

Structural Fragility and Inflammatory Response of Ruptured Cerebral Aneurysms

A Comparative Study Between Ruptured and Unruptured Cerebral Aneurysms

Kazuo Kataoka, MD; Mamoru Taneda, MD; Toshiharu Asai, MD; Akira Kinoshita, MD; Mamoru Ito, MD; Ryotaro Kuroda, MD

Background and Purpose—Despite technical advances in endovascular and microsurgical treatment, patients with aneurysmal subarachnoid hemorrhage still have a high mortality and morbidity rate. To improve the treatment results in patients with aneurysms, we must better understand the pathophysiology of cerebral aneurysms and the mechanisms leading to their rupture. Therefore, we studied the pathological differences between unruptured and ruptured aneurysms.

Methods—Ruptured (n=44) and unruptured (n=27) aneurysms were obtained at surgery. The aneurysmal endothelium was scored from 0 (normal) to 5 (complete disruption) by using a scanning electron microscope. The aneurysmal wall was evaluated by immunohistochemical methods. The wall structure was scored from 1 (dense collagen and rich, smooth muscle cells) to 5 (hyaline-like structure). The degree of inflammatory cell invasion into the wall was also scored from 0 (very few cells) to 3 (many cells).

Results—Ruptured aneurysms manifested significant endothelial damage (score of 3.7 versus 0.8; Mann-Whitney *U* test, $P < 10^{-3}$), significant structural changes of the wall (3.7 versus 1.7, $P < 10^{-5}$), and significant inflammatory cell invasion (2.2 versus 0.8, $P < 10^{-4}$) compared with unruptured aneurysms. There was a significant correlation between the score for wall structure and the score for inflammatory cell invasion ($R_s = 0.63$; Spearman rank correlation test, $P < 10^{-5}$). The pathophysiology of several symptomatic unruptured aneurysms was similar to that of ruptured aneurysms.

Conclusions—We conclude that the pathophysiology of unruptured, asymptomatic and ruptured aneurysms is different. The wall of ruptured aneurysms was found to be fragile, possibly because macrophage infiltration into the aneurysmal wall resulted in loss of smooth muscle cells and in degradation of matrix proteins. (*Stroke*. 1999;30:1396-1401.)

Key Words: atherosclerosis ■ cerebral aneurysm ■ macrophage ■ protease

Pathological studies¹⁻⁴ revealed that the structure of the aneurysmal wall varied from a simple, thin, membranous layer to a thick wall containing advanced atherosclerotic changes. Epidemiological studies concerning subarachnoid hemorrhage (SAH) documented that factors that affect atherosclerosis were related to the occurrence of SAH.⁵ The probability of bleeding from an unruptured aneurysm is relatively low.⁶⁻⁸ There is little understanding of the processes that lead to disruption of the aneurysmal wall, although an understanding of the pathological differences between ruptured and unruptured aneurysms may answer questions pertaining to the processes that lead to wall rupture. Because not many comparative studies of unruptured and ruptured aneurysms have been published, we performed such a study.

Hemodynamic stress initially affects the endothelium, and changes in the vascular endothelium induce atherosclerosis

that in turn affects the strength of the aneurysmal wall. We studied the endothelial cell layer covering the inside of aneurysms by using a scanning electron microscope (SEM). Vascular endothelial cells and smooth muscle cells produce extracellular matrix proteins; in the aneurysmal wall, they help to maintain the structural integrity of the aneurysm against intra-aneurysmal pressure.^{1,9} In atherosclerotic lesions, inflammatory cells such as macrophages and leukocytes affect the vascular pathology; they secrete many kinds of proteases that destroy the extracellular matrix proteins.¹⁰ By immunohistochemical studies, we determined the expression of collagen type IV, one of the extracellular matrix proteins, and smooth muscle actin (SMA), a marker of smooth muscle cells, in the aneurysmal wall. We also evaluated the macrophages and leukocytes in the wall and studied the expression of proteases produced by inflammatory cells.

Received January 27, 1999; final revision received March 30, 1999; accepted April 23, 1999.

From the Department of Neurosurgery (K.K., M.T., T.A.), Kinki University School of Medicine, Osaka-Sayama; the Department of Neurosurgery (A.K., M.I.), Izumisano Municipal Hospital, Izumisano; and the Department of Pathophysiology and Therapeutics (R.K.), Faculty of Pharmaceutical Sciences, Kinki University, Higashi-Osaka, Osaka, Japan.

Correspondence to Kazuo Kataoka, MD, Department of Neurosurgery, Kinki University School of Medicine, 377-2, Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan. E-mail kataoka@med.kindai.ac.jp

© 1999 American Heart Association, Inc.

Stroke is available at <http://www.strokeaha.org>

Methods

We examined 44 ruptured and 27 unruptured cerebral aneurysms obtained from 70 patients at surgery. The mean age of patients with ruptured aneurysms was 59 years and that of patients with unruptured aneurysms was 56 years. Of the 27 unruptured aneurysms, 22 were incidentally diagnosed when magnetic resonance imaging or magnetic resonance angiography was performed for reasons unrelated to the cerebral aneurysm. One incidentally discovered aneurysm was operated on after we obtained angiographic evidence that it had begun to grow. Three of the other 5 unruptured aneurysms were associated with SAH from other aneurysms. A patient with an unruptured internal carotid artery (ICA) aneurysm presented with ipsilateral oculomotor palsy. One unruptured and partially thrombosed middle cerebral artery aneurysm was complicated by cerebral infarction in the territory of the parent artery. In all cases, the aneurysmal sac was removed after clipping the neck. The specimens were gently washed out with saline to remove any blood and fixed in 10% formalin.

Scanning Electron Microscopy

Eighteen ruptured and 11 unruptured aneurysms were evaluated by SEM. The specimens were immersed in 2% glutaraldehyde solution for 24 hours at room temperature, followed by 1-hour immersion in 1% osmium solution at 4°C, and washed with distilled water. The specimens were gradually dehydrated by immersion in a graded series of ethanol solutions and isoamyl acetate (30 minutes each). After critical-point drying, they were mounted on aluminum stubs and coated with gold for 10 minutes; the inner surface of the aneurysmal sac was examined by SEM (JSM-840, JEOL).

Immunohistochemical Studies

Forty-three ruptured and 23 unruptured aneurysms were evaluated immunohistochemically. We used monoclonal mouse anti-human SMA (DAKO), monoclonal mouse anti-human collagen type IV (DAKO), monoclonal mouse anti-human macrophage CD68 (DAKO), monoclonal mouse anti-human leukocyte common antigen (DAKO), polyclonal rabbit anti-human cathepsin D (DAKO), and polyclonal rabbit anti-human cathepsin G (DAKO) antibody. For visualization of the primary antibody, we used the LSAB2 kit (DAKO) with a peroxidase- or alkaline phosphatase-streptavidin complex. Formalin-fixed, paraffin-embedded tissue sections (6- μ m thick) were cut and mounted on Silan-coated glass slides, and then deparaffinization and rehydration were performed. For monoclonal mouse anti-human macrophage and anti-human leukocyte common antigen, sections were dipped in target retrieval solution (DAKO) and autoclaved for 10 minutes at 120°C. When the peroxidase-streptavidin complex was used, the sections were dipped in 3%-H₂O₂ solution for 10 minutes to inactivate endogenous peroxidase. For monoclonal mouse anti-human collagen type IV, the sections were incubated with proteinase K solution (DAKO) for proteolytic digestion. The slides were incubated for 45 minutes at room temperature with these primary antibody solutions or a control solution containing nonspecific mouse or rabbit immunoglobulin. After being washed with buffer solution, sections were incubated with biotinylated anti-rabbit or anti-mouse immunoglobulin solution for 30 minutes at room temperature. The specimens were then incubated with the peroxidase- or alkaline phosphatase-streptavidin complex solution for 10 minutes at room temperature. TrueBlue (KPL) solution or diaminobenzidine tetrahydrochloride was used as the chromogen for peroxidase. For visualization of alkaline phosphatase, we used the new fuchsin system (DAKO).

Definitions

On the basis of previously reported SEM findings regarding endothelial damage in aneurysms and arteries,¹¹⁻¹⁴ we classified and scored our SEM findings from 0 to 5 (Table 1). The structure of the aneurysmal wall was classified and scored from 1 to 5 (Table 2) according to previous morphological studies.¹⁻³ The degree of inflammatory cell invasion into the aneurysm wall was classified and scored from 0 to 3, depending on the pathology of the arterioscle-

TABLE 1. Score for the Inner Surface of the Aneurysmal Sac

0	The endothelial cell layer inside the aneurysmal sac is almost normal; a few leukocytes may adhere at intercellular gaps.
1	Endothelial cells show varied shapes, intercellular filaments, and widened intercellular gaps. There is no obvious endothelial cell disruption.
2	In some areas, the endothelial cell layer is damaged and blood cell adhesion is noted. Other areas are covered with normal-appearing endothelial cells.
3	In an extended area, the endothelial cell layer is damaged and blood cell adhesion is detected. The area remote from endothelial disruption is covered with undamaged endothelial cells.
4	There is extensive damage of the endothelial cell layer. Blood cell adhesion is observed, and deformed endothelial cells may be present.
5	Almost the entire endothelial cell layer is damaged and covered with blood cells and a fibrin network.

rosis^{4,15} found in the cerebral aneurysm (Table 3). One of us (T.A.) assigned the SEM score, the structural score for the wall, and the score for inflammatory cell invasion into the wall without having information about the specimens. Statistical analysis was performed using the nonparametric Mann-Whitney *U* test and the Spearman rank correlation test.

Results

Ruptured Aneurysms

In some ruptured aneurysms, SEM study revealed that the endothelial cell layer was destroyed and the inner surface covered with fibrin and blood cells. On histological examination, the wall was almost totally replaced by a hyaline-like structure, and no layers of collagen type IV or smooth muscle cells were present. In some instances, there was diffuse invasion of macrophages and leukocytes into the wall. Cathepsin D and cathepsin G were also noted. In several ruptured aneurysms, the endothelial cell layer was extensively damaged (SEM score of 4). Even in areas with preserved endothelial cells, their arrangement was disrupted, and many blood cells were present in gaps between the damaged endothelial cells (Figure 1A). Histological study showed not only an aneurysmal wall that was thinly layered and that contained scattered smooth muscle cells and irregular layers of collagen type IV but also an aneurysmal wall that was thick with arteriosclerotic changes in areas with a

TABLE 2. Score for the Structure of the Aneurysmal Wall

1	There are dense, smooth muscle cells and regular layers of collagen type IV in the aneurysmal wall.
2	Dense, smooth muscle cells are noted, but the layers of collagen type IV are irregular. Alternatively, the layers of collagen type IV are relatively regular, although smooth muscle cells are scattered in the aneurysmal wall.
3	Smooth muscle cells are scattered, and irregular layers of collagen type IV are seen in the aneurysmal wall.
4	A part of the aneurysmal wall is replaced by a hyaline-like structure. In other areas, a collagen layer and smooth muscle cells may be present.
5	Most of the aneurysmal wall is replaced by a hyaline-like structure. In the wall, there are almost no smooth muscle cells or layers of collagen type IV.

TABLE 3. Score for Inflammatory Cell Invasion Into the Aneurysmal Wall

0	Very few macrophages are detected in the intima and media of the wall. There is no cathepsin G immunoreactivity. Cathepsin D may be present in smooth muscle cells and in the outer layer of the wall.
1	Macrophages are observed in the wall. No leukocytes are present. Cathepsin D but no cathepsin G is detected.
2	A cluster of macrophages and cells strongly positive for cathepsin D is observed in the wall, where smooth muscle cells are scattered and the collagen layer is disrupted. Leukocytes may be present.
3	There is diffuse invasion of the wall by many macrophages. Strong immunoreactivity for cathepsin D may be present. Leukocytes are observed in the wall, and cathepsin G may be present.

proliferation of smooth muscle cells. In some cases, a portion of the wall was replaced by a hyaline-like structure. Intramural hemorrhage was also noted in the wall. In most aneurysms with severe endothelial damage, the structural integrity of the wall was compromised. In ruptured aneurysms with a high structural score, invasion of the wall by macrophages and leukocytes was common. In some specimens, clusters of macrophages were seen in areas where the smooth muscle cells and the collagen layer were disrupted (Figure 2A and 2B). In other ruptured aneurysms, there was no endothelial erosion on the inner surface located at a distance from the rupture point (SEM score of 2 to 3). From the body of the aneurysm to its neck, the endothelial cells were longitudinal. Some specimens obtained from the aneurysmal fundus exhibited polygonal cells with filamentous intercellular extensions. In these aneurysms, portions of the wall were given a score of 1 to 3.

Unruptured Aneurysms

In most unruptured aneurysms, the inner surface of the aneurysmal sac was completely covered with normally shaped arterial endothelial cells (Figure 1B). These aneu-

rysms usually had a regular layer of collagen type IV, and smooth muscle cells were common in the wall (Figure 2C and 2D). A limited number of macrophages or cathepsin D-positive cells were observed. Usually, neither leukocytes nor cathepsin G-positive cells were noted in the wall. In 1 aneurysm that had increased in size during a 1-year observation period, the endothelial cell layer was partially disrupted. In the patient with an unruptured aneurysm that led to embolic infarction of the territory of the distal artery, the endothelium of the sac was completely disrupted, and part of the wall had been replaced by a hyaline-like structure. In the patient with an unruptured ICA aneurysm who presented with ipsilateral oculomotor palsy, the layer of collagen type IV was obscure and the smooth muscle cells were scattered. Instead, there was invasion of the wall by macrophages (Figure 2E and 2F). Among the other 24 asymptomatic aneurysms, we found 3 with structural weakness (inflammatory score ≥ 2 or structural score ≥ 3).

Quantitative Analysis

SEM studies of the vascular endothelium inside the aneurysmal sac revealed significant differences between ruptured and unruptured aneurysms (SEM score of 3.7 ± 1.2 [mean \pm SD] versus 0.8 ± 1.7 ; Mann-Whitney *U* test, $P < 0.001$; Figure 3). Also, there were differences in the structure of the aneurysmal wall (structural score of 3.7 ± 1.2 versus 1.7 ± 1.0 ; Mann-Whitney *U* test, $P < 10^{-5}$; Figure 4) and the degree of inflammatory cell invasion into the wall (inflammatory score of 2.2 ± 0.9 versus 0.8 ± 0.9 ; Mann-Whitney *U* test, $P < 10^{-4}$; Figure 5). Figure 6 suggests a causal relationship between the degree of inflammatory cell invasion and the level of structure of the wall ($n = 61$, $R_s = 0.63$, Spearman rank correlation test; $P < 10^{-5}$). In ruptured aneurysms, we assessed the effect of elapsed time between onset of rupture and harvest of the aneurysm specimens and the pathological findings on the aneurysmal wall. When the

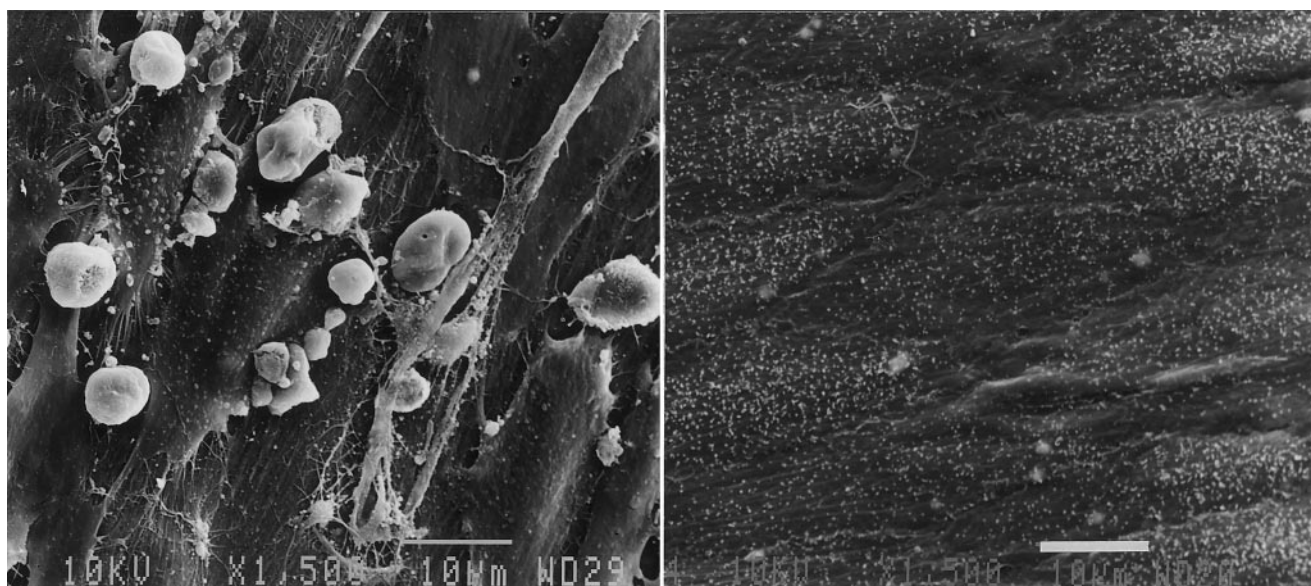


Figure 1. SEM of cerebral aneurysm. Left, Ruptured middle cerebral artery aneurysm. The arrangement of endothelial cells is disrupted. Blood cells adhere to interendothelial cell gaps. Right, Incidentally discovered middle cerebral artery aneurysm. Note the normal shape of the longitudinal endothelial cell layer. Bar = 10 μ m.

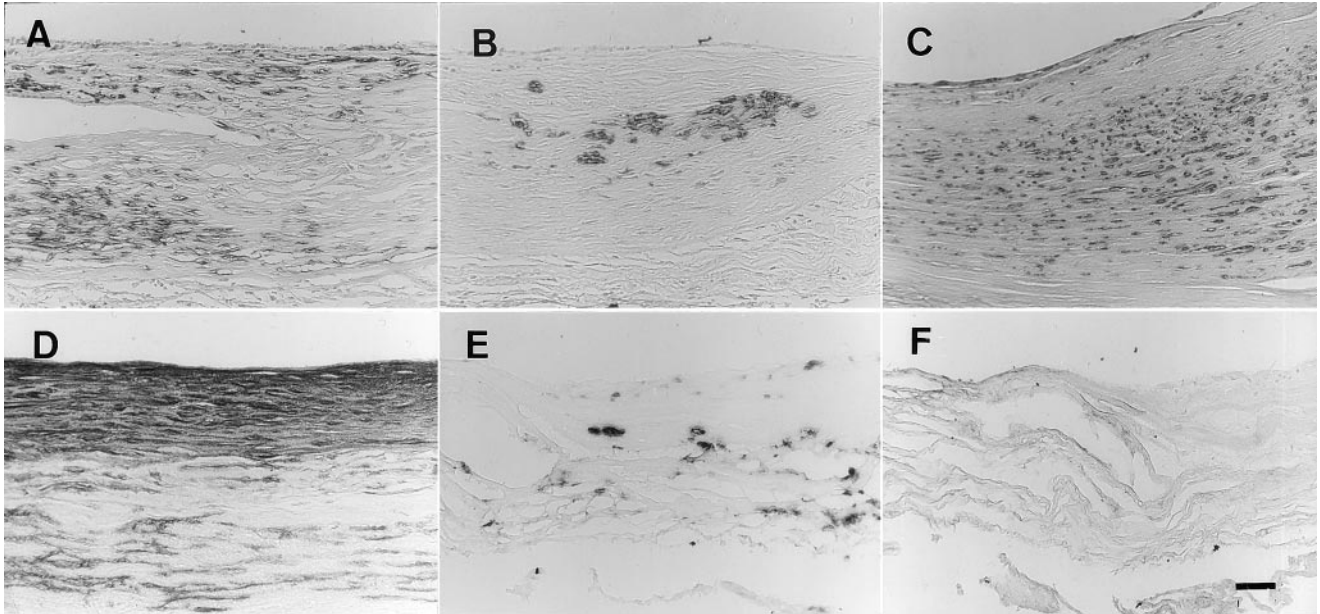


Figure 2. Immunohistochemistry of cerebral aneurysm. A and B, Ruptured anterior communicating aneurysms. Immunostaining against SMA (A) and cathepsin D (B). Note the cluster of cathepsin D-positive macrophages in the wall where few SMA-positive cells are seen. In other areas without cathepsin D-positive cells, many SMA-positive cells are present. C and D, Incidentally discovered middle cerebral artery aneurysm. Immunostaining against SMA (C) and collagen type IV (D) shows proliferation of smooth muscle cells and a dense layer of collagen type IV. E and F, Unruptured ICA aneurysm resulting in oculomotor nerve palsy. Immunostaining against macrophages (E) and collagen type IV (F). Note the irregular and poor staining of collagen type IV and macrophage infiltration into the wall. Bar=50 μ m.

elapsed time was between 6 and 12 hours, the structural score was 3.8 ± 1.3 and the inflammatory score was 2.3 ± 0.8 . When the elapsed time was from 13 to 24 hours, the structural score was 3.6 ± 1.3 and the inflammatory score was 2.1 ± 0.9 . When the interval was from 25 to 48 hours, the structural score was 4.0 ± 0.9 and the inflammatory score was 2.5 ± 0.8 . When >48 hours had passed, the structural score was 3.5 ± 1.4 and the inflammatory score was 2.5 ± 0.8 . We did not find a relationship: the length of time lapsed from rupture onset to specimen harvest had no effect on the wall scores.

Discussion

Wardlaw et al,^{16,17} who measured aneurysmal pulsation by using a color transcranial Doppler, found that in patients with multiple aneurysms, the recently ruptured aneurysm was more pulsatile than the asymptomatic one. This finding strongly suggests that the structural integrity is different in ruptured and unruptured aneurysms. Earlier morphological

studies of cerebral aneurysms had not identified these differences.^{2,4} Our study confirms that ruptured and unruptured aneurysms differ in their structural pathology. Serial angiographic study has revealed that aneurysms can enlarge rapidly.¹⁸ In our study, some ruptured aneurysms had a completely defective endothelium of the inner surface, and their walls tended to be replaced by a hyaline-like structure. These findings suggest that the wall of some aneurysms rapidly stretches after the development of structural fatigue due to intra-aneurysmal pulsatile pressure. These pathological findings show that surgical or endovascular manipulation of ruptured aneurysms, even at areas other than the initial bleeding point, carries a risk of bleeding.

Macrophages are the principal inflammatory cells in atherosclerosis. Leukocytes initially participate in the acute

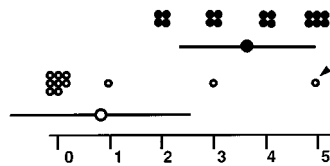


Figure 3. Score for the inner surface of the aneurysmal sac. Endothelial findings obtained from SEM studies were scored according to the scheme presented in Table 1. The score for ruptured aneurysms is 3.7 ± 1.2 (mean \pm SD); that for unruptured aneurysms is 0.8 ± 1.7 , and there is a significant difference between the 2 groups (Mann-Whitney *U* test, $P < 0.001$). The arrowhead indicates an unruptured and partially thrombosed aneurysm. The closed and open circles indicate ruptured and unruptured aneurysms, respectively.

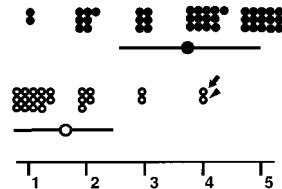


Figure 4. Score for the structure of the aneurysmal wall. Findings obtained from immunohistochemical studies with anti-SMA and anti-collagen type IV were scored according to the scheme presented in Table 2. The score for ruptured aneurysms is 3.7 ± 1.2 (mean \pm SD), that for unruptured aneurysms is 1.7 ± 1.0 , and there is a significant difference between the 2 groups (Mann-Whitney *U* test, $P < 10^{-5}$). The arrowhead indicates an unruptured and partially thrombosed aneurysm. The arrow shows an unruptured aneurysm; this patient presented with oculomotor nerve palsy. Closed and open circles indicate ruptured and unruptured aneurysms, respectively.

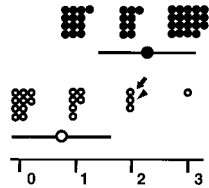


Figure 5. Score for inflammatory cell invasion into the aneurysmal wall. Findings of inflammatory cell invasion into the aneurysmal wall obtained by immunohistochemical studies with anti-macrophage, anti-leukocyte common antigen, anti-cathepsin D, and anti-cathepsin G were scored according to the scheme presented in Table 3. The score for ruptured aneurysms is 2.2 ± 0.9 (mean \pm SD), that for unruptured aneurysms is 0.8 ± 0.9 , and there is a significant difference between the 2 groups (Mann-Whitney *U* test, $P < 10^{-4}$). The arrowhead indicates an unruptured and partially thrombosed aneurysm. The arrow shows an unruptured aneurysm; this patient presented with oculomotor nerve palsy. Closed and open circles indicate ruptured and unruptured aneurysms, respectively.

inflammatory process. Invasion of the wall by macrophages and leukocytes was commonly observed in ruptured aneurysms with a fragile wall. Leukocytes in the wall could be associated with SAH. We found widespread disruption of the endothelial cell layer in ruptured aneurysms and blood cell adhesion to the damaged endothelium. We must point out that endothelial erosion enhances leukocyte invasion of the wall before rupture. An acute inflammatory response usually changes over time. In our study, there were almost no changes in either the degree of inflammatory responses or the degree of wall structure that could be attributed to the time elapsed between rupture onset and fixation of the specimens. Therefore, to a considerable degree, factors leading to the pathological findings made in ruptured aneurysms may exist before rupture occurs.

The patient with an unruptured ICA aneurysm who presented with oculomotor nerve palsy exhibited a fragile wall in association with macrophage infiltration. Oculomotor nerve palsy has been thought to be a warning that an unruptured ipsilateral ICA aneurysm has a high risk of bleeding.^{19,20} Among the other 26 unruptured aneurysms, we found 5 with structural weakness (SEM score ≥ 3 , inflammatory score ≥ 2 , structural score ≥ 3). Thus, these 6 unruptured aneurysms are thought to be at high risk for hemorrhage, and the pathological findings we made show the process leading to disruption of the wall before bleeding. The low probability of bleeding

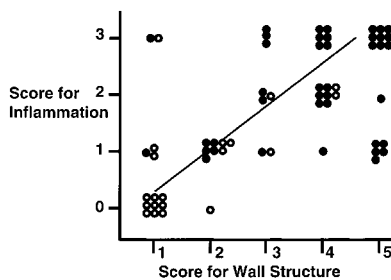


Figure 6. Correlation between structural changes of the wall and degree of inflammatory cell invasion into the wall. There is a significant correlation between the 2 kinds of scores ($n = 61$, $R_s = 0.63$, Spearman rank correlation test; $P < 10^{-5}$). Closed and open circles indicate ruptured and unruptured aneurysms, respectively.

from an unruptured aneurysm⁶⁻⁸ may coincide with the fact that we did not find many unruptured ones with structural weakness.

Not only proliferation of smooth muscle cells but also loss of smooth muscle cells was observed in atherosclerotic lesions.^{4,21,22} Loss of smooth muscle cells leads to rupture of atherosclerotic plaques, because these cells play an important role in preserving the structural integrity of atherosclerotic lesions.²³ Kockx et al^{22,24} suggested that macrophage-derived factors could kill the adjacent smooth muscle cells in the atherosclerotic plaque. We also confirmed that macrophages invaded the ruptured aneurysm wall where smooth muscle cells had been lost. Macrophages and leukocytes produce many kinds of biologically active substances such as proteases. Cathepsin is one of these proteases, and macrophages indeed secrete cathepsin D and leukocytes produce cathepsin G.^{25,26} These can digest extracellular matrix proteins in the aneurysmal wall. We found clusters of macrophages that expressed cathepsin D in the wall of ruptured aneurysms where the collagen layer was eroded. The layers of extracellular matrix proteins contributes to the tensile strength of the aneurysmal wall against the ceaseless hemodynamically induced vibrational stress between diastole and systole. Proteases derived from inflammatory cells associated with atherosclerosis may help to compromise the structural integrity of the aneurysm and lead to rupture. We found a significant correlation between the degree of inflammatory cell invasion and the level of fragility of the wall. Thus, macrophage infiltration into the aneurysmal wall plays a vital role in the process resulting in wall fragility.

Conclusions

The present study showed significant differences in ruptured and unruptured aneurysm with respect to their inner surface and their wall. Macrophage infiltration into the wall may play an important role in weakening aneurysmal structural integrity.

Acknowledgments

This work was supported in part by grants-in-aid for scientific research from the Ministry of Education (09470303, 10671332), Japan.

References

- Mimata C, Kitaoka M, Nagahiro S, Iyama K, Hori H, Yoshioka H, Ushio Y. Differential distribution and expressions of collagens in the cerebral aneurysmal wall. *Acta Neuropathol (Berl)*. 1997;94:197-206.
- Scanarini M, Mingrino S, Giordano R, Baroni A. Histological and ultrastructural study of intracranial saccular aneurysmal wall. *Acta Neurochir (Wien)*. 1978;43:171-182.
- Sahs AL. Observations on the pathology of saccular aneurysms. *J Neurosurg*. 1966;24:792-806.
- Kosierkiewicz TA, Factor SM, Dickson DW. Immunocytochemical studies of atherosclerotic lesions of cerebral berry aneurysms. *J Neuro-pathol Exp Neurol*. 1994;53:399-406.
- Bell BA, Symon L. Smoking and subarachnoid haemorrhage. *BMJ*. 1979; 1:577-578.
- Winn HR, Almaani WS, Berga SL, Jane JA, Richardson AE. The long-term outcome in patients with multiple aneurysms: incidence of late hemorrhage and implications for treatment of incidental aneurysms. *J Neurosurg*. 1983;59:642-651.
- Juvela S, Porras M, Heiskanen O. Natural history of unruptured intracranial aneurysms: a long-term follow-up study. *J Neurosurg*. 1993;79: 174-182.

8. Heiskanen O. Risk of bleeding from unruptured aneurysm in cases with multiple intracranial aneurysms. *J Neurosurg.* 1981;55:524–526.
9. Futami K, Yamashita J, Tachibana O, Higashi S, Ikeda K, Yamashita T. Immunohistochemical alterations of fibronectin during the formation and proliferative repair of experimental cerebral aneurysms in rats. *Stroke.* 1995;26:1659–1664.
10. Sukhova GK, Shi GP, Simon DI, Chapman HA, Libby P. Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. *J Clin Invest.* 1998;102:576–583.
11. Dirrenberger RA, Sundt JTM. Carotid endarterectomy. *J Neurosurg.* 1978;48:201–219.
12. Kirse DJ, Young PH. A scanning electron microscopic study of arterial endothelial repair under turbulent flow conditions. *Microsurgery.* 1992; 13:26–30.
13. Hassler O. Scanning electron microscopy of saccular intracranial aneurysms. *Am J Pathol.* 1972;68:511–520.
14. Scanarini M, Mingrino S, Zuccarello M, Trincia G. Scanning electron microscopy (sem) of biopsy specimens of ruptured intracranial saccular aneurysms. *Acta Neuropathol (Berl).* 1978;44:131–134.
15. Crompton MR. Mechanism of growth and rupture in cerebral berry aneurysms. *BMJ.* 1966;1:1138–1142.
16. Wardlaw JM, Cannon JC. Color transcranial ‘power’ Doppler ultrasound of intracranial aneurysms. *J Neurosurg.* 1996;84:459–461.
17. Wardlaw JM, Cannon J, Statham PF, Price R. Does the size of intracranial aneurysms change with intracranial pressure? observations based on color ‘power’ transcranial Doppler ultrasound. *J Neurosurg.* 1998;88:846–850.
18. Alcock JM, Canham PB. Angiographic study of the growth of intracranial aneurysms. *J Neurosurg.* 1976;45:617–621.
19. Hamer J. Prognosis of oculomotor palsy in patients with aneurysms of the posterior communicating artery. *Acta Neurochir (Wien).* 1982;66: 173–185.
20. Okawara SH. Warning signs prior to rupture of an intracranial aneurysm. *J Neurosurg.* 1973;38:575–580.
21. Ariyoshi H, Okahara K, Sakon M, Kambayashi J, Kawashima S, Kawasaki T, Monden M. Possible involvement of m-calpain in vascular smooth muscle cell proliferation. *Arterioscler Thromb Vasc Biol.* 1998; 18:493–498.
22. Kockx MM, De Meyer GR, Bortier H, de Meyere N, Muhring J, Bakker A, Jacob W, Van Vaeck L, Herman A. Luminal foam cell accumulation is associated with smooth muscle cell death in the intimal thickening of human saphenous vein grafts. *Circulation.* 1996;94:1255–1262.
23. Kockx MM, Herman AG. Apoptosis in atherogenesis: implications for plaque destabilization. *Eur Heart J.* 1998;19(suppl G):G23–G28.
24. Kockx MM, De Meyer GR, Muhring J, Jacob W, Bult H, Herman AG. Apoptosis and related proteins in different stages of human atherosclerotic plaques. *Circulation.* 1998;97:2307–2315.
25. Selak MA, Chignard M, Smith JB. Cathepsin G is a strong platelet agonist released by neutrophils. *Biochem J.* 1988;251:293–299.
26. Loscalzo J. The macrophage and fibrinolysis. *Semin Thromb Hemost.* 1996;22:503–506.