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PROTEINASE-ACTIVATED RECEPTOR-1 PLAYS A MAJOR ROLE IN THE VASOSPASM OF THE BASILAR ARTERY IN THE RABBIT DOUBLE SUBARACHNOID HEMORRHAGE MODEL

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Cerebral vasospasm is one of the major determinants of the prognosis in subarachnoid hemorrhage (SAH). However, the molecular mechanism of its pathogenesis still remains elusive. It has been suggested that thrombin plays an important role in the development of cerebral vasospasm in SAH. In the present study, we investigate the role of thrombin and its receptor, proteinase-activated receptor-1 (PAR-1), in the vasospastic responses of the basilar artery, using a rabbit double subarachnoid hemorrhage model. In the control rabbit, thrombin and PAR-1 activating peptide (PAR-1AP) slightly contracted the basilar artery at high concentrations (10 U/ml thrombin or 100 μM PAR-1AP). In SAH, the contractile responses to thrombin and PAR-1AP were greatly enhanced and observed at lower concentrations than those in the controls. Western blot analysis revealed that the expression of PAR-1 in the basilar artery was up-regulated in SAH. When PAR-1 antagonist was injected twice into the cisterna magna at the time of hemorrhage, the enhancement of the contractile response to thrombin was prevented, and the up-regulation of PAR-1 expression was attenuated. *Ex vivo* treatment with PAR-1 antagonist inhibited the contractile response to thrombin in the basilar artery of the untreated SAH. We thus conclude that PAR-1 plays a key role in the development of the vasospastic response of the cerebral artery to thrombin in subarachnoid hemorrhage. We propose PAR-1 antagonist to be a novel therapeutic strategy for prevention and treatment of vasospasm in SAH.

Key Words: smooth muscle cells, subarachnoid hemorrhage, thrombin PAR-1

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INHIBITORY ROLE OF REDOX FACTOR-1 ON THE VASCULAR INFLAMMATION

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Redox factor-1 (Ref-1) is a ubiquitous protein that is a redox-sensitive regulator of multiple transcription factors as well as an apurinic/apyrimidic endonuclase in the base excision repair pathway. Monocyte adhesion to vascular endothelial cells seems to be initial and crucial events in the pathological process of the inflammatory diseases, such as atherosclerosis. Recently, it has been uncovered extra-nuclear role of Ref-1 against the oxidative stress in the vascular cells. Therefore, we assessed the hypothesis that Ref-1 has an inhibitory action on the vascular inflammation in the vascular endothelial cells. Endothelial activation as a model of vascular inflammation in the cultured human umbilical vein endothelial cells was induced by the treatment of tumor-necrosis factor-alpha (TNF-α). Overexpression of Ref-1 in the endothelial cells was performed by the transfection with an adenovirus encoding human Ref-1 cDNA. Adenoviral overexpression Ref-1 significantly inhibited TNF-α-induced induction of vascular cell adhesion molecule-1 and monocyte adhesion on the endothelial cells. Ref-1-mediated suppression on the TNF-α-induced vascular cell adhesion molecule-1 was blocked by pretreatment of nitric oxide synthase inhibitor, L-nitroarginine methyl ester. Furthermore, overexpression of Ref-1 inhibited TNF-α-induced increase in the intracellular superoxide production by using the superoxide-sensitive fluorophore dihydroethidine. However, overexpression of Ref-1 did not affect the expression of copper/zinc superoxide dismutase in the endothelial cells. These data provide evidence that Ref-1 in the endothelial cells suppressed TNF-α-induced vascular cell adhesion molecule-1 and monocyte adhesion through a nitric oxide-dependent mechanism, and by inhibiting the intracellular oxidative stress in the endothelial cells. Considering the biological functions of Ref-1, endogenous vascular Ref-1 might serve an anti-inflammatory action.

Key Words: Ref-1, Vascular inflammation, ROS, VCAM-1