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Short Communication

Changes of Tight Junction Protein Claudins in Small Intestine and Kidney Tissues of Mice Fed a DDC Diet

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Abstract: DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine)-fed mice are widely used as a model for cholestatic liver disease. We examined the expression of tight junction protein claudin subspecies by immunofluorescent histochemistry in small intestine and kidney tissues of mice fed a DDC diet for 12 weeks. In the small intestine, decreases in claudin-3, claudin-7 and claudin-15 were observed in villous epithelial cells corresponding to the severity of histological changes while leaving the abundance of these claudin subspecies unchanged in crypt cells. Nevertheless, the proliferative activity of intestinal crypt cells measured by immunohistochemistry for Ki-67 decreased in the mice fed the DDC diet compared with that of control mice. These results suggest the possibility that DDC feeding affects the barrier function of villous epithelial cells and thus inhibits the proliferative activity of crypt epithelial cells. On the other hand, in the kidney, remarkable changes were found in the subcellular localization of claudin subspecies in a segment-specific manner, although histological changes of renal epithelial cells were quite minimal. These results indicate that immunohistochemistry for claudin subspecies can serve as a useful tool for detecting minute functional alterations of intestinal and renal epithelial cells. (DOI: 10.1293/tox.2013-0009; J Toxicol Pathol 2013; 26: 433–438)

Key words: claudins, tight junction, small intestine, kidney, DDC diet

A diet containing the liver toxin 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) induces liver cell necrosis and subsequent oval cell proliferation in mice[1]. DDC causes accumulation of protoporphyrin in the liver by inhibiting protoheme ferrolyase (ferrochelatase) activity[2]. Protoporphyrin has been shown to induce cholestasis in the isolated perfused rat liver[3]. Thus DDC-fed mice are widely used as a model for cholestatic liver disease[4], though direct toxic effects of DDC, especially on the intestinal mucosa, cannot be ruled out.

Experimental and clinical studies have shown that obstructive jaundice results in increased intestinal permeability[5]. Although altered expression of tight junction-related molecules such as occludin and ZO-1 in the intestines of jaundiced rats was reported[5], to our knowledge no report is available that examines the changes in the expression of claudin subspecies of the intestinal epithelial cells in mice fed a DDC diet. Patients with obstructive jaundice are susceptible to acute renal failure when undergoing major surgery[6], but little is known about the changes in the renal epithelial cell barrier function of mice fed a DDC diet.

The epithelial cells of the intestine and kidney are highly organized, connecting with each other via cell-to-cell junctional complexes. Among them, the tight junction is located most apically and functions as an intercellular barrier by inhibiting solute and water flow through the paracellular spaces[7].

The claudin family, which consists of at least 27 members, has four transmembrane domains and is solely responsible for intercellular barrier function[8,9]. It has been shown that the expression of claudin subspecies varies considerably among tissues[8], and specific limited sets of claudin subspecies are expressed in each organ[10]. It is also known that claudin subspecies are expressed in a region- or segment-specific manner in the epithelial cells of the intestine[11,12] and kidney[13,14]. While there are some conflicting published data, as far as immunohistochemistry is concerned, claudin-2 and claudin-3[11] and claudin-7, claudin-12 and claudin-15[12] are expressed in the epithelial cells of the mouse jejunum. On the other hand, in the mouse kidney, claudin-3, claudin-10, claudin-11, claudin-16 and claudin-19 are found in the thick ascending limb, and claudin-3 and claudin-8 are found in the distal tubules. Claudin-3, claudin-4 and claudin-8 are present in the collecting ducts, whereas the expression of claudin-1 and claudin-2 is limited to the proximal nephron[14]

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and the epithelial cells of the Bowman's capsule[13], respectively. In the present study, we compared the expression abundance and subcellular localization of these claudin subspecies in the intestine and kidney tissues of mice fed the DDC diet with those in control mice to elucidate the effects of DDC feeding on intestinal and renal epithelial cell barrier function. We demonstrated in the present paper the immunohistochemical localization of claudin-3, claudin-7 and claudin-15 in the mouse jejunum and claudin-1, claudin-2, claudin-3, claudin-8 and claudin-19 in the mouse kidney, as we were limited to using commercially available antibodies to claudin subspecies and no change in claudin-2 in the intestine and claudin-3 and claudin-7 in the kidney was observed.

C57BL/6 mice (Clea, Tokyo, Japan) were housed individually under specific-pathogen-free conditions at the Center for Animal Resources and Development, Sapporo Medical University School of Medicine. Six 8-week-old male mice were fed a diet containing 3,5-diethoxycarbonyl-1,4- dihydrocollidine (DDC) (Sigma-Aldrich, St. Louis, MO, USA) (0.1% wt/wt) for 12 weeks. Three control mice were fed a basal diet without DDC for 12 weeks. The mice were then anesthetized with diethyl ether, and specimens were obtained. All aspects of the study were approved by the Animal Care and Use Committee of Sapporo Medical University School of Medicine.

The liver, jejunum and kidney tissues of mice were fixed in 10% formalin in PBS and embedded in paraffin. Thin sections approximately 5 μm thick were stained with hematoxylin and eosin (H.E.). Additional paraffin-embedded tissue sections from the jejunum were cut approximately 5 μm thick, placed on MAS-coated slide glass and deparaffinized. The sections were incubated with a rat monoclonal anti-mouse Ki-67 antibody (Dako Japan) for 1 h at room temperature. Following this, they were incubated in biotinylated anti-rat IgG for 30 min at room temperature, then visualized with DAB solution and counterstained with hematoxylin. The proliferative index was derived by counting by the number of Ki-67-labeled nuclei in 10 full crypts of jejunal tissues divided by the total number of cells per crypt as described by Sukhotnik *et al*.

Rabbit polyclonal anti-claudin-1, rabbit polyclonal anti-claudin-2 and rabbit polyclonal anti-claudin-3 antibodies were obtained from Zymed Laboratories (San Francisco, CA, USA). Rabbit polyclonal anti-claudin-7, rabbit polyclonal anti-claudin-8 and rabbit polyclonal anti-claudin-15 antibodies were obtained from IBL Co., Ltd. (Tokyo, Japan). A rabbit polyclonal anti-claudin-19 antibody was obtained from Novus Biologicals (Littleton, CO, USA). Alexa 488 (green)-conjugated anti-rabbit IgG was purchased from Molecular Probes, Inc. (Eugene, OR, USA). The jejunum and kidney tissues were frozen in Neg-50 (Richard-Allan Scientific, Kalamazoo, MI, USA). Serial sections, each 7–8 μm thick, were cut with a cryostat (Leica CM1850, Heidelberg, Germany) and placed on MAS-coated slides (Matsunami, Tokyo, Japan). The sections were incubated with anti-claudin-1, anti-claudin-2, anti-claudin-3, anti-claudin-7, anti-claudin-8, anti-claudin-15 and anti-claudin-19 antibodies (1:100 dilution) at room temperature for 1 h. After washing with PBS, the sections were incubated with an Alexa 488 (green)-conjugated anti-rabbit IgG antibody (1:200) at room temperature for 1 h. For counterstaining of cell nuclei, we used 4,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich). The specimens were examined with an epifluorescence microscope (Olympus, Tokyo, Japan).

In the livers of mice fed the DDC diet for 12 weeks, many bile thrombi were observed intermingling with proliferated bile ducts and oval cells in the periportal areas by H.E. staining (Fig. 1Ab, c). No abnormality was seen in the control mouse liver (Fig. 1Aa).

In the jejuna of mice fed the DDC diet for 12 weeks, H. E. staining showed a slight edematous change in the lumina propria of villi in accordance with the data of Assimakopoulus *et al.*[5] in two mice out of six, whereas no significant change in length or villous epithelial cell morphology was observed. The above changes were regarded as mild injury (Fig. 1B, mild). On the other hand, shortened villi with atrophic crypts were seen in the other four mice fed the DDC diet. These changes were regarded as severe injury in the present study (Fig. 1B, severe).

In the jejuna of mice fed the DDC diet, the proliferative index of cryptic cells, indicated by Ki-67 immunohistochemistry (Fig. 1C), was 74.2% for mild injury and 48.9% for severe injury. These results were significantly lower than for the control (82.3%).

In the control mouse jejunum, claudin-3 and claudin-15 were expressed in the apical-most region in villous and crypt epithelia, whereas claudin-7 was observed throughout the lateral membrane of villous and crypt epithelia (Fig. 2). In the jejunum of mice fed the DDC diet, claudin-3, claudin-7 and claudin-15 decreased in the villous epithelium roughly in parallel to the severity of injury (Fig. 2). However, in the epithelium of the crypt, claudin-3, claudin-7 and claudin-15 were maintained (Fig. 2). These results suggested that DDC feeding mainly affected the permeability barrier function of villous epithelial cells and thus inhibited the proliferative activity of epithelial cells in the crypt, as shown by immunohistochemistry for Ki-67. These results were in accordance with the data of Sukhotonik *et al.* However, the molecular mechanism by which impaired barrier function of villous epithelial cells inhibits crypt cell proliferation and induces subsequent atrophy of crypts remains unsolved in the present study.

In the kidneys of mice fed the DDC diet for 12 weeks, H.E. staining revealed that some glomeruli were slightly enlarged compared with the control, whereas no change was observed in renal tubules (Fig. 3A).

In the kidneys of mice fed the DDC diet, claudin-1 in the Bowman's capsule markedly decreased when compared with that in the control, and claudin-2 in the proximal tubules, claudin-8 in the distal tubules and claudin-19 in the thick ascending limb of the loop of Henle were dispersed from the apical-most regions diffusely throughout the cytoplasm (Fig. 3B). No change in the localization of claudin-3 or claudin-7 in distal tubules was observed (Fig. 3B).

Claudin-1 is expressed preferentially in the epithe-

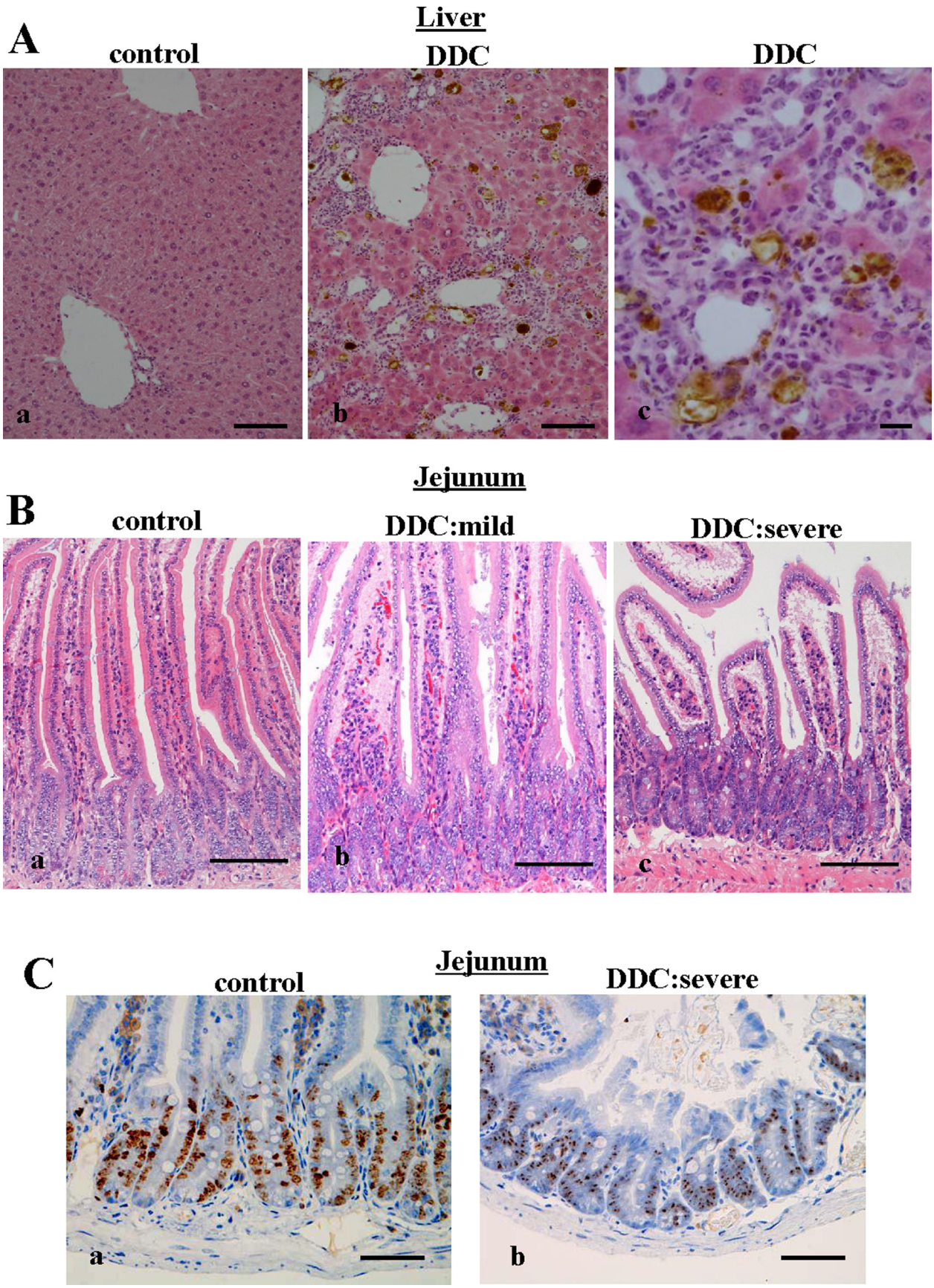

Fig. 1. (A) H.E. staining in the livers of mice fed the DDC diet for 12 weeks (b, c) and control (a). Bars=200 μm. (B) H.E. staining of the jejunal tissues of mice fed the DDC diet for 12 weeks with mild injury (b) and severe injury (c) and the control (a). Bars=500 μm. (C) Immunohistochemistry for Ki-67 in the jejuna of mice fed the DDC diet for 12 weeks (b: severe) and the control (a). Bars=50 μm.

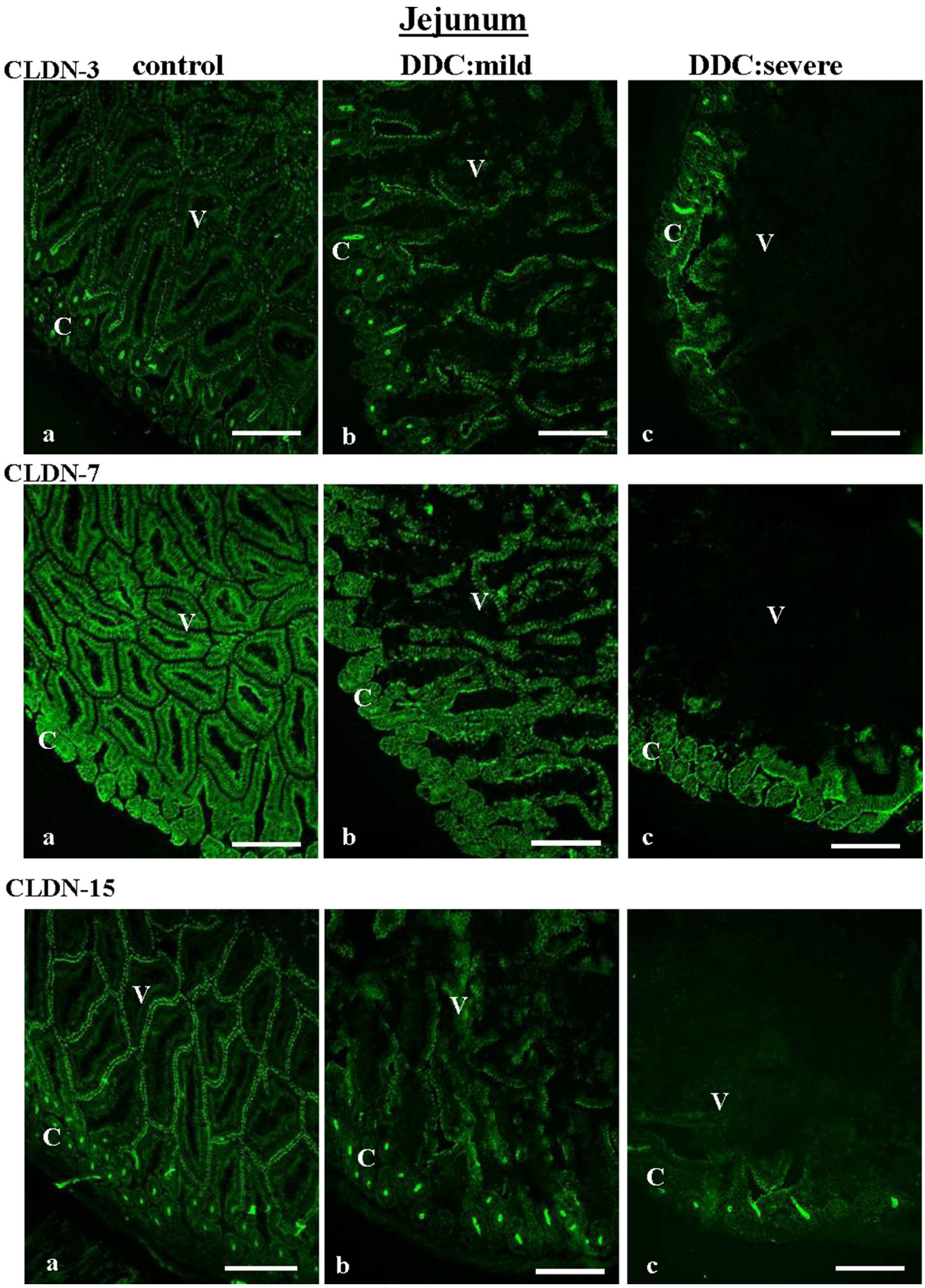

Fig. 2. Immunohistochemistry for claudin-3 (CLDN-3), claudin-7 (CLDN-7) and claudin-15 (CLDN-15) of the jejunum in mice fed the DDC diet for 12 weeks with mild injury (b: mild) and severe injury (c: severe) and the control (a). Bars=200 μm. C: crypts. V: villi.

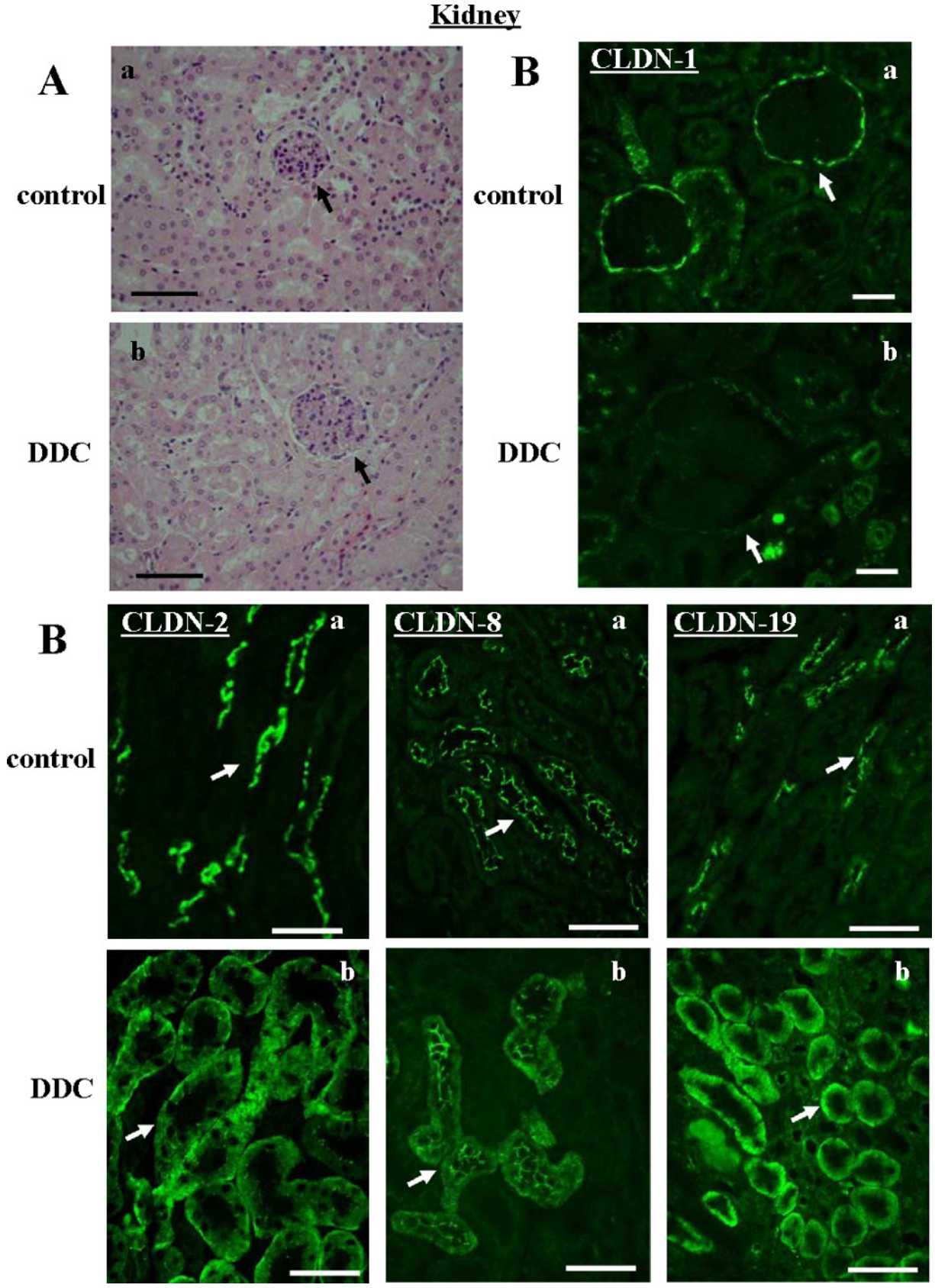

Fig. 3. (A) H.E. staining of the kidney in mice fed the DDC diet for 12 weeks (b) and the control (a). Bars=50 μm. (B) Immunohistochemistry for claudin-1 (CLDN-1), claudin-2 (CLDN-2), claudin-8 (CLDN-8) and claudin-19 (CLDN-19) in the kidney of mice fed the DDC diet for 12 weeks (b) and the control (a), Bars=100 μm. Arrows in control mouse kidney (a) show typical immunohistochemical localization of claudin subspecies in the epithelial cells of the Bowman's capsule (CLDN-1) and renal tubules (CLDN-2, CLDN-8 and CLDN-19). Arrows in the mouse kidney designated (b) show that of claudins in approximately identical portions of kidneys of a mouse fed the DDC diet for 12 weeks.

lial cells of the Bowman's capsule[13], whereas claudin-2 is expressed in the proximal tubule cells and constitutes the cation reabsorptive patheway[14]. On the other hand, claudin-14, claudin-16 and claudin-19 are expressed in the thick ascending limb of the loop of Henle, forming a complex that regulates calcium transport[15], whereas claudin-4, claudin-7 and claudin-8 are expressed in the collecting duct cells acting as chloride permeability determinants[14]. Therefore, the results of the present study suggested that DDC feeding affected selectively the barrier function of epithelial cells of the Bowman's capsule, proximal tubule, distal tubule and thick ascending limb of the loop of Henle.

Though a direct effect of DDC on the intestinal mucosa and renal epithelial cells cannot be ruled out, it is also possible that DDC-induced jaundice might impair tight junction-related molecules, because increased bilirubin in the serum inhibits mitochondrial respiration[2] and ATP depletion preferentially abolishes the barrier function of epithelial tight junctions[16].

In conclusion, in the present study, we showed for the first time that the expression and subcellular localization of claudin subspecies changed markedly in the intestinal and renal epithelial cells in a region- or segment-specific manner in mice fed a DDC diet for 12 weeks. The results of the present study also suggested that immunohistochemistry of claudin subspecies could be a useful tool for detecting functional alterations of intestinal and renal epithelial cells.

References

1. Preisegger KH, Factor VM, Fuchsbichler A, Stumptner C, Denk H, and Thorgeirsson SS. Atypical ductular proliferation and its inhibition by transforming growth factor beta1 in the 3,5-diethoxycarbonyl-1,4-dihydrocollidine mouse model for chronic alcoholic liver disease. Lab Invest. **79**: 103–109. 1999. [Medline]
2. Tephly TR, Gibbs AH, and De Matteis F. Studies on the mechanism of experimental porphyria produced by 3,5-diethoxycarbonyl-1,4-dihydrocollidine. Biochem J. **180**: 241–244. 1979. [Medline]
3. Avner DL, Lee RG, and Berenson MM. Protoporphyrin-induced cholestasis in the isolated in situ perfused rat liver. J Clin Invest. **67**: 385–394. 1981. [Medline]
4. Sukhotnik I, Kuscuoglu U, Altindag B, Tao GZ, Lehwald N, and Sylvester KG. Intestinal involvement during 3,5-diethoxycarbonyl-1,4-dihydrocollidine-induced chronic liver injury in a mouse model. J Pediatr Surg. **46**: 1495–1502. 2011. [Medline]
5. Assimakopoulos SF, Scopa CD, and Vagianos CE. Pathophysiology of increased intestinal permeability in obstructive jaundice. World J Gastroenterol. **13**: 6458–6464. 2007. [Medline]
6. Kramer HJ. Impaired renal function in obstructive jaundice: roles of the thromboxane and endothelin systems. Nephron. **77**: 1–12. 1997. [Medline]
7. Gumbiner BM. Breaking through the tight junction barrier. J Cell Biol. **123**: 1631–1633. 1993. [Medline]
8. Tsukita S, Furuse M, and Itoh M. Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol. **2**: 285–293. 2001. [Medline]
9. Mineta K, Yamamoto Y, Yamazaki Y, Tanaka H, Tada Y, Saito K, Tamura A, Igarashi M, Endo T, Takeuchi K, and Tsukita S. Predicted expansion of the claudin multigene family. FEBS Lett. **585**: 606–612. 2011. [Medline]
10. Morita K, Fruse M, Fujimoto K, and Tsukita S. Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. Proc Natl Acad Sci USA. **96**: 511–516. 1999. [Medline]
11. Tamagawa H, Takahashi I, Furuse M, Yoshitake-Kitano Y, Tsukita S, Ito T, Matsuda H, and Kiyono H. Characteristics of claudin expression in follicle-associated epithelium of Peyer's patches: preferential localization of claudin-4 at the apex of the dome region. Lab Invest. **83**: 1045–1053. 2003. [Medline]
12. Fujita H, Chiba H, Yokozaki H, Sasaki N, Sugimoto K, Wada T, Kojima T, Yamashita T, and Sawada N. Differential expression and subcellular localization of claudin-7, -8, -12, -13, and -15 along the mouse intestine. J Histochem Cytochem. **54**: 933–944. 2006. [Medline]
13. Kiuchi-Saishin Y, Gotoh S, Furuse M, Takasuga A, Tano Y, and Tsukita S. Differential expression patterns of claudins, tight junction membrane proteins, in mouse nephron segments. J Am Soc Nephrol. **13**: 875–886. 2002. [Medline]
14. Hou J. New light on the role of claudins in the kidney. Organogenesis. **8**: 1–9. 2012. [Medline]
15. Günzel D, and Yu ASL. Function and regulation of claudins in the thick ascending limb of Henle. Pflugers Arch. **458**: 77–88. 2009. [Medline]
16. Mandel LJ, Bacallao R, and Zampighi G. Uncoupling of the molecular 'fence' and paracellular 'gate' functions in epithelial tight junctions. Nature. **361**: 552–555. 1993. [Medline]