Deleterious Effect of All Nitric Oxide Synthases in Cerebral Infarction in Male Mice: Involvements of Sex Difference and Testosterone

Masato Tsutsui\textsuperscript{1}, Haruaki Kubota\textsuperscript{1,4}, Mayuko Sakanashi\textsuperscript{1}, Katsuhiko Noguchi\textsuperscript{1}, Toshihiro Matsuzaki\textsuperscript{1}, Junko Nakasone\textsuperscript{1}, Hiroaki Shimokawa\textsuperscript{2}, Yusuke Ohya\textsuperscript{3}, Kazuhiro Sugahara\textsuperscript{4}, Manabu Kakinohana\textsuperscript{4}

\textsuperscript{1}Department of Pharmacology, Graduate School of Medicine, University of the Ryukyus, Japan, \textsuperscript{2}Department of Cardiovasc Med, Tohoku Univ Grad Sch Med, Japan, \textsuperscript{3}Third Dept Intern Med, Ryukyu University Graduate School of Medicine, Japan, \textsuperscript{4}Department of Anesthesiology, Ryukyu University Graduate School of Medicine, Japan

Background: The role of each nitric oxide synthase (NOS) isoform in cerebral infarction (CI) has been studied in individual NOS isoform-deficient mice. In a model of middle cerebral artery occlusion (MCAO), neuronal and inducible NOSs exacerbate CI, whereas endothelial NOS conversely alleviates it. Although the role of all NOSs in CI has been examined in pharmacological studies with non-selective NOSs inhibitors, obtained results are inconsistent possibly because of non-specificity of the agents. We addressed this point in mice lacking all three NOSs.

Method and Results: We newly generated triple n/i/eNOSs-/ mice and wild-type (WT) littermates by crossbreeding each NOS-/ isoform-deficient mouse. In the male, CI size at 24 hours after transient (1 hour) MCAO was markedly smaller in the triple NOSs-/ genotype as compared with the WT genotype (P<0.05). Neurological deficit score and mortality rate were also significantly less in the triple NOSs-/ than in the WT genotype (each P<0.05). In contrast, in the female, the CI size at 24 hours after transient MCAO tended to be rather larger in the triple NOSs-/ than in the WT genotype. We compared the CI size between the male and the female in each genotype. In the WT genotype, the CI size was significantly larger in the male than in the female, whereas, in the triple NOSs-/ genotype, it was conversely significantly smaller in the male than in the female (each P<0.05). In the triple NOSs-/ genotype, ovariectomy had no effect on the CI size in the female, whereas orchiectomy significantly increased it in the male (P<0.05). This effect of orchiectomy was significantly inhibited by treatment with testosterone (P<0.05). Cyclopaedic and quantitative comparisons of mRNA expression levels in CI lesions between the male WT and the male triple NOSs-/ genotype (by RNA sequencing using a next generation sequencer) showed significant involvements of androgen signaling, mitochondrial dysfunction, and oxidative stress (each P<0.05).

Conclusions: These results provide the first evidence that all NOSs exert a deleterious effect in CI only in male mice through the testosterone-dependent pathway. Inhibition of the NOSs system may be a new therapeutic option in the treatment of male CI.