Sulfatase 1 mediates the inhibitory effect of angiotensin II type 2 receptor inhibitor on angiotensin II-induced hypertensive mediator expression and proliferation in vascular smooth muscle cells from spontaneously hypertensive rats

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Background: Extracellular sulfatases (Sulfs), sulfatase 1 (Sulf1) and sulfatase 2 (Sulf2), play a pivotal role in cell signaling by remodeling the 6-O-sulfation of heparan sulfate proteoglycans on the cell surface. The present study examined the effects of Sulfs on angiotensin II (Ang II)-induced hypertensive mediator expression and vascular smooth muscle cells (VSMCs) proliferation in spontaneously hypertensive rats (SHR).

Methods: Ang II receptors, 12-lipoxygenase (12-LO), and endothelin-1 (ET-1) messenger RNA (mRNA) expressions in SHR VSMCs were analyzed by real-time polymerase chain reaction and Western blotting. VSMCs proliferation was determined by [3H]-thymidine incorporation.

Results: Basal Sulfs mRNAs expression and enzyme activity were elevated in SHR VSMCs. However, Sulfs had no effect on the basal or Ang II-induced 12-LO and ET-1 mRNA expression in SHR VSMCs. The inhibition of Ang II-induced 12-LO and ET-1 expression by blockade of the Ang II type 2 receptor (AT2 R) pathway was not observed in Sulf1 siRNA-transfected SHR VSMCs. However, Sulf2 did not affect the action of AT2 R inhibitor on Ang II-induced 12-LO and ET-1 expression in SHR VSMCs. The down-regulation of Sulf1 induced a reduction of AT2 R mRNA expression in SHR VSMCs. In addition, the inhibition of Ang II-induced VSMCs proliferation by blockade of the AT2 R pathway was mediated by Sulf1 in SHR VSMCs.

Conclusion: These findings suggest that extracellular sulfatase Sulf1 plays a modulatory role in the AT2 R pathway that leads to an Ang II-induced hypertensive effects in SHR VSMCs.

Keywords: Sulfatases; Angiotensin II type 2 receptor; Hypertension

INTRODUCTION

Extracellular sulfatases (Sulfs) are hydrolytic enzymes that regulate cell metabolism and signaling [1]. There are 17 distinct sulfatases in humans and 14 sulfatases in rodents. Sulfa-

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Therefore, altered Sulfs activity plays an important role in both cell survival and proliferation. In addition to cell signaling, Sulfs modulate various cellular processes, such as cell development, tumor growth, muscle regeneration, neuromodulation, and immunomodulation [1,5-7]. Mice with double knockout Sulf1 and Sulf2 show significant developmental defects and reduced body weight [8,9]. Although Sulf1 and Sulf2 are structurally similar and modulate cell signaling, they have opposite effects in tumor cells. Sulf1 in cancer cells is known to inhibit angiogenesis and proliferation [10,11]. Contrarily, Sulf2 has pro-angiogenic and tumorigenic effects [12,13]. Therefore, Sulfs have been proposed to be novel reagents for modifying HSPGs or therapeutic targets for cancer treatment.

The pathophysiological roles of Sulfs in the development or maintenance of hypertension have not yet been fully elucidated. HSPGs are found in vascular walls, and reduced HSPG levels in the glomerular basement membrane are associated with essential hypertension [14]. Sulfs are known to regulate 6-O-sulfation of HSPGs; thus, they have been associated with the mediation of several functions of vascular smooth muscle cells (VSMCs) [13,15]. Sulfs may play a functional role in the development and maintenance of hypertensive vasculature since expression of hypertensive mediators and proliferation of VSMCs are important pathologic features of hypertension.

To the best of our knowledge, there have been no studies evaluating the relationship between Sulfs and the development and maintenance of hypertension. Although Sulfs have been established to mediate cell signaling and proliferation, the exact mechanism of Sulfs in hypertensive VSMCs have not been fully elucidated. Therefore, as a preliminary study to determine the relationship between Sulfs activity and pathophysiological process of hypertensive cells, we examined the effects of Sulfs on angiotensin II (Ang II)-induced hypertensive mediators, 12-lipoxygenase (12-LO), and endothelin-1 (ET-1) expression, as well as cell proliferation in VSMCs from spontaneously hypertensive rats (SHR).

MATERIALS AND METHODS

1. Reagents

Total RNA extraction kit was purchased from iNtRON (Biotechnology Inc, Seoul, Korea). Dulbecco’s Modified Eagle’s medium (DMEM), fetal bovine serum (FBS) and penicillin-streptomycin were purchased from Lonza (Walkersville, MD, USA). Ang II was purchased from Calbiochem (San Diego, CA, USA). Losartan and PD123319 were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). LightCycler FastStart DNA SYBR Green I Mix was purchased from Roche (Mannheim, Germany). Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA, USA). Primer oligomers for Ang II type 1 receptor (AT1 R), Ang II type 2 receptor (AT2 R), Sulf1, Sulf2, 12-LO, ET-1, and β-actin were synthesized at Bionics (Daejeon, Korea). 12-LO and ET-1 antibodies were purchased from Santa Cruz Biotechnology (California, USA). AT2 R antibody was purchased from Abcam (Cambridge, UK). Monoclonal anti-γ-tubulin antibody was purchased from Sigma-Aldrich (St. Louis, MO, USA). Rat Sulf1 small interfering RNA (siRNA) oligomers were purchased from Bioneer technology (Daejeon, Korea). Rat Sulf2 siRNA oligomers were purchased from Santa Cruz Biotechnology (California, USA). Negative control siRNA was purchased from Invitrogen (Carlsbad, CA, USA). All other reagents used in this study were pure-grade commercial preparations.

2. Animals

Specific pathogen-free, inbred normotensive, male Wistar-Kyoto rats (WKY) and SHR, all aged 22-weeks, were purchased from Japan SLC Inc. (Shizuoka, Japan). All experimental animals were fed autoclaved food and received bedding in order to minimize exposure to microbial pathogens. This experiment was approved by the Institutional Animal Care and Use Committee of the Yeungnam University College of Medicine (protocol no. YUMC-AEC2013-006).

3. Preparation of VSMCs

VSMCs were isolated from the thoracic aortas of these rats, following the explant method [16]. VSMCs were cultured in DMEM, which had been supplemented with 10% FBS and 1% penicillin-streptomycin. The cells were detached with 0.25% trypsin/EDTA and seeded into 75 cm² tissue culture flasks at a density of 105 cells/mL. All experiments were conducted during cell passages 3 to 7. Prior to stimulation, 95% confluent VSMCs were serum-starved overnight in