The changes of tight junction proteins by hydrogen peroxide in primary culture of bovine brain microvessel endothelial cells

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Directed by Professor Namkoong, Kee

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Abstract

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The homeostasis of microenvironment in central nervous system, essential for normal function, is maintained by blood-brain barrier (BBB). Occludin and ZO-1 in tight junctions (TJs) and actin play an important role in maintaining BBB endothelial ion and solute barriers. Malfunction of BBB by reactive oxygen species has been attributed to disruption of TJs. This study examined H_2O_2 effects on paracellular permeability and changes in actin and TJ proteins (occludin and ZO-1)

using primary culture of bovine brain microvessel endothelial cells.

The BBB permeability, measured as TER, increased in a dose- and time- dependent manner when treated H_2O_2 (0.01, 0.1 and 1.0 mM). Cytotoxicity test revealed that H_2O_2 did not cause cell death below 1 mM H_2O_2. H_2O_2 caused increased protein expression of occludin (1.17 ~ 1.29 fold) and actin (1.2 ~ 1.3 fold). ZO-1 maintained steady state levels of expression. H_2O_2 caused intermittent disruption and loss of occludin and ZO-1 at TJs and formation of actin stress fibers. Although ZO-1 did not show significant change in protein expression, permeability changes shown in the current study correlate with alterations in expression and localization of occludin, actin and ZO-1.

In conclusion, we report that H_2O_2 induces increased paracellular permeability of BBB that is accompanied with alterations in localization and protein expression of occludin, actin and ZO-1.

Key Words : H_2O_2, tight junctions, endothelial cell, blood-brain barrier

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I. Introduction

The brain functions within a well-controlled environment that is quite different from the general environment of the body. The surroundings of the central nervous system (CNS) are maintained in a constant states by the blood-brain barrier (BBB). The endothelial cells of the cerebral microvessel form the basis of BBB. The occlusive property of this barrier can be attributed, at least in part, to the

presence of a continuous ring of tight junctions (TJs) between neighboring endothelial cells[1] and delivery of ions and solutes from blood to CNS is limited by the selectivity of BBB[2].

TJs function as the fences separating apical from basolateral plasma membrane and are the major paracellular barrier. TJs are complex of plasma proteins connected to the cytoarchitecture via membrane associated accessory proteins. Claudin, copolymerized occludin and junctional adhesion molecule (JAM) are integral membrane proteins interacting those of neighboring plasma membrane and form TJ barrier[3, 4]. Cytoplasmic TJ accessory proteins [Zonular occludens (ZO)-1, ZO-2, cingulin] connect TJs to the actin cytoskeleton[5].

The main function of TJs is the control of paracellular permeability and polarity[6]. The restraint exerted by TJs to the paracellular diffusion of ions and solutes might be attributable to the transmembrane TJ proteins to seal together adjacent endothelial cells[7]. Permeability inversely correlates with the number of TJ strands, which contain occludin[7] and claudin[8]. However it is unknown whether occludin,

claudin and JAM mediate homophilic or heterophilic adhesion or whether they function independently or cooperatively[9].

Signal transduction pathways that are triggered by bacterial toxins and cytokines[10, 11] modulate the function of TJs. Reactive oxygen species (ROS) are implicated in the pathophysiology of variety of vascular diseases, ischemia-reperfusion and inflammation. As nitric oxide is known to serve as signaling molecule, ROS including superoxide (O_2^-) and hydrogen peroxide (H_2O_2) can alter the function of specific proteins and enzymes[12, 13]. In most cases, the mechanism by which these agents interact with their molecular targets in BBB is still unknown[14].

Although TJ complex has been identified, little is known about alteration in these proteins under pathologic insults producing ROS. In this study, the effects of H_2O_2 on paracellular permeability and expression of TJ proteins were studied within primary culture of bovine brain microvessel endothelial cell.

II. Materials and Methods

1. Materials

Transwell polycarbonate membrane inserts and cell culture plates were purchased from Corning Costar (Acton, MA, USA). Dispase II and collagenase/dispase were purchased from Roche Applied Science (Indianapolis, IN, USA). Minimal essential medium (MEM), DMEM/F12 and rat-tail collagen (type I) were purchased from Invitrogen (Carlsbad, CA, USA). Epithelial voltohm meter (EVOM) and electrode (STX-2) were purchased from World Precision Instruments (Sarasota, FL, USA). All other nutrients, salts, antibiotics and etc used in culture media were purchased from Sigma (St. Louis, MO, USA).

2. Antibodies

Rabbit polyclonal anti-occludin, anti-ZO-1, goat anti-rabbit-IgG-HRP, goat anti-rabbit-IgG-FITC and goat anti-mouse-IgG-FITC

were purchased from Zymed Laboratories (San Francisco, CA, USA). Mouse monoclonal anti-actin antibody was purchased from Sigma.

3. Isolation of microvessel endothelial cells from bovine brains

Bovine brain microvessel endothelial cells (BBMECs) were isolated from gray matter of Korean native cattle (Bos Taurus coreanae) by enzymatic digestion as described in other report[15] with minor modification. In short, fresh bovine brains were obtained from slaughtered cows and placed in ice-cold MEM (pH 7.4) buffered with 50 mM HEPES, containing 100 μg/ml penicillin/streptomycin. Meninges and large surface vessels were removed from brains. Cerebral gray matter was collected and minced. The minced gray matter was enzymatically dispersed during a 2 hr dispase digestion at 37 ^{0}C. The pellets separated by dextran centrifugation were further incubated for 3 hours at 37 ^{0}C with collagenase/dispase (1 mg/ml). Finally, BBMECs were separated by Percoll gradient centrifugation.

4. Culture of BBMECs

Cell viability was determined by Trypan-Blue exclusion test. BBMECs were seeded at 50,000 cells/cm^2 on 12-well plate and 300,000 cells/cm^2 on Transwell insert (0.4 μm pore). The culture surfaces were treated with rat-tail collagen (2 mg/ml). BBMECs were cultured in DMEM/F12 containing 10 % horse serum, 100 μg/ml penicillin/ streptomycin, 45 μg/ml polymyxin B and 1.25 mg/ml amphotericin B. The cells were grown in a 37 ^{0}C incubator in 5 % CO_2 and 95 % room air. The culture medium was changed on the third day and thereafter every other day with DMEM/F12 containing 10 % horse serum, 100 μg/ml penicillin/streptomycin and 100 μg/ml heparin. The growing cells were observed with phase-contrast microscope (CK2, Olympus, Tokyo, Japan). The properties of the cultured cells were confirmed as BBB endothelial cell in previous studies[16, 17].

5. Assessment of transendothelial electrical resistance (TER)

The isolated BBMECs were plated on 12 well Transwell inserts. The media were changed every other day until confluent by visual inspection. The resistances of monolayers were monitored every other day until they reached a steady state. Once stable resistances were obtained (>60 $\Omega \cdot cm^2$), the cells were treated with H_2O_2. TER across BBMECs monolayers was measured using EVOM. The TER ($\Omega \cdot cm^2$) was calculated from the displayed electrical resistance on the layout screen by subtraction of electrical resistance of a collagen-coated filter without cell and a correction for filter surface area. The initial value of TER was measured and at every 15 minutes. Measurements were taken in triplicate (mean±SD).

6. Cytotoxicity study of BBMECs after treatment with H_2O_2

Viability of BBMECs after treatment of H_2O_2 was assessed using AlamarBlue assay (Serotec, Kidlington, Oxford, UK). Confluent BBMECs

were exposed for 30 min to various concentrations of H_2O_2. The culture medium was replaced with fresh media containing 10 % AlamarBlue. The culture was returned to incubator for 15 minutes to 4 hours. The absorbance was measured at wavelength of 570 nm using Spectra MAX 340 (Molecular Devices, Sunnyvale, CA, USA). Background absorbance measured at 600 nm was subtracted from the 570 nm absorbance (n=5). Viability was expressed as a percentage of control.

7. Western blot analysis

After culture was treated with H_2O_2 or control condition, proteins were isolated from confluent BBMEC monolayers using Triton/ deoxycholate/SDS buffer (0.2 % SDS, 100 mM NaCl, 1 % Triton X-100, 0.5 % deoxycholic acid, 2 mM EDTA, 10 mM HEPES, pH 7.5, 10 mM NaFl, 1 mM $NaVO_4$, 1 mM benzamidine, 0.2 mM phenylmethylsulfonyl fluoride) along with 1 tablet of Complete mini protease inhibitor (Amersham, Biosciences, Piscataway, NJ, USA). The protein

concentration of lysate was determined with microBCA assay (Pierce, Rockford, IL, USA). Protein samples were separated on a 10 % SDS polyacrylamide gel at 100 V for 90 min. The proteins were transferred to polyvinylidene fluoride membrane with 300 mA at 4 ^{0}C for 60 min. The membranes were blocked using 5 % nonfat dry milk in TBST (20 mM Tris base, 137 mM NaCl, pH 7.2, 0.1 % Tween 20) and incubated for 60 min at room temperature (RT) with primary antibodies (1:1000, in TBST). The membranes were washed three times for 5 min each with TBST before incubation with the respective HRP conjugated secondary antibodies (1:2000, in TBST) for 60 min at RT. After three washes, the protein bands were visualized with enhanced chemilucence method (ECL plus, Amersham). The membranes were scanned with Intelligent Box II (Fugifilm, Tokyo, Japan) and LAS-1000 Lite image analysis software (Fugifilm). Optical density of the bands was calculated with Image Gauge 4.0 (Fugifilm). Experiments were taken in triplicate (mean±SD). The results are reported as a percent of control.

8. Confocal microscopy of BBMECs

BBMECs grown on glass cover were exposed to H_2O_2 or normal condition before immunofluorescence staining. After treatment, culture medium was removed and monolayer was washed with prewarmed phosphate buffered saline (PBS, 0.01M). Cells were fixed with 3 % paraformaldehyde (in PBS) for 20 min at RT and permeabilized with 0.1 % Triton X-100 (in PBS) for 10 min at RT. After fixing and permeabilization, monolayer was blocked with 1 % bovine serum albumin (BSA)/PBS for 60 min at RT. Confluent monolayers from each treatment group were incubated with anti-occludin (5 μg/ml), anti-ZO-1 (5 μg/ml) or anti-actin (1:200) primary antibody for 1 hr at RT. The cells were rinsed with 1 % BSA/PBS, followed by incubation with a FITC conjugated secondary antibody (5 μg/ml) for 1 hr at RT in the dark. The fluorescent-stained cells were rinsed three times with PBS before being mounted on a slide with 50 % glycerol-PBS and sealed. Photographs were taken with a confocal laser microscope (LSM-510

meta, Zeiss, Berlin, Deutschland).

9. Statistical methods

Data are expressed as mean ± S.D. of three individual monolayers. Differences between mean values were tested for the significance using Student's t-test ($p<0.05$).

III. Results

1. TER analysis

The effects of H_2O_2 on TER were measured in BBMEC monolayers over 2 hr (Fig. 1). A blank collagen-coated Transwell insert was used as an indicator of background effects on TER and was consistent at 102 ± 8 Ω · cm^2. Controls showed no significant change in TER over 2 hr. During prolonged exposure of H_2O_2, TER changed significantly over 2 hr. Under these conditions, monolayers demonstrated a gradual drop in resistance with the greatest decreases detected at 2 hr. This suggests that alterations of endothelial permeability are dependent on the length and severity of H_2O_2.

2. Cytotoxicity analysis of H_2O_2 treated BBMECs

To test the possibility that changes in TER result from the death of cells and the subsequent formation of holes in monolayer, the cell

viability was measured using AlamarBlue assay. H_2O_2 concentration of 10 μM, 100 μM and 1 mM did not decrease cell viability as compared to the control (Fig. 2). However after treatment of 5 mM H_2O_2, the cell viability greatly decreased to about 30 % respectively. These indicate that the H_2O_2 concentration of 1 mM and below is insufficient to cause cell death.

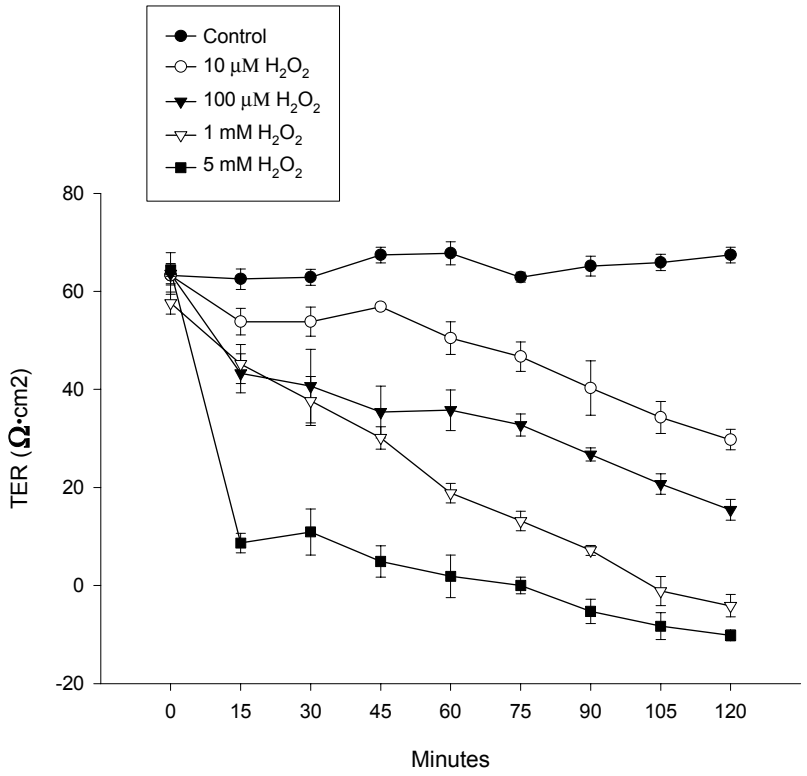

Fig. 1. H_2O_2 mediated transendothelial electrical resistance (TER, Ω · cm^2). TER of bovine brain microvessel endothelial cells was measured with EVOM. Treatment of 5 mM H_2O_2 (■) showed abrupt and significant decrease in TER at 15 minutes. Compared with control level (●), lower doses of H_2O_2, 10 μM(○), 100 μM(▼) and 1 mM(▽) showed gradual decrease with dose and time dependent pattern. Data are shown as mean ± SD and significantly different from the control values after 15 min with $p<0.05$ (n=3).

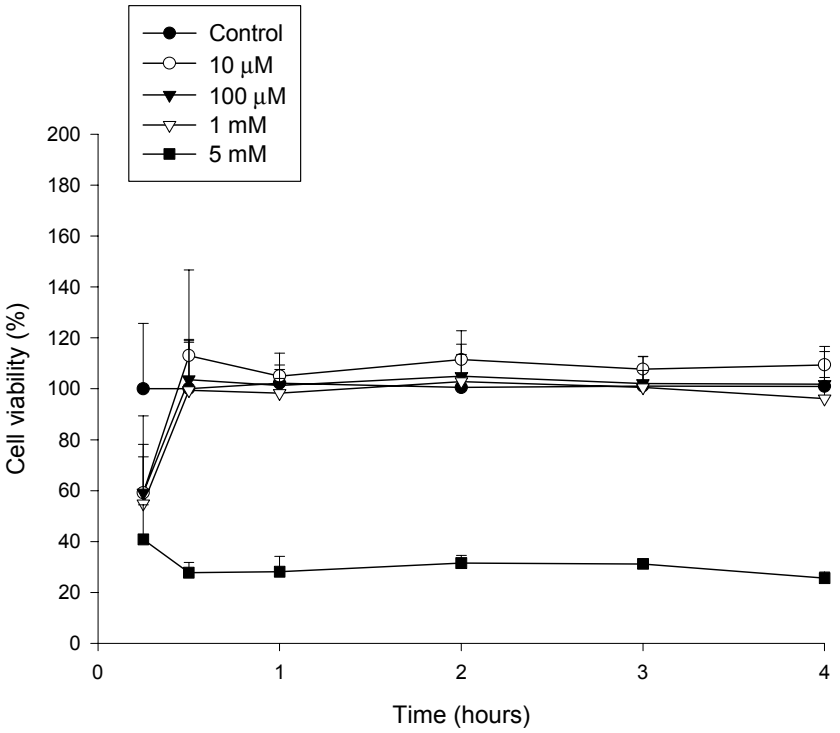

Fig. 2. Percentage of viability of bovine brain microvessel endothelial cells after H_2O_2 treatment. The cell viability was assessed using AlamarBlue assay. Treatment of 5 mM (■) H_2O_2 showed significant decrease in viability. Compared with control level (●), lower doses of H_2O_2, 10 μM(○), 100 μM(▼) and 1 mM(▽) did not affect the cell viability. Data are shown as mean ± SD. Only 5 mM data is significantly different from the control values after 1 hr with $p<0.05$ (n=5).

3. Western blot analysis of TJ proteins

By immunoblotting, alterations in expression of proteins that form TJs and cytoskeletal architecture were examined in 10 μM, 100 μM, 1 mM H_2O_2 treatment and control. Expression of occludin (~65 kD) increased about 1.17 (100 μM) ~ 1.29 (1 mM) fold after treatment of H_2O_2 (Fig. 3A, B). Increased expression (1.2 ~ 1.3 fold) of major cytoskeletal protein (actin) occurred after treatment of 100 μM and 1 mM H_2O_2 (Fig. 4A, B). But ZO-1 (220 kD) maintained steady state levels of expression (Fig. 5A, B). The results in table 1 were summary of western blot analyses of H_2O_2 treated BBMECs compared with controls.

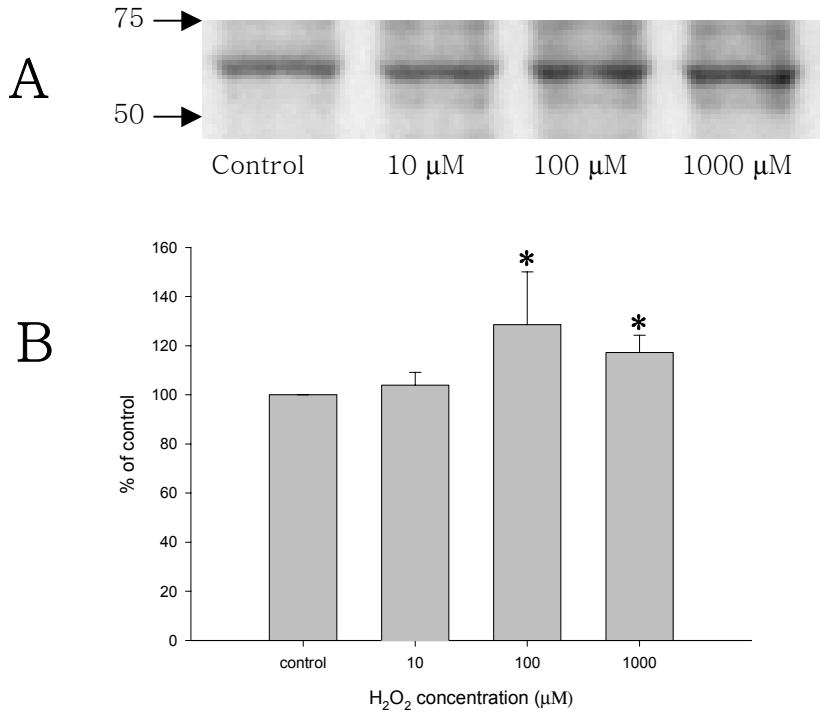

Fig. 3. Effect of H_2O_2 on occludin protein levels. Bovine brain microvessel endothelial cells were treated with H_2O_2 of 10 μM, 100 μM and 1 mM. Western blot analysis (A) shows the increased level of occludin (100 μM, 1 mM). Densitometry analysis (B) of blots showed significant statistical differences between the control and treatment groups (100 μM, 1 mM). Data are shown as the mean ± SD with $*p<0.05$ (n=3).

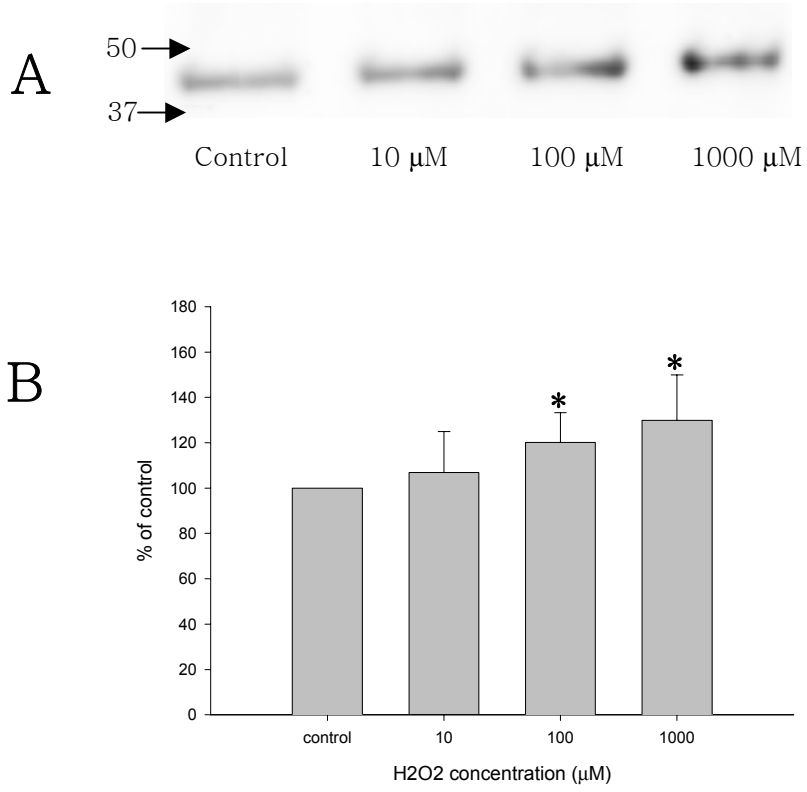

Fig. 4. Effect of H_2O_2 on actin protein levels. Bovine brain microvessel endothelial cells were treated with H_2O_2 of 10 μM, 100 μM and 1 mM. Western blot analysis (A) shows the increased level of actin (100 μM, 1 mM). Densitometry analysis (B) of blots showed significant statistical differences between the control and treatment groups (100 μM, 1 mM). Data are shown as the mean ± SD with $*p<0.05$ (n=3).

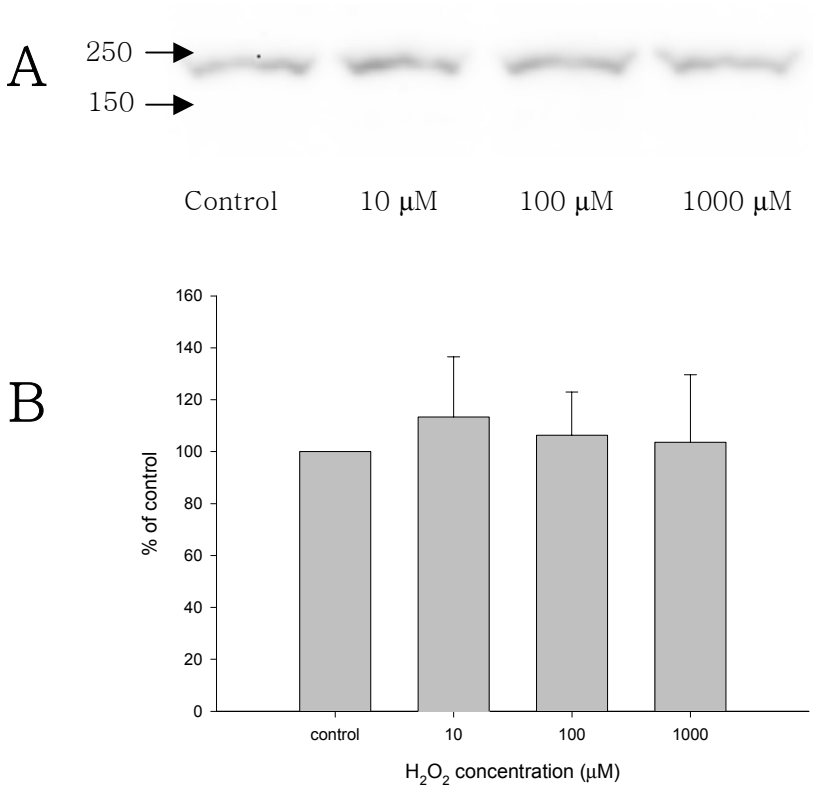

Fig. 5. Effect of H_2O_2 on ZO-1 protein levels. Bovine brain microvessel endothelial cells were treated with H_2O_2 of 10 μM, 100 μM and 1 mM. Western blot analysis (A) shows the steady-state level of ZO-1. Densitometry analysis (B) of blots showed no statistical differences between the control and treatment groups. Data are shown as the mean ± SD (n=3).

Table 1. Effects of H_2O_2 treatment on BBMEC protein expression

Treatment Group	Occludin, %	ZO-1, %	Actin, %
Control	100	100	100
10 μM	104 ± 17	113 ± 24	107 ± 17
100 μM	129 ± 13*	106 ± 18	120 ± 7*
1000 μM	117 ± 20*	104 ± 26	130 ± 18*

Effect of H_2O_2 on tight junction protein levels in bovine brain microvessel endothelial cells. ZO-1 shows the steady-state level of expression. Occludin and actin show significant increase of protein expression (100 μM, 1 mM). Data are percentage of control with means ± SD. *$p<0.05$ (n=3).

4. Confocal microscopy of BBMEC monolayer

The localization of occludin, ZO-1 and actin was examined after H_2O_2 treatment (Fig. 6). Control occludin staining demonstrates that the protein is localized at TJs (Fig. 6A). Control ZO-1 is localized at the periphery and cell junction of endothelial cells (Fig. 6C). After a 30 minutes exposure of 1 mM H_2O_2, the endothelial cell junctions exhibit a dramatic loss of occludin and ZO-1 (Fig. 6B, D), except for where TJs are observed. The endothelial cells exposed to H_2O_2 exhibit changes in actin localization with increased staining of filament and development of stress fiber formation (Fig. 6F), when compared to the control (Fig. 6E).

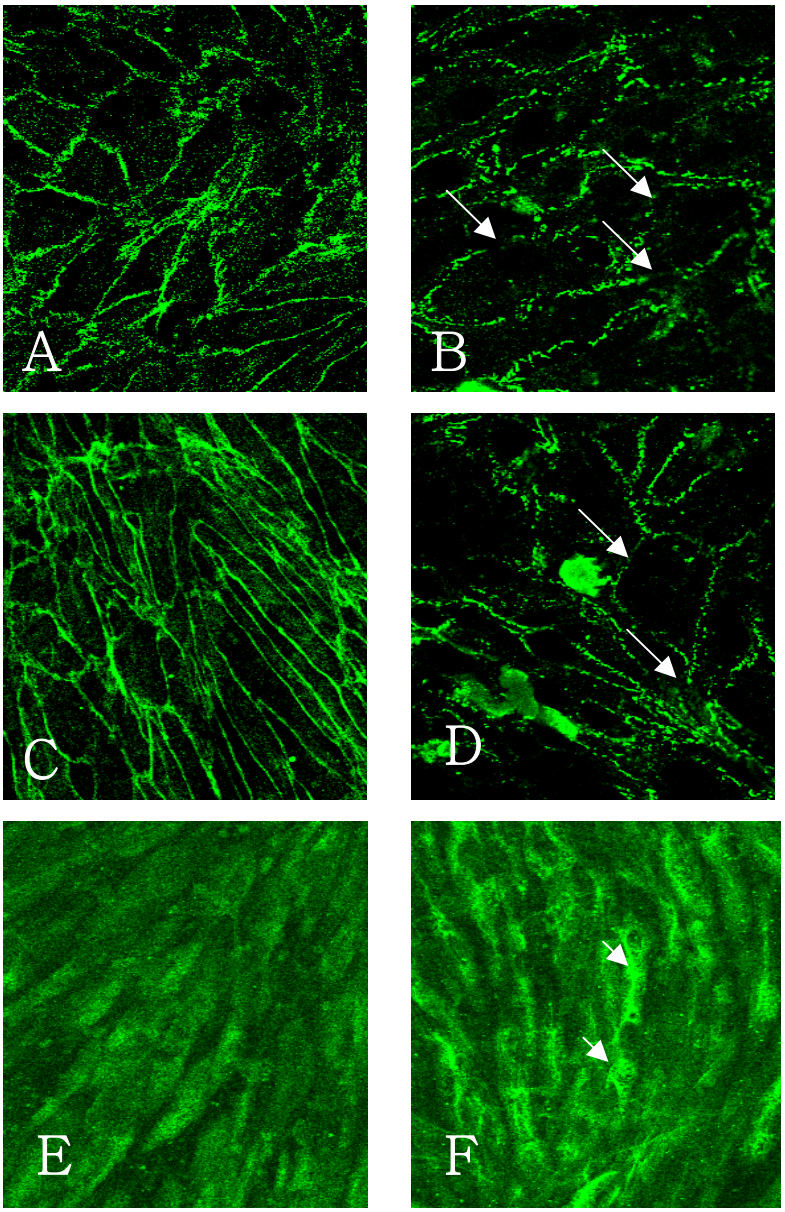

Fig. 6. Immunofluorescence staining showing protein localization in bovine brain microvessel endothelial cells. A, C and E show control staining for occludin, ZO-1 and actin, respectively. B, D and F exhibit occludin, ZO-1 and actin after 30 min exposure of 1 mM H_2O_2. Note that H_2O_2 causes a loss (long arrows) of occludin and ZO-1 at tight junctions and causes stress fiber formation (short arrows).

IV. Discussion

The homeostasis of microenvironment in CNS, essential for normal function, is maintained by BBB[18]. Recent reports have shown that tight junctional protein, occludin, plays an important role in maintaining endothelial solute barriers[19, 20]. In addition, ZO proteins and actin are important molecules regulating BBB functions[5, 21]. Several studies have shown that H_2O_2 is involved in permeability change of TJs in various cells[22, 23]. This study focuses on understanding how BBMECs respond to the treatment of H_2O_2, which is one of ROS.

We studied the effects of exposure to hydrogen peroxide on the TER across cultured monolayers of BBMECs. TER is an useful physiological marker of BBB integrity and is over 1000 $\Omega \cdot cm^2$ in vivo. It is well known that the establishment of TJ correlates with development of TER[24]. The resistance is about 60 $\Omega \cdot cm^2$ in our BBB model as in other model[25]. The BBB permeability, measured as TER,

increased in a dose- and time- dependent manner when treated with H_2O_2 (0.01, 0.1 and 1 mM). These data indicate that H_2O_2 increased permeability to a similar pattern as seen with other types of cells[26, 27]. H_2O_2 concentration of 1 mM and below did not affect cell viability as demonstrated by AlamarBlue assay. But 5 mM H_2O_2 caused significant decrease in cell viability. With these results, the H_2O_2 concentrations of 0.01, 0.1 and 1 mM were used in following studies. Under these concentrations, the permeability changes and decreased TER were not due to cytotoxicity.

Although ZO-1 did not show significant change in protein expression, permeability changes shown in the current study correlate with alterations in expression and localization of occludin, actin and ZO-1 as seen in other report[28]. Under normal condition, occludin has a continuous distribution pattern at cell-to-cell contact boundaries. Increased concentration of H_2O_2 caused intermittent disruption and loss of occludin at TJs. ZO-1, one of TJ accessory protein, disappeared

irregularly at TJs, as seen in occludin. These disruptions in TJ proteins at cell-to-cell contact sites correlate with increased paracellular permeability[28, 29]. There were increased expression of actin, a major cytoskeletal protein, and actin stress fibers. The increases in protein expression were found in occludin and actin. Increasing TJ protein synthesis to enhance weakened TJs may be an endothelial response to cytoarchitectural alterations and increased paracellular permeability.

Occludin with a molecular mass of ~65 kD has NH_2 and COOH termini in the cytoplasm with two extracellular loops projecting into paracellular space[21]. ZO-1, ZO-2 and ZO-3 are membrane-associated guanylate kinase-like homologues (MAGUKs). To date, three MAGUKs, ZO-1, ZO-2 and ZO-3, have been identified as component of TJs[30]. Members of this family are often found at the site of cell-to-cell contact and may function to couple extracellular signaling pathways with the cytoskeleton like actin[31]. It has known that the COOH terminal of occludin binds ZO-1, ZO-2 and ZO-3, indicating that occludin is a

membrane partner for ZO-1, ZO-2 and ZO-3[29]. But recent finding suggested that the three MAGUKs were recruited to claudin[5]. Many lines of evidence suggest that paracellular permeability is influenced by the state of perijunctional actin[32, 33].

Studies with cultured cell monolayers and intact tissue have identified some of the second messengers and signaling pathways important for the assembly of TJs. To date, multiple signaling transduction pathways have been implicated in TJ biogenesis including kinase[13], Ca^{2+}[,34], G proteins[35], cAMP[36]. In additions, generation of ROS from various pathophysiologic events suggests that ROS play a significant role in a variety of disease process such as heart attack, stroke, arthritis, circulatory shock and inflammatory disease, etc[37]. The development of oxidative stress achieved by exposing the cells to low concentration of H_2O_2 is a well-established technique. High concentration of H_2O_2 (250 ~ 1000 μM) causes endothelial cell injury[38]. As in other studies[26, 28], our data showed that the dose above did not

cause cytotoxicity. It is unknown whether H_2O_2 causes a direct effect on TJs or not. The alterations in expression and localization with such doses apparently suggest that H_2O_2 plays an important role in TJ function regulation. Besides, a lower concentration (121 μM) of H_2O_2 stimulates cell proliferation in fibroblasts[39]. Moreover, a proliferation of endothelial cells has been found to be associated with neovascular expansion. Endothelial cells, when exposed to lower concentration of H_2O_2 for 30 min, were found to stimulate angiogenesis[40]. Cancer cells also produce ROS including H_2O_2. Therefore, release of H_2O_2 might be one of the triggers of the angiogenic process in cancer cells[41].

Indeed, ROS are generated by specialized plasma membrane oxidases or by mitochondria in response to various growth factors or cytokines[42]. ROS have been traditionally regarded as toxic byproduct of metabolism and cells develop several antioxidant enzymes to protect themselves from these toxic species. However, recent data suggest that ROS are also essential participants in signaling[43, 44]. Besides the

activation of different members of signaling cascades, ROS may directly regulate the activity of transcription factors. Redox regulation has been reported for NF-κB[45], AP-1[46] and HIF-1[47]. However, the regulation of NF-κB and HIF-1 transcriptional activity by ROS seems far more complex. ROS impair endothelial cell functions not only by direct cellular injury but also eliciting signal cascades[48].

In conclusion, we report that H_2O_2 induces increased paracellular permeability of BBB that is accompanied with alterations in localization and protein expression of occludin, actin and ZO-1. Because redox-sensitive responses appear at times to be cell specific, much remains to be studied concerning the signaling pathways and genes that are regulated by ROS. Further studies are needed to elucidate how ROS produced by hypoxia-reoxygenation affect TJ functions in BBB. Therapeutic strategies aimed at controlling endothelial cell function by intervening ROS mediated signaling cascades have widely applicability in vessel involved diseases.

V. Conclusion

The homeostasis of microenvironment in CNS, essential for normal function, is maintained by BBB. Occludin and ZO-1 in TJs and actin play an important role in maintaining BBB endothelial ion and solute barriers. Malfunction of BBB by ROS has been attributed to disruption of TJs. This study examined H_2O_2 effects on paracellular permeability and changes in actin and TJ proteins (occludin and ZO-1) using primary culture of BBMECs.

The BBB permeability, measured as TER, increased in a dose- and time- dependent manner when treated H_2O_2 (0.01, 0.1 and 1.0 mM). Cytotoxicity test revealed that H_2O_2 did not cause cell death below 1 mM H_2O_2. H_2O_2 caused increased protein expression of occludin (1.17 ~ 1.29 fold) and actin (1.2 ~ 1.3 fold). ZO-1 maintained steady state levels of expression. H_2O_2 caused intermittent disruption and loss of occludin and ZO-1 at TJs and formation of actin stress fibers. Although

ZO-1 did not show significant change in protein expression, permeability changes shown in the current study correlate with alterations in expression and localization of occludin, actin and ZO-1.

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국문요약

소의 뇌 미세혈관 내피세포의 일차배양에서 hydrogen peroxide에 의한 치밀이음부 단백질의 변화

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뇌의 정상적인 활동에 필수적인 중추신경계 미세환경의 항상성은 혈액뇌장벽에 의해 유지된다. Actin과 치밀이음부 단백질인 occludin과 ZO-1은 혈액뇌장벽의 내피세포가 이온과 용질에 대한 장벽으로서 기능을 하는데 중요한 역할을 한다. 활성산소종으로 인한 혈액뇌장벽의 기능 이상은 치밀이음부의 기능 부전에 기인한다. 본 연구에서는 소의 뇌 미세혈관 내피세포를 일차 배양해서 생체외 혈액뇌장벽 모형을 만든 후, 활성산소종의 하나인 과산화수소에 의해 일어나는 세포간 물질 투과도의 변화와 actin 및 치밀이음부 단백질 (occludin과 ZO-1) 의 변화를 알아보고자 하였다.

전기저항으로 측정한 혈액뇌장벽의 투과도는 과산화수소의 농도(0.01, 0.1과 1.0 mM) 와 시간에 비례하여 증가하였다. 과산화수소의 농도가 1 mM 이하에서는 과산화수소의 독성에 의한 세포 생존율의 변화가 없었다. 과산화수소에 의해 occludin (1.17 ~ 1.29 배) 과 actin (1.2 ~ 1.3 배) 은 증가하였으나 ZO-1은 변화가 없었다. 치밀이음부에서는 occludin과 ZO-1은 과산화수소에 의해 불규칙하게 소실되었으며, 세포질에서는 actin stress fibers가 형성되었다. 본 실험에서 나타난 혈액뇌장벽 투과도의 변화는 occludin, actin, ZO-1 단백질의 양과 분포의 변화와 연관된 것으로 생각된다. 요약하면, 과산화수소는 혈액뇌장벽에서 세포간 투과도를 증가시키며 이는 occludin, actin, ZO-1 단백질의 양과 분포의 변화와 밀접한 관계가 있다고 볼 수 있다.

핵심되는 말 : H_2O_2, 치밀이음부, 내피세포, 혈액뇌장벽