Varicella zoster virus vasculopathy

Analysis of virus-infected arteries

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ABSTRACT

Objective: Varicella zoster virus (VZV) is an under-recognized yet treatable cause of stroke. No animal model exists for stroke caused by VZV infection of cerebral arteries. Thus, we analyzed cerebral and temporal arteries from 3 patients with VZV vasculopathy to identify features that will help in diagnosis and lead to a better understanding of VZV-induced vascular remodeling.

Methods: Normal and VZV-infected cerebral and temporal arteries were examined histologically and by immunohistochemistry using antibodies directed against VZV, endothelium, and smooth muscle actin and myosin.

Results: All VZV-infected arteries contained 1) a disrupted internal elastic lamina; 2) a hyperplastic intima composed of cells expressing α -smooth muscle actin (α -SMA) and smooth muscle myosin heavy chain (SM-myosin) but not endothelial cells expressing CD31; and 3) decreased medial smooth muscle cells. The location of VZV antigen, degree of neointimal thickening, and disruption of the media were related to the duration of disease.

Conclusions: The presence of VZV primarily in the adventitia early in infection and in the media and intima later supports the notion that after reactivation from ganglia, VZV spreads transaxonally to the arterial adventitia followed by transmural spread of virus. Disruption of the internal elastic lamina, progressive intimal thickening with cells expressing α -SMA and SM-MHC, and decreased smooth muscle cells in the media are characteristic features of VZV vasculopathy. Stroke in VZV vasculopathy may result from changes in arterial caliber and contractility produced in part by abnormal accumulation of smooth muscle cells and myofibroblasts in thickened neointima and disruption of the media. **Neurology® 2011;77:364-370**

GLOSSARY

α -SMA = α -smooth muscle actin; PBS = phosphate-buffered saline; SM = smooth muscle; VZV = varicella zoster virus.

Primary varicella zoster virus (VZV) infection usually causes varicella (chickenpox), after which virus becomes latent in ganglia along the entire neuraxis.¹⁻³ A natural decline in cell-mediated immunity to VZV with age or immunosuppression⁴⁻⁸ results in VZV reactivation, manifest as herpes zoster (shingles). Zoster is common. Approximately 50% of people will have had an episode by age 85.⁹

An uncommon but serious complication of virus reactivation is ischemic and hemorrhagic stroke produced by VZV vasculopathy that affects both immunocompetent and immunocompromised individuals and can present as headache and mental status changes with or without focal neurologic deficits. Both large and small vessels are involved, and MRI shows multifocal ischemic lesions, commonly at gray-white matter junctions. Importantly, the diagnosis of VZV vasculopathy is often missed because 1) symptoms and signs may occur months after zoster¹⁰; 2) up to one-third of patients do not have a preceding zoster rash; 3) up to one-third of patients do not have CSF pleocytosis; and 4) PCR analysis of CSF for VZV DNA is only 30%

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Table Clinical features of patients with VZV vasculopathy						
Subject	Age, y	Gender	Zoster rash	Interval from rash to stroke, wk	Interval from stroke to death, wk	Antigen
1	80	M	Yes	4	Not applicable	+
2	73	M	No	Not applicable	45	+
3	37	M	Yes	40	8	+

Abbreviation: VZV = varicella zoster virus.

sensitive; in fact, the best laboratory criterion for diagnosis is detection of anti-VZV antibodies in the CSF.¹¹

Since VZV is an exclusively human virus, no animal model to study VZV vasculopathy exists. Morphologic analyses have been limited to sporadic case reports which noted a wide range of vascular pathology, ranging from neointimal proliferation to necrosis with or without inflammation.¹² Herein, VZV-infected cerebral and temporal arteries from patients with VZV vasculopathy at autopsy and biopsy were analyzed histologically and immunohistochemically.

METHODS Clinical features. A normal cerebral artery and the temporal and middle cerebral arteries containing VZV antigen from 3 subjects with VZV vasculopathy were studied (table). Subject 1 was an 80-year-old man who developed left ophthalmic-distribution zoster followed by left ophthalmic artery occlusion 1 month later; even though the patient had no symptoms or signs of disease in the left temporal artery, giant cell arteritis was initially considered to have caused his left-sided loss of vision, and a temporal artery biopsy was obtained which revealed VZV vasculopathy.¹³ Magnetic resonance angiography revealed left ophthalmic artery occlusion. The CSF was negative for VZV DNA by PCR, but positive for anti-VZV immunoglobulin G and immunoglobulin M. The patient improved after treatment with IV acyclovir for 14 days followed by oral valacyclovir. Subject 2 was a 73-year-old man with no history of rash, who developed an ill-defined protracted multifocal vasculopathy from which he died 10 months later.^{14,15} He presented initially with fatigue, anorexia, somnolence, confusion, and headache followed 2 weeks later by anterior uveitis of the right eye. MRI revealed a right parieto-occipital infarct and a small infarct in the right midbrain. During his hospital course, he developed a transient internuclear ophthalmoplegia, then a left hemiplegia and lethargy that improved with IV acyclovir and prednisone. An angiogram revealed narrowing of the right anterior cerebral artery and the supraclinoid portion of the right carotid artery extending into the proximal segment of the right middle cerebral artery. During the remainder of his hospitalization, he developed anorexia, headaches, and fluctuating confusion complicated by pneumonia. He died 316 days from onset of symptoms. Antibody to varicella in the CSF was present at a titer of 1:8. The CSF was not tested for the presence of VZV DNA. Postmortem analysis of cerebral arteries revealed VZV DNA in the right posterior cerebral and basilar arteries, and VZV antigen was found in the right middle and posterior cerebral arteries. Subject 3 was

a 37-year-old man with AIDS who developed disseminated herpes zoster, followed 10 months later by a right homonymous hemianopia, confusion, and agitation. Brain imaging revealed focal disease involving the left midtemporal and inferior parietal gyri and subcortical white matter. He became increasingly confused and developed a right hemiparesis and left-sided ptosis. A CT scan showed new areas of low attenuation and swelling in the right parietal lobe and subcortical white matter with new petechiae and edema in the left frontal and right temporal lobes. Angiography revealed segmental narrowing of the supraclinoid portions of both internal carotid arteries at the proximal anterior cerebral arteries, as well as in the proximal and distal branches of the left middle cerebral artery, and in the right posterior communicating artery. His CSF was not examined for either VZV DNA or anti-VZV antibodies. Postmortem examination revealed VZV DNA in brain tissue and VZV antigen in the left middle cerebral artery.¹⁶

Cerebral and temporal arteries examined. The left temporal artery from subject 1 corresponded to the distribution of zoster and to the ipsilateral vision loss due to left ophthalmic artery occlusion. The right middle cerebral artery studied in subject 2 corresponded to the area of arterial narrowing seen in subject's angiogram and to the distribution of the bland infarct. The right middle cerebral artery studied in subject 3 corresponded to CT abnormalities in the right temporal lobe. The normal cerebral artery was an uninfected middle cerebral artery obtained from subject 3 that was negative for both VZV DNA and antigen. Since the morphologies of cerebral and temporal arteries are similar, including the absence of an external elastic lamina, no additional temporal artery controls were used.

Patient consents. Arteries from subjects 1 and 2 were archival autopsy material obtained in 1995 and 1996 and published as clinicopathological conferences in the *New England Journal of Medicine*^{14,16}; the temporal artery from subject 3 was sent to the neurovirology laboratory at the University of Colorado School of Medicine for virologic diagnostic evaluation.

Histopathology. Formalin-fixed, paraffin-embedded sections of cerebral arteries from 3 patients with VZV vasculopathy (table) were studied. Sections were cut (5 μ m), baked at 72°C for 30 minutes, and stained with hematoxylin & eosin and Verhoeff-Van Gieson for elastic fibers.

Immunohistochemistry. Primary antibodies used were as follows: 1:5,000 polyclonal rabbit anti-VZV 63¹⁷; 1:40 monoclonal mouse anti-CD31 (Dako, Carpinteria, CA); 1:500 mouse anti- α -smooth muscle cell actin (α -SMA; Ventana, Tucson, AZ); and 1:1,000 rabbit anti-smooth muscle myosin heavy chain (SM-MHC; a gift from Dr. R. Adelstein, NIH). Except when indicated, all incubations were at room temperature. Sections were deparaffinized 3 times for 5 minutes each time in 100% xylene and then in 100% ethanol. After sequential dipping in 95%, 70%, and 50% ethanol, sections were placed in distilled water, heated in 10 μ m citrate buffer for 20 minutes for antigen retrieval, and cooled in water. Sections were blocked in phosphate-buffered saline (PBS) containing 5% normal goat serum for 1 hour, washed 3 times with PBS, and incubated with primary antibodies against VZV 63 or myosin overnight at 4°C. After warming to room temperature, sections were rinsed 3 times with PBS, incubated with 1:100 biotinylated goat antirabbit secondary antibody (Dako) for 1 hour, rinsed 3 times in PBS, and incubated with prediluted alkaline phosphatase-conjugated streptavidin (BD Biosciences, Cat. 551008, San Diego, CA) for 1 hour. The color reaction was developed for 2 minutes using the

Figure 1 Morphology of normal cerebral artery

Movat pentachrome stain reveals the 3 layers of a normal cerebral artery. The intima, adjacent to the lumen, is composed of a single endothelial layer (arrows indicate endothelial cell nuclei [pink]). The media is composed of smooth muscle cells, and the adventitia contains collagen and fibroblasts. An internal elastic lamina (IEL) separates the intima and media. Unlike coronary and pulmonary arteries, cerebral arteries do not have an external elastic lamina between the media and adventitia.

fresh fuchsin substrate system (Dako) in the presence of levamisole at a final concentration 24 $\mu\text{g}/\text{mL}$.

For CD31 and α -SMA immunostaining, sections were deparaffinized, heated for 36 minutes for epitope retrieval, and slides were processed using an automated slide stainer according to the manufacturer's instructions (reagents/protocol in iVIEW DAB Detection Kit; Tucson, AZ). Slides were incubated with the corresponding primary antibody at 37°C for 30 minutes, rinsed, and incubated with biotinylated secondary antibody followed by horseradish peroxidase and DAB (iVIEW DAB Detection Kit). Slides were rinsed, dehydrated, and mounted on xylene-based medium.

All slides were viewed using a Nikon Eclipse E800 microscope with Axiovision digital imaging software.

RESULTS Histopathology. A normal middle cerebral artery from subject 3 (figure 1) was compared with VZV-infected temporal and middle cerebral arteries from subjects 1–3. In contrast to normal arterial structure (figure 2A), the intimal layer was thickened in the arteries of all 3 subjects with VZV vasculopathy (figure 2, B–D, vertical black lines). While Verhoeff-Van Gieson staining of the normal cerebral artery revealed an intact internal elastic lamina (figure 2E, arrow), it was duplicated or disrupted in all VZV-infected arteries (figure 2, F–H, arrows).

Immunohistochemistry. In contrast to the absence of VZV antigen in the normal cerebral artery (figure 2I), VZV antigen was seen in the adventitia of subject 1 at 4 weeks after zoster (figure 2J, arrow), in the media of subject 2 (who did not have zoster rash) after 45 weeks of VZV vasculopathy (figure 2K, arrow), and in the hyperplastic intima of subject 3 at 48 weeks after zoster (figure 2L, arrow).

To identify the cellular components of the hyperplastic intima in VZV-infected arteries, immunohistochemical techniques were used to detect endothelial cell antigen CD31, and α -smooth muscle actin (α -SMA) and smooth muscle myosin heavy chain (SM-MHC). A single cell layer of endothelium is present in a normal cerebral artery (figure 3A, arrow). In the 3 subjects with VZV vasculopathy (figure 3, B–D), a thin endothelium (arrows) was seen adjacent to the lumen, while no endothelial cells were detected in the thickened intima (vertical white lines). Analysis of smooth muscle cell distribution revealed cells expressing α -SMA (figure 3E, vertical black line, brown color) in the media of the normal cerebral artery. In the VZV-infected temporal artery of subject 1 at 4 weeks after zoster, cells expressing α -SMA were present in the media (figure 3F, vertical black line, brown color) but at a lower density than in the media of the normal artery; cells expressing α -SMA were also present in the hyperplastic intima (figure 3F, vertical white line, brown color). In contrast, the middle cerebral arteries of subjects 2 and 3 with protracted VZV vasculopathy showed a striking paucity of cells expressing α -SMA in the media (figure 3, G–H, vertical black lines, brown color) with a greater abundance of these cells in the hyperplastic intima (figure 3, G–H, vertical white lines, brown color). Cells expressing SM-MHC were present in the media of the normal cerebral artery (figure 3I, vertical black line, pink color). Cells expressing SM-MHC were seen in the media of subject 1 at 4 weeks after zoster, but at a lower density (figure 3J, vertical black line, pink

Figure 2 Histologic and virologic analysis of a normal human cerebral artery and varicella zoster virus (VZV)-infected temporal and cerebral arteries of patients with VZV vasculopathy

Hematoxylin & eosin (H&E) stain of a normal uninfectd middle cerebral artery from subject 3 (A); Verhoeff-Van Gieson (VVG) staining shows an intact internal elastic lamina (E, arrow) devoid of VZV antigen (I). In the temporal artery of subject 1 with early VZV vasculopathy, as well as the right middle cerebral artery of subjects 2 and 3 (both of whom died of protracted VZV vasculopathy), H&E staining reveals a hyperplastic intima in all 3 arteries (B-D, vertical black lines), and VVG staining shows duplication or frank disruption of the internal elastic lamina in the arteries of all 3 subjects with VZV vasculopathy (F-H, arrows). VZV antigen (pink) is seen in the adventitia of subject 1 at 4 weeks after zoster (J, arrow), in the media of subject 2 (without a history of zoster rash) after a 45-week course of VZV vasculopathy (K, arrow), and in the hyperplastic intima of subject 3 at 48 weeks after zoster (L, arrow). Magnification = $\times 100$ in panels A-H and $\times 600$ in panels I-L.

color) and were even less abundant in the media of subjects 2 and 3 with protracted VZV vasculopathy (figure 3, K through L, vertical black lines, pink color). Like α -SMA, SM-myosin was expressed by cells in the hyperplastic intima of the cerebral arteries of subjects 1–3 with VZV vasculopathy (figure 3, J–L, vertical white lines, pink color).

DISCUSSION We examined cerebral and temporal arteries, all of which contained VZV antigen, from 3 patients with VZV vasculopathy, as well as a control artery that was negative for VZV antigen. Findings on the VZV-infected arteries were compared to the uninfectd normal cerebral artery (figure 1) which was composed of a single layer of endothelial cells adjacent to the lumen (intima), an internal elastic lamina, a wall of smooth muscle cells (media), and an

outer layer consisting of collagen and adventitial fibroblasts (adventitia). Importantly, the arteries represented early and late infection: the artery from subject 1 was obtained 4 weeks after zoster before neurologic symptoms and signs relevant to that artery developed, while the arteries from subjects 2 and 3 were obtained at autopsy after 45 and 48 weeks of protracted neurologic illness, respectively. The presence of most VZV antigen in the adventitia of the early case, combined with a heavy antigenic burden in the media and intima of the 2 late cases, supports the notion that VZV spreads transmurally from the adventitia to the intima, presumably after transaxonal spread to the artery via ganglionic afferent fibers.^{18,19} Although it remains unknown why virtually all cases of VZV vasculopathy involve cerebral arter-

Figure 3 Immunohistochemical analyses of a normal cerebral artery and varicella zoster virus (VZV)-infected arteries from patients with VZV vasculopathy

A thin layer of endothelial cells expressing CD31 is seen in the normal cerebral artery (A, brown color, arrow). The thickened intima of cerebral arteries from subjects 1–3 with VZV vasculopathy (B–D, vertical white lines) does not contain endothelial cells expressing CD31; however, a thin endothelium is seen adjacent to the lumen (B–D, brown color, arrows). In the normal artery, α -smooth muscle actin (α -SMA) is present exclusively in smooth muscle cells of the media (E, vertical black line, brown color). In the VZV-infected artery of subject 1 at 4 weeks after zoster, cells expressing α -SMA are present but less dense in the media and also seen in the hyperplastic intima (F, vertical black and white lines, brown color, respectively); in contrast, the cerebral arteries of subjects 2 and 3 with protracted VZV vasculopathy revealed a striking paucity of cells expressing α -SMA in the media (G, H, vertical black lines, brown color) and a greater abundance of such cells in the hyperplastic intima (G, H, vertical white lines, brown color). Cells expressing smooth muscle myosin heavy chain (SM-MHC) are abundant in the media of the normal artery (I, vertical black line, pink color); such cells are also present but less dense in the arterial media in subject 1 at 4 weeks after zoster (J, vertical black line, pink color) and sparse in the media of subjects 2 and 3 with protracted VZV vasculopathy (K, L, vertical black lines, pink color). Like α -SMA, SM-MHC is expressed by cells in the hyperplastic intima of the cerebral arteries of subjects 1–3 with VZV vasculopathy (J–L, vertical white lines, pink color). Magnification = $\times 200$ in all panels.

ies rather than systemic arteries, it is possible that the absence of an external elastic lamina in cerebral arteries, unlike systemic arteries,²⁰ facilitates transmural spread of virus in cerebral arteries with continued virus production in a thickened intima.

Results of histologic and immunohistochemical analyses of cerebral and temporal arteries from subjects with VZV vasculopathy were similar. All arteries contained a hyperplastic intima with a duplicated or disrupted internal elastic lamina. The degree of neointimal thickening was greater in the late cases (subjects 2 and 3) than in the early case (subject 1), suggestive of vascular remodeling that continues for

months after initial VZV infection. Although a thin endothelial layer was readily seen on the luminal surface of the 3 VZV-infected arteries, the thickened intima did not contain CD31-positive cells, thus making endothelial cells an unlikely source of the hyperplastic intima. Instead, the thickened intima contained cells expressing both α -SMA and SM-MHC, indicating a smooth muscle cell origin. Furthermore, far fewer cells expressing α -SMA and SM-MHC were seen in the media of the late cases (subjects 2 and 3) compared to the early case (subject 1) or in the normal cerebral artery. Together, these findings suggest that some neointimal cells

originated from smooth muscle cells in the media after VZV infection. The thickened intima also contained abundant cells that expressed α -SMA but not SM-MHC (myofibroblasts). While dedifferentiated smooth muscle cells can retain α -SMA and lose SM-MHC expression, it is also possible that these myofibroblasts originated from resident or circulating progenitor cells or adventitial fibroblasts. Unfortunately, there are no specific markers to identify the origin of these neointimal myofibroblasts.

The VZV-infected arteries did not contain a distinct core of extracellular lipid in the thickened intima characteristic of atheromatous lesions²¹ or medial hypertrophy seen in hypertensive vascular disease.²² Although intimal hyperplasia and a fragmented internal elastic lamina may be seen in cerebral arteries of patients with HIV-associated vasculopathy, other diverse pathologic changes characteristic of HIV vasculopathy—perivascular space dilatation, rarefaction, pigment deposition with vessel wall mineralization, and perivascular inflammatory cell infiltrates²³—as well as aneurysmal formation and fibrosis²⁴ were conspicuously absent.

Analysis of the morphology and composition of the thickened intima and media, and the location of viral antigen in the adventitia in early VZV vasculopathy, revealed clues to the possible mechanisms of VZV-induced vascular remodeling that leads to stroke. Previous studies of pulmonary and coronary vascular wall remodeling revealed that the adventitia is a key regulator in vascular wall structure and function.^{25–36} After vascular injury (i.e., balloon injury,^{25,26,28} pulmonary hypertension,^{29,31,33} hypoxia^{30,32}), adventitial fibroblasts can differentiate into myofibroblasts that proliferate and migrate to the intima. In addition, these “activated” adventitial fibroblasts can 1) secrete factors that create a proinflammatory environment, further contributing to vascular wall remodeling,^{29,30,32–34} and 2) affect adjacent adventitial fibroblasts and medial smooth muscle cells such that they acquire a proliferative, migratory, and invasive phenotype.^{27,32,35} Alternatively, adventitial dendritic cells have been shown to become activated and contribute to a proinflammatory environment leading to vascular wall remodeling, as seen in giant cell arteritis.^{36,37} It is possible that VZV infection of adventitial cells might lead to cerebrovascular wall remodeling in a similar manner. Further studies are under way to analyze the inflammatory environment and dendritic cell activation in VZV-infected arteries.

AUTHOR CONTRIBUTIONS

Dr. Nagel: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition

of data, study supervision. I. Traktinskiy: study concept or design, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data, study supervision. Dr. Azarkh: analysis or interpretation of data. Dr. Kleinschmidt-DeMasters: drafting/revising the manuscript, analysis or interpretation of data. Dr. Hedley-Whyte: drafting/revising the manuscript, contribution of vital reagents/tools/patients. Dr. Russman: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data. Dr. VanEgmond: analysis or interpretation of data. Dr. Stenmark: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, obtaining funding. Dr. Frid: drafting/revising the manuscript, study concept or design, contribution of vital reagents/tools/patients, study supervision. Dr. Mahalingam: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data. M. Wellish: study concept or design. A. Choe: study concept or design, analysis or interpretation of data, acquisition of data, statistical analysis. Dr. Cordery-Cotter: analysis or interpretation of data, contribution of vital reagents/tools/patients, study supervision. Dr. Cohrs: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data, obtaining funding. Dr. Gilden: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data, study supervision, obtaining funding.

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