Involvement of Acid-Sensing Ion Channel 1a Contribute to The Effect of Extracellular Acidosis on Vascular Endothelialcell Damage of Henoch-Schönlein Purpura Patients

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Short Report

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Abstract

Background: Henoch-Schönlein purpura (HSP) is a common kind of systematic vasculitis in children characterized by rash, joint pain, abdominal symptoms and renal disease, the detail pathogenesis of HSP has not been elucidated. Acid-sensing ion channels (ASICs) is a proton-gated sodium selective channel that belongs to Degenerin /epithelial Sodium Channel (DEG/ENaC) superfamily, previous research found the expressions of ASIC1a and ASIC3 in the vascular endothelial cells of HSP

This study aims to investigate the molecular mechanisms of silencing of acid-sensing ion channel 1a (ASIC1a) protects the vascular endothelial cells from Henoch-Schönlein purpura (HSP) patients.

Findings: Human dermal microvascular endothelial cells (HDMVEC) were cultured in vitro, siRNA sequences were designed for the coding region of human ASIC1a gene and HDMVEC cells were transfected with recombinant lentivirus (LV) -sh-ASIC1a. The control group (NC group) without virus transfection and LV-sh-ASIC1a transfection group (si-ASIC1a group) were set up. The expression of transfected ASIC1a gene was detected by RT-PCR. After virus transfection 72 h, serum IgA1 from HSP patients and serum IgA1 of normal children were added into HDMVEC cells. ASIC1a and cytoskeleton protein (sm- α f-actin, **MLCK**) mRNA and protein expressions were detected by real-time PCR and Western blotting Methods. The binding activity of NF- κ B with DNA in HDMVEC was determined by electrophoretic mobility shift assay (EMSA). The whole-cell patch-clamp technique was used to record the current changes and electrophysiological characteristics of ASICs in HDMVEC.

Cytoskeleton protein (sm- α f-actin, **MLCK**) mRNA and protein expressions in the group of si-ASIC1a group were significantly increased compared with the NC control group ($P < 0.01$). The HSP serum and silencing of ASIC1a had no significant effect on the binding activity of NF- κ B with DNA in HDMVEC. Compared with the NC control group, the ASICs current and calcium overload of the si-ASIC1A group were reduced ($P < 0.01$)

Conclusions: Silencing ASIC1a can protect HSP vascular endothelial cell injury by inhibiting ASIC1-related calcium influx and reducing the overload of calcium, not the NF- κ B signaling pathway.

Introduction

Henoch-Schönlein purpura (HSP) is a common kind of systematic vasculitis in children characterized by rash, joint pain, abdominal symptoms and renal disease [1]. The pathogenesis of HSP is mainly abnormal IgA1-mediated immune complexes deposition in capillary walls, which results in vascular inflammation and ischemia [2]. Inflammation and ischemia are usually accompanied by tissue acidification. Acid-sensing ion channels (ASICs) are cation channels activated by extracellular acidification and play an important role in the pathological process of many diseases such as cerebral ischemia, rheumatoid arthritis and systemic sclerosis. ASIC1a is one member among 6 subunits of ASICs, which is responsible for Ca^{2+} transportation and is involved in inflammation, tumor, and ischemic injury in central nervous system and non-neuronal tissues [3]. Our previous research found the expression of ASIC1a in vascular

endothelial cells could be stimulated by serum IgA1 from HSP patients under acidic environment, and acid-inhibiting agents omeprazole can significantly improve the clinical manifestations of HSP patients[4]. These studies suggested that ASIC1a was involved in the the pathogenesis of HSP. However, its precise role in the vascular endothelial cell injury of HSP hasn't been elucidated.

In this present study our aim was to investigate the role of ASIC1a channels in IgA1-mediated immunue inflammation of the small blood vessel in HSP patients.

Materials And Methods

Isolation of IgA1

Serum of 6 HSP patients with (6 ~ 13 years old, 3 males and 3 females) and 6 healthy children of the same age were collected. IgA1 was extracted by Jacalin-agarose affinity chromatography and protein purification^[5].

Cell Culture and si-ASIC1a RNA transfection

HDMVEC purchased from ATCC was cultured in an incubator at 37°C with 5% CO₂. We used lentivirus vectors containing ASIC1a-siRNA (constructed by GenePharma, Shanghai, China), and the lentivirus-expressing blank served as a negative control(NC). After 72h of infection, cell transfection was observed under a fluorescence microscope, and the expression of the ASIC1a was determined by RT-PCR. At 5 days after infection, cells were divided into six groups: ①negative control cells(NC group), ②negative control cells subjected to normal healthy children serum IgA1 treatment for 6h(NC+serum group), ③negative control cells subjected to serum IgA1 from HSP patients for 6h(NC+HSP group), ④cells with si-ASIC1a(si-ASIC1a group),⑤cells with si-ASIC1a subjected to normal healthy children serum IgA1 treatment for 6h(si-ASIC1a+serum group),⑥cells with si-ASIC1a subjected to serum IgA1 from HSP patients for 6h(si-ASIC1a + HSP group). And then the cells were subjected to acid exposure.

Real-time PCR and RT-PCR analysis

Total RNA was extracted from the collected cells using TRIzol reagent, and then reverse transcribed into complementary DNA (cDNA) according to the manufacturer's instructions,the cDNA was used for the real-time polymerase chain reaction (PCR) process. Primer sequences are listed in **Table 1**. Real-time polymerase chain reaction (RT-PCR) was performed using SYBR Green PCR Master Mix (Roche Applied Science) in a StepOnePlus PCR System (Applied Biosystems). Ct values of the sample were calculated, and transcript levels were analyzed by the $2^{-\Delta\Delta Ct}$ method.

Table 1 Gene-Specific Primer Sequences

primer	primer sequence	length(bp)
SM- α	F- AGGGAGTGATGGTTGGAATG R- GATGATGCCGTGTTCTATCG	200
MLCK	F-GCTGCCTGACCACGAATATAAR- CATCTGACACCTCCACTTCATC	137
β -tubulin	F-GCCAGATCTTTAGACCAGACAAR- CCACATCCAGGACAGAATCAA	114

Western blotting

The protein of collected cells was extracted with 500 μ L RIPA lysis solution (10 μ L PMSF per 1000 μ L RIPA), the concentration of the extracted protein was detected by the BCA assay kit. Proteins were separated by 10% SDS polyacrylamide gels transferred to PVDF membranes. After blocking, blots were probed with antibodies against SM- α , **F-actin and β -tubulin** (1:1000 rabbit anti-human polyclonal) followed by HRP conjugated secondary antibodies (1:5000 goat anti-rabbit IgG). The signals were visualized by Thermo ECL. The results were analyzed with Quantity One software.

Electrophoretic mobility shift assay(EMSA)

DNA binding activity of NF- κ B was detected by EMSA in HDMVEC, the nuclear protein extracts were prepared from HDMVEC according to kit instructions. Nuclear proteins were incubated with electrophoretic mobility shift assay (EMSA) buffer on ice before the probe was added. The DNA-protein complexes were electrophoresed on the 0.5 $\times$ TBE non-denaturing polyacrylamide gels, and then electroblotted to a NC membrane at 380 mA for 30 min. The transferred DNA was crosslinked to the membrane by UV light, detected according to the manufacturer's instructions.

Whole-cell patch clamp in HDMVEC

The whole-cell patch-clamp recordings were performed to observe the ASIC1a current as previously described[6,7]. The pipette resistance was 8 to 10 M Ω . The current signals were acquired at a sampling rate of 10 kHz using an EPC-10 amplifier (HEKA, Lambrecht, Germany) and Pulse/PulseFit software (HEKA, Southboro, Germany). All the recordings were made at room temperature (20–22°C).

Statistical analysis

All data were analyzed using SPSS 16.0, the results were expressed as $X \pm S$, T - test was used for comparison between groups, $P < 0.05$ was considered significant.

Results

Our previous study has proved that ASIC1a is expressed in the vascular endothelium cells of HSP patients. To further investigate whether ASIC1a have a function in inflammatory vascular damage of HSP, the currents were observed by whole-cell patch-clamp recording. The result showed extracellular pH6.0

elicited inward ASIC-like currents of vascular endothelial cell incubated with serum IgA1 from HSP patients and silencing ASIC1a inhibited this ASIC-like currents (**Fig. 1**). This finding indicates that ASIC1a was functionally expressed in vascular endothelium cells.

Previous studies have shown that acidosis promotes vascular endothelial cell damage induced by serum IgA1 from HSP patients. In this present study we found that serum IgA1 from HSP patients could inhibit expression of cytoskeletal protein SM- α , F-actin and MLCK in HDMVEC under extracellular acidosis environment(**Fig. 2**). However, the role of ASIC1a in vascular injury of HSP patients has not been determined. Calcium is considered as a crucial mediator of vascular endothelial injury. As shown in **Fig. 1b**, extracellular acidosis significantly increased Ca^{2+} flow in vascular endothelial cell incubated with serum IgA1 from HSP patients. Silencing of ASIC1a could inhibit the Ca^{2+} flow (**Fig. 1**) compared with the NC control group ($P < 0.05$). NF- κB pathway is an important pathway in inflammatory response, which has been showed to be closely related to Ca^{2+} flow[8]. EMSA result in this present study showed serum IgA1 from HSP and inhibiting ASIC1a had no significant effect on the binding activity of NF- κB with DNA in vascular endothelial cells(**Fig. 3**). These indicated that ASIC-calcium signal contribute to the vascular endothelial cell injury of HSP, but not via NF- κB pathway.

Discussion

The present study demonstrated that ASIC1a is functionally expressed in vascular endothelial cells(VEC) and contribute to VEC injury of HSP in low pH condition.

HSP is a form of vasculitis with IgA1-dominant immune deposited on small vessels. The vascular endothelial cell inflammatory responses induced by IgA1 in HSP is important for its pathophysiological conditions[9]. Endothelial cell inflammatory responses promote the adherence of blood cells to vessel wall, which may lead to vaso-occlusion and tissue ischemia. And then substantial lactic acid and carbon dioxide accumulated around small vessel and lead to acidification of local tissues. A myriad of studies show that localized interstitial acidosis caused by dysregulated cell metabolism and/or defective blood perfusion to remove acidic metabolic byproducts is a biochemical hallmark in inflammatory tissues, ischemic organs, and solid tumors[10,11].

It has been shown that tissue acidity aggravates organ injury and exacerbates the progression of acidosis-associated diseases such as inflammation, ischemic diseases[12]. Our previous study has also proved that extracellular acidosis stimulated IL-8, NO and TM release of VEC incubated with IgA1 from HSP patients. In this study, our results showed that cytoskeletal protein SM- α , F-actin, MLCK expression in VEC was significantly decrease in pH 6.0 medium when incubated with IgA1 from HSP. These results indicated that tissue acidity may be an important risk factor that increases the incidence of vascular injury in HSP patients. However, the molecular mechanism of vascular endothelial cells respond to acidosis are largely unknown.

ASICs are cation channels activated by extracellular acidification, and studies have shown that they play an important role in the pathological process of cerebral ischemia and tumor accompanied by tissue acidification. Inflammation is usually associated with tissue acidification. Our previous study found that ASIC1a was expressed at the mRNA and protein levels in vascular endothelial cells of HSP patients. In this study, we examined the function of ASIC1a in vascular endothelial cells of HSP. We found that low extracellular pH elicits inward ASIC currents in vascular endothelial cells, which were reversibly inhibited by silencing ASIC1a. Moreover, silencing ASIC1a could inhibit the mRNA and protein expression reduction of cytoskeletal protein F-actin, MLCK in these cells. These data suggested that vascular endothelial cells expressed functional ASIC1a. ASIC1a can permeate Ca^{2+} and intracellular calcium overload is associated with injury of HSP vascular endothelial cells. Now we investigated whether increasing intracellular Ca^{2+} mediated by ASIC1a is associated with IgA1-induced vascular damage in HSP patients. Our results demonstrated that IgA1 from HSP patients prompted Ca^{2+} flow into cells in low pH condition, which were reversibly inhibited by silencing ASIC1a.

NF- κ B, a member of the Rel family, is a multidirectional regulatory nuclear transcription factor that widely exists in various cells. It participates in the regulation of immune response and inflammatory response, and can induce the gene expression of various cytokines, adhesion factors and chemokines[13]. Both intracellular calcium overload and extracellular acidification can activate NF- κ B, leading to the development of endothelial dysfunction[8]. However NF- κ B and DNA binding activity in vascular endothelial cells in HSP patients was not observed after acid-induced calcium signal activation. And inhibiting ASIC1a expression had no significant effect on NF- κ B and DNA binding activity.

In summary, this study demonstrated that ASICs are functionally expressed in vascular endothelial cells of HSP patients. ASIC1a-mediated elevation of $[\text{Ca}^{2+}]$ contribute to the effect of acidic microenvironment on vascular injury in HSP patients. Thus, ASIC1a may provide a molecular basis for explaining HSP pathogenesis, and targeting ASIC1a may be a novel therapeutic target for HSP treatment. However, ASIC1a activation induces vascular injury not through NF- κ B transcriptional activity, which will be further studied in our future research.

Abbreviations

ASIC1a: Acid-sensing ion channel 1a; ASICs: Acid-sensing ion channels ;

HSP: Henoch-Schönlein purpura; HDMVEC: Human dermal microvascular endothelial cells (HDMVEC); LV: lentivirus; EMSA: Electrophoretic mobility shift assay; VEC: vascular endothelial cells

Declarations

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Availability of data and materials

All the available data, pertinent to this topic has been presented in this manuscript.

Authors' contributions

Jin-kun Wang :First author,he designed this study,collected and analyzed the data ,literature search and manuscript writing;

Yan Bo:revised the test and edited the manuscript,

Qi-lian Zhou:conducted experiments and edited the manuscript;

Li-ping Yuan:critical revision and final Approval;

All authors approved the final manuscript.

Competing interests

The authors declared that they have no competing interests.

Consent for publication

All the authors have reviewed the final manuscript and given their consent for publication.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University.

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Figures

Figure 1

The whole-cell patch-clamp results. a: ASICs current. b: Intracellular Ca2+ detection.

Figure 2

Detection of cytoskeleton protein sm-α f-act in and MLCK mRNA and protein expressions. c: the mRNA expression of cytoskeletal protein sm-α and MLCK d: Protein expression of sm-α, Tubul in β and f-act in. e: Protein expression gray of sm-α and f-actin

Figure 3

The binding activity of NF- κ B with DNA in HDMVEC