DAMAGING EFFECT OF TIGHT JUNCTIONS AS BARRIER FUNCTION IN CACO-2 CELLS INDICATED BY DIOXINS AND THEIR RELATED COMPOUNDS

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Introduction

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. 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)].

In this paper, we evaluated the effects of organic environmental pollutants such as TCDD and B(a)P on epithelial barrier function by trans-epithelial electrical resistance (TEER) and paracellular permeability.

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 (TCDD), benzo(a)pyrene (B(a)P), tetrabromobisphenol A (TBBPA), hexachlorocyclohexane (α-HCH), and hexachlorobenzene (HxCB) were dissolved in dimethyl sulfoxide

(DMSO).

3) *Measurement of trans-epithelial electrical resistance (TEER)*

Caco-2 cells were seeded in Transwell™ chambers (Corning, NY) 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 (Promega) 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. 4-40 kDa Fluorescein isothiocyanate-dextran (FITC-dextran) (Sigma) 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.

Result and discussion

To examine whether the organic environmental pollutants have the activities on damaging effect of barrier function in a cell-based context, we measured trans-epithelial electrical resistance (TEER) of polarized epithelial cells from human. 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. When TCDD and B(a)P were added on human intestinal cells (Fig. 1), a time dependent reduction of the TEER was observed. After 72 h of treatment of Caco-2 cells with 100 nM TCDD or 10 μM B(a)P, TEER decrease reached 40%. 10 μM TBBPA, 10 μM α-HCH, and 10 μM HxCB did not observed the TEER decrease. To determine whether the reduction in TEER induced by TCDD and B(a)P was due to cell death, the effect of 100 nM TCDD and 10 μM B(a)P on the

viability of the Caco-2 cell monolayers was examined using WST-8 assay and LDH release assay. These concentrations of TCDD and B(a)P did not significantly alter the cell viability up to 48 h after treatment (data not shown). These results indicate that non-cytotoxic dose of TCDD and B(a)P decrease the TEER of human intestinal cells in a time dependent manner.

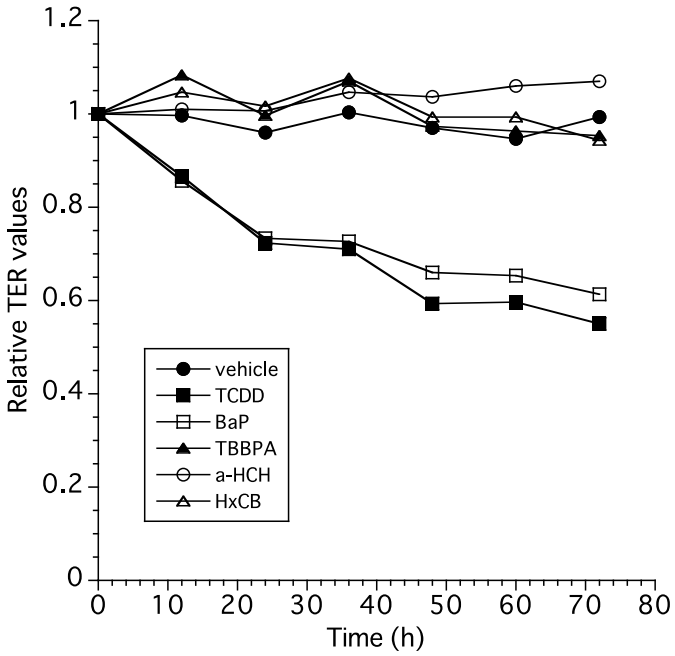

Fig. 1 Effects of organic environmental pollutants on TJ barrier in Caco-2 cells.
Relative TEER values were calculated as the ration of TEER values at 0 h. Data are from n=4.

Table 1 Effect of TCDD on paracellular flux of FITC-dextran in Caco-2 cell monolayers.

FITC-dextran MW (Da)	Vehicle (DMSO)	TCDD (100 nM)
4,000 (r=1.4 nm)	122 ± 18.4	877 ± 29.7
10,000 (r=2.3 nm)	106 ± 33.9	127 ± 11.3
20,000 (r=3.3 nm)	41.5 ± 9.19	45.5 ± 12.0
40,000 (r=4.5 nm)	44.0 ± 2.83	41.5 ± 2.13

Caco-2 monolayers were treated with TCDD (100 nM) during 48 h. FITC-dextran was then added in the apical compartment. After 1 h, the fluorescent intensity was measured in the basolateral compartment. Values represent the mean ± SD (n=3)

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 TCDD before adding FITC-dextran in the apical compartment: one hour later, the fluorescence intensity was measured in the basolateral compartment. Untreated 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 TCDD, the monolayer became significantly more permeable with a 7.2-fold increase in the trace flux (Table 1). On the other hand, FITC-dextran with molecular masses of 10,000, 20,000, and 40,000 Da were not permeable.

Further study is needed to clarify their barrier disruption mechanism by TCDD and B(a)P.

Acknowledgements

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