

Short Peptide Modules for Enhancing Intestinal Barrier Function

Soichi Tanabe*

Hiroshima University, Graduate School of Biosphere Science, Higashi-hiroshima, 739-8528, Japan

Abstract: The intestinal epithelial barrier is indispensable to our immune system. Defects in this barrier function have been observed in intestinal disorders such as inflammatory bowel diseases, food allergies, and celiac diseases. Therefore, the modulation of the barrier function is currently viewed as a potentially positive pharmacological outcome. This review describes a unique peptide, Asn-Pro-Trp-Asp-Gln (NPWDQ), which can finely adjust the intestinal barrier. It is obtained by the hydrolysis of casein, a major milk protein, and considerably inhibits the permeation of ovalbumin, one of the food allergens, in Caco-2, a human intestinal cell line. Using DNA microarray, we observed that NPWDQ only up-regulated expression of the occludin gene, whereas the levels of other genes, such as those of the claudin and zonula occludens families, remained unchanged. Increased protein expression of occludin was also observed. The fact that milk-derived peptide(s) can enhance intestinal barrier function gives a new significance to lactation because it plays an important role in promoting the maturation of the intestinal barrier. In this context, it is highly probable and worthy of considerable attention that various bioactive peptides with this type of activity are yet to be observed in the bovine and/or human casein sequence. Moreover, milk-derived peptides could be considered as potential candidates for the prevention of certain intestinal disorders.

Keywords: Peptides, casein peptides, intestinal barrier, tight junction, inflammatory bowel disease (IBD), food allergy, microarray.

1. INTRODUCTION

The intestinal tract is not only responsible for digestion and absorption of nutrients, but also has a barrier function that is a critical component of the innate immune system [1-3]. Only a single layer of epithelial cells separates the luminal contents from the effector immune cells located in the lamina propria and the rest of the body. A breach of this single layer of epithelium can expose the highly immunoreactive subepithelium to antigens and a vast number of microbes that are present in the lumen. The breakdown of this barrier is implicated in bacterial invasion, which can lead to sepsis, and in the pathogenesis of acute illnesses [4]. Increased permeability early in life has been implicated in the pathogenesis of several diseases that manifest later in life. These diseases are called leaky gut syndrome and include inflammatory bowel disease (IBD), food allergies, atopy, and celiac enteropathy [3, 5]. Therefore, modulation of the epithelial barrier function is currently viewed as a potentially positive pharmacological outcome.

Both physical and biological barriers constitute a first line of defense. The biological barrier comprises mucin, immunoglobulin A, digestive enzymes, and antibacterial substances such as defensins and lysozymes. The physical barrier consists of the epithelial cell layer with overlying mucus. The integrity of intestinal epithelial cells is maintained by intercellular junctional complexes, such as the tight junction, which comprises several proteins, provides continuous belt-like cell-cell junctions, and acts as a barrier to regulate paracellular permeability [5, 6]. Zonula occludens (ZO) proteins bind to transmembrane proteins, such as occludins and claudins of which there are at least 24 members, and links them to cytoskeletal actin [Fig. (1)].

This article focuses on milk-derived short peptides which can enhance the function of the tight junction. The following section provides a brief introduction of dietary components that are involved in its modulation.

2. ENHANCEMENT OF INTESTINAL BARRIER FUNCTION BY DIETARY COMPONENTS

Dysfunction of the intestinal barrier can result in enhanced

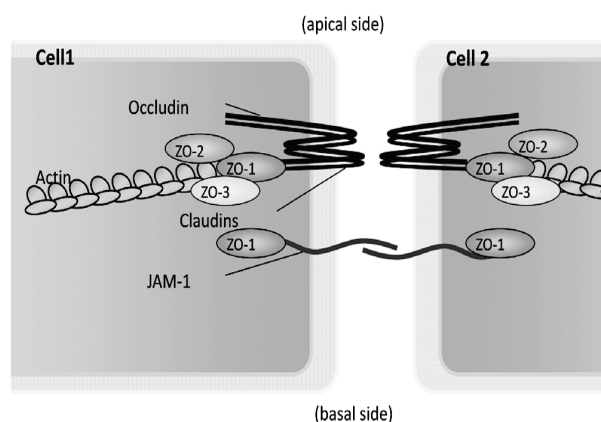


Fig. (1). Schematic drawing of the structure of a tight junction Tight junctions are composed of many components. Occludin has four transmembrane domains and two extracellular loops and associates with ZO-1 through the C-terminal intracellular domain of occludin. Three proteins, ZO-1, ZO-2, and ZO-3, have been identified as members of the ZO protein family. ZO-1 binds to transmembrane proteins, such as claudins and occludin, and links them to cytoskeletal actin. There are at least 24 types of claudins. They have four transmembrane domains similar to occludin, although little homology is apparent between occludin and claudins.

antigen uptake, bacterial translocation, and abnormal immune responses. IBD, which comprises the two major conditions (ulcerative colitis and Crohn's disease), is one of the most significant diseases characterized by an abnormal immune response [7]. Therapeutically, anti-inflammatory remedies, such as tumor necrosis factor (TNF)- α antibody (infliximab, commercial name "Remicade"), are most effective in improving the symptoms of active IBD and simultaneously repairing intestinal barrier function [8]. Other agents can also directly affect the barrier function. Recent investigations have focused on the development of new therapeutic agents, including dietary components, because patients wish to avoid the long-term use of drugs.

*Address correspondence to this author at Hiroshima University, Graduate School of Biosphere Science, 1-4-4 Kagamiyama, Higashi-hiroshima, 739-8528, Japan; Tel: +81-82-424-7932; Fax: +81-82-424-7916; E-mail: stanabe@hiroshima-u.ac.jp

Probiotic bacteria, such as lactobacilli and bifidobacteria, can reduce intestinal inflammation and restore the balance of intestinal microbiota [9]. Evidences from animal models of IBD indicate that probiotics can alter intestinal microbiota and improve the symptoms of the disease [10]. In addition, the clinical efficacy of probiotics in IBD has been investigated in a number of clinical studies, and key findings from clinical trials have recently been reviewed comprehensively by Hedin *et al.* [11].

It was demonstrated that administration of *Bidifobacterium longum* BB536 was effective in inducing remission in patients with ulcerative colitis [12]. Kato *et al.* [13] reported the effects of administering fermented milk supplemented with bifidobacteria for management of active ulcerative colitis. VSL#3, *Escherichia coli* Nissle 1917, and *Lactobacillus rhamnosus* GG have also been tested extensively. VSL#3 is a mixture of eight species of lactobacilli (*L. plantarum*, *L. acidophilus*, *L. casei* and *L. delbrueckii* subsp. *bulgaricus*), bifidobacteria (*B. longum*, *B. infantis* and *B. breve*), and streptococcus. In general, clinical efficacy was achieved in the treatment of ulcerative colitis; however, the results for Crohn's disease were less positive.

Multiple mechanisms of action have been suggested to explain the protective effects of probiotics in intestinal inflammation. These can be broadly classified into the following: (1) improvement of the epithelial barrier function, (2) suppression of growth or epithelial binding/invasion by pathogenic bacteria, and (3) immune regulation [10].

Among the dietary factors involved in the improvement of symptoms of IBD, polyphenols have been proposed as protective agents in distinct models of colon inflammation [14,15]. For example, quercetin can strengthen the epithelial barrier, which has been shown to promote the assembly of ZO-2, occludin, and claudin-1 and up-regulate claudin-4 within the epithelial tight junction in a human epithelial cell line [14]. In addition, amino acids, e.g., glutamine [4, 16] and histidine [17], have been proposed as candidates for barrier strengthening therapy.

The prevalence of allergic diseases is increasing in most developed countries. Food allergy has been thought to involve an excessive immune reaction to allergens that permeate from the intestinal tract. After ingesting a specific food allergen, a sensitized individual may experience gastrointestinal symptoms, such as diarrhea and nausea, and extra-intestinal symptoms can occur in the skin and airways. The intestinal epithelium theoretically acts as a barrier to restrict the permeation of macromolecules. However, a small proportion (10^{-3} to 10^{-4}) of dietary proteins can cross the epithelium to access and activate effector cells, which results in food allergy [18]. Moreover, it has been reported that the permeability of antigens through the intestinal tract is enhanced in patients with allergies [19]. In the light of these facts, it is conceivable that enhancement of the barrier function of the intestinal epithelium could help prevent food allergies.

A large number of different types of peptides are formed in the intestine after digestion of dietary proteins. It is therefore probable that these peptides interact with the intestinal epithelium and affect the barrier function. However, to our knowledge, no studies on the relationship between the barrier and dietary peptides have been previously reported. In the following section, interesting results from our ongoing work on milk-derived peptides are presented.

3. NPWDQ FINELY ADJUSTS THE EPITHELIAL BARRIER

3.1. A Casein-derived Peptide that Inhibits the Permeation of Allergens into the Intestinal Epithelial Barrier (*In vitro*)

To investigate milk protein-derived peptides that inhibit the permeation of allergens into the intestines, we chose enzyme-modified cheese (EMC) as a base. EMCs are generally produced by the hydrolysis of cheese with commercial proteases and are used in food, e.g., as the sole source of cheese flavor in a product, to intensify an existing cheesy taste, or to give a specific cheese character to a blander cheese product [20]. In addition, the enzymatic treatment of cheese can potentially produce bioactive peptides that may provide nutritional and medical benefits. For example, Haileselassie *et al.* [21] isolated antihypertensive peptides, such as YFPGPPI that inhibits an angiotensin I-converting enzyme, from EMC. In our experiment, we prepared "EMC2" from a Danish skimmed milk cheese by a series of treatments with Protease S, Newlase A, and Umamizyme [22].

To investigate the ability of EMC2 to inhibit the permeation of allergens, we used Caco-2 cells, a human colon carcinoma cell line that differentiates under standard culture conditions to form confluent monolayers and acquires many features of absorptive intestinal cells during culture. Caco-2 cells spontaneously exhibit various enterocytic characteristics, including the expression of brush border enzymes and nutrient transporters, and the formation of intercellular tight junctions [23].

Our group established an *in vitro* system to evaluate the permeation of allergens using differentiated Caco-2 cells grown on a permeable filter [22]. In this system, Caco-2 cell monolayers were used as a model of the small intestinal tract, and ovalbumin (OVA) was used as a typical allergen [Fig. (2)].

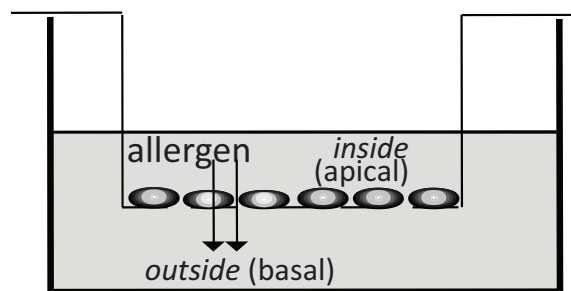


Fig. (2). Schematic drawing of the evaluation of intestinal permeability using human Caco-2 cells seeded onto Transwell. Each compartment was placed in a well of a 12-well cluster plate containing medium outside (basolateral side) and inside (apical side). Fluorescein isothiocyanate (FITC)-OVA was added to the apical side, and the test sample (e.g., EMC2 or NPWDQ) was added to the basolateral side. The monolayers were incubated for 2 h, after which the outside medium was collected. The concentration of FITC-OVA in the basolateral medium was determined by measuring fluorescence intensity, and permeability was calculated.

Because the water extract of EMC2 demonstrated inhibitory activity, it was fractionated by Sep-pak cartridge and reversed-phase HPLC to isolate an active peptide, Gly-Pro-Ile-Val-Leu-Asn-Pro-Trp-Asp-Gln (GPIVLNPWDQ). This amino acid sequence corresponds to the sequence of amino acids 102–111 of α_2 -casein. The decapeptide GPIVLNPWDQ was then synthesized and its activity–concentration relationship was measured [Fig. (3A)]. It demonstrated a concentration-dependent inhibition of OVA permeation. We further identified portions of the decapeptide that are essential for the inhibitory activity. For this, the two pentapeptide halves GPIVL (aa102–106) and NPWDQ (aa107–111) were synthesized, and their activities were evaluated. Interestingly, the inhibitory activity of NPWDQ, but not GPIVL, was found to be almost identical to that of GPIVLNPWDQ [Fig. (3B)]. Therefore, it was concluded that only the C-terminal half peptide is essential for this activity.

At least two possible mechanisms existed by which GPIVLNPWDQ and NPWDQ could inhibit the permeation of OVA in Caco-2 cells: the enhancement of tight junction functions and the inhibition of OVA endocytosis. To test the former, the effect of the addition of NPWDQ on the integrity of Caco-2 monolayers was evaluated by measurements of transepithelial electrical resistance

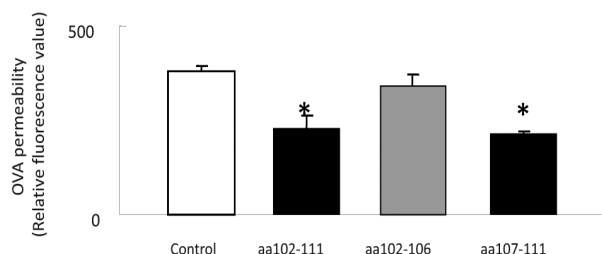


Fig. (3). Effects of GPIVLNPWDQ (aa102-111) and its two pentapeptide fragments, GPIVL (aa102-106) and NPWDQ (aa107-111), on OVA permeability through Caco-2 monolayers [ref 22]. Data represent means $\pm$ standard deviation (n = 3).

*Significantly different ($p < 0.05$) from the control sample

(TER). TER increased in response to the addition of increasing concentrations of NPWDQ, which suggested that this peptide enhanced the epithelial barrier function [Fig. (4)].

3.2. Microarray Analysis of Tight Junction-Related Molecules

DNA microarray analysis was performed to investigate the effect of the addition of NPWDQ in detail [24]. NPWDQ was added to the apical side of Caco-2 cells; these were then incubated for 24 h, after which RNA was extracted and analyzed by microarray. Changes in 48,000 types of gene expression by NPWDQ were evaluated. Genes that demonstrated an abundant level of expression (more than 1,000 arbitrary fluorescence units; a total of 7,108 genes) were chosen for further analysis. Among these, only two [*fos* (GenBank accession number NM_005252) and *egr1* (NM_001964)] were up-regulated by more than 2-fold and three [*mgcl4376* (NM_032895), *grb2* (NM_002086) and *dusp1* (NM_004417)] were up-regulated by 1.5–2-fold. Moreover, none of the genes was down-regulated by more than 1.5-fold (Table 1). Therefore, it was found that NPWDQ did not drastically affect mRNA expression in Caco-2 cells.

Differences in gene expression of the major tight junction-related molecules, occludin, claudins, ZO families, and junctional adhesion molecule (JAM) families, were analyzed. As shown in (Table 2), occludin was up-regulated by nearly 1.5-fold. In contrast, gene expressions of claudin-1, -2, -3, -4, -7, -12, -14, -15, -19, and -23, ZO-1, -2 and -3 and JAM-1 did not change remarkably (0.88–1.13-fold changes). From this data, it was clear that NPWDQ up-regulated occludin mRNA expression only. The up-regulation of occludin mRNA by NPWDQ was also confirmed by a real-time PCR.

The effect of NPWDQ on expression of the major tight junction-related proteins occludin, claudin-1 and ZO-1 was evaluated by western blotting. NPWDQ significantly increased the level of occludin by 1.5-fold [Fig. (5)], although the levels of claudin-1 and ZO-1 did not change; these results were consistent with those of the microarray (Table 2). This data suggested that NPWDQ particularly increased the level of occludin and finely adjusted the tight junction barrier.

4. NPWDQ AND EMC SUPPRESS THE PERMEATION OF ALLERGENS

4.1. NPWDQ Suppresses OVA Permeation *Ex Vivo*

We determined whether NPWDQ could inhibit the permeation of allergens *ex vivo* in a rat intestinal loop [25]. The rats were

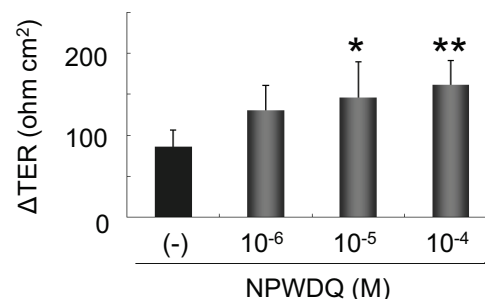


Fig. (4). Changes in TER by the addition of NPWDQ to Caco-2 cells [ref 24]. NPWDQ was added to the apical side, and cells were incubated for 24 h, after which TER was measured.

Data represent means $\pm$ standard deviation (n = 6).

*, **, Significantly different ($p < 0.05$ and $p < 0.01$, respectively) from the control sample

Table 1. DNA Microarray Revealed that NPWDQ did not Generally Affect mRNA Expression in Caco-2 cells [ref 24]

Change Fold	Gene Numbers
> 1.5	5
1.5 – 0.67	7,103
< 0.67	0

NPWDQ (10⁻⁶ M) was added to the apical side, and cells were incubated for 24 h. RNA was extracted from Caco-2 cells with or without incubation with NPWDQ. Changes in 48,000 types of gene expression were evaluated. This table is the summary of 7,108 genes that showed an expression of more than 1,000 (arbitrary fluorescence units). Only five genes, *fos*, *egr1*, *mgcl4376*, *grb2* and *dusp1* were up-regulated by more than 1.5-fold; the fold changes were 2.93, 2.92, 1.54, 1.53 and 1.51, respectively. The mRNA expression of occludin was up-regulated by nearly 1.5-fold (Table 2).

subjected to intestinal injury by two subcutaneous injections of indomethacin (7.5 mg/kg each, with a 24 h interval). The major pathogenesis of indomethacin-induced intestinal impairment is basically caused by the inhibition of cyclooxygenase, depletion of endogenous and protective prostaglandins in the mucosa, and subsequently impairment of the mucosal barrier function. Twenty-four hours after the second injection of indomethacin, jejunal and ileac segments of the small intestine were excised. OVA was injected alone or with NPWDQ into the closed loop, and the loop was then placed into a 15-mL tube that contained PBS heated to 37°C [Fig. (6A)]. The tube was incubated for 2 h, and the outside solution was collected to measure OVA permeation.

As shown in [Fig. (6B)], administration of indomethacin almost doubled the permeation of OVA into the jejunal loop; however, addition of NPWDQ restored this to normal levels. OVA permeation into the ileac segment of normal rats was extremely low, while that in indomethacin-administered rats was remarkably high Fig. (6C). This indomethacin-induced increase in permeation of OVA was more prominent in the ileac segment than that in the jejunal segment, which suggests that the ileum was more heavily damaged than the jejunum. However, the addition of NPWDQ into the ileac loop effectively inhibited OVA permeation.

4.2. EMC2 Suppresses OVA Permeation *In vivo*

We next performed an *in vivo* experiment to determine whether EMC2, which contains various peptides such as NPWDQ, could inhibit allergen permeation in indomethacin-treated rats. The blood

Table 2. Changes in mRNA Expressions of Tight Junction-related Molecules by the Addition of NPWDQ [ref 24]

Gene	NPWDQ		Change Fold
	(-)	(+)	
occludin	164±34	245±43	1.49
claudin-1	23,834±640	27,039±790	1.13
claudin-2	1,150±76	1,041±43	0.91
claudin-3	2,470±115	2,557±105	1.04
claudin-4	624±57	588±35	0.94
claudin-7	11,205±319	10,881±329	0.97
claudin-12	3,117±130	3,225±121	1.03
claudin-14	3,770±139	3,303±141	0.88
claudin-15	3,306±92	3,487±133	1.05
claudin-19	531±35	587±52	1.11
claudin-23	4,389±168	4,492±220	1.02
ZO-1	5,398±133	5,536±150	1.03
ZO-2	2,070±87	2,223±79	1.07
ZO-3	9,363±286	8,851±204	0.95
JAM-1	8,142±352	7834±264	0.96

The expressions of claudin-5, -6, -8, -9, -10, -11, -16, -17, -18, -20, and -22 and JAM-2 and -3 were less than 100 (arbitrary fluorescence units). It was clearly observed that NPWDQ up-regulated occludin mRNA expression only.

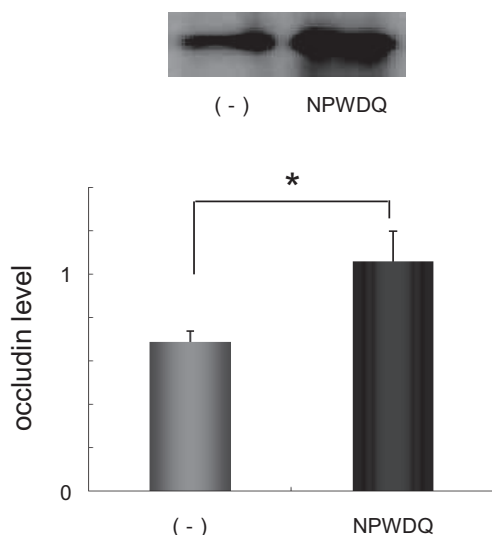


Fig. (5). Changes in expression of occludin by the addition of NPWDQ to Caco-2 cells [ref 24] NPWDQ was added to the apical side, and cells were incubated for 24 h. Protein was extracted from Caco-2 cells with or without incubation with NPWDQ. Protein expression was detected by western blotting, and the figure shows a representative blotting pattern. Data represent means ± standard deviation (n = 7).

concentration of OVA was measured in normal (healthy control) rats after its oral administration. Although OVA was detected at

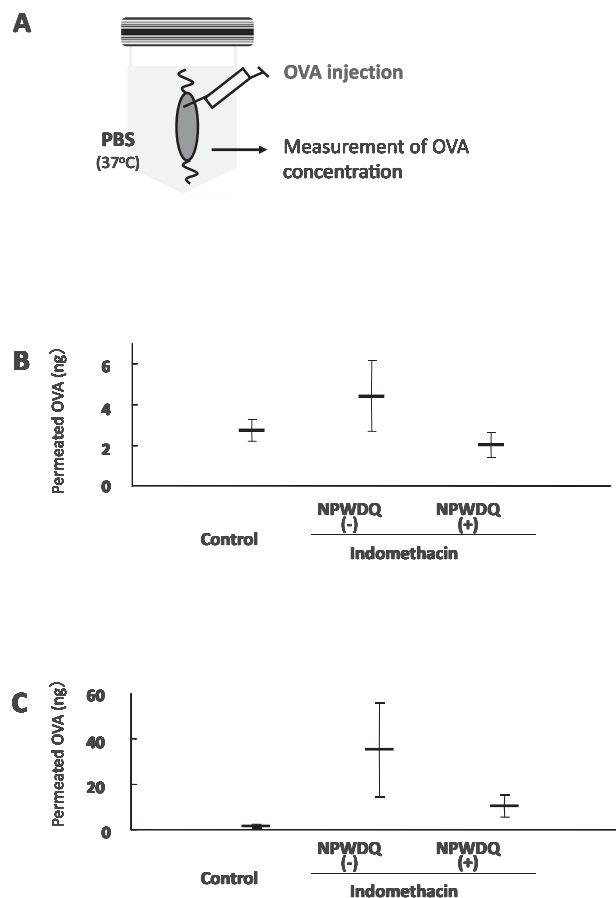


Fig. (6). Effects of NPWDQ on OVA permeation [ref 25] (A) Schematic drawing of the ex vivo loop experiment. OVA was injected into the closed loop from normal and indomethacin-treated rats in the presence or absence of NPWDQ, and the loop was then placed into a tube that contained phosphate buffer solution. The tube was incubated at 37°C for 2 h, and the outside solution was collected to measure the OVA concentration by a sandwich enzyme-linked immunosorbent assay (ELISA). (B,C) Effects of NPWDQ on OVA permeation into the inflamed jejunal (B) and ileac (C) loops. Data represent means ± standard error (n = 4, 5).

low levels 0.5 h after an OVA challenge in normal rats, no remarkable transfer of OVA into the peripheral blood was observed after 1 h. Thus, it is possible that OVA is rarely absorbed by the intestines of normal rats in the intact form. In contrast to normal rats, undigested OVA was detected at high levels in the peripheral blood of indomethacin-treated rats [Fig. (7)]. Indeed, the area under the OVA concentration-time curve for indomethacin-treated rats was approximately six times higher than that for normal rats [25].

Rats were administered EMC2 twice orally (0.1 g or 0.5 g each with a 24-h interval) concomitantly with two doses of indomethacin. Twenty-four hours after the second administration, the rats were given OVA orally. As shown in [Fig. (7)], EMC2 effectively lowered the blood concentration of OVA in a dose-dependent manner and inhibited OVA permeation *in vivo*. The inhibitory activity of EMC2 on OVA permeation was also confirmed by the time to reach maximum concentration, which was delayed in the EMC2-treated groups [25].

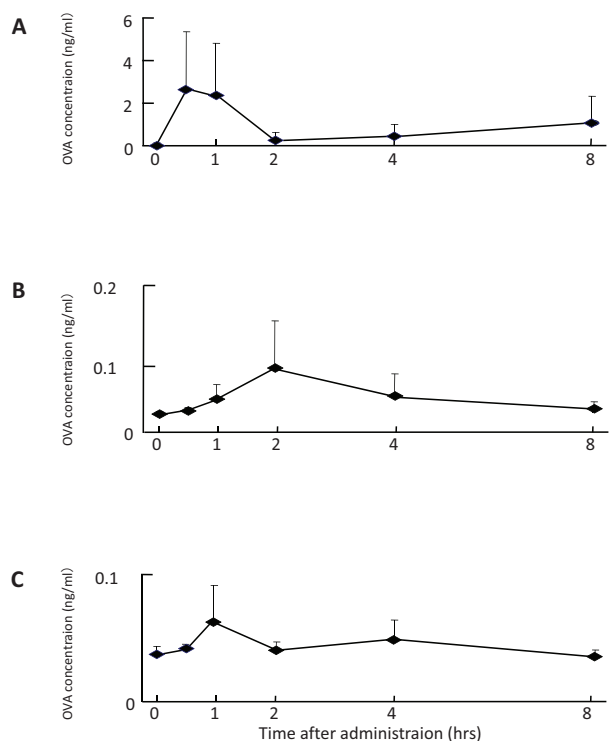


Fig. (7). Effects of oral administration of EMC2 on blood OVA concentrations in indomethacin-treated rats [ref 25] Rats were administered 200 mg OVA orally after overnight fasting. Blood samples were collected from the portal vein of control (A), 0.1 g EMC-fed (B) rats, and 0.5 g EMC-fed (C) rats, and plasma OVA concentration was measured by sandwich ELISA. Note: Scales of the Y-axis are different. Data represent means $\pm$ standard error ($n = 3$).

4.3. Can Barrier Recovery *In Vivo* be Achieved with NPWDQ and EMC?

From the data described above, it can be observed that NPWDQ can finely adjust the epithelial barrier by modulating the level of expression of occludin in Caco-2 cells in which the tight junction is well formed. However, it should be clarified whether or not the effect of NPWDQ on occludin expression is dependent on this peptide sequence, and whether NPWDQ can repair the barrier when the tight junction is impaired. Generally, the *in vitro* barrier-impairment model is achieved by the treatment of Caco-2 cell monolayers with TNF- α alone or TNF- α plus interferon- γ . It is frequently observed that treatment with TNF- α decreases TER, and this is partially mediated by an increased expression of myosin light-chain kinase protein [26]. TNF- α also induces a remarkable increase in inflammatory interleukin-8 secretion. It is therefore interesting to examine whether NPWDQ can repair TNF- α -induced damage in Caco-2 cells.

In addition, it is still unclear how EMC2 limits the permeation of OVA in indomethacin-treated rats. Further research is required to directly evaluate whether EMC2 as well as NPWDQ can repair the barrier function *in vivo*, and to clarify the putative mechanisms involved.

5. MILK CASEIN, A SOURCE OF BIOACTIVE PEPTIDES

Previously, we isolated the peptide Asp-Lys-Ile-His-Pro-Phe (DKIHPF) with the same activity as NPWDQ from Edam cheese. DKIHPF corresponds to the amino acid sequence of residues 47–52 of bovine β -casein and markedly inhibits the permeation of β -lactoglobulin, one of the potent milk allergens. The human β -casein amino acid sequence has a highly homologous peptide, Asp-Lys-Ile-Tyr-Pro-Ser-Phe (DKIYPSF), at the same position as that of bovine β -casein. Although it has not been demonstrated that DKIYPSF from human β -casein has similar activity to that of bovine β -casein in human epithelial cells, it is highly possible that this type of short peptide module is produced from casein by proteases in infants and could enhance the tight junction barrier and prevent invasion by allergens. These findings present a novel significance for lactation, which plays an important role in promoting the maturation of the intestinal barrier. In this context, it is highly probable and worthy of considerable attention that various bioactive peptides in the casein sequence have not yet been found. Furthermore, milk-derived peptides could contribute to the design of pharmaceutical lead-compounds.

ACKNOWLEDGEMENT

All data on EMC2 and NPWDQ were results of experiments performed by Ms. N. Isobe and Ms. H. Yasumatsu in our laboratory. I appreciate their continuous efforts toward this work.

ABBREVIATIONS

EMC	=	Enzyme modified cheese
IBD	=	Inflammatory bowel disease
NPWDQ	=	Asn-Pro-Trp-Asp-Gln
OVA	=	Ovalbumin
TER	=	Transepithelial electrical resistance
ZO	=	Zonula occludens

REFERENCES

- [1] Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* 2009; 9: 799-809.
- [2] Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 2009; 124: 3-20.
- [3] Catalioto RM, Maggi CA, Giuliani S. Intestinal epithelial barrier dysfunction in disease and possible therapeutical interventions. *Curr Med Chem* 2011; 18: 398-426.
- [4] Li N, Lewis P, Samuelson D, Liboni K, Neu J. Glutamine regulates Caco-2 cell tight junction proteins. *Am J Physiol Gastrointest Liver Physiol* 2004; 287: G726-G733.
- [5] Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiol Rev* 2011; 91: 151-75.
- [6] Steed E, Balda MS, Matter K. Dynamics and functions of tight junctions. *Trends Cell Biol* 2010; 20: 142-9.
- [7] Ngo B, Farrell CP, Barr M, *et al.* Tumor necrosis factor blockade for treatment of inflammatory bowel disease: efficacy and safety. *Curr Mol Pharmacol* 2010; 3: 145-52.
- [8] Fries W, Muja C, Crisafulli C, Cuzzocrea S, Mazzon E. Dynamics of enterocyte tight junctions: effect of experimental colitis and two different anti-TNF strategies. *Am J Physiol Gastrointest Liver Physiol* 2008; 294: G938-47.
- [9] Prisciandaro L, Geier M, Butler R, Cummins A, Howarth G. Probiotics and their derivatives as treatments for inflammatory bowel disease. *Inflamm Bowel Dis* 2009; 15: 1906-14.
- [10] Sartor RB. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology* 2004; 126: 1620-33.
- [11] Hedin C, Whelan K, Lindsay JO. Evidence for the use of probiotics and prebiotics in inflammatory bowel disease: a review of clinical trials. *Proc Nutr Soc* 2007; 66: 307-15.
- [12] Takeda Y, Nakase H, Namba K, *et al.* Upregulation of T-bet and tight junction molecules by *Bifidobacterium longum* improves colo-

- nic inflammation of ulcerative colitis. *Inflamm Bowel Dis* 2009; 15: 1617-8.
- [13] Kato K, Mizuno S, Umesaki Y, *et al.* Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. *Aliment Pharmacol Ther* 2004; 20: 1133-41.
- [14] Suzuki T, Hara H. Quercetin enhances intestinal barrier function through the assembly of zonula occludens-2, occludin, and claudin-1 and the expression of claudin-4 in Caco-2 cells. *J Nutr* 2009; 139: 965-74.
- [15] Suzuki T, Tanabe S, Hara H. Kaempferol enhances intestinal barrier function through the cytoskeletal association and expression of tight junction proteins in Caco-2 cells. *J Nutr* 2011; 141: 87-94.
- [16] Hering NA, Schulzke JD. Therapeutic options to modulate barrier defects in inflammatory bowel disease. *Dig Dis* 2009; 27: 450-4.
- [17] Son DO, Satsu H, Shimizu M. Histidine inhibits oxidative stress- and TNF- α -induced interleukin-8 secretion in intestinal epithelial cells. *FEBS Lett* 2005; 579: 4671-7.
- [18] Kaminogawa S, Hachimura S, Nakajima-Adachi H, Totsuka M. Food allergens and mucosal immune systems with special reference to recognition of food allergens by gut-associated lymphoid tissue. *Allergol Int* 1999; 48: 15-23.
- [19] Majamaa H, Isolauri E. Evaluation of the gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. *J Allergy Clin Immunol* 1996; 97: 985-90.
- [20] Tonouchi H, Suzuki M, Uchida M, Oda M. Antihypertensive effect of an angiotensin converting enzyme inhibitory peptide from enzyme modified cheese. *J Dairy Res* 2008; 75: 284-90.
- [21] Haileselassie SS, Lee BH, Gibbs BF. Purification and identification of potentially bioactive peptides from enzyme-modified cheese. *J Dairy Sci* 1999; 82: 1612-7.
- [22] Tanabe S, Isobe N, Miyauchi E, Kobayashi S, Suzuki M, Oda M. Identification of a peptide in enzymatic hydrolyzate of cheese that inhibits ovalbumin permeation in Caco-2 cells. *J Agric Food Chem* 2006; 54: 6904-8.
- [23] Kobayashi S, Tanabe S, Sugiyama M, Konishi Y. Transepithelial transport of hesperetin and hesperidin in intestinal Caco-2 cell monolayers. *Biochim Biophys Acta* 2008; 1778: 33-41.
- [24] Yasumatsu H, Tanabe S. The casein peptide Asn-Pro-Trp-Asp-Gln enforces the intestinal tight junction partly by increasing occludin expression in Caco-2 cells. *Br J Nutr* 2010; 104: 951-6.
- [25] Isobe N, Suzuki M, Oda M, Tanabe S. Enzyme-modified cheese exerts inhibitory effects on allergen permeation in rats suffering from indomethacin-induced intestinal inflammation. *Biosci Biotechnol Biochem* 2008; 72: 1740-5.
- [26] Mashukova A, Wald FA, Salas PJ. Tumor necrosis factor α and inflammation disrupt the polarity complex in intestinal epithelial cells by a posttranslational mechanism. *Mol Cell Biol* 2011; 31: 756-65.