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Valsartan reduces AT1-AA-induced apoptosis through suppression oxidative stress mediated ER stress in endothelial progenitor cells

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Abstract. – OBJECTIVE: Valsartan has been reported to have the function of treating hypertension and improving the prognosis of patients. Many studies indicated that valsartan can also increase angiotensin II, andosterone and plasma renin activity (PRA). Autoantibodies against the angiotensin II type 1 receptor (AT1-AA) have been showed to increase reactive oxygen species (ROS) and calcium (Ca^{2+}) and result in apoptosis in vascular smooth muscle cells. In this study, we attempted to explore the effect of valsartan on AT1-AA-induced apoptosis in endothelial progenitor cells.

MATERIALS AND METHODS: Endothelial progenitor cells (EPCs) were cultured. The cytotoxicity was determined by MTT assay. EPCs apoptosis was determined by DAPI staining and flow cytometry. Reactive oxygen species, intracellular calcium concentration and calpain activity were measured using Fluos and Omega Spectrofluorimeter. The expression of ERK, p-eIF-2α, CHOP, Bcl-2 and caspase-3 were detected by Western blot.

RESULTS: MTT assays showed that valsartan significantly inhibited AT1-AA- induced decline of the viability of EPCs. DAPI staining and flow cytometry results indicated valsartan inhibited AT1-AA induced decrease of the viability of EPCs via inhibiting AT1-AA induced apoptosis. Furthermore, the increasing of reactive oxygen species, intracellular calcium and calpain activity induced by AT1-AA in EPCs were also recovered by pre-treated with valsartan. Meanwhile, the up-regulation of p-ERK, p-eIF-2α and down-regulation of Bcl-2, and activation of caspase-3 induced by AT1-AA were reversed after re-incubated with valsartan.

CONCLUSIONS: Valsartan could inhibit AT1-AA induced apoptosis through inhibiting oxidative stress mediated ER stress in EPCs.

Keywords:
Valsartan, AT1-AA, Apoptosis, Oxidative stress, ER stress.

Introduction

Coronary heart disease (CHD) is one of the main diseases to harm human health[1]. CHD, which usually damages the dynamic imbalance between repairment and impairment of the endothelium, results from the excessive proliferation of neo-endothelium and the dysfunction of endothelium after the impairment of the vessel[2,3]. Endothelial progenitor cells (EPCs) are a kind of cells that can proliferate and differentiate into endothelial cell and yet do not express hematopoietic vascular cells, which is precursor cell of vessel. EPCs involve in repairing endothelial injury and the disorder of this process is one of the reasons for coronary heart disease[4-6]. Autoantibodies against the angiotensin II type 1 receptor (AT1-AA), which belongs to the G-protein-coupled receptor, are a membrane receptor and have all features of hormone receptors[7,8]. Many reports showed that AT1-AA has been found in refractory hypertension, preeclampsia and renal-allograft rejection patients. AT1-AA has been detected by the chronotropic responses to AT1-AA-mediated stimulation of cultured rat cardiomyocytes[9]. Previous studies[10] have found that AT1-AA can increase reactive oxygen species (ROS) and calcium (Ca^{2+}) and result in apoptosis in vascular smooth muscle cells. Although AT1-AA has been recognized to induce apoptosis in vascular smooth muscle cells, little is known about its effects on EPCs and underlying mechanism. It is necessary to look for effective drugs to antagonism the effect of AT1-AA on EPCs. Angiotensin II receptor blockers (ARBs) is a member of drugs for hypertension and the mechanism of ARBs is lowering blood pressure by blocking angiotensin II effect[11,12]. ARB has

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been used to initialize and maintain the therapy of antihypertensive for the five classes of antihypertensive drugs[13]. ARBs have recently been reported to suppress atrial fibrillation (AF) recurrence in both paroxysmal and persistent AF[14]. Valsartan is one of the ARBs, which has been reported to treat hypertension and improve the prognosis of patients. Valsartan is a selective antagonist of the angiotensin II type 1 receptor[15,16]. Many reports have shown a significant decrease of caspase-3 and BAX in valsartan-treated group compared to active control group in myocardial level. Meanwhile, the myocardial apoptosis index also has a significant decrease in a valsartan-treated group. Valsartan has been showed to inhibit apoptosis and reduce caspase-3 activity to treat hypertension and improve the prognosis of patients[17-19]. Also, it has been shown to have the abilities to increase angiotensin II, aldosterone and plasma renin activity (PRA)[20]. Given the function of valsartan, we try to explore whether valsartan could inhibit AT1-AA- induced apoptosis in EPCs and the underlying mechanism. In this study, we verified the effect of valsartan on AT1-AA-induced apoptosis on EPCs. The results showed valsartan could inhibit AT1-AA induced apoptosis through inhibiting oxidative stress mediated ER stress in EPCs. Therefore, valsartan has great potential as a drug against coronary heart disease.

Materials and Methods

Reagents and Chemicals

EGM-2 medium and fetal bovine serum (FBS) were obtained from GIBCO (Grand Island, NY, USA). 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Cell Signaling (Beverly, MA, USA). DAPI staining was from Phosphogen-Becton Dickinson (San Diego, CA, USA). Annexin-V-FITC apoptosis detection kit was obtained from Sigma-Aldrich (St. Louis, MO, USA). Western blot buffer, BCA protein kit and enhanced chemiluminescence kit were from Beyotime Institute of Biotechnology (Shanghai, China). ERK1/2, p-eIF-2α antibodies were purchased from Abcam (Cambridge, MA, USA). CHOP antibodies were from Cell Signaling (Beverly, MA, USA). Antibodies against Bcl-2, caspase-3 and α-tubulin were purchased from Beyotime Institute of Biotechnology (Nantong, China). p-ERK and ERK were obtained from Bioword (New York, NY, USA). AT1-AA was obtained from Abcam (Cambridge, MA, USA). Valsartan was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell Culture

EPCs were obtained from the Institute of Cell Research (Shanghai, China). EPCs were cultured in EGM-2 medium, which was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Beyotime, Beijing, China). Cells were incubated at 37°C in a humidified atmosphere with 5% CO$_2$.

MTT Assay

The cell viability was detected by MTT assay. EPCs (8000 cell) were seeded in flat-bottomed 96-well plates. After 24 h, cells were exposed in AT1-AA (2.5 and 10 μM) for 24 h or 10 μM for 6, 9, 12 and 24 h. Based on previous dose-response studies, effective AT1-AA (10 μM for 12 h) was chosen as optimum for further study. Then valsartan (0.5, 1 and 2 μM) for 24 or 1 μM for 2, 4, 6, 8, 12 and 24 h were added to the wells before pre-treatment efficacy[20]. Control group cultured with phosphate-buffered saline (PBS) in medium. 20 μL MTT (0.5 mg/mL PBS) was added to each well and the plates were incubated for 4 h at 37°C. Then the medium was removed and 150 μL DMSO was added to extract intracellular formazan in EPCs cells for 10 min to solute the purple formazan crystals. Absorbance was detected with a microplate reader Uscn Kit Inc (Wuhan, China) at 570 nm. The percentage of cell growth inhibition was calculated based on the following formula: (A_{570}Control-A_{570} Experiment) / A_{570} Control × 100%. All assays were executed in three independent experiments.

DAPI Staining of Apoptotic Cells

The apoptosis of EPCs was detected by DAPI staining. Briefly, EPCs were seeded at a density of 2×10^5 cells/well in flat-bottomed 12-well plates with glass slides. After 24 h, EPCs were treated with AT1-AA (2.5, 5 and 10 μM), valsartan (1 μM) in medium alone or composite processing. Control group was treated with PBS in medium. EPCs were washed with PBS twice and stained with DAPI in the dark at room temperature for 20 min. Then, EPCs were washed with PBS and cell nuclei were observed under laser confocal scanning microscope (LCSM) (Hiobio, Beijing, China). The apoptosis rate was measured by calculating the number of cells with nuclear phenotypic changes in 20 different microscopic fields.

Flow Cytometric Analysis

AT1-AA-induced apoptosis in EPCs were measured by flow cytometry using the annexin V-FITC and propidium iodide (PI) staining method. Briefly, EPCs were plated at a density of 1×10^6 cells/dish into 60 mm dishes. After 24 h, EPCs were exposed in AT1-AA (2.5, 5 and 10 μM), valsartan (1 μM) in medium alone or composite processing. Control group was treated with PBS in medium. Then cells were trypsinized, washed with cold PBS and re-suspended in binding buffer containing annexin V-FITC (5 μL) and of PI (2.5 μL). Cells were cultured at room temperature in the dark for 30 min. Finally, apoptosis was detected with a FAC Sort flow cytometer (Becton, Franklin Lakes, NJ, USA).

Measurement of Reactive Oxygen Species

Reactive oxygen species was detected by cell permeant probe CM-H2DCFDA. Briefly, EPCs were seeded in 6-well plates overnight and pre-treated with valsartan (1 μM) for 4 h and incubated with AT1-AA (10 μM) for 12 h. Cells were incubated with fluorescent probe 2', 7'-dichlorofluorescein diacetate (DCF-DA) 20 μM for 30 min. Then cells were trypsinized, with PBS (PH 7.4) and monitored at an excitation wavelength of 488 nm and emission of 530 by Fluostar Omega Spectrofluorimeter (Bio-ge Hong Kong, China).

Measurement of Intracellular Calcium Concentration

The level of intracellular EPCs determined by Fluo AM sta Briefly, cells were plated in 96 s and incub with Fluo-4 AM fluoresc for 30 min. Hanks' Balanced Salt olution SS), which was used in experi s, included NaCl, 142; KCl, 5.6; M 1; $CaCl_2$, 2; Na_2H , 0.34; KH_2PO_4, 0.44 PES, 1 lucose, 5.6; buffered to pH 7.4 wi OH. T a^{2+}-free HBSS contained (mM): NaCl, , 5.6: Cl_2, 1; Na_2HPO_4, 0.34; PO_4, HEP 10; glucose, 5.6;EGTA, 1. scenc ements were determined at 488 n for ex ion and 522 nm for emission by Omega Spectrofluorimeter (Bio-gene, hina).

pain Activity Assays

s were seeded in 24-well plates and pre-treated with Tetrakis (acetoxymethyl) 1,2-Bis(2-aminophenoxy) ethane-N,N,N',N'-tetraacetate(BAPTA), a calcium chelator or calpeptin and an inhibitor of calpain for 1 h. Then cells were loaded with Suc-Leu-Leu-Val-Tyr-AMC calpain protease substrate (40 M) and incubated with shikonin to the indicated time under a humidified sphere with 5% CO_2 at 37°C. The fluor detected by Fluostar Omega Spect orimeter (Bio-gene, H.K, China) at 360 n excitation and 460 nm for emission.

Western Blotting

Treated cells were ed wi PBS and lysed with West ysis b containing 1% PMSF for 15 in uged 000 rpm at 4°C for in to bitate nsoluble material. n concent measured with a BC kit. Eight rograms of each prot were d on 12% SDS-PAGE and transferred to poly ene fluoride (PVDF) mem The memb as blocked in TBS (T uffered saline cont ning Tween 20 and milk) for 1 h d incubated with anti-p-ERK, ERK, anti-p -2α, anti-eIF-2α, anti-CHOP, a cl-2, anti spase-3 and anti-α-tubulin at 4 ern Flowing this, the membranes were w th Tris-buffered saline containing Tween20 (TBST) and incubated with the appro- HRP-conjugated secondary antibody (Ab- n, mbridge, UK). Finally, the amount of protein was detected by chemiluminescence.

Statistical Analysis

Results were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) for Windows and expressed as the mean ± standard error of the mean. Statistical significance of differences was analyzed with one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests. A value of less than 0.05 ($p < 0.05$) and 0.01 ($p < 0.01$) were considered significant and highly significant, respectively.

Results

Valsartan Inhibits AT1-AA-Induced Decline of Cell Viability in EPCs

To investigate whether valsartan could inhibit AT1-AA-induced decline of cell viability in EPCs, we firstly detected the effect of AT1-AA on the viability of EPCs. EPCs were treated with AT1-AA (2.5, 5 and 10 μM) for 24 h or 10 μM for 3, 6, 9, 12 and 24 h. Results showed AT1-AA inhibited growth of EPCs in dose- and time-dependent (Figure 1A and B). Meanwhile, effective AT1-AA (10

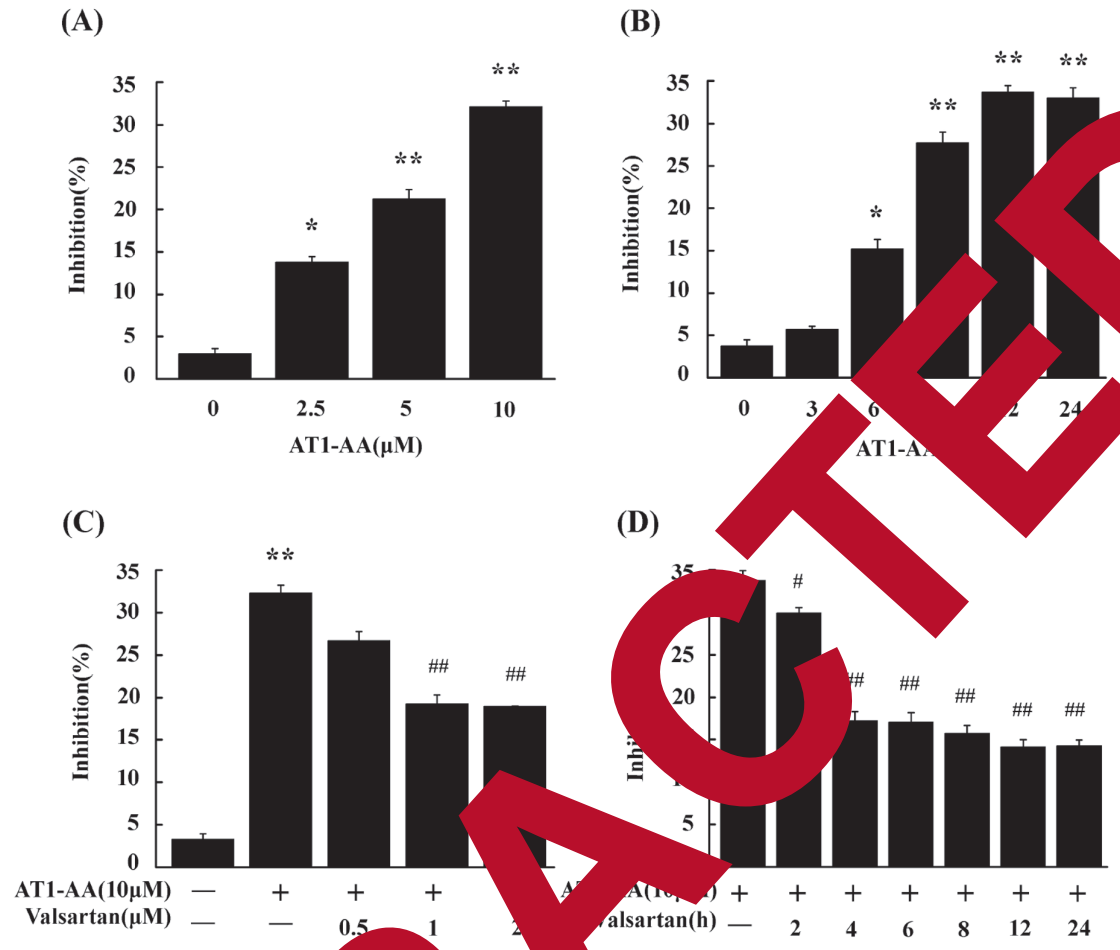

Figure 1. Effect of valsartan on ...A ...ed decli... cell viability in EPCs. ***(A)*** EPCs were treated with various concentrations of AT1-AA (2.5, 5 ... 0 μM) ... 4 h and ce... ability was determined by MTT assay; ***(B)*** EPCs were treated with AT1-AA (10 μM) for 3, ... 2, 24 h ... cell viability was detected by MTT assay; ***(C)*** EPCs were pre-treated with various concentrations of ... n (... for 24 h and then incubated with AT1-AA (10 μM) for 12 h. The cell viability was detecte... MT... ***(D)*** EPCs were pre-incubated with valsartan (1 μM) for 2, 4, 6, 8, 12, 24 h and then treated with AT1-... 0 μM) fo... Cell viability was determined by MTT assay. In ***(A)***, ***(B)***, ***(C)*** and ***(D)***, values are percent as the mean... f three indep... experiments. 0.01 <*p <0.05 and **p < 0.01 *vs.* control; 0.01 < $^{\#}p$ < 0.05 and $^{\#\#}p$ < 0.01 *vs.* AT1...

μM for 12... was chosen ... timum for further studies... n valsartan (0.5, ... d 2 μM) for 24 or 1... for 2, ... 8, 12 and 24 h was added to the... to d... mine the pre-treatment efficacy. The i... icated ... decline of cell viability ...PCs, ... h wa... uced by AT1-AA, was el- ... signi... y valsartan and the effective dos... d time ... μM for 4 h (Figure 1C and D). A... results suggested valsartan could inhibit ...AA... ed decline of viability of EPCs.

...sartan inhibits AT1-AA-Induced Cell A... tosis in EPCs

To verify whether valsartan inhibited AT1-AA-induced decline of cell viability in EPCs is related to apoptosis, EPCs were treated with AT1-AA (2.5, 5 and 10 μM for 12 h), valsartan (1 μM for 4 h) alone or composite processing. The apoptosis was determined by DAPI staining and flow cytometric analysis. As shown in Figure 2A and B, EPCs exhibited the apoptotic characteristics of chromatin condensation with typical apoptotic bodies after treated with AT1-AA and the percentage of apoptotic cells was significantly increased in a dose-dependent manner. After pre-treated with valsartan, the apoptosis rate of EPCs was significantly reverse compared with AT1-AA alone. The apoptosis of EPCs was further determined by flow cytometry using the annexin V-FITC and propidium iodide (PI) staining method (annexin V/PI double-staining). As suggested in Figure 2C and D, the apoptosis rate of EPCs induced by

AT1-AA (10 μM) alone increased to 30.3% but reversed to 13.7% after pre-treated with valsartan. These results indicated valsartan inhibited AT1-AA- induced apoptosis in EPCs.

Valsartan inhibits AT1-AA-Induced Cell Apoptosis Through Decreasing ROS Generation and Intracellular Calcium in EPCs

Both ROS and Ca^{2+} have been reported to involve in apoptosis signal transduction pathways and they have a close relationship in this pathway[21]. To detect whether valsartan inhibited AT1-AA-induced apoptosis in EPCs via decreasing ROS generation and intracellular calcium, EPCs were pre-treated with valsartan (1 μM) for 4 h and incubated with AT1-AA (10 μM) for 12 h or treated with (10 μM) for 6, 12 and 24 h alone. R[illegible]s showed AT1-AA increased ROS generation[illegible]re 3A) and intracellular calcium as early as 90 [illegible]igure 3B and C) and the effect of AT1[illegible]A on [illegible]llular calcium in EPCs in time-de[illegible]ent manner[illegible] 3B). Meanwhile, after EP[illegible]re-trea[illegible]d with v[illegible]tan, the increasing of R[illegible]nd int[illegible]ular calcium

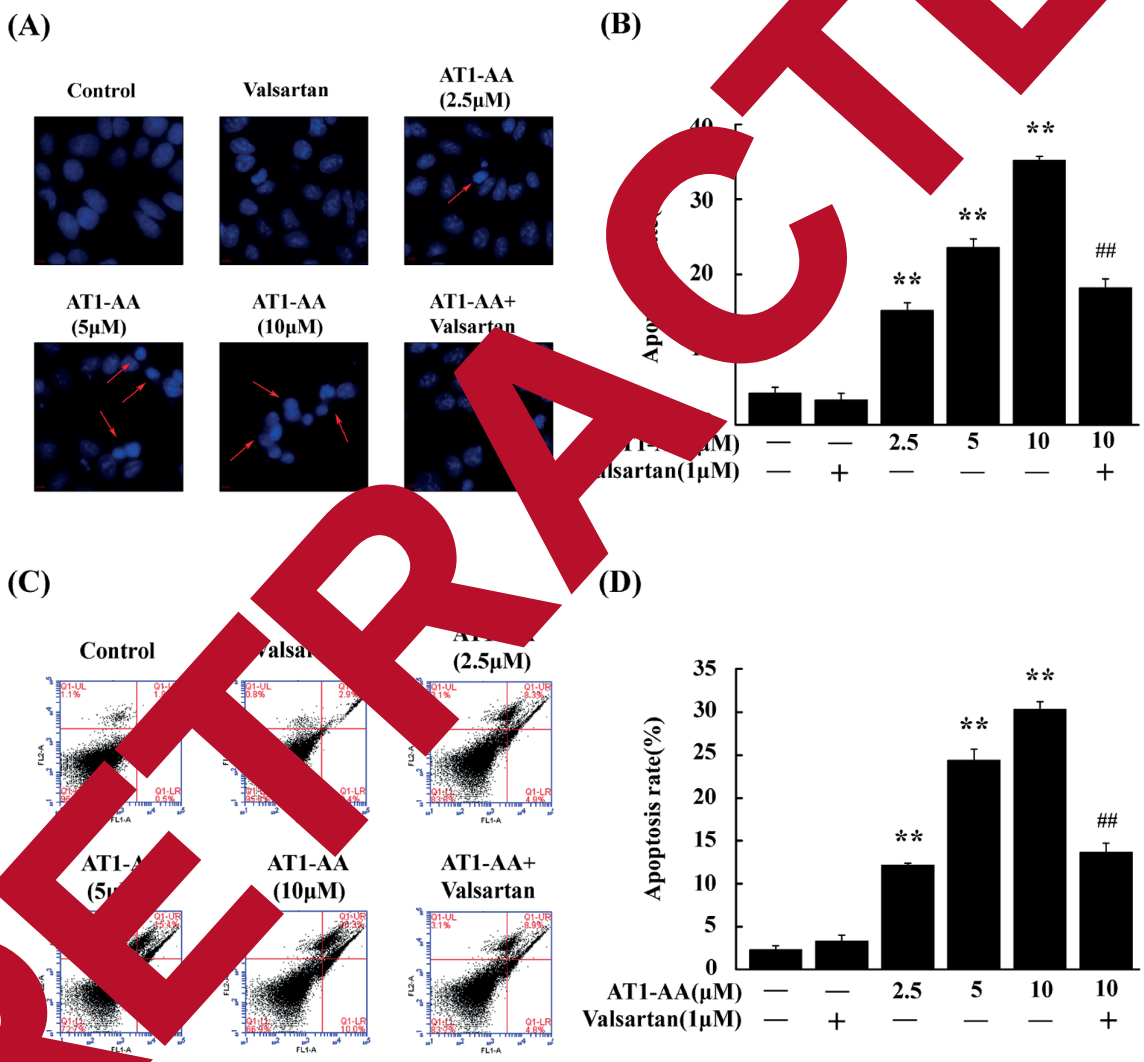

Figure 2. [illegible]ctive effect of valsartan on AT1-AA induced apoptosis in EPCs. ***(A)*** EPCs were pre-treated with valsartan (1 [illegible]M) for 4 h and incubated with various concentrations of AT1-AA (2.5, 5 and 10 μM) for 12 h. Then cell nuclei were stained [illegible]DAPI and images were captured under a fluorescence microscope; ***(B)*** The percentage of apoptotic cells was measured; [illegible]Cs were pre-treated with valsartan (1 μM) for 4 h and incubated with various concentrations of AT1-AA (2.5, 5 and 10 μM) [illegible]r 12 h and then cell apoptosis were determined by flow cytometry analysis using annexin V/propidium iodide double-staining; ***(D)*** Apoptosis ratio from flow cytometry were shown in the bar graph. The data in ***(B)*** and ***(D)*** were expressed as mean ± SD and acquired from three biologically independent experiments. $0.01 < {}^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* control; $0.01 < {}^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* AT1-AA alone.

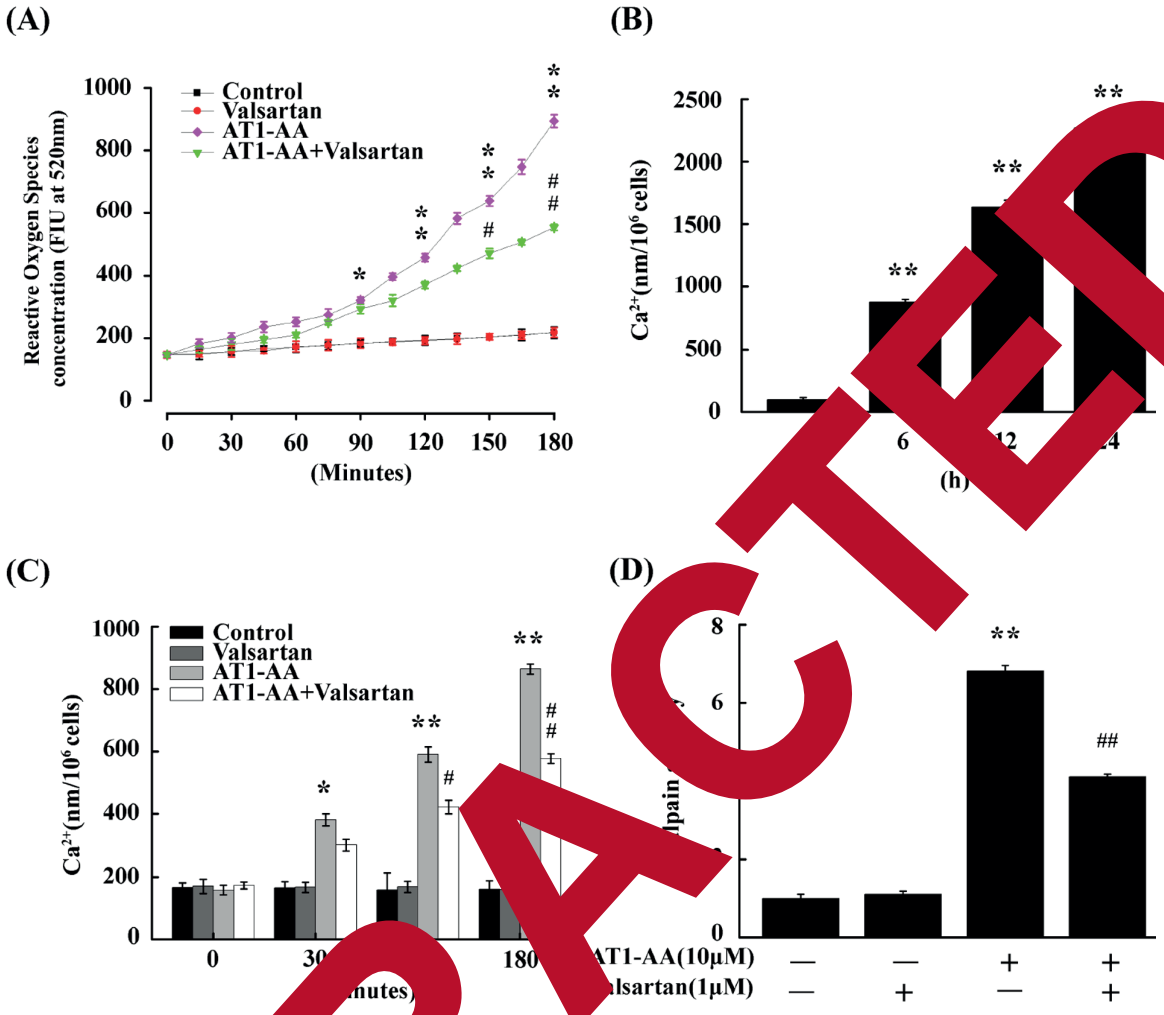

Figure 3. Valsartan inhibits A-in ation, increase in intracellular Calcium in EPCs. ***(A)*** Valsartan inhibits AT1-AA-induced ge EPCs. EP were seeded in flat-bottomed 6-well plates overnight and pre-treated with valsartan (1 μM) f n and in with AT1-AA (10 μM) for 12 h. Then, EPCs were incubated DCF-DA (50 μM) for 30 min and wash h PBS (4°C) mes. Finally, the generation of ROS was detected by fluorimetry according to standard protocol A induces in in intracellular calcium in EPCs. EPCs were plated in 96-well plates and exposed with AT (10 r 12 h. Then Cs were stained with Fluo-4AM (50 μM/mL) dye and the level of intracellular calcium meas by Fluost ga Spectrofluorimeter. ***(C)*** Valsartan inhibits AT1-AA-induced increase in intracellular calcium in EPCs were se 96-well plates and pre-treated with valsartan (1 μM) for 4 h and incubated with AT1-AA (10 r 12 h. The level of ellular calcium in EPCs was also determined by Fluostar Omega Spectrofluorimeter. ***(D)*** V an inhi AT1-AA-induced calpain activity in EPCs. EPCs were pre-treated with valsartan (1 μM) for 4 h and inc with A A (10 μM) for 12 h and calpain activity analyzed, as described in Material and Methods. The data were expr ± SD an quired from three biologically independent experiments. $0.01 < *p < 0.05$ and $**p < 0.01$ *vs.* control, < 0.05 a < 0.01 *vs.* AT1-AA alone.

was cued (F 3A and C). Calcium-activated ne tease (Calpain) activity was also detected with AT1-AA and results suggested a arked increase. After pre-treated with valsartan, AA-induced increase of calpain activity was re d (Figure 3D). All these results indicated valsartan inhibited AT1-AA-induced cell apoptosis through decreasing ROS generation and intracellular calcium in EPCs.

Valsartan inhibits AT1-AA-induced Cell Apoptosis Through Oxidative Stress Mediated ER Stress

ROS-mediated free intracellular Ca^{2+} is known to be involved in ER stress-induced apoptotic signaling[22]. Many reports have shown drug-induced increase in ROS generation, intracellular calcium and activation of ER leads to apoptosis in cells[23-25]. As the previous results suggested, valsartan inhib-

ited AT1-AA-induced increase of ROS generation and intracellular in EPCs. Therefore, we explored whether valsartan inhibited AT1-AA-induced apoptosis via inhibiting ROS dependent ER stress and examined ER stress-associated proteins in EPCs. The key factors of the ER stress include p-ERK, p-eIF-2α, CHOP, Bcl-2 and caspase-3. EPCs were pre-treated with valsartan (1 μM) for 4 h and incubated with AT1-AA (10 μM) for 12 h or exposed with AT1-AA (10 μM) for 0.5, 1, 3, 6, 9 and 12 h alone. Results revealed AT1-AA stimulated ERK1/2 phosphorylation in time-dependent manner and had a marked increase as early as 6 h (Figure 4A and B). As showed in Figure 4C, D and Figure 5A, B, C, AT1-AA effectively increased p-ERK, p-eIF-2α, CHOP, and caspase-3 protein levels along with decreased Bcl-2 expression. After pre-treated with valsartan, the increase of p-ERK, p-eIF-2α, CHOP and Caspase-3 and decrease of Bcl-2 induced by AT1-AA were all reversed. These data indicated valsartan inhibited AT1-AA-induced apoptosis through inhibiting oxidative stress mediated ER stress in EPCs.

Discussion

EPCs play an important role in coronary heart disease. EPCs can proliferate and differentiate from endothelial cells and participate in repairing endothelial injuries. EPCs dysfunction is the reason for coronary heart disease. AT1-AA has been

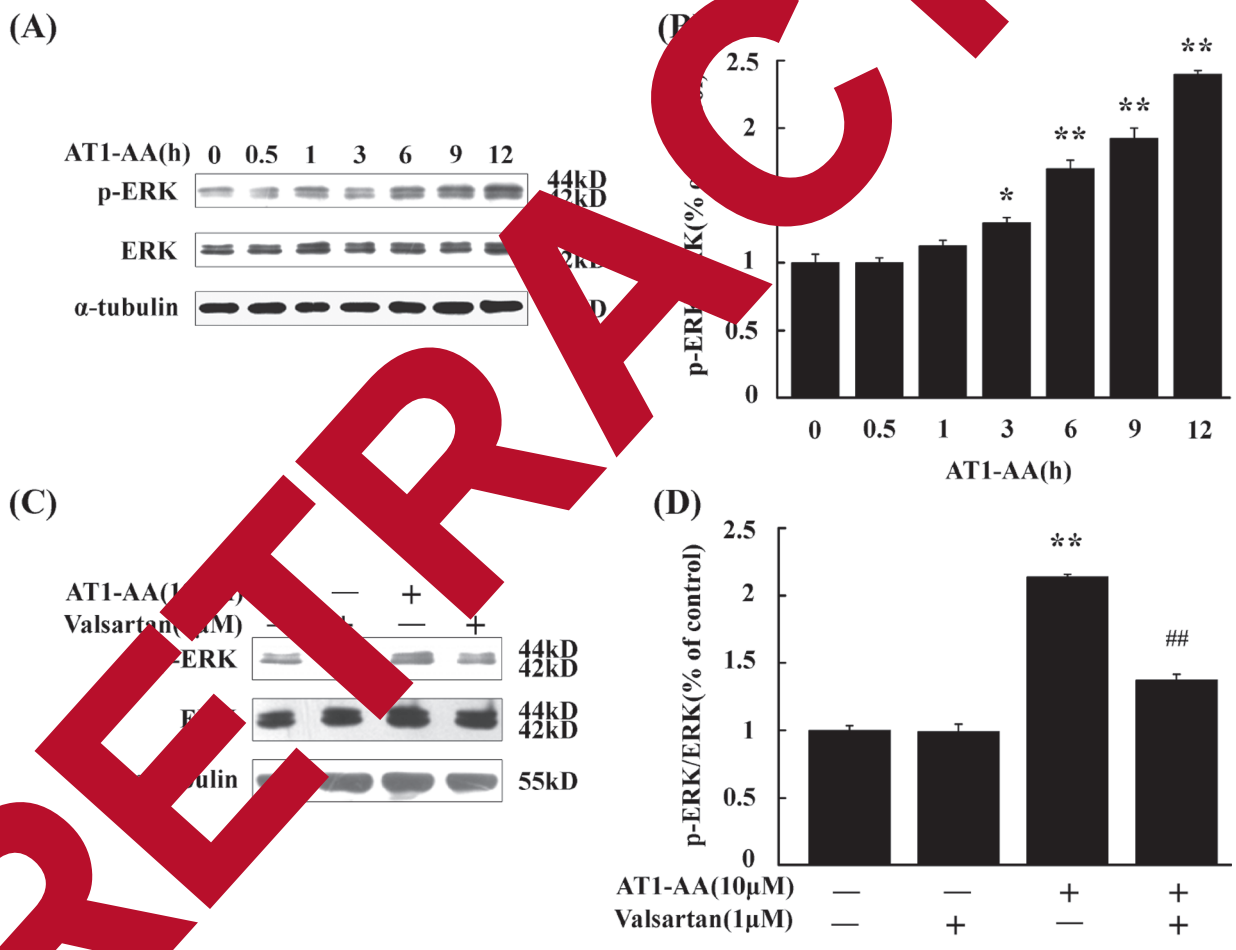

Figure 4. Valsartan inhibits AT1-AA induced apoptosis by ERK pathway in EPCs. ***(A)*** EPCs were treated with AT1-AA (10 μM) for 0.5, 1, 3, 6, 9 and 12 h. Western blotting was performed to analyze the protein levels of p-ERK and ERK. ***(B)*** The expression of p-ERK and ERK were quantified using grayscale scans analysis. ***(C)*** EPCs were pre-treated with valsartan (1 μM) for 4 h and incubated with AT1-AA (10 μM) for 12 h. Western blotting was performed to analyze the protein levels of p-ERK and ERK. ***(D)*** The expression of p-ERK and ERK were quantified using grayscale scans analysis. In ***(D)***, values are percent as the mean ± SD of three independent experiments. $0.01 < {}^{*}p < 0.05$ and $^{**}p < 0.01$ *vs*. control; $0.01 < {}^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs*. AT1-AA alone.

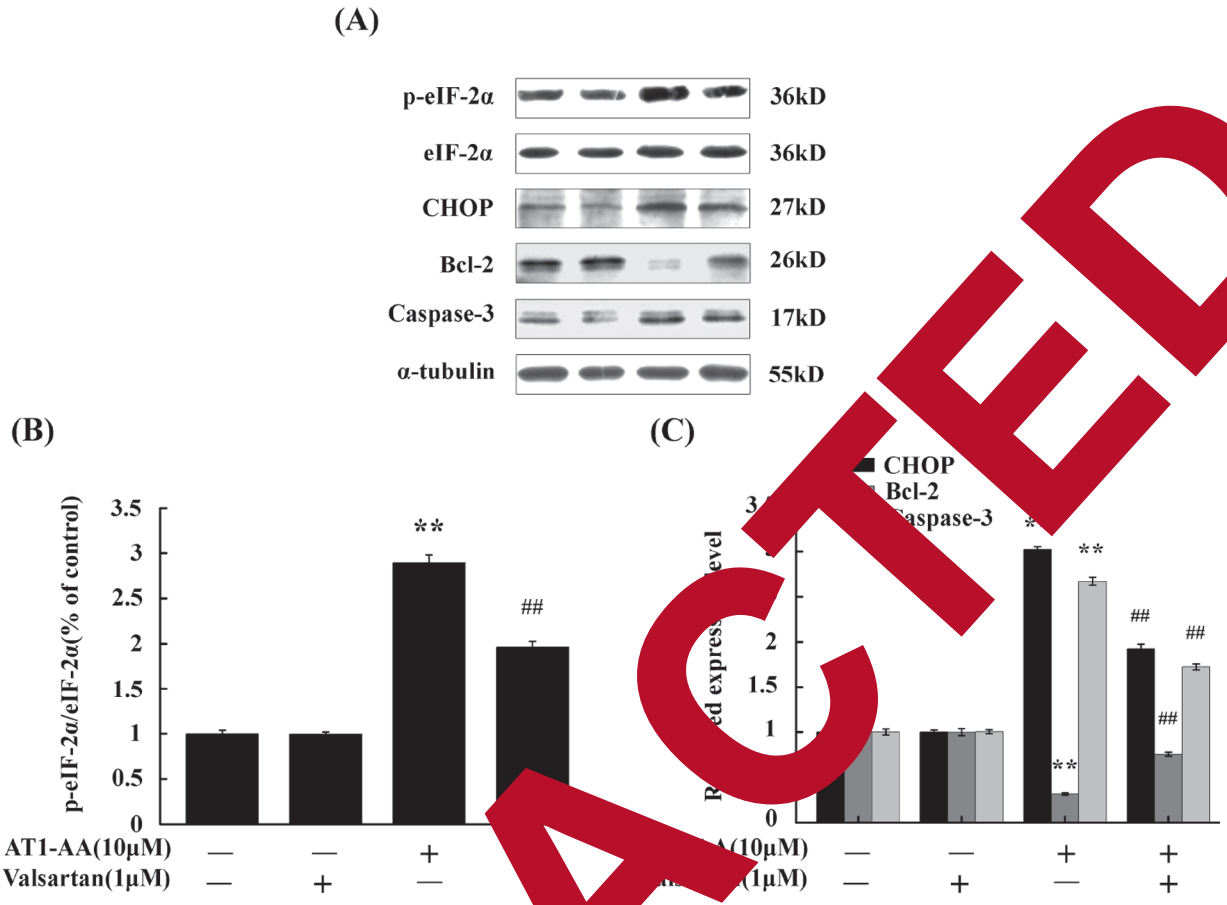

Figure 5. Effects of valsartan on AT1-AA altered-apo protein. ***(A)*** EPCs were pre-treated with valsartan (1 μM) for 4 h and incubated with AT1-AA (10 h. Weste ting was performed to analyze the protein levels of p-eIF-2α, eIF-2α, CHOP, Bcl-2 and caspase-3 The of value eIF-2α/eIF-2α was detected using grayscale scans analysis. ***(C)*** The expression of CHOP, B nd casp were qua ed using grayscale scans analysis. In ***(B)*** and ***(C)***, values are percent as the mean ± SD of t ndepend ents. $0.01 < {}^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* control; $0.01 < {}^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* AT1-AA alone.

showed to induc ptosis in e elial progenitor cells[9]. e, valsartan reduce drugs-induced poptos regulating oxidative stress me d ER stress

Apo s is an important on in drugs-induce death Therefore, we explore whether va de ed AT1-AA-induced decline of EPCs by apo s. MTT results revealed -AA ited th of EPCs in dose- and t epen valsartan could inhibit AT1-AA duced de e of viability of EPCs. Results of staining and flow cytometry showed pto es and apoptosis ratio were both gnificantly decreased after pre-treatment with rtan compared with AT1-AA alone. Many st [28] showed both ROS and Ca^{2+} involve in apoptosis signal transduction pathways and they have a close relationship in this pathway. Therefore, we explored the relationship between ROS, Ca^{2+} and AT1-AA- induced apoptosis. Results indicated AT1-AA increased ROS generation and intracellular calcium and the level of ROS generation and intracellular calcium were decreased after pre-treated with valsartan in EPCs. These results showed valsartan inhibited AT1-AA- induced cell apoptosis through decreasing ROS generation and intracellular calcium in EPCs. Oxidative stress mediated ER stress have been reported to play an important role in apoptosis process[28]. Therefore, we investigated the effect of oxidative stress mediated ER stress on AT1-AA-induced in EPCs and the effect of valsartan was further explored. The activation of ERK, which mainly depends on the stimuli and cell types involved, has been showed to cause cell apoptosis[29,30]. eIF2α and CHOP are important proteins in ER stress apoptotic pathway. Bcl-2 protein family is the central regulator of cell apoptosis. Anti-apoptosis protein Bcl-2 is a

crucial member of Bcl-2 family. Caspases, which responses to pro-apoptosis signals, are a family of cysteine proteases. Caspase-3 was activated in ER stress-dependent apoptosis cells. To further detect valsartant inhibited AT1-AA-induced apoptosis by ER stress-dependent pathway in EPCs, the level of p-ERK, p-eIF2α, CHOP, Bcl-2 and caspase-3 were detected after treated with AT1-AA alone and pre-treated with valsartan. Western blot results showed valsartan reduced the up-regulation of p-ERK, p-eIF2α, CHOP and caspase-3 and decreased the down-regulating of Bcl-2 after pre-treatment with valsartan. All these results indicated valsartan inhibited AT1-AA-induced apoptosis inhibiting oxidative stress mediated ER stress in EPCs.

Conclusions

Valsartan inhibits AT1-AA-induced apoptosis in EPCs by regulating ROS and Ca^{2+} and inhibiting ER stress-dependent apoptosis pathway. Valsartan decreases AT1-AA-induced activation of ERK and in turn modulates p-eIF2α, CHOP, Bcl-2 and caspase-3 and reduced ROS and [illegible]. This study revealed valsartan could antag[illegible]m AT1-AA-induced injury on EPCs and it has g[illegible] potential in against coronary heart disease.

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Conflict of In[illegible]st

The Authors d[illegible]la[illegible]e that the[illegible] no conflict of interests.

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1) Zh[illegible] Z, Lou[illegible]u G, Huang W, Zhang S, Liu F. Po[illegible] ov[illegible]ndrome (PCOS) and the risk coron[illegible] disease (CHD): a meta-analy-. Oncota[illegible]2016; 7: 33715-33721.

2) [illegible]K, Fu C, Nie S, You Y. The index and improve-[illegible] of using Danhong injection to patients with atherosclerosis symptoms of coronary heart disease (CHD). Pak J Pharm Sci 2014; 27: 1699-1704.

3) [illegible]anu Js, Gu Y, Zhi S, Yu M, Lu Y, Cong Y, Liu Y, Li Y, Yu Y, Cheng Y, Liu Y. Single nucleotide polymorphism rs3774261 in the AdipoQ gene is associated with the risk of coronary heart disease (CHD) in Northeast Han Chinese population: a case-control study. Lipids Health Dis 2016; 15: 6.

4) Altabas V, Altabas K, Kirigin L. Endothelial progenitor cells (EPCs) in ageing and age-related diseases: how currently available treatment [illegible] affect EPC biology, atherosclerosis [illegible] vascular outcomes. Mech Ageing [illegible] 2016; 159: 49-62.

5) Ankeny RF, Ankeny CJ, Nerem RM, [illegible] Maturing EPCs into endothelial cells[illegible]y the [illegible] with the EPCs: focus on "Fl[illegible]near stres[illegible] differentiation of circu[illegible] phenotype e[illegible] lial progenitor cells[illegible] J Ph[illegible] Cell Ph[illegible] 2012; 303: C589-5[illegible]

6) Lacquaniti A, G[illegible] S, Mes[illegible], Bue-mi A, Risitano[illegible] Chirico[illegible] M, [illegible] A. Neutrophil ge[illegible]se-associat[illegible] (NGAL) and end[illegible]progenitor ce[illegible]Cs) evaluation in[illegible] a[illegible]m repair. Cu[illegible] Vasc Pharmacol 201[illegible], 11: 100[illegible]

7) [illegible]ham MW, W[illegible] JM, Amaral L, Usry N, [illegible]t G, Dechend R, [illegible]Marca B. Agonistic autoantibodies to the angiotensin II type 1 receptor enhance[illegible]iotensin ii-induced renal vascular sensitivity[illegible]d reduce renal function during [illegible]regnancy. H[illegible]rtension 2016; 68: 1308-1313.

8) [illegible]Yan[illegible]ang X, Zheng Y, Jin Z, Zhi J. Role of ag[illegible]antibodies against type-1 angiotensin II receptor in the pathogenesis of retinopathy in preeclampsia. Sci Rep 2016; 6: 29036.

9) [illegible]V, Zhang W, Jin Z, Feng Y, Kuang Y, Zhi J. Angiotensin II type I receptor agonistic autoantibody-induced apoptosis in neonatal rat cardiomyocytes is dependent on the generation of tumor necrosis factor-α. Acta Biochim Biophys Sin 2012; 44: 984-990.

10) Dechend R, Viedt C, Müller DN, Ugele B, Brandes RP, Wallukat G, Park JK, Janke J, Barta P, Theuer J, Fiebeler A, Homuth V, Dietz R, Haller H, Kreuzer J, Luft FC. AT1 receptor agonistic antibodies from preeclamptic patients stimulate NADPH oxidase. Circulation 2003; 107: 1632-1639.

11) Sestito A. Hypertension therapy and cardiovascular protection. Effects of angiotensin II receptor block with Valsartan. Eur Rev Med Pharmacol Sci 2011; 15: 1247-1255.

12) Czechowska G, Celinski K, Korolczuk A, Wojcicka G, Dudka J, Bojarska A, Madro A, Brzozowski T. The effect of the angiotensin II receptor, type1 receptor antagonists, losartan and telmisartan, on thioacetamide-induced liver fibrosis in rats. J Physiol Pharmacol 2016; 67: 575-586.

13) Huang LY, Shau WY, Chen HC, Su S, Yang MC, Yeh HL, Lai MS. Pattern analysis and variations in the utilization of antihypertensive drugs in Taiwan: a six-year study. Eur Rev Med Pharmacol Sci 2013; 17: 410-419.

14) Liu L, Geng J, Zhao H, Yun F, Wang X, Yan S, Ding X, Li W, Wang D, Li J, Pan Z, Gong Y, Tan X, Li Y. Valsartan reduced atrial fibrillation susceptibility by inhibiting atrial parasympathetic remodeling

through MAPKs/Neurturin pathway. Cell Physiol Biochem 2015; 36: 2039-2050.

15) Ramadan R, Dhawan SS, Binongo JN, Alkhoder A, Jones DP, Oshinski JN, Quyyumi AA. Effect of angiotensin II type I receptor blockade with valsartant on carotid artery atherosclerosis: a double blind randomized clinical trial comparing valsartan and placebo (EFFERVESCENT). Am Heart J 2016; 174: 68-79.

16) Xu WP, Yao TQ, Jiang YB, Zhang MZ, Wang YP, Yu Y, Li JX, Li YG. Effect of the angiotensin II receptor blocker valsartan on cardiac hypertrophy and myocardial histone deacetylase expression in rats with aortic constriction. Exp Ther Med 2015; 9: 2225-2228.

17) Wu B, Lin R, Dai R, Chen C, Wu H, Hong M. Valsartan attenuates oxidative stress and NF-kappaB cativation and reduces myocardial apoptosis after ischemia and reperfusion. Eur J Pharmacol 2013; 705: 140-147.

18) Jung KH, Chu K, Lee ST, Kim SJ, Song EC, Kim EH, Park DK, Sinn DI, Kim JM, Kim M, Roh JK. Blockade of AT1 receptor reduces apoptosis, inflammation, and oxidative stress in normotensive rats with intracerebral hemorrhage. J Pharmacol Exp Ther 2007; 322: 1051-1058.

19) Giles TD, Bakris G, Oparil S, Weber MA, Li H, Mallick M, Bharucha DB, Chen C, Ferguson WG. Correlations of plasma renin activity and aldosterone concentration with ambulatory blood pressure responses to nebivolol and valsartan, alone and in combination, in hypertension. J Am Soc Hypertens 2015; 9: 845-854.

20) Konoshita T, Kaeriyama S, Urabe M, Nakaya T, Yam... M, Ichikawa M, Yamamoto K, Sato ...gawa M, F... M, Makino Y, Zenimaru Y, Waka...ki J, Ishizu...ka T, Nakamura H. On the t... ARB ... type Ca channel blocker leads t... elevat... of aldosterone. Biosci Rep 201... pii: e0...

21) Li WJ, Li Z, Chen Y... Li S... Zhou W..., Liao MY, Zhu F, Z... EH, Che... Zeng QT, Liao YH, Wei YM. A... tibodies tar... AT1 receptor from pati... acute coron... yndrome upregulate... infla... ory cytokine... expression in endothelial cells in... NF-κB pathway. Clin Dev I... ol 2014; 10:... 167.

22) Yuan Q, Lyu C, Wu C, Lei S, Shao Y, Wang Q. Valsartan inhibits angiotensin II-notch signaling of mesangial cells induced by high glucose. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2016; 32: 5-9.

23) Zhang S, Ong CN, Shen HM. Critical r... tracellular thiols and calcium in pa... duced apoptosis in human col... al cancer cells. Cancer Lett 2004; 208: 14...

24) Prasad A, Bloom MS, Carpenter D... of calcium and ROS in cell dea... induced ...lyunsaturated fatty acids in n... thymocy... Physiol 2010; 225: 82...

25) Ding W, Yang L, Zh... l, Gu Y... active oxyg... species-mediated ...lasm... eticulum stress contributes to ...dos... duced ...ptosis in tubular e... al cells ...hem ...lys Res Commun ... 418: 451-45...

26) Liu H, T... ng Y, Wu J, ... H, Fan J, Liao J. Re... e oxy... pecies-mediated endoplasmic reticulum str... d mitochondrial dysfunction ...tribute to po... -induced apoptosis in ... nasopharyngea... rcinoma CNE cells. J Cell Biochem 2011; 112: 3695-3703.

... Peng PA, Wa... Ma Q, Xin Y, Zhang O, Han HY, ...iu XL, Ji QW, ...u YJ, Zhao YX. Valsartan protects ...-2 cells fr... contrast media-induced apoptosis ... g endoplasmic reticulum stress. C... 015; 39: 1408-1417.

28) Lim JY, Kim D, Kim BR, Jun JS, Yeom JS, Park JS, Seo ...H, Park CH, Woo HO, Youn HS, Baik SC, Lee WK, ...MJ, Rhee KH. Vitamin C induces apoptosis in A... cells via production of ROS of mitochondria. Oncol Lett 2016; 12: 4270-4276.

29) Suntiparpluacha M, Tammachote N, Tammachote R. Triamcinolone acetonide reduces viability, induces oxidative stress, and alters gene expressions of human chondrocytes. Eur Rev Med Pharmacol Sci 2016; 20: 4985-4992.

30) Cerezo-Guisado MI, Zur R, Lorenzo MJ, Risco A, Martín-Serrano MA, Alvarez-Barrientos A, Cuenda A, Centeno F. Implication of Akt, ERK1/2 and alternative p38MAPK signalling pathways in human colon cancer cell apoptosis induced by green tea EGCG. Food Chem Toxicol 2015; 84: 125-132.