（第３号様式）

学 位 論 文 要 旨

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論　文　名　　アンジオテンシン II ２型受容体によるPPARγ活性化を介した虚血性脳障害抑制作用

学位論文要旨

Background: The renin-angiotensin system (RAS) plays a crucial role in brain damage after ischemic stroke. Angiotensin (Ang) II, a key player of the RAS binds with high affinity to two distinct receptors: Ang II type 1 (AT1) receptor and Ang II type 2 (AT2) receptor. The AT2 receptor was shown to be up-regulated in the rat brain after transient ischemic stroke, and the stimulation of AT2 receptor has a protective effect against brain injury after ischemia. A newly developed selective and potent non-peptide direct AT2 receptor agonist, compound 21 (C21) has contributed to revealing neuroprotection in experimental ischemic stroke. In the pre-clinical phase, C21 showed strong potential to be developed as treatment for stroke because of its oral availability and anticipated minimal effect on blood pressure. Peroxisome proliferator-activated receptor-gamma (PPAR-γ) which is a nuclear transcription factor and plays an important role in diabetes and atherosclerosis development, has been suggested to exert neuroprotective role after ischemic stroke recently, confirming by both animal and clinical studies. We recently demonstrated that AT2 receptor stimulation by C21 inhibited vascular intimal proliferation with activation of PPAR-γ, and that AT2 receptor-interacting protein maybe involved in AT2 receptor-induced PPAR-γ complex formation. We and others have suggested that AT1 receptor blockade mediated its beneficial effect on ischemic stroke through PPAR-γ pathway, in addition to activate relative AT2 receptor signaling. Therefore, we examined the possibility that direct AT2 receptor stimulation by C21 protects against ischemic brain injury at least by stimulating PPAR-γ activation.

Methods: Eight-week-old male C57BL/6J mice were subjected to middle cerebral artery (MCA) occlusion. Two weeks before MCA occlusion, they were administered C21 intraperitoneally at 10 μg/kg/day with or without GW9662, a PPAR-γ antagonist in drinking water at 0.35 mg/kg/day. Body weight and systolic blood pressure (SBP) were measured two weeks after treatment with C21 with or without GW9662. The brain samples were obtained 24 hours after MCA occlusion. Before brain sampling, neurologic deficit was evaluated using the neurologic score by Longa method. The brain samples were

sliced into seven coronal sections with 1 mm- thickness and immediately stained with 2% 2,3,5-triphenyltetrasodium chloride to measure ischemic area. Superoxide anion production was detected by dihydroethidium staining by employing frozen brain sections. Superoxide dismutase (SOD) activity was investigated by using a SOD assay kit-WST. mRNA expressions of AT1 receptor, AT2 receptor, SOD (SOD1, SOD2, SOD3), NADPH oxidase subunits ($p22^{phox}$, $p40^{phox}$, $p47^{phox}$, $p67^{phox}$, gp91), blood brain barrier (BBB) stabilization genes (Occludin, Claudin-5, ZO-1), eNOS and VEGF were measured by real-time RT-PCR. Cerebral blood flow (CBF) was monitored in the core and periphery of the MCA territory before, immediately after, 1 hour and 24 hours after MCA occlusion with a two-dimensional laser speckle blood flow imager.

Results: Body weight and SBP were not significantly changed by C21 with or without GW9662 treatment. After MCA occlusion, ischemic area in brain section was observed in the non-treated mice, and the maximal ischemic area was about 25% of the total area around section 3, which is the main territory of the MCA. The mice with C21 treatment exhibited significant decreases in neurological score and ischemic area compared with the non-treated mice. CBF decreased immediately after MCA occlusion, and this reduction continued for at least 24 hours in non-treated mice. Treatment with C21 significantly ameliorated CBF 24 hours after MCA occlusion both in the core and peripheral region compared with the non-treated group. Moreover, the mice with C21 treatment showed a decrease in superoxide anion production, an increase in BBB stabilization genes and a ratio of SOD activity on the ipsilateral to contralateral side compared with the non-treated mice. Co-administration of GW9662 partially attenuated such ameliorative effects of C21 on neurologic deficit and ischemic size by inhibiting the decreased superoxide anion production and the increased CBF, SOD activity and BBB stabilization, while GW9662 treatment alone had no significant effects on neurologic deficit and ischemic size. C21 with or without GW9662 administration did not significantly affect mRNA expression of AT1 receptor, AT2 receptor, SOD and NADPH oxidase subunits.

Conclusion: These results suggested that direct AT2 receptor stimulation by C21 has a preventive effect on stroke-induced brain injury partly due to activation of PPAR-γ.

キーワード（３～５）	angiotensin II type 2 receptor compound 21 oxidative stress peroxisome proliferator-activated receptor-gamma ischemic brain injury