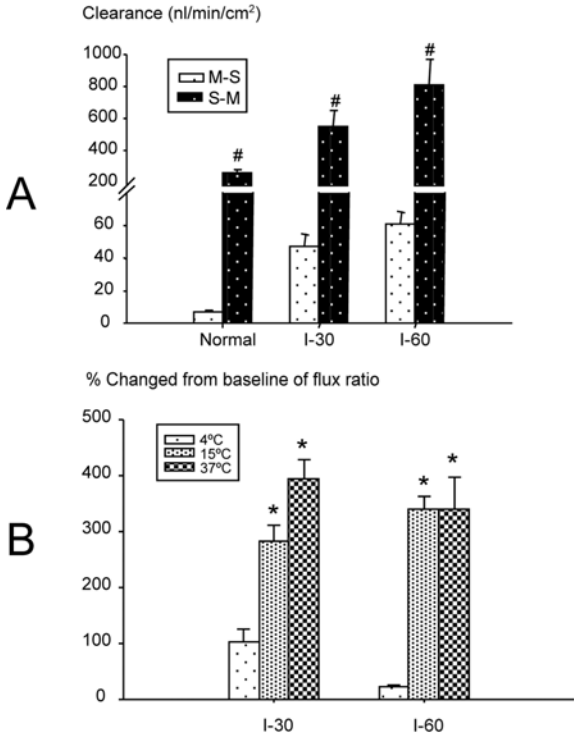

Fig. 5 Bidirectional flux of FD4 through the normal and graded ischemic conditions of everted gut sacs which were incubated in 37°C water bath, as described in Methods and Materials. Clearance of both directions were depicted as A, and percent changed from respective baseline value at each temperature of mucosal-to-serosal: serosal-to-mucosal clearance (or flux ratio) were depicted as B. $^{\#}$ $p < 0.001$, S→M vs. M→S clearance, and * $p < 0.001$ vs. normal using unpaired t-test

Table 1. The contribution of the mucosa to the total barrier function of the intestinal wall

		M-to-S clearance			S-to-M clearance		
		4°C	15°C	37°C	4°C	15°C	37°C
Normal	C_{normal}	2.3 ± 0.7	4.3 ± 0.6	7.2 ± 0.9	15.1 ± 5.5	112.4 ± 29.2	258.2 ± 25.2
I-30	CI-30	8.7 ± 0.8	15.7 ± 1.9	46.8 ± 7.7	24.9 ± 3.4	221 ± 50.6	549.3 ± 104.6
	Ccal	3.13	5.94	8.47	38.21	228.63	487.08
	CMB	73.53%	72.58%	84.69%	39.49%	49.15%	53%
I-60	CI-60	20.1 ± 2.1	23.7 ± 2.1	61.2 ± 7.1	162.7 ± 10.4	300.9 ± 58.3	815.5 ± 157.5
	Ccal	2.59	5.27	8.12	16.63	179.39	377.76
	CMB	88.53%	81.82%	88.28%	90.73%	62.65%	68.34%

Note: Clearance of fluorescein isothiocyanate-dextran (FD4; M.W. = 4kDa) was expressed as mean ± SEM (nl/min/cm^2), n = 5-6/each. The expected clearance was estimated by means of the formula: $(C_{normal})^{-1} = (C_{calc})^{-1} + (C_{isc})^{-1}$. CMB = percent of contribution of the mucosa to the total barrier function of the intestinal wall

mucosal epithelium to the total barrier of the intestinal wall (CMB) was 72-84% and 81-88% for the I-30 and I-60 in the M-S direction, respectively (Table 1). In the S-M direction under 15°C and 37°C, it was whereas it was 49-53% and 62-68%for the I-30 and I-60, respectively, whereas it was only 39% in I-30, but up to 90% in I-60.

ATP concentration in the intestinal tissue (n = 21 specimens/each condition) subjected to ischemia are shown in Fig. 6. ATP levels significantly dropped from normal of 19.8 ± 1.4 to 15.1 ± 0.7 ($p < 0.05$) and 5.6 ± 0.4 ($p < 0.05$) nmol/mg prot when ischemia was induced for 30- and 60-min duration, respectively. In addition, by histological examination (n = 20 specimens/each

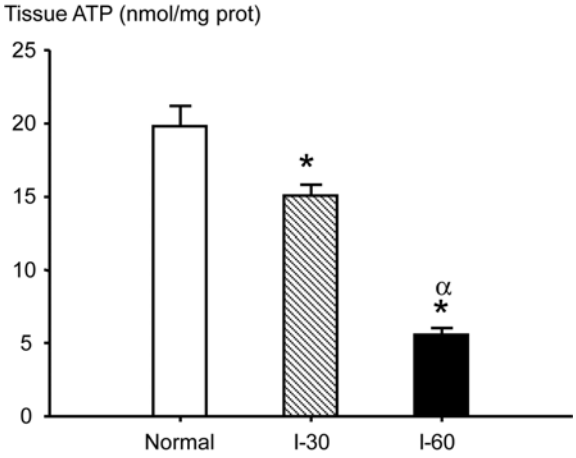

Fig. 6 Intestinal tissue ATP contents were measured in rat small intestine. Normal, I-30 and I-60 samples were taken from 21 rats and immediately frozen in liquid nitrogen and stored in -70°C (n = 21 specimens/each condition). Tissue ATP levels were determined using the luciferin/luciferase method, as described in the Methods and Materials. Differences within group was detected by ANOVA test with *post hoc* Dunnett's method; * $p < 0.05$ vs. normal, and $^{\alpha}$ $p < 0.05$, I-30 vs. I-60 using unpaired t-test

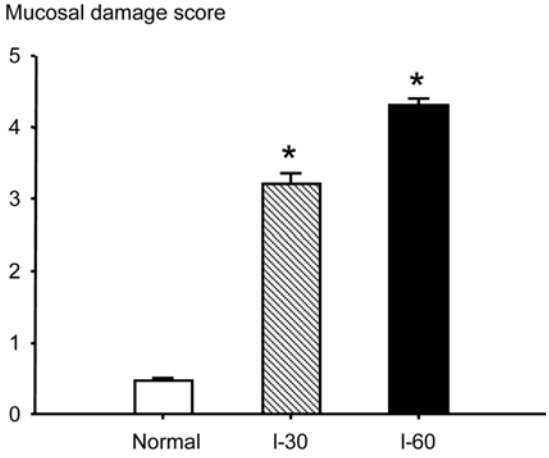

Fig. 7 Mucosal damage score was examined in rat small intestine specimens. Normal, I-30 and I-60 samples were taken from 21 rats and immediately fixed in 10% buffered formaldehyde, paraffin sectioned and stained with hematoxylin and eosin (n = 20 specimens/each condition). Score system (0-was described in Methods and Materials. Differences within group was detected by ANOVA test with *post hoc* Dunnett's method; * $p < 0.05$ vs. normal using unpaired t-test

condition), significant higher mucosal damage score (0-5) was found in both I-30 and I-60 groups (normal vs. I-30 vs. I-60; 0.5 $\pm$ 0.05 vs. 3.2 $\pm$ 0.1 vs. 4.3 $\pm$ 0.1, ($p < 0.05$). However, mucosal damage score of I-30 was less, but not significant, than that of I-60.

Discussion

We employed an everted gut sac technique in this study to assess the bidirectional flux of intestinal permeability. In addition to better exposure to the well-oxygenated suspended medium, the eversion reduces the thickness of the sac wall[9], especially an unstirred water layer and mucus layer which act as potential barriers of the intestine[12]. It was observed in our laboratory that lag period of this technique was similar to that of Ussing chamber (0-10 min). Due to weaker muscular wall of small intestine, stripping away the underlying seromuscular coat was considerably difficult to successfully perform, especially ischemic gut. By using this technique, there was no significant difference of clearance in the presence of regional differences. This result agrees with Yassin's study of the same technique[13], but conflicting results of regional differences were shown using Ussing chamber[14].

With regard to S-to-M cumulative permeability, the passage of FD4 was higher than the reverse direction. This is in accordance with our observation in Caco-2 cells and colonic mucosal mounted in the Ussing chamber (unpublished data). This is also consistent with the bidirectional flux study of FD3 through the small intestinal wall[11]. Most of markers used to demonstrate the bidirectional flux (in a polarized fashion) usually showed higher rate of S-to-M direction than the reverse direction[11]. However, the mechanistic basis of these bidirectional differences remained unclear. The results obtained with graded ischemia and incubation at three different temperatures indicated that bidirectional flux under physiologic or sublethal ischemia (I-30, mucosal damage score = 3) was energy-dependent process. Since there was significantly higher S-to-M clearance in I-60 than normal and I-30 gut sacs even incubated in 4°C, it is more likely to be the consequence of passive diffusion process, concomitant with a decrease in ATP contents and near-total loss villous epithelial mucosa (mucosal damage score = 4-5). In other words, I-60 insult would permit FD4 to get through the paracellular rather than transcellular route. Since the gut sac is incubated under a higher temperature, the FD4 probe would permeate (S-to-M) through both transcellular and paracellular routes. Moreover, S-to-M clearance was shown to be less dependent to the presence of intact mucosa (≈50%), whereas the M-to-S clearance of ischemia-induced gut injury depended on villous epithelial structure (≈70-80%). The reason that high CMB of I-60 (90%) when incubated in 4°C was obtained from calculation, as shown in Table 1, was a result of energy-independent passive diffusion due to transmural necrosis, thereby totally impaired membrane integrity of the intestinal wall. Accordingly, the reliability of plasma-to-lumen measurement[7,15] is questioned because it may not be represent mucosal barrier function, especially in an ischemia/reperfusion-induced gut injury.

There was, obviously, a temperature-dependent bidirectional flux in this present study. Decrease in clearances of both directions may be an adaptive mechanism for the survival of intestinal epithelial cells. Several studies have shown that ATP depletion directly affects tight junction and paracellular permeability[16-18]. Reduction of ATP contents below 30% of the initial value was found to relate to hyperpermeability[19,20]. This present study showed that I-30 and I-60 had reduction of ATP contents to 72% and 25% of their initial value, respectively. It was suggested that normal and I-30 guts had enough energy storage to regulate the bidirectional permeability, whereas ice cooling stopped those processes. When ATP depletion is induced by prolonged ischemia, leading to disruption of actin cytoskeleton, and the tight junction integrity is sequentially compromised[19,21].

Even though a relatively high S-to-M clearance was apparently found in most of macromolecule probes, ischemia-induced mucosal injury potentially increased mucosal permeability by increasing % flux ratio. A preferential flux in the S-to-M direction might serve as a useful pump of waste product from the body, whereas a high permeability in the opposite direction is harmful to the host. It would be questioned if the everted gut sac technique underestimates the mucosal permeability in a variety of diseases. Using the same concentration of FD4 in the serosal side (20 micro g/mL), there was no preferential flux in the S-to-M direction detected, except using FD4 concentration of 5 mg/mL (data not shown). It seems likely that S-to-M permeability may be dose-dependent process or concentration gradient process (passive diffusion).

In conclusion, the data presented here indicate that permeability of the M-to-S direction is more relevant to the actual mucosal barrier function under physiologic and pathologic conditions. The S-to-M permeability was shown to be both an

energy-requiring process and a passive diffusion. Since basal ATP is not enough for the cells to survive, the passive diffusion may be the predominant mechanism of solutes transportation.

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กลไกพึ่งพาอุณหภูมิของกระบวนการซึมผ่านสองทิศทางของผนังเยื่อบุลำไส้หนูที่ต่างระดับของการขาดเลือดมาเลี้ยง

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การศึกษาผลกระทบจากอุณหภูมิ และภาวะขาดเลือดมาเลี้ยงต่อการซึมผ่านผนังลำไส้หนูของสารเด็กซ์แทรนที่เคลือบด้วยฟลูออเรสเซนท์ (FD4) การวัดปริมาณการซึมผ่านจากเยื่อบุสู่ชั้นซีโรซา (M⟶S) และจากชั้นซีโรซาสู่เยื่อบุ (S⟶M) ด้วยวิธีปล้นถุงลำไส้เอาเยื่อบุออกด้านนอก ณ จุดตั้งต้น (B) ภาวะขาดเลือด 30 นาที (I-30) และ 60 นาที (I-60) และทิ้งไว้ที่อุณหภูมิ 37°ซ, 15°ซ และ 4°ซ เป็นเวลานาน 30 นาที แล้วคำนวณปริมาณสารที่ผ่านช่องระหว่างเซลล์เยื่อบุได้จากปริมาณสาร FD4 ณ ที่ 30 นาที ทิศทางการซึมผ่านทั้ง M⟶S และ S⟶M จะเพิ่มขึ้นเมื่ออุณหภูมิสูงขึ้นในทุกสถานะ (B, I-30, I-60) กรณีที่ลำไส้ขาดเลือด (ทั้ง 30 และ 60 นาที) พบว่ามีสัดส่วน (M⟶S)/(S⟶M) สูงกว่าลำไส้ปกติถึง 3 เท่า ($p < 0.001$) แต่ที่อุณหภูมิ 4°ซ ไม่พบความแตกต่างของ M⟶S และ S⟶M เมื่อเปรียบเทียบระหว่าง B กับ I-30 แต่จะเพิ่มขึ้นอย่างมีนัยสำคัญในสภาวะที่ขาดเลือดนาน 60 นาที ซึ่งแสดงถึงการซึมผ่านนี้เป็นกลไกพาสซีฟ และยังพบว่ามีการเพิ่มขึ้นของทั้งสองทิศทางในกรณีขาดเลือด (ทั้ง 30 และ 60 นาที) ที่อยู่ในสภาวะที่อุณหภูมิ 37°ซ จากผลการทดลองสรุปได้ว่าสาร FD4 ถูกนำส่งผ่านจากชั้นซีโรซาไปยัง ผนังเยื่อบุลำไส้ด้วยกลไกแอคตีฟที่ต้องใช้พลังงาน แต่ในกรณีที่ลำไส้ขาดเลือดมาเลี้ยงจะใช้กระบวนการนำส่งสารนี้ผ่านกลไกพาสซีฟเพิ่มมากขึ้น