EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 5
464-469
Year: 2019

VALSARTAN INHIBITS PROLIFERATION OF MESANGIAL CELLS BY DOWN-REGULATING mRNA AND PROTEIN EXPRESSION OF ANGIOTENSIN II TYPE 1 RECEPTOR

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Article Received on 21/03/2019 **Article Revised on 10/04/2019** **Article Accepted on 01/05/2019**

ABSTRACT

Background and aims. Angiotensin II type 1 receptor activation has been shown to be associated with glomerular injury and glomerular disease. Therefore, the aim of the present study was to investigate the effect of the angiotensin II type 1 receptor antagonist valsartan on angiotensin II type 1 receptor expression in cultured rat mesangial cells. **Methods:** Rat mesangial cells were treated with one of the following: (1) control; (2) solvent; (3) valsartan (10−6mol/L, 10−5mol/L) for 14 hr. The proliferation of rat mesangial cells was assessed by 3- (4,5-dimethyltiazol-2-yl)-2,5-diphenyl tetrazolium bromide. The expression of angiotensin II type 1 receptor mRNA was determined by semi-quantitative real-time PCR assay and protein levels were detected by western blotting. **Results:** Valsartan inhibited cell proliferation and down-regulated mRNA and protein expression of angiotensin II type 1 receptor in rat mesangial cells. These levels were significantly lower than control group ($P<0.01$). **Conclusion:** Because angiotensin II type 1 receptor has a central role in the development and progression of glomerular diseases, valsartan down-regulation of angiotensin II type 1 receptor expression of mesangial cells may provide beneficial effects to the glomerular injury.

KEYWORDS: Valsartan, Angiotensin II type-1 receptor, Mesangial cells, Proliferation, Expression.

INTRODUCTION

The renin-angiotensin system (RAS) has been recognized as playing an inmportant role in the progression of renal diseases. Its primary effector hormone, angiotensin II (Ang II) plays a central role in renal tissue damage by activating Ang II type I receptor (AT1R).[1,2] Activation of RAS, renal inflammation and oxidative stress are characteristic findings in the pathogenesis of renal diseases. Ang II promotes inflammatory response and reactive oxygen species (ROS) production via AT1R).[3-5]

Currently, there is accumulating evidence that pharmacological interference with the chronic activation of Ang II represents an important therapeutic target, which may provide incremental end-organ protection. In particular, the use of AT1R blockers, solely or in combination with other regimens, may prove beneficial in opposing the detrimental effects of AT1R in the progression of renal diseases.[6] It has been reported that Candesartan suppresses chronic renal inflammation by a novel antioxidant action independent of AT1R blockade.[7]

Valsartan is an antihypertensive drug that blocks AT1R.[8,9] Previous studies have shown that valsartan alleviates cyclosporine A-induced tubular toxicity by upregulation of renal glutathione peroxidase expression and by altering oxidative stress in rats.[10]

Our recent study demonstrated that valsartan significantly reduced ROS formation and protected kidneys from injury in doxorubicin-induced glomerular toxicity.[11] In order to further understand whether valsartan plays a role on proliferation and AT1R expression of mesangial cells, we examined the effects of valsartan on the proliferation and AT1R expression by utilized culture rat mesangial cells in the present study. To the best of our knowledge this is the first study of its kind to observe the effects of valsartan on the proliferation and AT1R expression of culture mesangial cells.

MATERIALS AND METHODS

Cell culture

Sprague–Dawley rat mesangial cell lines (China Type Culture Collection. Wuhan, China) were maintained at

37^0C in a humidified incubator with 5% CO_2/95% air and propagated in Dulbecco's Modified Eagle Medium containing 100 mg/dl d-glucose, 10% fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mmol/l supplemental glutamine.Cell lines were passaged once per week after treatment with trypsin-ethylenediaminetetraacetic acid. Cells were used for experiments from passages 5–15.[12]

Cells proliferation assay

The viability of rat mesangial cells was assessed by 3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyl tetrazolium bromide. In brief, cells were plated in 96-well tissue culture plates at a density of 4×104 cell/ml. Cell cultures were washed twice with phosphate buffered saline (PBS), followed by a final incubation in serum-deprived medium. During this final incubation, mesangial cells were treated with one of the following: (1) control; (2) solvent (methanol solution); (3) valsartan (10^{-6}mol/L, 10^{-5}mol/L) (St. Louis, MO, USA) for 14 hr. Each concentration was tested for at least six replicates. At the end of each treatment, 10 µl 3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyl tetrazolium bromide (5mg /ml) was added to each well and the plates were incubated at 37 °C for another 3 hr. The purple formazan crystals were dissolved in 50 µl sodium dobecyl sulphate (10%), and absorbance was determined at 565 nm using a Stat Fax 2100 microplate reader. Cell viability was calculated as follows: Inhibition rate (%) = (Absorbance of control wells-Absorbance of treated wells) /Absorbance of treated wells×100.[12]

Semi-quantitative real-time PCR analysis

Rat mesangial cells were treated with one of the following: (1) control; (2) solvent; (3) valsartan (10^{-6}mol/L, 10^{-5}mol/L) for 14 hr. Each concentration was tested for at least three experiments. RNA was extracted with the One Step Trizol kit. Primer sequences were as follows: AT1R (sense: 50′-CCCGGATGCGTACCTAAGGA-30′, antisense: 50′-CGGACGTTGCTTCGCTGT-30′) and β-actin (sense: 50′-AGCCAACTCTCACTGAAGCC-30′, antisense: 50′-GCCAACACGTGGATGCTC-30′). β-actin was used as an internal control for sample normalization. cDNA steps were omitted. PCR reaction: reaction system was the total system 25µL including SYBR Green mix 12.5µL, primer (5pmol/µL) 2µL, cDNA (10-fold dilution) 2.5µL, ddH_2O 8µL; the conditions were as follows: 50℃ for 2min, 95℃ for 2min, 95℃ for 15s, annealing for 15s, 72℃ for 45s, 72℃ for 10min, 40 cycles.[13, 14]

Western blotting analysis

Cell groups and treatments were the same as in real-time PCR analysis. Cells were washed in PBS and then lysed in 1 ml of 1% Nonidet P-40, 25 mM Tris-HCl, 150 mM NaCl, 10 mM EDTA, pH 8.0, containing a 1:50 dilution of a protease inhibitor cocktail for 30 min on ice. Samples were centrifuged at 14,000 g for 5 min to pellet cell debris. Samples (20µg) were mixed with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, boiled for 5min, electrophoresed on a 10% SDS polyacrylamide gel, and electro-blotted onto Hybond-ECL nitrocellulose membrane. The membrane was blocked in PBS containing 5% skimmed milk powder and 0.02% Tween 20. To detect AT1R, the membrane was incubated for1hr with goat polyclonal antibody (Santa Cruz, California, USA) to AT1R. After washing, the membrane was incubated with a 1:20,000 dilution of peroxidase-conjugated goat anti-mouse IgG (Santa Cruz, California, USA) in PBS containing 1% normal goat serum and 1% fetal calf serum. The blotting was then developed using the ECL detection kit to produce a chemiluminescence signal, which was captured on x-ray films. Equal loading of proteins was confirmed based on immunoblotting with an antibody against β-actin (Santa Cruz, California, USA).[13, 14]

Statistical analysis: The results are presented as means ± standard error of mean (Mean ± SEM). One-way analysis of variance (ANOVA) and Tukey's tests were used to analysis the data. $P<0.05$ was considered significant.

RESULTS

Effects of valsartan on the proliferation of rat mesangial cells

As shown in Figure 1, valsartan at both concentrations (10^{-6}, 10^{-5}mol/L) significantly suppressed cell proliferation in rat mesangial cells as compared with the control group (*P<0.01*).

Valsartan down-regulated mRNA and protein expression of AT1R in rat mesangial cells

Figure 2 showed that AT1R mRNA expression was significantly decreased in rat mesangial cells after treatment for 14 hr with valsartan at both concentrations (10^{-6}, 10^{-5}mol/L) (*P<0.01*). The level of protein expression of AT1R in valsartan group was also significantly lower in comparison with control group (*P<0.01*) (Figures 3).

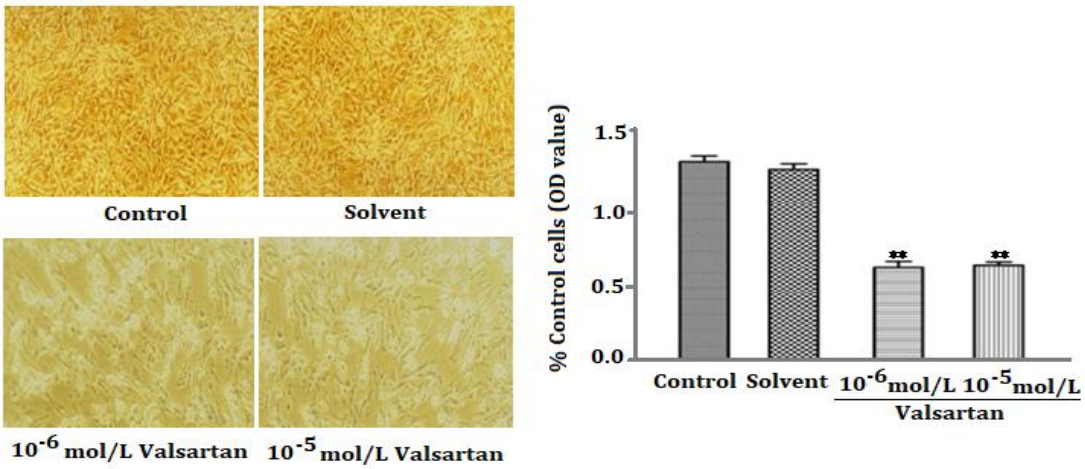

Figure 1: Effects of valsartan on the proliferation of rat mesangial cells. The viability of rat mesangial cells was assessed by 3-(4, 5-dimethyltiazol-2-yl) -2, 5-diphenyl tetrazolium bromide. Data were expressed as mean ± SD for six replicates of each concentration. $^{**}P < 0.01$ compared with control group.

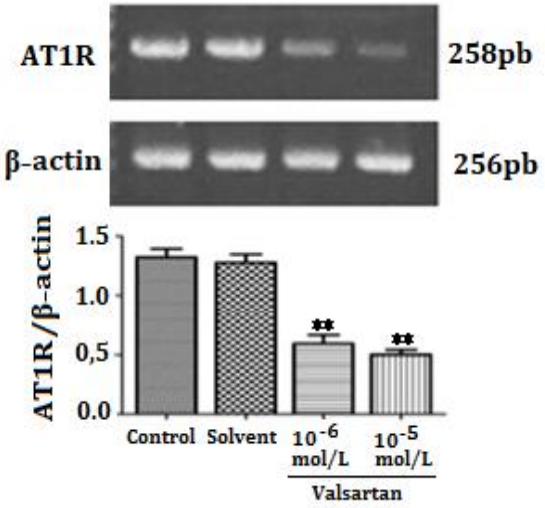

Figure 2: AT1R mRNA expression of rat mesangial cells by RT-PCR analysis. Data were expressed as mean ± SD for groups of three experiments. $^{**}P < 0.01$ compared with control group.

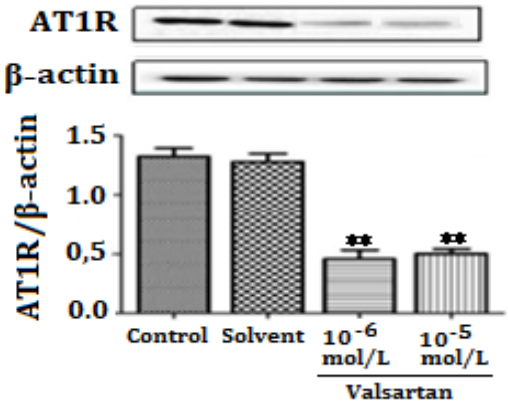

Figure 3. AT1R protein expression of rat mesangial cells by western blotting analysis. Data were expressed as mean ± SD for groups of three experiments. $^{**}P < 0.01$ compared with control group.

DISCUSSION

The kidney glomerulus has three intrinsic cell types: mesangial cells, endothelial cells and splanchnic wall epithelial cells. Mesangial cells are the most active cells among the three types of cells. They have many functions, including maintenance of glomerular capillary architecture and permeability of the glomerular filtration membrane, phagocytosis of macromolecules and immune complexes, production of bioactive substances, synthesis of an extracellular matrix, and secretion of variety of cytokines. In pathological conditions, abnormal proliferation, accumulation of extracellular matrix and collagen of mesangial cells are leading causes of glomerular injury.[15-17]

Ang II was previously reported as a stimulator of collagen and extracellular matrix synthesis in many cell lines such as mesangial cells and rat, porcine and human vascular smooth muscle cells.[18-20] Ang II signaling through AT1R has a critical role in the pathogenesis of nephropathy.[21] The blocking of AT1R by losartan decreases collagen expression at mRNA and protein levels, which corroborates clinical investigations indicating a beneficial effect of losartan in patients with vascular diseases and in experimental models of aortic pathology, since it reduces aortic hypertrophy and collagen accumulation in rat.[18, 22] Every known component of RAS is contained within mesangial cells, Ang II/AT1R can induce extracellular matrix accumulation and cell proliferation in rat renal mesangial cells.[23, 24] Previous studies showed that chronic activation of Ang II/AT1R is critical in the development of chronic kidney disease. Ang II/AT1R activates mesangial cells and stimulates the synthesis of collagen and extracellular matrix.[25] Additional research has also shown that angiotensin II induces extracellular matrix metalloproteinase inducer expression via an AT1R dependent pathway in aortic atherosclerotic plaque in apolipoprotein E knockout mice, valsartan could inhibit the effect of Ang II.[26]

Several previous reports indicate that valsartan plays a pivotal role in protecting against progressing renal tubule injury.[10, 27, 28] Our recent study demonstrated that valsartan significantly reduced ROS formation and protected kidneys from injury in doxorubicin-induced glomerular toxicity.[11] The results of our current study indicated that valsartan inhibited cell proliferation and down-regulated mRNA and protein expression of AT1R in rat mesangial cells. Valsartan inhibited cell proliferation by down-regulating AT1R expression in mesangial cells and that may provide beneficial effects by protecting the kidneys from glomerular injury.

CONCLUSION

The results of the present study, in combination with results that we reported earlier[11] suggest that valsartan may play a renoprotective role in glomerular injury, and that the mechanisms of this effect may involve down-regulation of AT1R expression in mesangial cells. Whether or not this mechanism also related to its antioxidant properties needs further investigation.

CONFLICT OF INTERESTS: The authors have no conflicts of interest regarding the content of this article.

ACKNOWLEDGEMENT: This work was supported by a research grant (No. 2017CFB210) from the Natural Science Foundation of Hubei Province of China. The results described in this paper were part of a student thesis.

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