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Claudin-3 expression in radiation-exposed rat models: A potential marker for radiation-induced intestinal barrier failure

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ABSTRACT

The molecular events leading to radiation-induced intestinal barrier failure are not well known. The influence of the expression of claudin proteins in the presence and absence of neurotensin was investigated in radiation-exposed rat intestinal epithelium. Wistar rats were randomly divided into control, irradiation, and irradiation + neurotensin groups, and bacterial translocation to the mesenteric lymph node and expression of claudins were determined. Irradiation led to intestinal barrier failure as demonstrated by significant bacterial translocation. In irradiated terminal ilea, expression of claudin-3 and claudin-4 was significantly decreased, and claudin-2 expression was increased. Administration of neurotensin significantly reduced bacterial translocation and restored the structure of the villi as seen by histologic examination. Among the three subtype of claudins, only claudin-3 expression was restored. These results suggest that the therapeutic effect of neurotensin on the disruption of the intestinal barrier is associated with claudin-3 alteration and that claudin-3 could be used as a marker in evaluating radiation-induced intestinal injury.

1. Introduction

Disruption of the intestinal barrier by radiation exposure (Wang et al. 2006) causes bacterial translocation that potentiates the development of septicemia, one of several causes of death following radiation exposure [1]. Bacterial translocation through the intercellular pathway in epithelial cells is controlled by tight junction molecules. Among the various molecular components of tight junctions, it is generally accepted that claudins play a crucial role in tightening cell–cell contacts [2–6]. In addition, alteration of claudins is closely related with pathophysiologies like inflammatory bowel diseases (IBD), chronic enteropathy, and tumorigenesis [4,7–9]. Previously published, there has been no previously published study investigating the fate or role of claudins in radiation-induced intestinal injury.

Neurotensin (NT) has various biologic actions on small and large bowel gastrointestinal tissues [10–13]. Administration of NT stimulates intestinal growth and adaptation and plays a protective role in preserving gut barrier integrity after injuries [14]. Collectively, these data suggest an important role for NT as a potent enterotrophic factor and as a contributing factor in the growth of other gastrointestinal tissues [10]. There has been no previously published investigation of the tight junction molecules regulated by NT in radiation-induced intestinal injury.

The purpose of this study was to answer two important questions in the field of radiation-induced intestinal damage. First, we determined whether or not any changes take place in claudin expression in the intestinal epithelium after radiation exposure. Second, we tested whether NT has any effect on claudin expression during the reconstruction of the intestinal barrier in an irradiated intestine.

2. Materials and methods

2.1. Animals

Male Wistar rats aged 6 weeks were obtained from Central Laboratory Animals (Seoul, Korea). The rats were kept under controlled conditions, with a constant temperature, and were

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allowed free access to regular chow and 3-stage filtered water. The Animal Investigation Committee of the Korea Institute of Radiological and Medical Sciences approved all animal experiments.

2.2. Irradiation (IR) and administration of neurotensin

Rats were irradiated in a single exposure of 12 Gy-whole abdominal irradiation (WAI) at a dose rate of 1 Gy/min using an X-RAD 320 X-ray irradiator (Softex, Korea). After exposure, animals were injected with an intraperitoneal dose of 300 μg/kg/day of NT (Sigma, St. Louis, MO) for the duration of the experimental periods [14].

2.3. Bacterial translocation

Detection of viable bacteria in mesenteric lymph nodes (MLN), harvested under sterile conditions, represents bacterial translocation from the lumen of the intestine. An equal aliquot of each homogenate was plated onto MacConkey agar (Becton Dickson, Franklin Lakes, NJ) and incubated at 37 °C, and then the number of colonies was counted on all plates [15].

2.4. Western blot of intestine

Equal proteins were separated on sodium dodecyl sulphate (SDS)–polyacrylamide gels and electrotransferred to Immuno-Blot polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA). Antibodies were purchased from the following sources: claudin-2, claudin-3, and claudin-4 were obtained from Invitrogen (Carlsbad, CA), and β-actin was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The claudin proteins/β-actin expression ratio was compared by densitometry using i-solution software.

2.5. Histologic examination of intestine

Terminal ileum samples were fixed with a 10% formalin solution, embedded in paraffin wax, and sectioned at a thickness of 4 mm. Sections were stained with hematoxylin and eosin. For immunohistochemistry, antigen was retrieved and treated with 0.3% hydrogen peroxide in methyl alcohol for 20 min to block endogenous peroxidase activity. After three washes in phosphate-buffered saline (PBS), the sections were blocked with 10% normal goat serum (Vector ABC Elite Kit, Vector Laboratories, Burlingame, CA) and allowed to react with claudin-3 (Invitrogen, Carlsbad, CA). After three washes in PBS, sections were incubated with horseradish peroxidase (HRP)-conjugated antibody (Dako, Carpinteria, CA). The peroxidase reaction was developed using a diaminobenzidine substrate (Dako, Carpinteria, CA) prepared according to the manufacturer's instructions.

2.6. Statistical analysis

All data are expressed as the mean ± SD, and the statistical significance of the differences in the values were evaluated via Student's t test ($p < 0.05$ was considered statistically significant).

3. Results

3.1. Bacterial translocation

Bacterial translocation was examined in MLN on days 4 and 6 (Fig. 1). The IR group presented significantly elevated bacterial translocation values compared with the control group on day 6 ($p < 0.05$). With NT treatment, bacterial translocation values were significantly reduced ($p < 0.05$).

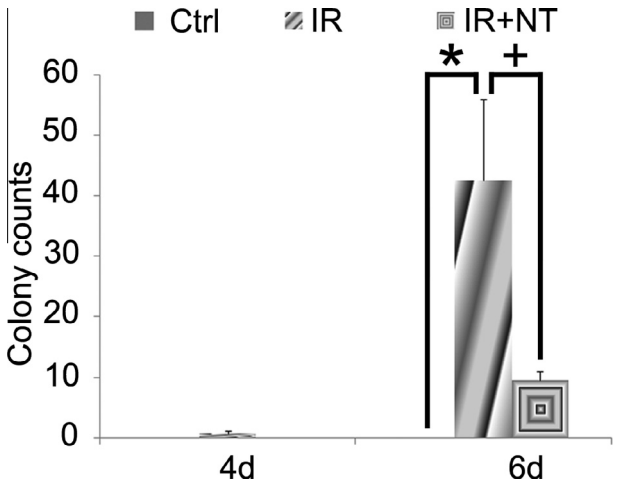

Fig. 1. Bacterial translocation in mesenteric lymph nodes. Bacterial translocation data are presented as the mean ± SD of the total colony forming units ($n = 4$ rats per group; $^{*}p < 0.05$ versus control group; $^{+}p < 0.05$ versus irradiation group).

3.2. Histology

The normal crypt-villus architecture was well preserved in the control group (Fig. 2A). In contrast, there were marked radiation-induced changes, including goblet cell depletion, shortening of the villi, and decreased number of crypt cells in the IR group (Fig. 2B). The villous structure in the NT-treated group was similar to that in the control group, and recovery of crypt and goblet cells was observed when compared with the IR group (Fig. 2C).

3.3. Evaluation of claudins expression

Obvious alterations of claudin-2, claudin-3, and claudin-4 protein levels in the intestinal tissue were observed using Western blot analysis on day 6 after irradiation. The expressions of claudin-3 and claudin-4 were decreased and the expression of claudin-2 was increased in the IR group as compared with the control group ($p < 0.05$; Fig. 3). In contrast, only claudin-3 expression was recovered ($p < 0.05$ as compared to the IR group; Fig. 3C) and claudin-2 and claudin-4 were not affected by NT administration (Fig. 3B and D).

3.4. Expression pattern of claudin-3

The immunohistochemical expression of claudin-3 in the intestinal epithelium was also consistent with Western blot results. In the control group, claudin-3 was expressed throughout the membrane in the villous surface epithelial cells; this staining was obvious in every villous but was weakly expressed in crypt (Fig. 4A). The frequency of claudin-3 positive cells was decreased in most epithelial cells of the villi and crypt in the IR group (Fig. 4B). In the NT group, claudin-3 expression was obvious throughout the membrane in both villi and crypt (Fig. 4C).

4. Discussion

Increases in intestinal permeability have been demonstrated in IR-induced intestinal injury, and permeability alterations are positively correlated with bacterial translocation. However, the molecules responsible for permeability alterations in radiation-induced intestinal injury are poorly understood.

The present study offered further insight into tight junction alterations in the intestinal mucosa with IR-induced injury. Our

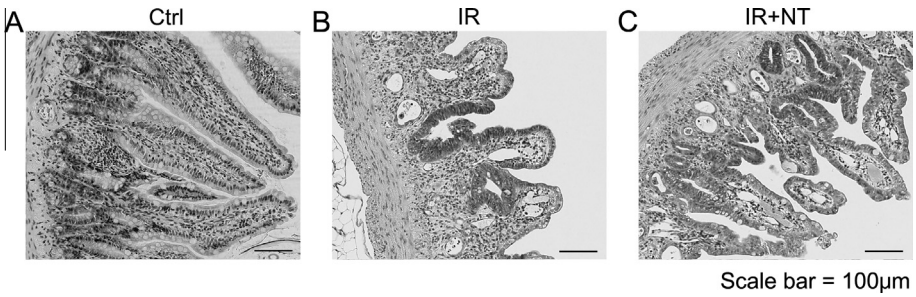

Fig. 2. Photomicrographs of hematoxylin and eosin-stained terminal ileal sections. Histologic sections stained with hematoxylin–eosin. (A) Non-irradiated tissue shows well-shaped crypt/villus axes and muscular layers. (B) Shortened villi and depleted crypts were observed. (C) The heights of the villi were similar to those in the control group, and goblet cells were observed in the villus. Original magnifications, 100×.

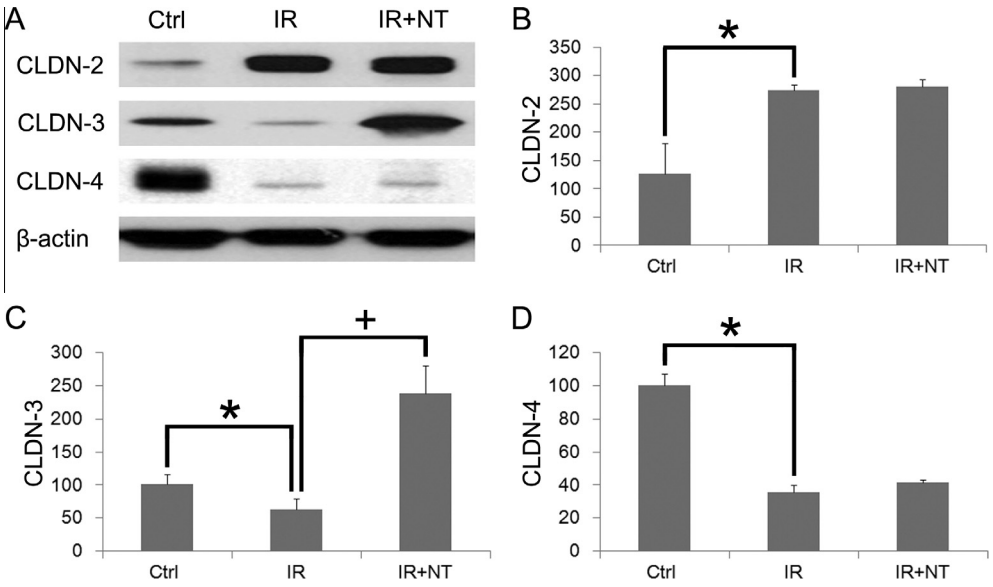

Fig. 3. Effects of neurotensin (NT) on intestinal claudin proteins in the terminal ileum on day 6. (A) Detection of claudin proteins using Western blots. Claudin-2, claudin-3, claudin-4, and β-actin were detected. (B–D) Densitometry graph representing claudin proteins expressed in terminal ileum. Irradiation significantly induced the protein level of claudin-2 (B), whereas the levels of claudin-3 (C) and claudin-4 (D) were reduced. NT restored the protein level of claudin-3 (C). Data are presented as the mean ± SD ($n = 4$ rats per group; $^{*}p < 0.05$ versus control group; $^{+}p < 0.05$ versus irradiation group).

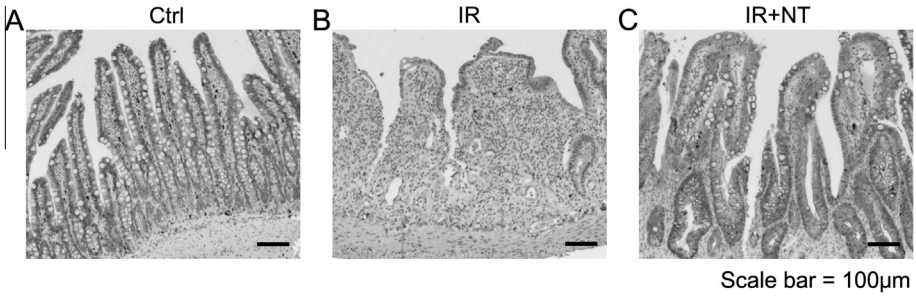

Fig. 4. Immunohistochemical expression of claudin-3 in epithelial cells of terminal ilea on day 6. Terminal ilea obtained 6 days post irradiation were stained for claudin-3. (A) Ileum from a control rat: the total epithelial cells of villi expressing claudin-3. (B) Ileum from irradiated rats with 12 Gy of X-ray: loss of claudin-3 expression in enterocytes indicates a region of tight junction incongruity. (C) Ilea from NT-treated rats showing restoration of claudin-3 expression in almost all villi and crypts. Original magnifications 100×.

results demonstrated significant bacterial translocation to MLN, an important marker of intestinal barrier disruption after radiation exposure with shortened villi and goblet cell depletion. In contrast, NT administration eliminated bacterial translocation and regenerated the structure of villi in the IR + NT group (Figs. 1 and 2). Our findings are in agreement with those of other investigators who have demonstrated reduced bacterial translocation to MLN following sufficient treatment in IR rat models [16–18]. Recently, Assimakopoulos and colleagues [14,19] reported that NT modulates the expression of tight junction proteins, thereby allowing enhanced barrier function of the intestinal epithelium in rats with experimental obstructive jaundice. Therefore, it is possible that NT could elicit beneficial effects via modulating tight junction proteins such as claudins in IR rats.

Immunoblotting data showed that the expression levels of claudins were altered in terminal ilea after IR (Fig. 3). Because claudin-2, claudin-3, and claudin-4 are highly expressed in intestinal epithelial junctions in both humans and rats [3,6], we stained these three subtypes of claudins. Irradiation had demonstrable effects on the expression of claudin-2, claudin-3, and claudin-4. Claudin-3 and claudin-4 were decreased and claudin-2 expression was increased significantly. Recent studies have demonstrated that the reductions of claudin-3 and claudin-4 are strongly associated with intestinal barrier disruption in the rodent ileum [6,20,21], and claudin-2 plays an opposing role to other claudins [5,8]. In the NT group, only claudin-3 expression was restored in the terminal ileum of IR rats. Previous studies have shown that claudin-3 is expressed in gradients along the crypt-to-villous axis of the rodent ileum [5,22]. Thus, we provided further insight into the claudin-3 expression pattern using an immunostaining method. In control rats, all the epithelial cells lining villi were positive for claudin-3 (Fig. 4A), though in IR rats we recorded a great loss of claudin-3 expression along the length of the villi (Fig. 4B). In the NT group, claudin-3 was expressed in epithelial cell surfaces, and this staining was observed in every villous and crypt (Fig. 4C). Thus, it seems possible that claudin-3 expression contributes to the formation of intercellular junctions along the crypt-to-villous axis.

In conclusion, the present study demonstrates that the therapeutic effect of NT on radiation-induced intestinal barrier dysfunction is associated with claudin-3. NT treatment reduced bacterial translocation to the MLN of rats. Moreover, a decreased level of claudin-3, one of the tight junction proteins, is also recovered by NT treatment after radiation exposure. These findings suggest that claudin-3 may be used as a marker in evaluating radiation-induced intestinal injury.

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