Role of a Decreased Expression of the Local Renin-Angiotensin System in the Etiology of Cerebral Aneurysms

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Background—Local renin-angiotensin systems (RAS) have been implicated as playing an important role in vascular remodeling. The relationship of this system to the etiology of cerebral aneurysm was investigated.

Methods and Results—The aneurysmal wall from patients with a ruptured or unruptured cerebral aneurysm and the cortical cerebral artery in control patients with head trauma or a glioma were taken during surgery for study. Local RAS were evaluated by reverse transcription-polymerase chain reaction (RT-PCR) and/or immunohistochemistry. RT-PCR analysis revealed a significantly decreased expression of angiotensin-converting enzyme (ACE), angiotensin type 1 (AT1) receptor, basic fibroblast growth factor, platelet-derived growth factor-AA, and tissue inhibitor of matrix metalloproteinases-1 mRNA in the aneurysmal wall as compared with the control cortical arterial wall. Immunohistochemistry also revealed a decreased expression of ACE, AT1 receptor, and angiotensin II in the aneurysmal wall.

Conclusion—Expression of local RAS was decreased in the aneurysmal wall, which may induce aneurysm formation caused by a lack of vascular remodeling that prevents the arterial wall from thickening under increased hemodynamic stress. This is the first report that suggests that a decreased expression of local RAS plays a part in the pathogenesis of any disease. (Circulation. 2003;108:785-787.)

Key Words: aneurysm ▪ angiotensin ▪ arteries

Local renin-angiotensin systems (RAS) have been implicated in playing an important role in several cardiovascular diseases by affecting cardiovascular remodeling via smooth muscle cell (SMC) migration, proliferation, and hypertrophy. However, the downregulation of this system has not, up to now, been associated with the pathogenesis of any disease.

Currently, subarachnoid hemorrhage caused by a rupture of cerebral aneurysms has a high morbidity and mortality. The etiology of cerebral aneurysms has been attributed to a disrupted balance between local hemodynamic stress and arterial wall strength. Under increased hemodynamic stress, the local RAS is usually activated, and this induces vascular remodeling resulting in a thickened arterial wall. However, paradoxical findings are seen in cerebral aneurysms. A thinning of the arterial medial smooth muscle layer due to a decrease in the number of SMCs coexists with the increased hemodynamic stress. This could be due to an abnormal conversion rate in the local RAS. The aim of this study was to investigate the expression of the local RAS in cerebral aneurysms and to evaluate their possible role in aneurysm formation.

Methods

Clinical Material
Thirty-three patients with ruptured or unruptured cerebral aneurysms who underwent direct microsurgical aneurysm repair were eligible for this study. During surgery, to confirm that the aneurysm was completely isolated from the circulation, the aneurysm dome was opened by dissecting the aneurysm wall, which was used for this study. Eighteen age-matched patients with head trauma or glioma served as controls. Their cerebral cortical arteries were taken for study. Eighteen age-matched patients with head trauma or glioma served as controls. Informed consent was obtained from each patient or the patient’s family, and the protocol was approved by the Institutional Human Ethics Committee.

mRNA Measurement for ACE, AT1 Receptor, Basic Fibroblast Growth Factor (bFGF), Platelet-Derived Growth Factor-AA (PDGF-AA), and Tissue Inhibitor of Matrix Metalloproteinases-1 (TIMP-1)
The dissected aneurysmal wall and the cortical cerebral artery were lysed and homogenized. Extracted total RNA was used for the reverse transcription (RT) reaction. The 576-µL reaction mixture containing 6 µL of the first-strand cDNA library and 15 units of Taq DNA polymerase were constructed and divided into 96-µL aliquots. Two microliters 25 µmol/L each of sense and antisense primer for ACE, AT1 receptor, bFGF, PDGF-AA, TIMP-1, and GAPDH mRNA were added to 1 of the 96-µL reaction mixtures, and polymerase chain reaction (PCR) was performed for 30 cycles under conditions in accordance with previous studies. RT-PCR reaction
mixture (20 μL) was size-fractioned by 2% agarose gel electrophoresis, using a DNA marker. The amplified bands were detected by ethidium bromide staining, and the images were transferred to a computer system by a charge-coupled device imaging system. The intensity of the ethidium bromide fluorescence of each band was measured by the NIH Image analysis system.

**Immunohistochemistry for ACE, AT1 Receptor, and Angiotensin II**

The specimens were sectioned into 4-μm slices and fixed in acetone. Sections were treated with 0.3% H2O2 solution for 30 minutes and were then incubated overnight at 4°C with the specific primary antibodies. The primary antibodies used in this study were rabbit polyclonal antibody against human angiotensin II (1:1000, IgG Corp, Nashville, Tenn), mouse monoclonal antibody against human ACE (1:1000, Chemicon International, Temecula, Calif), and rabbit polyclonal antibody against human AT1 receptor (1:1000, Santa Cruz, Calif). Additionally, to identify SMCs and endothelial cells, mouse monoclonal antibody against human α-smooth muscle actin (1:50, Dako Laboratories, Carpinteria, Calif) or rabbit polyclonal antibody against human von Willebrand factor (1:200, Dako Laboratories) was used as a primary antibody. The labeled streptavidin biotin method (Dako Laboratories) was used. The sections were counterstained with hematoxylin.

**Serum Renin Activity and Serum Angiotensin II, and Other Lipid Profiles**

Serum renin activity and serum angiotensin II were measured by radioimmunoassay using blood taken on admission. Serum HDL cholesterol, LDL cholesterol, and triglyceride were also examined on admission.

**Results**

**Serum Concentration of Renin and Angiotensin II and Lipid Profile**

The serum renin activity and the serum angiotensin II value showed no statistically significant differences among the patients with unruptured cerebral aneurysms, those with ruptured aneurysms, and the control patients. There were no statistically significant differences in HDL cholesterol, LDL cholesterol, and triglyceride among the 3 groups.

**mRNA Measurement for ACE, AT1 Receptor, bFGF, PDGF-AA, and TIMP-1**

The ratios of ACE, AT1 receptor, bFGF, PDGF-AA, and TIMP-1 mRNA to GAPDH mRNA in the unruptured and ruptured aneurysmal wall were statistically significantly lower as compared with the same ratio in the control cortical arterial wall (Table). The ratio of ACE mRNA to GAPDH mRNA in the ruptured aneurysmal wall was statistically significantly lower as compared with the same ratio in the ruptured aneurysmal wall.

**Immunohistochemistry for ACE, AT1, and Angiotensin II**

In the control cortical cerebral arteries, von Willebrand factor immunostaining for endothelial cells and SMC α-actin immunostaining for medial SMCs were well demarcated (Figure, A and B). In the unruptured aneurysmal wall, immunostaining was decreased (1 and J). In the ruptured aneurysmal wall, von Willebrand factor immunostaining was diminished (Q) and SMC α-actin immunostaining was markedly decreased (R). The control arterial wall revealed a distinct expression of ACE in endothelial cells that corresponded to the von Willebrand factor staining (C and D). Its immunostaining was stronger in the cases without hypertension (C) than in the cases with hypertension (D). ACE expression decreased in the unruptured aneurysmal wall (K) and diminished in the ruptured aneurysmal wall (P). Its expression showed no differences between the cases without hypertension and the cases with hypertension in the aneurysmal wall.
(data not shown). AT1 receptor and angiotensin II expressions were diffusely seen in the control cortical cerebral arteries (E through H), and their expressions were more marked in the cases without hypertension (E and G) than in the cases with hypertension (F and H). AT1 receptor and angiotensin II expression were decreased in the unruptured aneurysmal wall (L and M) and markedly decreased in the ruptured aneurysmal wall (Q and R) as compared with the control cortical arteries. Their expressions showed no differences between the cases without hypertension and the cases with hypertension in the aneurysmal wall (data not shown).

Discussion

The histological findings of cerebral aneurysms have been characterized as having a decreased number or degeneration of endothelial cells, degeneration of the internal elastic lamina, and thinning of the medial layer. The internal elastic lamina and vascular extracellular matrix are considered to be the main contributors to the structural integrity of vessel walls, and there have been several reports indicating that a decrease of the extracellular matrix is related to the etiology of cerebral aneurysms. Increased circulating levels of serum gelatinase or elastase in patients with cerebral aneurysms11 and increased matrix metalloproteinase in the aneurysmal walls3 have been found. As well, a genetic locus for cerebral aneurysms has been found to lie within or close to the elastin gene locus on chromosome 7.12

Another characteristic finding of aneurysmal walls is thinning of the medial layer consisting of a decrease in the number of SMCs.1,4 Recently, in both experimental1 and clinical5 studies, apoptosis of SMCs has been found to be a causative factor for the decreased SMC number. The results of this study also confirm a decrease of SMCs, which was the main causative factor for a decreased expression of local RAS. The etiology of aortic aneurysms has been associated with atherosclerosis induced by an upregulated RAS.13 However, there have been no reports supporting this association of atherosclerosis with the etiology of cerebral aneurysm. Nevertheless, there are some situations in which cerebral aneurysms could be associated with an upregulation of local RAS. Animal experimental studies have suggested that an initiating episode in cerebral aneurysm formation is degeneration and reduction in number of the endothelial cells,14 which is thought to be caused by maximum wall shear stress.15 The development of endothelial cell degeneration usually causes a proliferative response of the SMCs induced by upregulated local RAS. Furthermore, augmented hemodynamic stress, which has been implicated as an important causative factor for aneurysm formation, is thought to contribute to the upregulation of the local RAS. However, this study suggests that expression of local RAS is not increased. In situations in which local RAS should be upregulated as described above, if the expression of RAS expression is decreased, because of a decreased number of endothelial cells and SMCs, this decrease of RAS expression can further accelerate the reduction in the number of SMCs and the thinning of the medial layer. As well, angiotensin II is known to increase the level of bFGF, PDGF-AA, and TIMP-1.16 This study also revealed decreased expression of these mRNAs, which seems to result in inhibiting an increased response of extracellular matrix and SMC proliferation and hypertrophy. The mechanism of decreased expression of local RAS should be examined in future studies.

References

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