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# Protective effects of hydrogen-rich medium on lipopolysaccharide-induced monocytic adhesion and vascular endothelial permeability through regulation of vascular endothelial cadherin

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**ABSTRACT.** We observed the effect of hydrogen-rich medium on lipopolysaccharide (LPS)-induced human umbilical vein endothelial cells (HUVECs), hyaline leukocyte conglutination, and permeability of the endothelium. Endotheliocytes were inoculated on 6-well plates and randomly divided into 4 groups: control, $H_2$, LPS, LPS+$H_2$, $H_2$, and LPS+$H_2$ in saturated hydrogen-rich medium. We applied Wright's staining to observe conglutination of hyaline leukocytes and HUVECs, flow cytometry to determine the content of vascular cell adhesion protein 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1), enzyme-linked immunosorbent assay to measure the E-selectin concentration in the cell liquor, the transendothelial electrical resistance (TEER) to test the permeability of endothelial cells, and Western blot and im-

 

munofluorescence to test the expression and distribution of vascular endothelial (VE)-cadherin. Compared with control cells, there was an increase in endothelium-hyaline leukocyte conglutination, a reduction in VCAM-1, ICAM-1, and E-selectin, and the TEER value increased obviously. Compared with LPS, there was an obvious reduction in the conglutination of LPS+$H_2$ cells, a reduction in VCAM-1, ICAM-1, and E-selectin levels, and a reduction in the TEER-resistance value, while the expression of VE-cadherin increased. Fluorescence results showed that, compared with control cells, the VE-cadherin in LPS cells was incomplete at the cell joints. Compared with LPS cells, the VE-cadherin in LPS+$H_2$ cells was even and complete at the cell joints. Liquid rich in hydrogen could reduce LPS-induced production of adhesion molecules and endothelium-hyaline leukocyte conglutination, and influence the expression and distribution of VE-cadherin to regulate the permeability of the endothelium.

**Key words:** Hydrogen; Lipopolysaccharide; Sepsis; Endothelial cells; Vascular endothelial-cadherin

## INTRODUCTION

Sepsis is a syndrome that is caused by various kinds of infective agents, centered on inflammation in the body, which is one of its common clinical complications (Rubenfeld and Herridge, 2007) in critical disease and the main cause leading to death (Hotchkiss and Karl, 2003). There are over 180 million sepsis patients globally and 1.4 of them will die daily due to the illness (Russell, 2006). The nature of sepsis is an uncontrolled inflammatory response, of which the pathophysiological process is complicated involving a series of overall responses, including various cell factors, inflammatory mediators, and activation of the blood coagulation system, while the central link in this process is a change in the function of vascular endothelial (VE) cells (Gerszten et al., 1999). Sepsis, as the first line of defense, involves activation of VE cells, which subsequently release many inflammatory factors and adhesion molecules that mediate the migration of inflammatory cells from blood vessels to the sight of inflammatory injuries, where they bind to the infectious site and are activated by tyrosine phosphorylation of a series of plasmosins, which accentuates the inflammatory effects and can cause multiple organ failure (Matsuda and Hattori, 2007). The primary phenomenon in this process is the attachment of the hyaline leukocytes to the VE cells; various kinds of adhesion molecules and chemotactic factors are able to coordinate the transfer, adhesion, and accumulation of hyaline leukocytes to the endothelium, which finally destroys the endothelial cells to increase their permeability. A significant pathological process in the death of a patient from sepsis is the increase in permeability of the endothelial cells. The barrier of endothelial cells is the main physiological function of the permeability of the endothelium and its functional change is the basis for the destruction of microcirculatory hemodynamics and the occurrence of tissue edema. Therefore, protection of the blood vessel endothelium barrier is key in curing sepsis (Curry and Adamson, 2010).

Cadherin, a kind of transmembrance protein, is part of the intercellular adhesion molecule family. Its function depends on the concentration of calcium ions in cells, and is primar-

ily involved in forming adhesion junctions (Tekeichi, 1995), while VE cells are able to specifically express VE-cadherin, the most significant factor in the formation of adhesion junctions in the endothelium (Abbruscato and Davis, 1999). Adhesion junctions mediated by VE-cadherin play an important role in regulating the permeability of blood vessels, and factors that can restrain its expression can cause formation of intercellular space in the endothelium, which leads to increased permeability of the endothelium. On the contrary, factors that increase its expression can reduce permeability of the endothelium and protect it (Birdsey et al., 2008).

Hydrogen ($H_2$) is distributed widely in nature and is the richest element in the universe, and as an alternative antioxidant, it has antioxidant, anti-inflammatory, and anti-apoptosis functions (Chen et al., 2013). At present, $H_2$ and hydrogen-rich saline (HS) have been certified as providing protection against many kinds of illnesses, including ischemia-reperfusion injury (Huang et al., 2011), sepsis (Xie et al., 2010a), multiple organ dysfunction syndrome (Xie et al., 2010b), type II diabetes mellitus (Kajiyama et al., 2008), and neurodegenerative disorders (Fu et al., 2009). Furthermore, our previous tests have shown that $H_2$ can provide protection for mice with sepsis and suppress apoptosis (Xie et al., 2012), while its effects on the integrity of junctions between endothelial cells require further research.

## MATERIAL AND METHODS

### Preparation of hydrogen-rich medium

We referred to previously reported methods (Ohsawa et al., 2007) and made some improvements, using a GCH-300 high-purity hydrogen generator to produce $H_2$ (Tianjin Tongpu Analytic Instrument Technology Co., Ltd.) and adding Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) including 10% fetal bovine serum (FBS; Gibco) to a high-purity hydrogen (purity, >99.9999%) environment that was exposed under pressure at 0.4 MPa for 4 h until the $H_2$ dissolved in the medium and reached saturation. We adopted a hydrogen electrode (Unisense, Denmark) to test the concentration of $H_2$ to 0.6 mM and sealed it in a special aluminum bag after filtration and sterilization (no dead space) for storage at 4°C. To ensure the concentration of $H_2$ in the medium, HS needs to be freshly prepared.

### Cell culture and treatment

After human umbilical vein endothelial cells (HUVECs; ATCC, USA) and U937 cells (ATCC) recovered, they were put in 10% FBS, 1% DMEM culture with penicillin streptomycin solution (Gibco), and RPMI1640 medium (Gibco), into a 5% $CO_2$ incubator at 37°C to develop. When the cells reached 80%, subculture was conducted and the second and third generations of cells were used in the test.

The HUVECs were divided into 4 groups at random: control, $H_2$, lipopolysaccharide (LPS), and LPS+$H_2$, in which control and LPS were developed in normal medium, and $H_2$ and $H_2$+LPS in HS medium. LPS (1 μg/mL; #4394, Sigma, USA) was added to the LPS and LPS+$H_2$ groups and an equal amount of normal saline was added to the control and $H_2$ cells.

### Wright-Giemsa staining

HUVECs were inoculated to 6-well plates (2 mL/well, cell concentration 2 x $10^6$/

mL), and given the corresponding treatments. Then, HUVEC-12 cells were mixed into one layer, adding U937 (2 mL/well, cell concentration 5 x $10^6$/mL) at 6, 12, and 24 h. These two kinds of cells were co-cultured for 90 min and then 5 wells of cells from groups of different time points were randomly selected to continue the experiment. The cells were then fixed with formaldehyde for 30 min and washed with phosphate-buffered saline (PBS) twice. Wright-Giemsa A (Beijing Far Western Science and Technology Co., Ltd.) was then dripped onto the surface of the cells and left to develop for 1 min. Wright Giemsa B was added 2-3 times to the cells and mixed completely, dying for 5-10 min, washing, and drying, then reversed under the microscope to observe injury of the cells.

## Flow cytometry test

The HUVEC cells were collected 24 h after each corresponding treatment and suspended in binding buffer and the cell concentration adjusted to 1 x $10^6$/mL. A 100-μL aliquot of cell suspension buffer was added to a 5-mL flow tube and 20 μL fluorescein isothiocyanate (FITC) mouse anti-human CD106 [vascular cell adhesion protein 1 (VCAM-1), BD, USA] or FITC Mouse Anti-human CD54 [intercellular adhesion molecule 1 (ICAM-1), BD] was added and mixed smoothly and developed for 30 min at room temperature and protected from light, before adding 400 μL PBS to the reaction tube and testing with flow cytometry (BD).

## Enzyme-linked immunosorbent assay (ELISA)

The HUVEC cells were collected 24 h after each corresponding treatment and centrifuged at 4°C and 10,000 *g* for 10 min, and the supernatant collected. The ELISA kit (Wuhan Boshi Biological Engineering Co., Ltd.) was used to test the concentration of E-selectin in the supernatant, according to manufacturer instructions.

## Transendothelial electrical resistance (TEER)

Cells were prepared and HUVECs were inoculated on the Transwell basement membrane of the upper chamber at a concentration of 1 x $10^4$/mL and complete medium added to the lower chamber to ensure an equal liquid level inside and outside the chamber. An equal amount of the same medium sample was added to uninoculated cells as a vacuity contrast. Changes in TEER value were monitored daily and the overall procedure was conducted under a constant temperature (23°C), taking 3 points in different directions in each chamber and repeating the measurement 3 times. TEER = (sample reading from inoculated cells - sample reading from blank contrast) x 0.33. After the TEER values became stable, it was considered that the HUVECs had formed one compact layer on the Transwell membrane. After the corresponding treatments were given, the TEER values were measured at 6, 12, and 24 h.

## Western blot

Western blotting was used to measure the expression of VE-cadherin in the HUVECs

24 h after each corresponding treatment. Cells were collected and centrifuged, 1000 *g* for 5 min, the supernatant discarded, and the cells washed in PBS 3 times. RIPA lysate was used to split the cells and bicinchoninic acid (BCA) to measure the concentration of protein after centrifugation to remove the supernatant. Fifty micrograms of protein sample/group was separated with SDS-PAGE assay (10%) and the electrophoretic bands were transferred to PVDF membranes (Millpore, USA) and blocked with 5% fat-free milk for 2 h. Rabbit anti-human VE-cadherin (Abcam, UK) was added to the PVDF membranes with mildly consistent shake overnight on a table at 4°C, then, we incubated them with 1:5000 diluted goat-anti-rabbit IgG (Sigma, USA) for 1 h at room temperature. The membrane was then washed in Tris-buffered saline and Tween 20 (TBST), enhanced chemiluminescence (ECL) used to develop and fix it, and scanned using an image analysis system (Bio-Rad, USA) and the Gel-pro analyzer (Media Cybernetics, USA) to measure the integrated optical density.

### Immunofluorescence

Immunofluorescence was used to measure the expression of VE-cadherin 24 h after each corresponding treatment. The samples were then fixed in 80% alcohol for 30 min and absolute ethyl alcohol for 30 min, then penetrated with 0.5% Triton-100 for 10 min and blocked with 10% of donkey serum for 1 h at room temperature before adding rabbit anti-human VE-cadherin (Abcam), leaving overnight at 4°C, with recovery at room temperature for 30 min, then adding two goat anti-rabbit IgG/TRITC (Beijing Zhong Shan Golden Bridge Bio-Technology Co., Ltd.), and developing for 1 h at room temperature protected from light. Subsequently the samples were dyed with 4',6-diamidino-2-phenylindole (DAPI) for 1-2 min, washed and mounted for observation and filming under a fluorescence microscope (Leica, Germany), in order to measure the OD value and expression of VE-cadherin in the reaction tube using the Image-Pro Plus 6.0 software.

### Statistical analysis

Statistical analysis was conducted using the SPSS21.0 statistics software. Data are reported as means ± standard deviation. Components were compared using one-way analysis of variance (ANOVA) and the homogeneity of variance assessed using least significant difference (LSD). $P < 0.05$ was considered to be statistically significant.

## RESULTS

### Influence of HS on LPS-induced hyaline leukocyte adhesion

The primary phenomenon in response to LPS treatment is that hyaline leukocytes attach to VE cells. Observed using Wright-Giemsa staining, hyaline leukocyte adhesion is extremely low in control and $H_2$ cells, while in LPS cells, endothelium-hyaline leukocyte conglutination increases substantially ($P < 0.05$), in a time-dependent manner and mostly within 24 h. In contrast to the LPS group, hyaline leukocyte adhesion in the LPS+$H_2$ group substantially reduced ($P < 0.05$; Figure 1A-D).

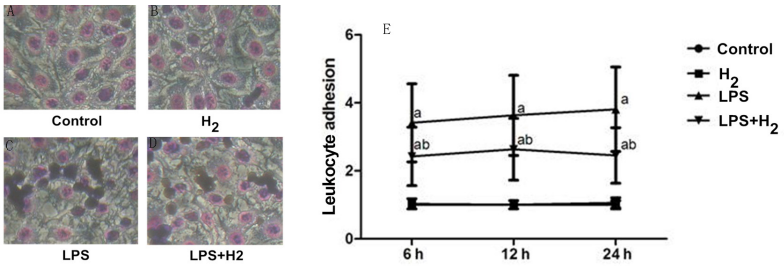


**Figure 1.** Influence of HS medium on hyaline leukocyte adhesion induced by lipopolysaccharide (LPS). **A.-D.** Wright-Giemsa staining of cells after 24 h and hyaline leukocyte attachment to endotheliocytes. **E.** Data analysis on hyaline leukocyte adhesion at 6, 12, and 24 h. Results are reported as means ± SD (N = 3 for each group at each time point. [a]P < 0.05 *vs* control group, [b]P < 0.05 *vs* LPS group.

## Influence of saturated HS medium on VCAM-1 and ICAM-1 expression in LPS-stimulated VE cells

Flow cytometry was used to measure the VCAM-1 and ICAM-1 expression 24 h after treatment (Figure 2A-H). There was little VCAM-1 expression in the control and $H_2$ groups, and no obvious difference between the two (P > 0.05); 24 h after LPS treatment, VCAM-1 expression significantly increased on the surface of VE cells (P < 0.05), and hydrogen-rich medium was able to reduce LPS-induced VCAM-1 expression (P < 0.05; Figure 2I). Compared with the control and $H_2$ cells, ICAM expression in both the LPS and LPS+$H_2$ cells increased (P < 0.05). Compared with the LPS cells, ICAM-1 expression in the LPS+$H_2$ cells reduced (P < 0.05; Figure 2J).

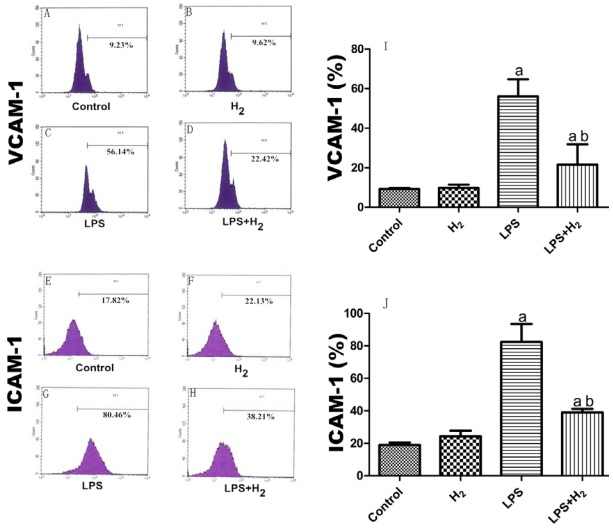


**Figure 2.** Influence of saturated HS medium on lipopolysaccharide (LPS)-induced vascular endothelial cell expression of vascular cell adhesion protein 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). Flow cytometry was used to observe the content of VCAM-1 (**A.-D.**) and ICAM-1 (**E.-H.**) and software was used to analyze the percentage of VCAM-1 (**I**) and ICAM-1 (**J**). Results are reported as means ± SD (N = 3 per group). [a]P < 0.05 *vs* control group, [b]P < 0.05 *vs* LPS group.

## Influence of saturated HS medium on LPS-stimulated endotheliocyte expression of E-selectin

Compared with the LPS, control, and $H_2$ groups, E-selectin expression in both the LPS and LPS+$H_2$ cells significantly increased ($P < 0.05$), and compared with the LPS group, E-selectin expression in the LPS+$H_2$ group significantly reduced ($P < 0.05$; Figure 3).

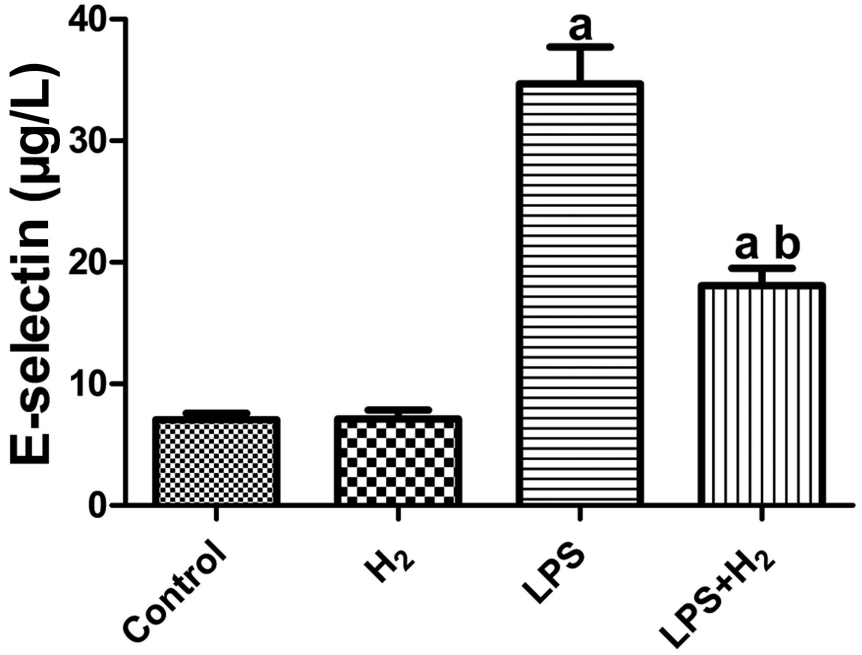


**Figure 3.** Influence of saturated HS medium on LPS-stimulated expression of vascular endothelial cell E-selectin. Enzyme-linked immunosorbent assay (ELISA) was used to measure the concentration of E-selectin and data analysis was conducted. Results are reported as means ± SD (N = 5 per group). [a]$P < 0.05$ *vs* control group, [b]$P < 0.05$ *vs* LPS group.

## Influence of HS on TEER value induced by LPS

We cultured endotheliocytes in Transwell cells, and measured TEER daily to reflect conjunction among endotheliocytes and changes in barrier functions. It was shown that the TEER value of VE cells increases with development time and became stable after 3 h, it was verified that after development for 3 days in Transwell cells, the cells had formed closed connections, which made them suitable for use in the test (Figure 4A). On the fourth day of development, the corresponding treatments were given after 6, 12, and 24 h and the TEER value was measured. It was shown that after LPS stimulation, the permeability of VE cells significantly increased ($P < 0.05$), and they were destroyed with increasing time, reaching a peak after 24 h. Compared with LPS cells, the TEER value in LPS+$H_2$ cells significantly increased ($P < 0.05$; Figure 4B).

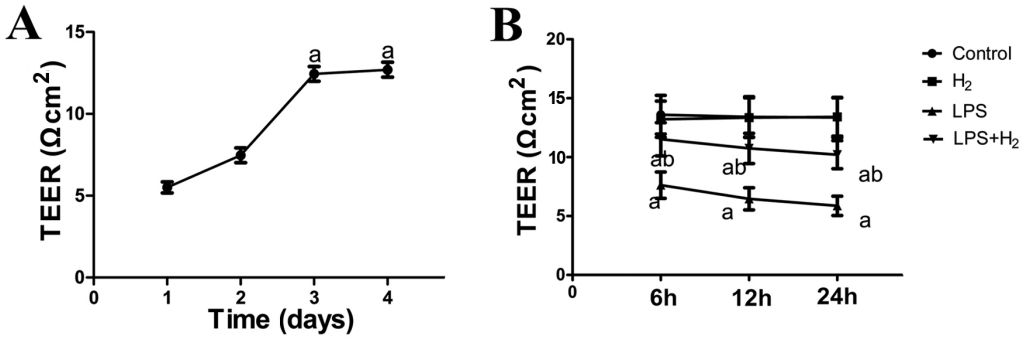


**Figure 4.** Influence of HS liquid on the TEER value of injured vascular endothelial cells induced by LPS. Transwell cells were used to develop human umbilical vein endothelial cells (HUVECs) and measure their TEER value (**A**) daily, and various interventions were conducted after the value became stable (4 days). TEER values were measured in all groups at 6, 12, and 24 h and data analysis conducted (**B**). Results are reported as means ± SD (N = 3 per group). [a]P < 0.05 *vs* control group, [b]P < 0.05 *vs* LPS group.

## Influence of saturated HS medium on the expression and distribution of VE-cadherin in response to LPS-injury of HUVEC cells

Western blot results showed that LPS could induce a reduction in the expression of VE-cadherin (P < 0.05); however, the HS liquid group showed significant restriction of the reduction of VE-cadherin (P < 0.05; Figure 5A and B). In the fluorescence results, red linear fluorescence represents the expression of VE-cadherin and blue fluorescence represents the nucleus. VE-cadherin in the control and $H_2$ groups surrounded the nucleus to form a successive one layer barrier, while VE-cadherin in the LPS group was not complete in the cell connections. Fluorescence intensity was weak; however, after HS liquid was given, VE-cadherin in the LPS group was relatively complete at the cell connections and distributed evenly with an obvious increase in fluorescence intensity, (Figure 5C-F).

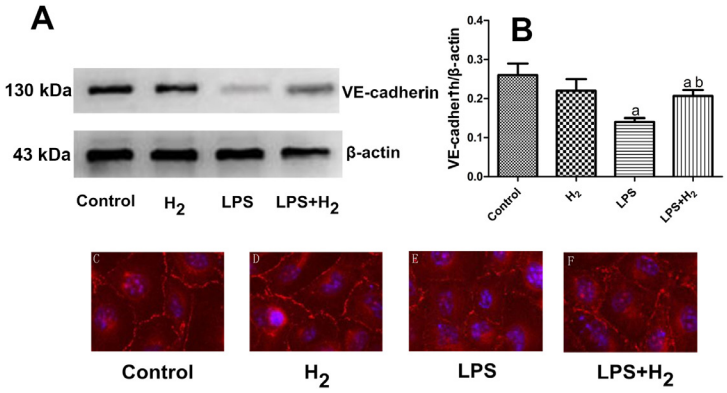


**Figure 5.** Influence of saturated HS medium on the expression and distribution of vascular endothelial (VE)-cadherin induced by lipopolysaccharide (LPS). Western blotting was used to measure VE-cadherin and β-actin (**A**), using the IOD values of VE-cadherin/β-actin to represent expression of VE-cadherin (**B**). Results are reported as means ± SD (N = 3 per group). [a]P < 0.05 *vs* control group, [b]P < 0.05 *vs* LPS group. **C.-F.** Intercellular expression of VE-cadherin shown using immunofluorescence.

## DISCUSSION

VE-cadherin is a specific cadherin of endotheliocytes and also the transmembrane compound of endotheliocyte cohesion. It is connected with an F-actin cytoskeleton through tiles in the cytoplasm and plays an important role in maintaining the compactness of one layer endotheliocyte (Dejana et al., 2008). Our previous test highlighted that HS liquid is able to reduce apoptosis of endotheliocytes induced by LPS significantly and strengthen the vigor of endotheliocytes (Han et al., 2012), while $H_2$ can provide protection for mice with sepsis and reduce production of inflammatory factors and has obvious anti-inflammatory effects (Xie et al., 2012). The test results show that LPS can induce the release of adhesion molecules and enhance hyaline leukocyte attachment to endotheliocytes, and reduce the TEER value and expression of VE-cadherin leading to an increase in permeability of the endothelium and cell injury. However, after HS liquid treatment, intercellular adhesion and permeability of blood vessel endothelium are reduced, which has protective effects on the compactness of the endotheliocytes.

The primary and most significant pathologic process for death of patients with sepsis is the attachment of hyaline leukocytes to VE cells leading to an increase of permeability in blood vessel endothelium and is the pathologic basis leading to successive changes. On one hand, this can cause edema in the intercellular space, increase the distance among cells leading to cell hypoxia; while, on the other hand, massive accumulation of hyaline leukocytes can cause direct injury to tissues and organs (Srinivasan et al., 2011). It is known that VE-cadherin can adjust the permeability of endothelium via 3 mechanisms as follows, including tyrosine phosphorylation, endocytosis, and dissociation of VE-cadherin; however, the specific mechanism is required in further research. Saturated HS liquid can increase the expression of VE-cadherin and reduce dissociation participation in the adjustment of permeability of the endothelium. VCAM-1 and ICAM-1 are the major regulatory factors in inflammatory reactions; they mediate leukocyte attachment to endotheliocytes and participate in signal transmission, which plays an important role in inflammatory reactions (Lu et al., 2012). While the intercellular adhesion molecule, E-selectin, expressed on the surface of the endothelium during inflammation mainly mediates initial rolling on the surface of the endothelium, adhesion, and transmembrane interaction. Vascular cell adhesion molecules and intercellular adhesion molecules not only play an important role in the process of leukocyte adhesion but also can be a kind of signal molecule, which can cause accumulation of calcium in the cytoplasm, activate and adjust relevant small GTP enzymes such as those involved in cell proliferation and adhesion. They can adjust the phosphorylation of VE-cadherin, which disturb the adhesion connection of cells and regulate the process of leukocytes crossing endotheliocytes that lead to an increase in permeability of the endothelium (Van Rijssel et al., 2012). Accumulated adhesion molecules can also induce VE-cadherin to separate from F-actin in the cytoskeleton, adjust cell shrinkage, and increase the permeability of endotheliocytes. $H_2$ was shown to increase expression of VE-cadherin and protect permeability of the endotheliocytes from destruction and this might relate to $H_2$ adjustment of the expression of adhesion molecules. It is known that small GTP enzymes participate in combinational joining regarding cytoskeletal actin, which is a regulator of cell adhesion, and changes the permeability of endotheliocytes (Spindler et al., 2010). It is yet to be researched whether the small GTP enzymes participate in $H_2$ adjustment of endothelium-hyaline leukocyte conglutination or influence the permeability of endotheliocytes.

$H_2$ is able to reduce the release of many inflammatory factors and injuries caused by inflammatory effects on the nucleus, and participates in the adjustment of inflammatory reactions. The test results showed that $H_2$ can restrain endothelium-hyaline leukocyte conglutination, increase expression of VE-cadherin, and participate in the adjustment of the permeability of blood vessel endothelium during inflammatory reactions through reducing LPS-stimulated release of adhesion molecules, thus providing new insight into the use of $H_2$ as an anti-inflammatory.

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## REFERENCES

Abbruscato TJ and Davis TP (1999). Protein expression of brain endothelial cell E-cadherin after hypoxia/aglycemia: influence of astrocyte contact. *Brain Res.* 842: 227-286.

Birdsey GM, Dryden NH, Amsellem V, Gebhardt F, et al. (2008). Transcription factor Erg regulates angiogenesis and endothelial apoptosis through VE-cadherin. *Blood* 111: 3498-3506.

Chen HG, Xie KL, Han HZ, Wang WN, et al. (2013). Heme oxygenase-1 mediates the anti-inflammatory effect of molecular hydrogen in LPS-stimulated RAW 264.7 macrophages. *Int. J. Surg*. 11: 1060-1066.

Curry FR and Adamson RH (2010). Vascular permeability modulation at the cell, microvessel, or whole organ level: toward closing gaps in our knowledge. *Cardiovasc. Res.* 87: 218-229.

Dejana E, Orsenigo F and Lampugnani MG (2008). The role of adherens junctions and VE-cadherin in the control of vascular permeability. *J. Cell. Sci.* 121: 2115-2122.

Fu Y, Ito M, Fujita Y, Ito M, et al. (2009). Molecular hydrogen is protective against 6-hydroxydopamine-induced nigrostriatal degeneration in a rat model of Parkinson's disease. *Neurosci. Lett.* 453: 81-85.

Gerszten RE, Garcia-Zepeda EA, Lim YC, Yoshida M, et al. (1999). MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature* 398: 718-723.

Han H, Xie K, Chen H, Liu D et al. (2012). Effects of hydrogen gas on lipopolysaccharide-induced apoptosis in human umbilical vein endothelial cells *in vitro*. *Chin. J. Anesthesiol*. 32: 973-975.

Hotchkiss RS and Karl IE (2003). The pathophysiology and treatment of Sepsis. *N. Engl. J. Med*. 348: 138-150.

Huang Y, Xie K, Li J, Xu N, et al. (2011). Beneficial effects of hydrogen gas against spinal cord ischemia-reperfusion injury in rabbits. *Brain Res.* 1378: 125-136.

Kajiyama S, Hasegawa G, Asano M, Hosoda H, et al. (2008). Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. *Nutr. Res.* 28: 137-143.

Lu Y, Zhu X, Liang GX, Cui RR, et al. (2012). Apelin-APJ induced ICAM-1, VCAM-1 and MCP-1 expression via NF-κB/JNK signal pathway in human umbilical vein endothelial cells. *Amino Acids* 43: 2125-2136.

Matsuda N and Hattori Y (2007). Vascular biology in sepsis: pathophysiological and therapeutic significance of vascular dysfunction. *J. Smooth Muscle Res*. 43: 117-137.

Ohsawa I, Ishikawa M, Takahashi K, Watanabe M, et al. (2007). Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat. Med.* 13: 688-694.

Rubenfeld GD and Herridge MS (2007). Epidemiology and outcomes of acute lung injury. *Chest* 131: 554-562.

Russell JA (2006). Management of sepsis. *N. Engl. J. Med.* 355: 1699-1713.

Spindler V, Schlegel N and Waschke J (2010). Role of GTPases in control of micro vascular permeability. *Cardiovasc. Res.* 87: 243-253.

Srinivasan R, Ozhegov E, van den Berg YW, Aronow BJ, et al. (2011). Splice variants of tissue factor promote monocyte-endothelial interactions by triggering the expression of cell adhesion molecules via integrin-mediated signaling. *J. Thromb. Haemost*. 9: 2087-2096.

Tekeichi M (1995). Morphogenetic roles of classic cadherins. *Curr. Opin. Cell. Biol*. 7: 619-627.

Van Rijssel J, Kroon J, Hoogenboezem M, van Alphen FP, et al. (2012). The Rhoguanine nucleotide exchange factor Trio controls leukocyte transendothelial migration by promoting docking structure formation. *Mol. Biol. Cell* 23: 2381-2844.

Xie K, Yu Y, Pei Y, Hou L, et al. (2010a). Protective effects of hydrogen gas on murine polymicrobial sepsis via reducing oxidative stress and HMGB1 release. *Shock* 34: 90-97.

Xie K, Yu Y, Zhang Z, Liu W, et al. (2010b). Hydrogen gas improves survival rate and organ damage in zymosan-induced generalized inflammation model. *Shock* 34: 495-501.

Xie K, Yu Y, Huang Y, Zheng L, et al. (2012). Molecular hydrogen ameliorates lipopolysaccharide-induced acute lung injury in mice through reducing inflammation and apoptosis. *Shock* 37: 548-555.