Dioxins Disrupt the Biological Barrier Fanction by Aryl Hydrocarbon Receptor

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Introduction

Polyaromatic compounds like PCDD/DF, PBDD/DF and B(a)P are not intentionally produced but are generated as undesired by-products in various processes. As a result, such compounds have also found throughout the environment. Then, the intake of these contaminants from mainly food is the primary route of human exposure, and a highly accumulation in human body is accomplished with enterohepatic circulation.

The intestine is the most important route of entry for foreign antigens. The antigens that may ingest into the body from the intestine include food proteins, natural toxins, commensal gut flora, and invading pathogens. The intestinal tract also represents the first barrier to ingested chemicals such as organic environmental pollutant. As a consequence of their exposed location, intestinal epithelial cells have developed a variety of mechanisms besides the maintenance of barrier function. Such mechanisms include those that act directly to inhibit bacterial colonization along the exposed surface of the monolayer and those that function through an interactive process with components of the underlying immune system.[1)] The intrinsic mechanisms of immunity are derived from the presence of a physical barrier formed by the specialized epithelial cells. Epithelial integrity is critical in maintaining a physical but selective barrier between external and internal environments (Fig. 1). This barrier function is maintained by well-organized intercellular structures including tight junctions (TJs), adherence junctions (AJs), and desmosomes surrounding the apical region of epithelial cells.[2)] Damaging effect of the above intestinal barrier allows increased penetration of normally excluded luminal substances that may promote intestinal disorders.[3, 4)]

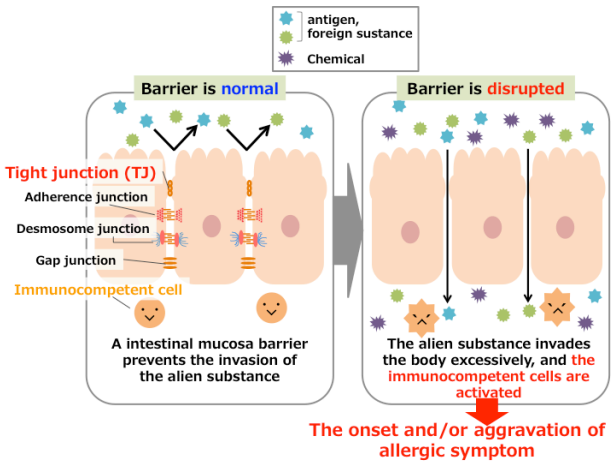

Fig. 1 Biogenic defense mechanism of intestinal mucosa barrier.

Our previous studies were clarified that environmental pollutants with TJ disrupted capability were a group of compounds with aromatic hydrocarbon receptor (AhR) activity. Therefore, we reported on the involvement of AhR in TJ disruption.

Material and methods

1) *Cell culture*

Caco-2 cells, a human intestinal cell line, were cultured in Dulbecco's modified Eagle's medium (DMEM)

containing 10% fetal bovine serum (FBS) at 37 ˚C.

2) *Chemicals*
2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378), 1,3,6,8-TCDD (1368) and CH223191 were dissolved in dimethyl sulfoxide (DMSO).

3) *Measurement of trans-epithelial electrical resistance (TEER)*
Caco-2 cells were seeded in Transwell™ chambers at a subconfluent density. TEER of the Caco-2 monolayer cell sheets on the chamber, a sign of TJ integrity, was measured using a Millicell-ERS epithelial volt-ohmmeter plateau and the TJs were well developed, the Caco-2 monolayers were treated with dioxins and their related compounds on the apical side of the chamber, and the TEER values were measured. The TEER value of a blank Transwell™ chambers (background) was subtracted.

4) *Cytotoxic Activity*
Cell survival and viability were determined using a tetrazolium-based colorimetric assay or lactate dehydrogenase (LDH) assay. Briefly, cells (1×10^4 cells) cultures in a 98-well culture dish for 24 h, and were treated with dioxins and their related compounds for 48 h. After incubation, WST-8 was added to the wells, mixed thoroughly, and further incubated for 1 h. After incubation, the absorbance measured at 450 nm. The release of LDH from the cells was analyzed using a CytoTox96 NonRadioactive Cytotoxicity Assay kit according to the manufacturer's protocol. LDH release was calculated using the following equation: percentage of maximal LDH release LDH in the cultured medium/total LDH in the culture dish.

5) *Paracellular tracer flux assay*
Caco-2 cells were grown in Transwell™ chambers and treated with TCDD for 48 h as described above. 4 kDa Fluorescein isothiocyanate-dextran (FITC-dextran) was dissolved in cell culture medium and used at a final concentration of 5 mg/mL in the apical cell compartment. After 1 h of incubation the amount of fluorescence was measured in the basolateral compartment with TriStar LB 941. The excitation and emission wavelengths were 485 and 535 nm, respectively.

5) *Quantification of tight junction mRNA level by real-time reverse transcription polymerase chain reaction (RT-PCR)*
Total RNA from Caco-2 cells treated with dioxins and their related compounds was extracted with ISOGEN and reverse transcribed with the PrimeScript RT Master Mix. PCR reactions were performed using KAPA SYBR FAST Universal qPCR kit, and assayed on the Thermal Cycler Dice. The transcript number of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was quantified, and each sample was normalized on the basis of GAPDH content.

Result and discussion
To determine whether 2,3,7,8 and 1,3,6,8 show cytotoxicity at the concentaraiton in this study, the effect of 100 nM 2,3,7,8 and 1,3,6,8 on the viability of the Caco-2 cell monolayers was examined using WST-8 assay and LDH release assay. These concentrations of 2,3,7,8 and 1,3,6,8 did not significantly alter the cell viability up to 48 h after treatment (data not shown). To examine whether AhR is involved in barrier function, we compared the TEER values using 2,3,7,8 and 1,3,6,8. The TEER of cell monolayers could be considered as a good indicator of the degree of organization of the TJs within the cell monolayer and epithelial integrity. As indicated by a decreased TEER, we could predict that several physical and chemical factors damage against the TJs. Fig.2 shows the results of TEER values by 2,3,7,8- and 1,3,6,8-TCDD. Addition of 2,3,7,8 from the apical side to the Caco-2 cell monolayer showed a decrease in the TEER value in a dose-dependent manner. On the other hand, 1,3,36,8 have no effect on TEER values. These results indicate that non-cytotoxic dose of 2,3,7,8-TCDD decrease the TEER of human intestinal cells via AhR.
Alterations in TEER are related to the damaging degree in epithelial barrier function. To address the physiological significance of TEER decrease in polarized monolayers, we performed effects of TCDD on permeability to FITC-dextran. Caco-2 cell monolayers were treated for 48 h with 100 nM 2,3,7,8 and 1,3,6,8

before adding FITC-dextran in the apical compartment: one hour later, the fluorescence intensity was measured in the basolateral compartment. Untreated and 1,3,6,8 treated Caco-2 inserts were not readily permeable to FITC-dextran with a molecular mass of 4,000 Da; however, upon 48 h treatment with 100 nM 2,3,7,8, the monolayer became significantly more permeable with a 7.2-fold increase in the trace flux (Fig. 3). These results suggest that 2,3,7,8-TCDD disrupted cell adhesion through activation of AhR and promotes invasion of foreign foreign substances such as allergens.

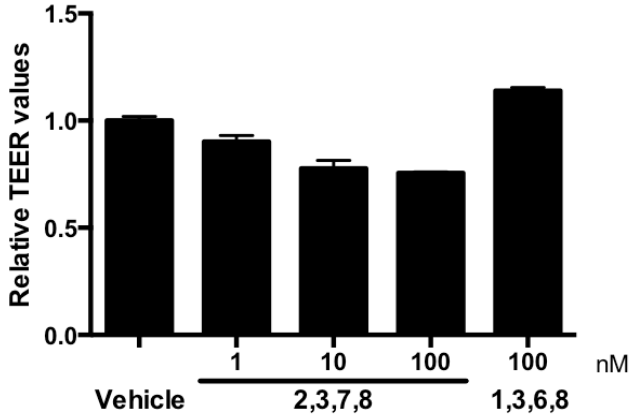

Fig. 2 Effects of TCDD on TJ barrier in Caco-2 cells.
Caco-2 cells were seeded in Transwell. TJs were well developed, the Caco-2 monolayers were treated with 2,3,7,8 or 1,3,6,8-TCDD on the apical side of the chamber, and the TEER values were measured. Relative TEER values were calculated as the ration of TEER values at 0 h. Data are from n=4.

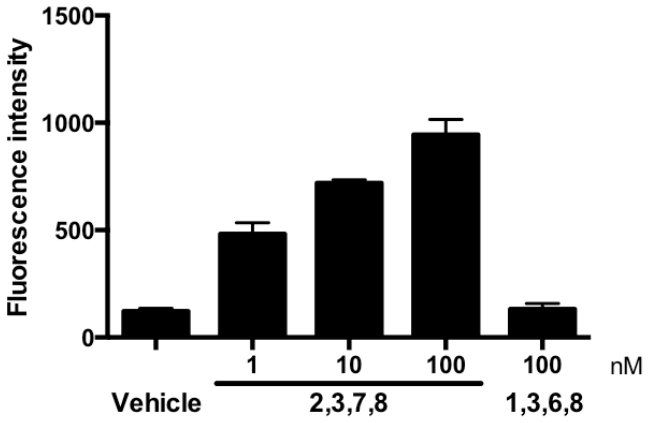

Fig 3 Effect of TCDD on paracellular flux of FITC-dextran in Caco-2 cell monolayers.
Caco-2 cells were grown in Transwell chambers and treated with 2,3,7,8- or 1,3,6,8- TCDD for 48 h. 4 kDa Fluorescein isothiocyanate-dextran (FITC-dextran) was dissolved in cell culture medium and used at a final concentration of 5 mg/mL in the apical cell compartment. Fluorescence intensity in the basal was measured. Data are from n=4.

Claudin-4, a member of a tetra-transmembrane protein family that comprises 27 members, is a key functional and structural component of the tight junction-seal in epithelium. To confirm the mechanism by which TCDD decrease the epithelial barrier function in Caco-2 cells, we performed claudin-4 mRNA level by quantitative real-time PCR. As shown in Fig. 4, 2,3,7,8 was decreased by 40% of vehicle-treated cells. On the other hand,

1,3,36,8 have no effect on CL-4 mRNA expression level. These results suggested that disruption of organic environmental pollutant depends on claudin-4 expression.
Further study is needed to clarify their barrier disruption mechanism by TCDD.

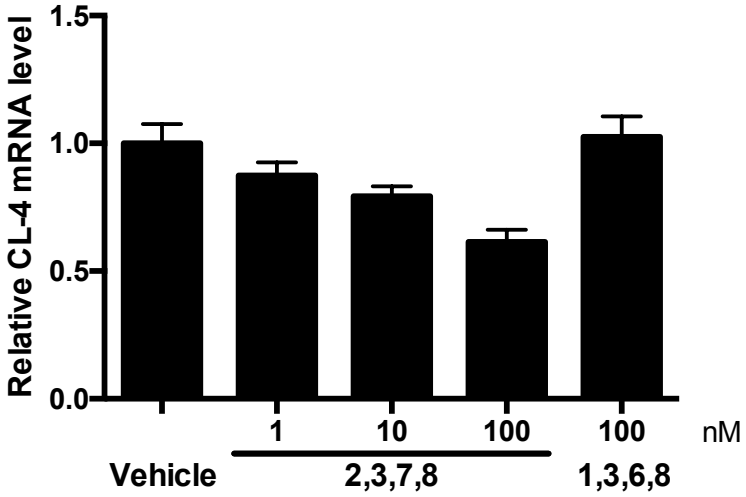

Fig. 4 Expression of claudin-4 in Caco-2 cells treated with TCDD.
Relative claudin-4 mRNA levels were measured by quantitative real-time PCR. Claudin-4 mRNA level was normalized on the basis of GAPDH content. The data are presented as means ± SD (n=4).

Acknowledgements

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Reference

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