Anti-inflammatory Effect of Spironolactone on Human Peripheral Blood Mononuclear Cells

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Abstract. We evaluated the effect of alacepril, CV-11974, and spironolactone on the production of monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-alpha (TNF-α) in cultured human peripheral blood mononuclear cells stimulated with angiotensin (Ang) II. Alacepril, CV-11974, and spironolactone significantly reduced the enhanced production of MCP-1 and TNF-α induced by exogenous Ang II. Specifically, 10 μM of spironolactone significantly reduced cytokine production, compared to the same dose of alacepril or CV-11974. These findings indicate that spironolactone may contribute to ameliorate the prognosis of patients with cardiovascular diseases by reducing Ang II-induced inflammation, although further exploration including determining the mechanisms would be required.

Keywords: spironolactone, angiotensin II, peripheral blood mononuclear cell

Inflammation is one of the critical pathogenetic factors of cardiovascular diseases, including heart failure (1), myocarditis, atherosclerosis, and rupture of atherosclerotic plaques (2, 3). Therefore, therapeutic strategies to reduce inflammation should be preferentially explored to ameliorate the prognosis of patients with cardiovascular diseases (2, 4). Blockers of renin-angiotensin-aldosterone (R-A-A) system have improved their outcomes (5–8), and anti-inflammatory potencies of R-A-A blockers partly contribute to the results (4). In the present study, anti-inflammatory potencies of three R-A-A blockers, alacepril (an angiotensin (Ang)-converting enzyme inhibitor (ACEI)), CV-11974 (an Ang II type 1 receptor (AT1R) antagonist (AT1A)), and spironolactone (a mineralocorticoid receptor (MR) antagonist) were compared by the reduction rates of enhanced monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-alpha (TNF-α) production induced by Ang II in cultured human peripheral blood mononuclear cells (PBMCs).

Human PBMCs were obtained from 10 healthy adult volunteers (6 men and 4 women, aged from 26 to 37). The Cardiovascular Research Ethics Committee of Okayama University Graduate School of Medicine, which conforms to the Declaration of Helsinki, approved the protocol and all volunteers gave written informed consent. PBMCs were isolated by density centrifugation with lymphocyte separation medium (Cappel, Aurora, CA, USA). The collected cells were washed 3 times with PBS, resuspended in a conditioned medium consisting of RPMI 1640 (Sigma, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (Sigma), 50 μM of 2-mercaptoethanol, and 100 μg/ml of kanamycin, and then they were incubated at 37°C in humidified air with 5% CO2 (9). Alacepril (an ACEI) was provided by Dainippon Pharmaceutical Co. (Osaka); CV-11974 (an AT1A, an active metabolite of candesartan) by Takeda Pharmaceutical Co. (Osaka); and spironolactone (a MR antagonist) by Fujisawa Pharmaceutical Co. (Osaka). Ang II was purchased from Sigma. Alacepril, CV-11974, and spironolactone were dissolved in methanol at 1 mM, and Ang II was dissolved in sterile water at 1 mM. Finally, each reagent
was further diluted to the desired concentrations and added to the conditioned medium.

PBMCs (5 × 10^7/ml) were incubated with 0, 100 nM, 1 µM, or 10 µM of alacepril, CV-11974, or spironolactone for 30 min, and then 10 µM of Ang II was administered for subsequent inflammatory reactions (10, 11). After incubation for 48 h, the supernatants were collected, filtered with 0.45-µm millipore filters and stored at −80°C until use. Concentrations of MCP-1 and TNF-α were measured by using BioSource ELISA kits in accordance with the manufacturer’s instructions (BioSource, Camarillo, CA, USA) and the results are expressed as means ± S.E.M. Statistical analysis was performed by ANOVA, with comparison of different treatment groups by Fisher’s protected least significant difference test. Values of P<0.05 were considered to be significant.

Ang II enhanced the production of MCP-1 and TNF-α in cultured human PBMCs. CV-11974 and spironolactone significantly reduced the enhanced MCP-1 production compared to alacepril (Fig. 1). In addition, CV-11974 and spironolactone significantly reduced the enhanced TNF-α production compared to alacepril at the doses of 1 and 10 µM (Fig. 2). Furthermore, spironolactone reduced the production of MCP-1 and TNF-α, compared to CV-11974 at the dose of 10 µM (Figs. 1 and 2).

Spironolactone significantly reduced Ang II-induced cytokine production in cultured human PBMCs no less efficaciously than CV-11974. To our knowledge, this is the first report about anti-inflammatory effect of spironolactone on human PBMCs stimulated with Ang II. Spironolactone blocks mineralocorticoids, especially aldosterone, with subsequent reduction of mineralocorticoids-induced cardiovascular damage and inflammation. Another important property of spironolactone, that is, anti-inflammatory effect was revealed in the present study.

Clinical trials have shown that ACEI, AT1A, and/or MR antagonist therapy improved clinical outcomes of patients with cardiovascular diseases (5 – 8), and numerous investigators have reported the anti-inflammatory potencies of ACEI, AT1A, and MR antagonist. However, the first choice among the three R-A-A blockers remains controversial and there are only a few studies that compare the anti-inflammatory potencies.
of the three R-A-A blockers. Our results suggest that MR antagonist might have priority over ACEI or AT1A from the viewpoint of anti-inflammation.

Ang II induces the activation of NF-κB via phosphorylation of Akt (protein kinase B) and IκBζ in human PBMCs through Ang II receptors (12). NF-κB enhances gene expressions involved in inflammation with subsequent cytokine production such as MCP-1 and TNF-α. At the beginning of this study, our hypothesis was that AT1A would have the strongest anti-inflammatory effects because AT1A directly blocks Ang II to bind AT1R and reduces the downstream signals. The differences in the effectiveness between ACEI and AT1A are explained by the blockade of AT1R-dependent Ang II signals. However, this convincing mechanism cannot explain the strongest anti-inflammatory effect of spironolactone, that does not directly compete with Ang II for Ang II receptors. Alternatively, spironolactone competes with mineralocorticoids for MR and reduces gene activation mediated by NF-κB. Mechanisms via MR might contribute to anti-inflammatory effect of spironolactone, although we could not reveal the involvement of mineralocorticoids in the present study. Meanwhile, glucocorticoids bind to the glucocorticoid receptor (GR) and demonstrate anti-inflammatory effect via inhibition of gene activation mediated by NF-κB on human PBMCs. Spironolactone has a steroidal structure and may also bind to and interact with GR, resulting in an agonist or antagonist glucocorticoid activity. Couette et al. reported that spironolactone bound to GR with low affinity and behaved as an antagonist of GR (13). Whatever the case, the mechanisms still remain unclear how spironolactone reduces Ang II-induced inflammation and further exploration should be required.

Recent studies have shown that inflammation is one of the key players in the pathogenesis of atherosclerosis and rupture of atherosclerotic plaques (2, 3). A common finding in atherosclerotic lesions (especially ruptured sites) is the infiltration of inflammatory cells, including monocytes, macrophages, and lymphocytes, as well as the proliferation of smooth muscle cells (SMCs). In these cells, Ang II enhances the production of cytokines such as MCP-1 and TNF-α (12), which are deeply involved in inflammation. MCP-1, one of the C-C chemokines, is a powerful monocyte chemoattractant and plays roles in the activation and migration of monocytes towards inflammatory lesions (12), as well as in the proliferation and dedifferentiation of SMCs (14). TNF-α is one of the proinflammatory cytokines that enhances the production of other inflammatory cytokines including MCP-1 (15). Both TNF-α and MCP-1 make atherosclerotic plaques unstable, resulting in acute coronary syndrome (3). Spironolactone might suppress the progression of atherosclerosis and the instability of atherosclerotic plaques in vivo because it decreases the production of MCP-1 and TNF-α.

In conclusion, spironolactone efficaciously reduced Ang II-induced inflammation in human PBMCs, although the mechanisms still remain unclear and further exploration will be required.

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References