Clinical Trial/Experimental Study

Medicine®

OPEN

RhACE2 – playing an important role in inhibiting apoptosis induced by Ang II in HUVECs

Hongli Zhang, MD, Xiaocui Zhang, MD, Zhaoyu Hou, MD, Fang Deng, MD*

Abstract

Background: Henoch-Schonlein purpura (HSP) is a common hemorrhagic disease, which manifests the inflammation in the body's most microvasculars. Angiotensin II (Ang II) can induce the damage and apoptosis of vascular endothelial cells while angiotensin converting enzyme 2 (ACE2) can antagonist the action of Ang II. However, the effect of ACE2 on Ang II-induced endothelial damage remains unknown.

Objective: To evaluate the effect of recombinant human angiotensin converting enzyme 2 (rhACE2) on the Ang II-induced damage of human umbilical vein endothelial cells (HUVECs) and the release of inflammatory mediator in vitro.

Methods: Cultured HUVECs were randomly divided into 6 groups: the control group, rhACE2 group, Ang II group, and Ang II+ rhACE2 groups (3 subgroups). The cell vitality, cell cycle, apoptosis rate of the HUVECs and the levels of reactive oxygen species (ROS), interleukin 8 (IL-8), tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1) and lactate dehydrogenase (LDH) were measured, respectively.

Results: Compared with the control group, the cell viability and the rate of S phase cells in Ang II group significantly decreased ($P < .05$) while the apoptosis percentage and the levels of ROS, IL-8, TNF-α, TGF-β1, and LDH in Ang II group significantly increased ($P < .05$). There were no significant differences between the control group and rhACE2 group. Compared with the Ang II group, the cell viability and the rate of S phase cells in Ang II+rhACE2 groups were higher ($P < .05$) and the apoptosis percentage, the level of ROS, IL-8, TNF-α, TGF-β1, LDH in Ang II+rhACE2 groups were lower ($P < .05$).

Conclusions: Ang II can induce the apoptosis of HUVECs and the release of inflammatory mediator, while rhACE2 can inhibit the detrimental effects of Ang II. The results of this study suggest that rhACE2 has a protective effect on HSP, which is probably a new way for the prevention and treatment of HSP.

Abbreviations: ACE2 = angiotensin converting enzyme 2, ACE-Ang II-AT1R = angiotensin converting enzyme-angiotensin II-angiotensin II type 1 receptor, Ang II = angiotensin II, ANOVA = analysis of variance, AT1 = angiotensin II type 1, DCFH-DA = 2,7-dichlorodihydrofluorescein diacetate, DMEM = Dulbecco's modified eagle medium, ELISA = enzyme-linked immunosorbent assay, ET-1 = endothelin 1, FBS = fetal serum bovine, FITC = fluorescein isothiocyanate, HSP = Henoch-Schonlein purpura, HUVECs = human umbilical vein endothelial cells, IL-8 = interleukin 8, LDH = lactate dehydrogenase, MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyletrazolium bromide, NADPH = nicotinamide adenine dinucleotide phosphate, NF-κB = nuclear factor kappa-B, PBS = phosphate buffer saline, PI/RNase = propidium iodide/ribonuclease, RAS = renin-angiotensin system, rhACE2 = recombinant human angiotensin converting enzyme 2, ROS = reactive oxygen species, TGF-β1 = transforming growth factor-β1, TNF-α = tumor necrosis factor-α.

Keywords: Ang II, apoptosis, HUVECs, inflammatory mediator, rhACE2

Editor: Undurti N. Das.

This study was funded by Anhui Public Welfare Technology Application Research Linkage Project in 2017: Clinical and related basic research of angiotensin converting enzyme 2 in children with Henoch-Schonlein purpura nephropathy (No. 1704f0804027), as well as The Outstanding Talents Cultivation Project of the Department of Education of Anhui Provincial in 2017 (No. gxbjZD07).

The authors declare no competing financial interests. All the images, drawings, and photographs were created by the author of the paper, Hongli Zhang.

Department of Pediatrics, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China.

** Correspondence: Fang Deng, Department of Pediatrics, The First Affiliated Hospital of Anhui Medical University, 69 Jixi Road, Hefei City, Anhui Province 230032, China (e-mail: dengfang1997@126.com).*

Medicine (2019) 98:22(e15799)

Received: 27 December 2018 / Received in final form: 12 April 2019 / Accepted: 2 May 2019

http://dx.doi.org/10.1097/MD.0000000000015799

1. Introduction

Henoch-Schonlein purpura (HSP) is a common hemorrhagic disease, which manifests the inflammation in the body's most microvasculars.[1] The vascular endothelial cells have important metabolic and endocrine functions as well as contribute to the deterioration of HSP when the vascular endothelial cells are injured.[2] The renin-angiotensin system (RAS) is undisputedly one of the most important endocrine (tissue-to-tissue), paracrine (cell-to-cell) and intracrine (intracellular/nuclear) vasoactive systems in the physiological regulation of cardiovascular, blood pressure and kidney function.[3] When the balance of RAS is broken, it may result in the development of cardiovascular diseases, hypertensive and renal diseases. In recent studies, angiotensin converting enzyme 2 (ACE2) is found to be a new member of RAS, which is the key enzyme of the angiotensin converting enzyme-angiotensin (1–7)-mas receptor axis, playing a protective role in diseases such as blood pressure decompression, cardiovascular disease, and kidney disease.[4] As we all know that angiotensin II (Ang II) can induce the damage and apoptosis of vascular endothelial cells while ACE2 can antagonist

the action of Ang II.[5] However, the effect of ACE2 on Ang II-induced endothelial damage remains unknown. In this study, the human umbilical vein endothelial cells (HUVECs) were cultured in vitro and used to explore whether recombinant human angiotensin converting enzyme 2 (rhACE2) could inhibit the damage and inflammatory mediator of HUVECs induced by Ang II, which may provide new ways to prevent or treat HSP.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Medical Research Ethics Board of the First Affiliated Hospital of Anhui Medical University and it strictly followed the relevant regulations of Biological Research Ethics in China. We are willing to receive review and supervision.

2.2. Cells, reagents, and instruments

HUVECs (Jennio, China), Dulbecco modified eagle medium (DMEM) with low sugar (Hyclone, USA), fetal serum bovine (FBS, BI, India), penicillin-streptomycin (Bi YT, China), 0.25% pancreatin (Bi YT, China), rhACE2 (R&D Systems, USA), Ang II (Torics, UK), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyletrazolium bromide (MTT) cell proliferation and cytotoxicity assay kit (Sigma, USA), propidium iodide/ribonuclease (PI/RNase) cycle detection kit (BD, USA), fluorescein isothiocyanate (FITC) annexin V apoptosis detection kit 1 (BD, USA), reactive oxygen species (ROS) detection kit (Bi YT, China), interleukin (IL)-8 enzyme-linked immunosorbent assay (ELISA) kit (R&D, USA), tumor necrosis factor α (TNF-α) ELISA kit (R&D, USA), transforming growth factor-β1 (TGF-β1) ELISA kit (Abcam, USA), lactate dehydrogenase (LDH) ELISA kit (Jiancheng, China). HF90/HF240 cell incubator (Heal Force, China), Multiskan GO 1510 spectrophotometer (Thermo Fisher, USA), BD FACS Aria III flow cytometer (BD, USA).

2.3. Cell culture and drug treatments

HUVECs were cultured in DMEM with low sugar, 10% fetal serum bovine, 1% penicillin-streptomycin at 37°C under a CO_2 atmosphere (5%) to logarithmic phase, and then the cells were cultured in 96-well plates for 24 hours to a density of 5×10^5 cells per well for the examination of cell viability, while the cells were cultured in 12-well plates for 24 hours to a density of 5×10^5 cells per well for the examination of cell cycle, apoptosis rate, ROS and the levels of LDH, IL-8, TNF-α and TGF-β1. There were 18 wells of cells cultured for each item of examination. Cultured HUVECs were then randomly divided into 6 groups (3 wells per group): the control group, rhACE2 group, Ang II group and Ang II+ rhACE2 groups (3 subgroups). Ang II or/and rhACE2 at different concentrations were added to the plates for 24 hours. In the control group, HUVECs were treated with 100 μl culture medium. In the rhACE2 group, HUVECs were treated with 100 μl 0.1 ng/μl rhACE2. In Ang II group, HUVECs were treated with 100 μl 1.106 ng/μl (10^{-6} mol/L) Ang II. In Ang II+ rhACE2 groups, HUVECs were treated with 50 μl 2 × 1.106 ng/μl Ang II as well as 50 μl 0.002, 0.02, or 0.2 ng/μl rhACE2. Three parallel tests had been done in each group.

2.4. Examination of cell viability

The cultured HUVECs were incubated with 5 mg/ml MTT for 4 hours first, then the medium was removed and 150 μl dimethylsulfoxide was added to dissolve formazan crystals. The cell viability was determined by measuring the absorbance at 490 nm using an ELISA microplate reader.

2.5. Examination of cell cycle of HUVECs

HUVECs were collected by trypsinization, washed twice with phosphate buffer saline (PBS) and fixed by 70% ethanol at −20°C for at least 2 hours. Then the cells were spun down (1000 rpm, 5 minutes) and rinsed twice with PBS. Finally, the cells were re-suspended in PBS containing 50 μg/ml of propidium iodide in the presence of 100 μg/ml RNase A and incubated for 15 minutes at room temperature without light. The distribution of HUVECs in different phases were determined by BD FACS flow cytometer.

2.6. Examination of apoptosis rate of HUVECs

HUVECs were collected by trypsinization, washed twice with PBS, re-suspended in 100 μl binding buffer (1×) with 5 μl propidium iodide in the presence of 5 μl FITC Annexin V and incubated for 15 minutes at room temperature without light. The apoptosis of HUVECs was determined by BD FACS flow cytometer.

2.7. Examination of ROS of HUVECs

HUVECs were collected by trypsinization, washed twice with PBS, re-suspended in 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) 10 μm and incubated for 20 minutes, then the HUVECs washed by DMEM for 3 times. The ROS in the HUVECs was determined by BD FACS flow cytometer.

2.8. Examination of LDH, IL-8, TNF-α, TGF-βof HUVECs

The cell culture supernatant fluid was collected and the levels of IL-8, TNF-α and TGF-β1 were determined by ELISA kits and Multiskan GO 1510 spectrophotometer according to the manufacturer's instructions.

2.9. Statistical analysis

Data were expressed as (mean ± standard deviation) and data analysis was conducted by SPSS 25.0. One-way analysis of variance (ANOVA) was used to compare the data of control group, rhACE2 (0.1 ng/μl) group and Ang II (1.106 ng/μl) group as well as the data of Ang II (1.106 ng/μl) group, Ang II (1.106 ng/μl) + rhACE2 (0.001 ng/μl) group, Ang II (1.106 ng/μl) + rhACE2 (0.01 ng/μl) group and Ang II (1.106 ng/μl) + rhACE2 (0.1 ng/μl) group. Simultaneously, Turkey test was used to compare every 2 groups. The test level was set with $\alpha = 0.05$ (2-sided) and $P < .05$ was considered statistically significant.

3. Results

3.1. The effect of rhACE2 on the viability of HUVECs

The results of the viability of HUVECs in different groups showed that Ang II decreased cell viability, while rhACE2 inhibited the decrease of cell viability induced by Ang II. Different concentrations of rhACE2 reduced the effect of Ang II on HUVECs, increased the viability of HUVECs, which was demonstrated in a dose-dependent manner. The results of the cell viability of HUVECs in different groups were shown in Table 1.

Table 1

The results of the cell viability of HUVECs in different groups (n = 3, $\bar{x} \pm s$).

Group	OD	F	P	q	P′
Control group	0.91 ± 0.05	19.629	.002	–	–
rhACE2 (0.1 ng/μl) group	0.98 ± 0.08			2.049	.377
Ang II (1.106 ng/μl) group	0.69 ± 0.04▲			6.441	.009
Ang II (1.106 ng/μl) group	0.69 ± 0.04	13.607	.002	–	–
Ang II (1.106 ng/μl) + rhACE2 (0.001 ng/μl) group	0.73 ± 0.01			1.469	.733
Ang II (1.106 ng/μl) + rhACE2 (0.01 ng/μl) group	0.79 ± 0.06			3.672	.118
Ang II (1.106 ng/μl) + rhACE2 (0.1 ng/μl) group	0.92 ± 0.06•			8.445	.002

There was no significant difference between control group and rhACE2 (0.1 ng/μl) group in cell viability. Compared with the control group, the cell viability in Ang II (1.106 ng/μl) group significantly decreased (▲, $P < .05$). Compared with Ang II (1.106 ng/μl) group, the cell viability in Ang II (1.106 ng/μl) + rhACE2 (0.1 ng/μl) group significantly increased (•, $P < .05$). There was an increase trend in the cell viability in the Ang II+rhACE2 groups with different concentrations of rhACE2.

3.2. The effect of rhACE2 on the cell cycle distribution of HUVECs

The results of the cell cycle distributions of HUVECs in different groups showed that the Ang II inhibited the cell cycle from G1 phase to S phase, increasing the number of G0/G1 phase cells and decreasing the number of S phase cells. Different concentrations of rhACE2 reduced the effect of Ang II on HUVECs, decreasing the number of G0/G1 phase cells and increasing the number of S phase cells, which promoted the cell cycle progression in a dose-dependent manner. The results of the cell cycle distributions of HUVECs in different groups were shown in Figure 1.

3.3. The effect of rhACE2 on the cell apoptosis rate of HUVECs

The results of the cell apoptosis rates of HUVECs in different groups showed that Ang II increased the apoptosis rate, while rhACE2 inhibited the increase of cell apoptosis rate induced by Ang II, which was demonstrated in a dose-dependent manner. The results of the cell apoptosis rates of HUVECs in different groups were shown in Table 2 and Figure 2.

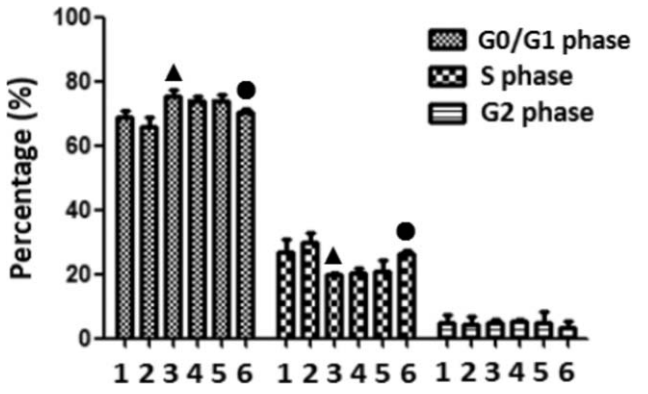

Figure 1. The results of the cell cycle distributions of HUVECs in different groups (n = 3, $\bar{x} \pm s$). 1, 2, 3, 4, 5, and 6 represent the Control group, rhACE2 (0.1 ng/μl) group, Ang II (1.106 ng/μl) group, Ang II (1.106 ng/μl) + rhACE2 (0.001 ng/μl) group, Ang II (1.106 ng/μl) + rhACE2 (0.01 ng/μl) group and Ang II (1.106 ng/μl) + rhACE2 (0.1 ng/μl) group, respectively. There was no significant difference between control group and rhACE2 (0.1 ng/μl) group in the cell cycle distribution. Compared with the control group, the number of G0/G1 phase cells in the Ang II (1.106 ng/μl) group significantly increased (▲, $P < .05$) and the number of S phase cells in the Ang II (1.106 ng/μl) group significantly decreased (▲, $P < .05$). Compared with the Ang II (1.106 ng/μl) group, the number of G0/G1 phase cells in the Ang II (1.106 ng/μl) + rhACE2 (0.1 ng/μl) group significantly decreased (•, $P < .05$) and the number of S phase cells in the Ang II (1.106 ng/μl) + rhACE2 (0.1 ng/μl) group significantly increased (•, $P < .05$). There was an increase trend in the number of S phase cells in the Ang II+rhACE2 groups with different concentrations of rhACE2.

3.4. The effect of rhACE2 on the level of ROS in HUVECs

The results of the level of ROS in HUVECs in different groups showed that Ang II increased the level of ROS, while rhACE2 inhibited the increase of the level of ROS induced by Ang II, which was demonstrated in a dose-dependent manner. The results of the levels of ROS in HUVECs in different groups were shown in Figure 3.

3.5. The effect of rhACE2 on the levels of IL-8, TNF-α, TGF-β1, and LDH in HUVECs

The results of the levels of IL-8, TNF-α, TGF-β1, and LDH in HUVECs in different groups showed that Ang II increased the levels of IL-8, TNF-α, TGF-β1, and LDH, while rhACE2 inhibited the increase of the levels of IL-8, TNF-α, TGF-β1, and LDH induced by Ang II, which was demonstrated in a dose-dependent manner. The results of the level of IL-8, TNF-α, TGF-β1, and LDH in HUVECs in different groups were shown in Table 3.

4. Discussion

HSP is a common microvascular allergic hemorrhagic disease, which can invade various organs, especially the small blood vessels of the skin and kidney, mainly manifested as skin purpura, arthralgia, gastrointestinal bleeding, and hematuresis.[6] It was reported that the vascular endothelial cell injury and vascular tissue damage were the bases of the pathophysiology in HSP children.[6]

As is known, RAS is one of the most important factors in regulating blood pressure, blood flow, and internal environment homeostasis. Angiotensin converting enzyme-angiotensin II-angiotensin II type 1 receptor (ACE-Ang II-AT1R), which is involved in vascular damage and tissue reconstruction, has been considered as the only metabolic pathway for RAS to work for a long time.[7] However, experimental and clinical studies demonstrated that the ACE2-Ang (1–7)-Mas axis was relatively antagonistic to the function of ACE-Ang II-AT1R axis, which made us recognize that the regulatory mechanism of RAS was dynamically balanced.[8] Ang II is the key enzyme of ACE-Ang II-AT1R axis, by acting on angiotensin II type 1 (AT1) receptor, Ang II cannot only lead to blood pressure elevating,

Table 2

The results of the cell apoptosis rates of HUVECs in different groups (n=3, $\bar{x} \pm s$).

Group	Cell apoptosis rate (%)	F	*P*	*q*	*P'*
Control group	2.32±0.31	1843.894	<.001	–	–
rhACE2 (0.1 ng/μl) group	1.88±0.03			3.669	.091
Ang II (1.106 ng/μl) group	11.01±0.18▲			72.473	<.001
Ang II (1.106 ng/μl) group	11.01±0.18	193.943	<.001	–	–
Ang II (1.106 ng/μl) + rhACE2 (0.001 ng/μl) group	8.64±0.37•			14.972	<.001
Ang II (1.106 ng/μl) + rhACE2 (0.01 ng/μl) group	7.24±0.33•			23.816	<.001
Ang II (1.106 ng/μl) + rhACE2 (0.1 ng/μl) group	5.84±0.15•			32.660	<.001

There was no significant difference between control group and rhACE2 (0.1 ng/μl) group in the cell apoptosis rates. Compared with the control group, the cell apoptosis rate in the Ang II (1.106 ng/μl) group significantly increased (▲, $P<.05$). Compared with the Ang II (1.106 ng/μl) group, the cell apoptosis rates in all the three Ang II (1.106 ng/μl) + rhACE2 groups significantly decreased (•, $P<.05$). There was a decrease trend in the cell apoptosis rate with different concentrations of rhACE2.

cardiovascular remolding and kidney damage, but also induce the proliferation of vascular smooth muscle cells and the damage and apoptosis of vascular endothelial cells.[9] Also, experimental and clinical studies have demonstrated a role for the ACE2-Ang (1–7)-Mas axis in the evolution of hypertension, the regulation of renal function, the progression of renal disease (including diabetic nephropathy) and the progression of cardiovascular disease.[10,11] Recently, ACE2, a homolog of ACE, which can hydrolysis Ang II into Ang (1–7), has been discovered as an important modulator of RAS by reducing Ang II levels and increasing Ang (1–7) levels, indicating its potential application in the treatment of vasculitis.[12]

In normal tissues, cell proliferation and cell apoptosis are in a dynamic equilibrium throughout the whole life, inhabitation of cell proliferation or excessive cell apoptosis will break the equilibrium.[13] In our study, Ang II inhibited the viability, proliferation and cycle progression of endothelial cells and induced cell apoptosis, while rhACE2 could antagonist the

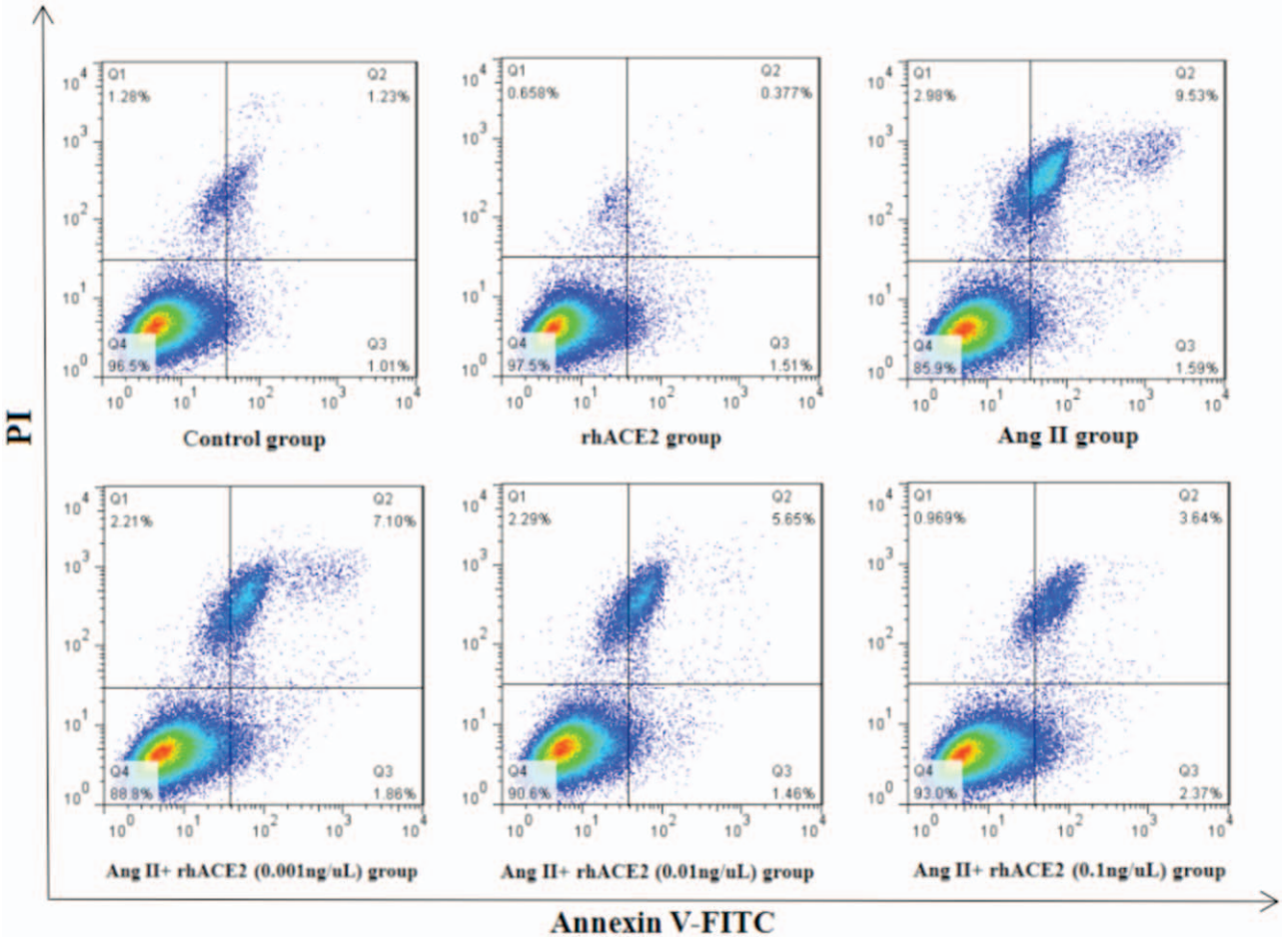

Figure 2. The results of the cell apoptosis rates of HUVECs in different groups. The cell apoptosis rate in the Ang II (1.106 ng/μl) group was higher compared with the control group and the cell apoptosis rates in all the three Ang II (1.106 ng/μl) + rhACE2 groups were lower than those in the Ang II (1.106 ng/μl) group with a decreasing trend in different concentrations of rhACE2.

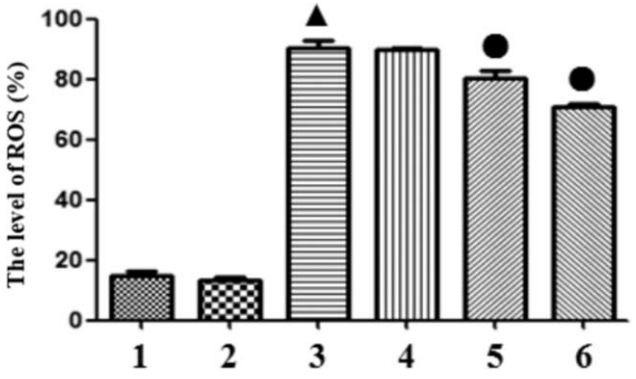

Figure 3. The results of the level of ROS in HUVECs in different groups (n = 3, $\bar{x} \pm s$). 1, 2, 3, 4, 5, and 6 represent the Control group, rhACE2 (0.1 ng/μl) group, Ang II (1.106 ng/μl) group, Ang II (1.106 ng/μl) + rhACE2 (0.001 ng/μl) group, Ang II (1.106 ng/μl) + rhACE2 (0.01 ng/μl) group and Ang II (1.106 ng/μl) + rhACE2 (0.1 ng/μl) group, respectively. There was no significant difference between control group and rhACE2 (0.1 ng/μl) group in the level of ROS. Compared with the control group, the level of ROS in HUVECs in the Ang II (1.106 ng/μl) group significantly increased (▲, $P < .05$). Compared with the Ang II (1.106 ng/μl) group, the level of ROS in both the Ang II (1.106 ng/μl) + rhACE2 (0.01 ng/μl) group and the Ang II (1.106 ng/μl) + rhACE2 (0.1 ng/μl) group significantly decreased (•, $P < .05$). There was a decrease trend in the level of ROS with different concentrations of rhACE2.

damaging effects of Ang II (Tables 1 and 2, Fig. 3). Kidney disease or nephrectomy can lead to decreased expression of ACE2, further promoting the damage to kidney induced by Ang II.[14] It was reported that Ang (1–7) could inhibit Ang II-induced endothelial cell apoptosis and the release of LDH and Endothelin 1 (ET-1), protecting the endothelial cell from damage.[15] It is considered that the protective effect of rhACE2 may be related to that rhACE2 could hydrolysis Ang II into Ang (1–7), reducing the level of Ang II, with the generated Ang (1–7) in turn inhibiting the Ang II-induced endothelial damage.[5]

Ang II, a potential inflammatory pre-reaction substance, can mediate the release of pre-inflammatory substances by activating the transcription factors nuclear factor kappa-B (NF-κB) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, producing ROS.[16] The increasing ROS in cells, in turn, initiates the transcriptional activity of transcription factor NF-κB, further causing high expression of LDH, IL-8, TNF-α, and TGF-β1.[16] ROS is an oxygen-centered group or non-group derivatives and NADPH oxidase is the key enzyme in the produce of ROS.[17] Besides, Ang II induces an increase of ROS by activating the NADPH oxidase complex.[17] In the study, we found that Ang II increased the level of ROS, while rhACE2 inhibited the increase of the level of ROS induced by Ang II (Fig. 3).

Erythrocytes are rich in LDH, when the vascular endothelial cells are damaged or destroyed, or the permeability of cell membrane increases, LDH will be released into the serum, leading to the level of LDH in the serum increasing significantly.[18] So, the examination of LDH level can reflect the degree of cell damage. IL-8, TNF-α, and TGF-β1 are important cytokines in the body, playing an important role in producing damage to vascular endothelial cells. It was reported that IL-8 could promote adhesion and migration of neutrophils and vascular endothelial cells, leading to the permeability of cell membrane increasing.[19] TNF-α is a pro-inflammatory factor, which acts on vascular endothelial cell directly, leading to damage to the vascular endothelial cell, increasing permeability to endothelial cell and small vasculitis.[20] TGF-β1 is synthesized and secreted by endothelial cells, it cannot only inhibit the growth, proliferation, and differentiation of normal cells, but also play an important role in promoting cell angiogenesis and apoptosis.[21] The results of this study showed that Ang II could induce the increase of ROS in endothelial cells and promote the release of IL-8, TNF-α, TGF-β1, and LDH; while rhACE2 could inhibit the endothelial cell damage induced by Ang II (Table 3). The mechanism of the protective effect of rhACE2 on Ang II-induced endothelial cell damage may be that rhACE2 inhibits the increase of intracellular ROS induced by Ang II, weakens the activation of NF-κB factor and reduces the release of inflammatory mediators such as LDH, IL-8, TNF-α, and TGF-β1, which has a protective effect on endothelial cell damage.[22]

HSP is a systemic disease involving multiple factors with endothelial cell damage and inflammation response playing an important role in its occurrence and development. The RAS regulating mechanism is unbalanced in children with HSP.[23] The rising of Ang II reduces the activity of vascular endothelial cell and increases the activity of NADPH oxidase, producing a large amount of ROS which aggravates the oxidative stress of vascular endothelial cell. Then, the endothelial cells release inflammatory mediators, inducing endothelial cell damage and eventually leading to cell apoptosis.[24] The permeability of vascular in children with HSP increases, involving small vasculitis in various organs of the body, manifesting as skin purpura, joint pain, gastrointestinal bleeding, and hematuria. Since rhACE2 has protective effects on Ang II-induced endothelial damage, we speculate that the use of rhACE2 in the treatment of children with HSP perhaps can reduce intracellular ROS production and release of inflammatory mediators, thereby protecting endothelial cells and relieving the development of vasculitis, which will be a

Table 3

The results of the levels of IL-8, TNF-α, TGF-β1, and LDH in HUVECs in different groups (n = 3, $\bar{x} \pm s$).

Group	IL-8 (pg/ml)	TNF-α (pg/ml)	TGF-β1 (pg/ml)	LDH (U/L)
Control group	46.00 ± 3.60	49.45 ± 0.66	27.38 ± 2.43	227.91 ± 7.99
rhACE2 (0.1 ng/μl) group	46.13 ± 1.97	48.44 ± 0.44	30.18 ± 1.21	214.07 ± 7.99
Ang II (1.106 ng/μl) group	184.13 ± 2.28▲	153.50 ± 0.25▲	97.41 ± 1.21▲	490.89 ± 15.98▲
Ang II (1.106 ng/μl) + rhACE2 (0.001 ng/μl) group	183.47 ± 3.00	152.63 ± 1.33	93.21 ± 6.42	477.04 ± 15.98
Ang II (1.106 ng/μl) + rhACE2 (0.01 ng/μl) group	142.80 ± 2.12•	145.68 ± 0.25•	88.31 ± 5.29	444.75 ± 21.21•
Ang II (1.106 ng/μl) + rhACE2 (0.1 ng/μl) group	98.93 ± 0.83•	102.34 ± 0.44•	46.29 ± 5.29•	292.50 ± 7.99•

There was no significant difference between control group and rhACE2 (0.1 ng/μl) group in the level of levels of IL-8, TNF-α, TGF-β1, and LDH. Compared with the control group, the levels of IL-8, TNF-α, TGF-β1, and LDH in HUVECs in the Ang II (1.106 ng/μl) group significantly increased (▲, $P < .05$). Compared with the Ang II (1.106 ng/μl) group, the level of IL-8, TNF-α, TGF-β1, and LDH in both the Ang II (1.106 ng/μl) + rhACE2 (0.01 ng/μl) group and the Ang II (1.106 ng/μl) + rhACE2 (0.1 ng/μl) group significantly decreased (•, $P < .05$). There was a decrease trend in the level of levels of IL-8, TNF-α, TGF-β1, and LDH with different concentrations of rhACE2.

new research direction for HSP treatment, while the application of rhACE2 still needs further study.

Acknowledgments

We wish to acknowledge the Department of Pediatrics of the First Affiliated Hospital of Anhui Medical University for supporting this scientific research. We would also like to thank the Key Laboratory Zoonses of Anhui Province and the Department of Clinical Laboratory of the First Affiliated Hospital of Anhui Medical University for their assistance and advice in providing detection reagents and instruments.

Author contributions

Formal analysis: Xiaocui Zhang, Zhaoyu Hou.
Funding acquisition: Fang Deng.
Investigation: Fang Deng.
Methodology: Hongli Zhang.
Project administration: Hongli Zhang, Xiaocui Zhang, Zhaoyu Hou.
Resources: Hongli Zhang.
Supervision: Fang Deng.
Validation: Hongli Zhang.
Writing – original draft: Hongli Zhang.
Writing – review & editing: Hongli Zhang.

References

[1] Zhang Q, Guo Q, Gui M, et al. Henoch-Schönlein purpura with acute pancreatitis: analysis of 13 cases. BMC Pediatr 2018;18:159.
[2] Lei WT, Tsai PL, Chu SH, et al. Incidence and risk factors for recurrent Henoch-Schönlein purpura in children from a 16-year nationwide database. Pediatr Rheumatol 2018;16:25.
[3] Liu Z, Liu J, Xiao M, et al. Mesenchymal stem cell derived microvesicles alleviate pulmonary arterial hypertension by regulating renin angiotensin system. J Am Soc Hypertens 2018;12:470–8. [Epub ahead of print].
[4] Klempin F, Mosienko V, Matthes S, et al. Depletion of angiotensin-converting enzyme 2 reduces brain serotonin and impairs the running-induced neurogenic response. Cell Mol Life Sci 2018;75:3625–34. [Epub ahead of print].
[5] Williams VR, Scholey JW. Angiotensin-converting enzyme 2 and renal disease. Curr Opin Nephrol Hypertens 2018;27:35–41.
[6] López-Mejías R, Castañeda S, Genre F, et al. Genetics of immunoglobulin-A vasculitis (Henoch-Schönlein purpura): an updated review. Autoimmu Rev 2018;17:301–15.
[7] Nozaki T, Ura H, Takumi I, et al. The angiotensin II type I receptor antagonist losartan retards amygdala kindling-induced epileptogenesis. Brain Res 2018;1694:121–8. pii: S0006-8993(18)30284-1. [Epub ahead of print].
[8] Ac SES, Teixeira MM. ACE inhibition, ACE2 and angiotensin-(1-7) axis in kidney and cardiac inflammation and fibrosis. Pharmacol Res 2016; 107:154–62.
[9] Nunes-Silva A, Rocha GC, Magalhaes DM, et al. Physical exercise and ACE2-Angiotensin-(1-7)-Mas receptor axis of the Renin Angiotensin System. Protein Pept Lett 2017;24:809–16.
[10] Borém LMA, Neto JFR, Brandi IV, et al. The role of the angiotensin II type I receptor blocker telmisartan in the treatment of non-alcoholic fatty liver disease: a brief review. Hypertens Res 2018;41(1 Suppl):394–405. [Epub ahead of print].
[11] Ali Q, Dhande I, Samuel P, et al. Angiotensin type 2 receptor null mice express reduced levels of renal angiotensin II type 2 receptor/angiotensin (1-7)/Mas receptor and exhibit greater high-fat diet-induced kidney injury. J Renin Angiotensin Aldosterone Syst 2016;17:1–8.
[12] Nomura S, Tsujimoto H, Aosasa S, et al. Impact of angiotensin-converting enzyme 2 levels on postoperative pneumonia after esophagectomy. J Surg Res 2018;224:200–6.
[13] Aspect IT, Prodrug FIA. More than just an immunosuppressant: the emerging role of FTY720 as a novel inducer of ROS and apoptosis. Oxid Med Cell Longev 2018;2018:4397159eCollection.
[14] Santos RAS, Sampaio WO, Alzamora AC, et al. The ACE2/angiotensin-(1-7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1-7). Physiol Rev 2018;98:505–53.
[15] Magalhaes GS, Barroso LC, Reis AC, et al. Angiotensin-(1-7) promotes resolution of eosinophilic inflammation in an experimental model of asthma. Front Immunol 2018;9:58eCollection 2018.
[16] Lin N, Ji Z, Huang C. Smad7 alleviates glomerular mesangial cell proliferation via the ROS-NF-κB pathway. Exp Cell Res 2017;361: 210–6.
[17] Aviello G, Knaus UG. NADPH oxidases and ROS signaling in the gastrointestinal tract. Mucosal Immunol 2018;11:1011–23. [Epub ahead of print].
[18] Mali AV, Bhise SS, Katyare SS, et al. Altered kinetics properties of erythrocyte lactate dehydrogenase in type II diabetic patients and its implications for lactic acidosis. Indian J Clin Biochem 2018;33: 38–45.
[19] Kim JE, Woo YJ, Sohn KM, et al. Wnt/β-catenin and ERK pathway activation: a possible mechanism of photobiomodulation therapy with light-emitting diodes that regulate the proliferation of human outer root sheath cells. Lasers Surg Med 2017;49:940–7.
[20] Salomon BL, Leclerc M, Tosello J, et al. Tumor necrosis factorα and regulatory T cells in oncoimmunology. Front Immunol 2018;9: 44400444. eCollection 2018.
[21] Zhang S. The role of transforming growth factor β in T helper 17 differentiation. Immunology 2018;155:24–35. [Epub ahead of print].
[22] Wang W, McKinnie SM, Farhan M, et al. Angiotensin-converting enzyme 2 metabolizes and partially inactivates pyr-apelin-13 and apelin-17: physiological effects in the cardiovascular system. Hypertension 2016;68:365–77.
[23] Nalbantoglu S, Tabel Y, Mir S, et al. Association between RAS Gene polymorphisms (ACE I/D, AGT M235T) and Henoch-Schönlein purpura in a Turkish population. Dis Markers 2013;34:23–32.
[24] Yue Y, Ma K, Li Z, et al. Angiotensin II type 1 receptor-associated protein regulates carotid intimal hyperplasia through controlling apoptosis of vascular smooth muscle cells. Biochem Biophys Res Commun 2018;495:2030–7.