

# Lesions of the Tunica Media in Traumatic Rupture of Vertebral Arteries: Histologic and Biochemical Studies

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**ABSTRACT:** Discontinuous non-circumferential lesions of tunica media were observed in four cases of traumatic rupture of the vertebral artery. We hypothesize that these lesions were due to mechanical disruption of smooth muscle cells and the liberation of catabolic enzymes with subsequent degradation of the arterial media. To test this hypothesis, healthy vertebral arteries were incubated with crude extracts of bovine smooth muscle cytosol in attempt to reproduce the histological changes of the arterial media in traumatized vertebral arteries. We observed cytosol-induced degradation of tunica media, characterized by pallor of staining with the Masson's Trichrome method, which was due to catabolic enzyme activity that was effectively inhibited by heat inactivation of the cytosol. The cytosol-induced tinctorial changes were similar to the lesions of the tunica media in naturally-occurring cases of traumatic vertebral artery rupture. We conclude that although vertebral arteries can be ruptured by physical distortion alone, associated lesions of the tunica media are due to *in situ* trauma-associated release of heat-labile catabolic enzymes.

**RÉSUMÉ:** Lésions de la média dans la rupture traumatique des artères vertébrales: études histologiques et biochimiques. Des lésions discontinues non-circonférentielles de la média ont été observées dans quatre cas de rupture traumatique de l'artère vertébrale. Nous émettons l'hypothèse que ces lésions étaient dues à une rupture mécanique des cellules musculaires lisses et à la libération d'enzymes cataboliques avec dégradation subséquente de la média artérielle. Pour vérifier cette hypothèse, des artères vertébrales saines ont été incubées avec des extraits bruts de cytosol provenant de muscle lisse bovin pour tenter de reproduire les changements histologiques de la média artérielle dans les artères vertébrales traumatisées. Nous avons observé une dégradation de la média induite par le cytosol, caractérisée par une pâleur de la coloration par la méthode trichrome de Masson, qui était due à l'activité enzymatique catabolique qui était effectivement inhibée par l'inactivation du cytosol par la chaleur. Les changements de coloration induits par le cytosol étaient similaires aux lésions de la média dans les cas de rupture traumatique de l'artère vertébrale survenant naturellement. Nous concluons que, même si les artères vertébrales peuvent être rupturées par une distorsion physique seule, des lésions associées de la média sont dues à la libération traumatique *in situ* d'enzymes cataboliques thermolabiles.

*Can. J. Neurol. Sci.* 1992; 19: 53-56

Massive fatal subarachnoid hemorrhage has been associated with traumatic rupture of a vertebral artery and is usually seen in young, healthy men who are intoxicated and collapse following craniocerebral trauma.<sup>1-4</sup> Death follows within minutes to days after the initial assault, and at autopsy, subarachnoid hemorrhage cannot be associated with underlying vascular anomalies. While many reports suggest an extracranial site of rupture with intracranial subarachnoid spread of blood, more recent studies have drawn attention to rupture of the intracranial portion of the vertebral artery.<sup>5</sup> Usually, the rupture is seen as a longitudinal tear of the posterior aspect of an intracranial vertebral artery. Several mechanisms have been offered to explain the biophysics of traumatic vertebral artery rupture.<sup>6</sup> The most frequently discussed mechanism relies on the observation that the

vertebral artery is tethered to the dura mater at the point where the vessel enters the cranium.<sup>7</sup> Accordingly, it is postulated that stretching the vessel or distortions due to other traumatic forces coming to bear on the vessel result in rupture due to the relative immobility of the artery. Another proposed mechanism suggests that transient occlusion of vertebral arteries due to mechanical displacement of the head results in an acute rise in intra-arterial pressure due to reversed blood flow from the basilar artery.<sup>8</sup> Using an experimental model of the flow reversal hypothesis the characteristic longitudinal tears of the vertebral artery have been reproduced using cadaveric vertebral arteries.<sup>9</sup>

We herein describe four cases with traumatic rupture of a vertebral artery and fatal subarachnoid hemorrhage in which we have found another histologic change in traumatized vertebral

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Received July 18, 1991. Accepted in final form October 10, 1991

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arteries. In addition to the characteristic longitudinal tear, we have observed discontinuous, non-circumferential, apparently necrotic regions of the tunica media.

We hypothesize that necrosis of medial smooth muscle could be due to two possible mechanisms. First, physical distortion of the vertebral artery could result in the disruption of medial smooth muscle, resulting in rupture of cell membranes, exudation of cytoplasm into the extracellular space, and alteration in the usual tinctorial properties of the arterial media. Second, disruption of the smooth muscle cell membrane may release compartmentalized cytosol containing degradative enzymes which catabolize cell-derived and medial matrix macromolecules causing alteration in characteristic smooth muscle staining. We devised two experimental models to test these hypotheses. In the first model, cadaveric vertebral arteries were exposed to distortional forces in an attempt to reproduce alteration in vertebral arterial media staining. In the second model, cadaveric vertebral arteries were treated with crude smooth muscle cytosol extracts with and without inhibition of cytosolic catabolic enzyme activity to determine if histological changes in the media could be reproduced by *in situ* degradation from smooth muscle-derived enzymes.

#### REPORT OF CASES

The histologic changes in the vertebral artery are described in four cases of traumatic rupture of the vertebral artery with extensive fatal basal subarachnoid hemorrhage. Three of these cases (Patients 1-3) have been previously described and details are available elsewhere (case 1, 3 and 4 of reference 5). The remaining case (Patient 4) was a 31-year-old man who was assaulted with multiple head injuries and was found dead in an alley way. At autopsy, there were several recent scalp contusions and abrasions and a laceration at the level of the right mastoid region. Extensive basal subarachnoid hemorrhage was found in the posterior fossa with cerebellar tonsillar herniation. The posterior aspect of the right vertebral artery near the origin of the posterior-inferior cerebellar artery was the site of a transmural 7.0 mm longitudinal tear (Figure 1). Histological examination of the vertebral artery in all cases showed recent platelet-rich thrombus at the site of the transmural tear. At the edges of the tear, the arterial media showed changes consistent with necrosis characterized by amorphous eosinophilia with an absence of nuclear staining. Similar eosinophilic regions were found in several



Figure 1 — Inferior surface of the posterior fossa in a case of traumatic rupture of the right vertebral artery showing massive subarachnoid hemorrhage (case 4). A metal probe exposes the dorsal surface of the vertebral artery showing a 7 mm longitudinal tear.

sections of the non-ruptured portions of the vertebral artery in a multifocal and non-circumferential pattern. In places, the changes involved at most one-half of the vessel circumference, but were more commonly found as discontinuous patches. Histological changes were seen to advantage using the elastic-Martius Scarlet blue, Movat's pentachrome, and Masson's Trichrome stains, where the eosinophilic regions corresponded to pallor of smooth muscle cytoplasmic staining (Figure 2a) with some preservation of myofibrils as indicated by staining with phosphotungstic acid hematoxylin. Immunohistochemical analysis of traumatized vessels with anti-desmin, and anti-muscle specific actin showed no alteration in normal confluent staining. The characteristic histologic changes of the tunica media were only observed in cases of traumatic vertebral artery rupture with survival time of over one hour. In other cases with well-documented near instantaneous death, similar changes could not be identified. In the cases reported here, survival time ranged from one hour to two days.

#### MATERIAL AND METHODS

##### Physical Distortion of Cadaveric Vertebral Arteries

Vertebral arteries were removed from short post-mortem interval autopsies (less than 6 hours post-mortem) and washed in distilled water. One end of the vertebral artery was tied with silk suture and immobilized by pinning to a wooden surface. The free end of the artery was grasped with hemostats and the vessel was stretched manually until near rupture or rupture. Cadaveric vertebral arteries were also distorted using the method of Farag et al.<sup>9</sup> with minor modifications. Following distortion, the mid portion of the vessel and the site of rupture was cut into sequential cross sections, fixed in neutral buffered formalin, processed for paraffin embedding, sectioned and stained using hematoxylin and eosin, and Masson's Trichrome.

##### Smooth Muscle Cytosol Preparation

Bovine myometrium was obtained from a local abattoir from a grossly normal uterus, and packed in ice prior to arrival at the laboratory. All subsequent steps were performed at 4°C. Myometrium was washed and minced in 50 mM TRIS, 150 mM NaCl, 2 mM CaCl<sub>2</sub>, pH 7.4, and homogenized using a Waring blender (7g tissue/mL buffer). Collagen fibers were separated from the homogenate by filtration through a 150 µm mesh. Nuclei, cell fragments and extraneous debris were separated from the filtrate by differential centrifugation at 2000 g for 30 minutes (JS-13.1 Rotor, J2-21 M Beckman Induction Drive Refrigerated Centrifuge). The supernatant was used as a crude smooth muscle cytosol fraction and stored at -70°C until use.

##### Cytosol Treatment of Healthy Cadaveric Vertebral Arteries

Histologically normal vertebral arteries were removed from short post-mortem interval autopsy cases (less than 6 hours post-mortem) and washed in distilled water. Vertebral arteries were sequentially cut into 2.0 mm cross sections, affixed to a cryostat, frozen, 5-7 µm sections cut, and sections mounted on poly-L-lysine coated slides. Cryostat sections were incubated with the crude smooth muscle cytosol fraction containing 50 mM TRIS, 150 mM NaCl, 2 mM CaCl<sub>2</sub>, pH 7.4, or in buffer alone for 6 hours at 37°C. In tandem experiments, vertebral artery sections were also incubated in heat-inactivated crude smooth muscle cytosol to inhibit enzymatic activity in the cytosol preparation (100°C for 15 minutes). Following incubation, slides were fixed in ice-cold ethanol and stained with Masson's Trichrome.



## RESULTS

Physical distortion of cadaveric vertebral arteries failed to reproduce the characteristic changes of medial smooth muscle found in naturally-occurring cases of traumatized vertebral arteries (not shown), and were histologically similar to previous experimental reports.<sup>9</sup> However, incubation of cadaveric vertebral arteries with smooth muscle cytosol extracts resulted in histologic changes of the tunica media similar to traumatized vertebral arteries (Figures 2b and 2d). Histologic changes were typified by the lack of characteristic smooth muscle cytoplasmic staining (Figure 2d) when compared to controls (Figure 2c). Cytosol-induced histologic changes were abolished by heating cytosol extracts to 100°C.

## DISCUSSION

Discontinuous non-circumferential lesions of the tunica media of the intracranial portion of traumatized vertebral arteries are apparently a frequent histologic feature in traumatic subarachnoid hemorrhage. Although previous reports have not identified the lesions which we currently describe, this is likely due to the infrequency of detailed histologic study of the traumatized vertebral artery due to the more common application of post mortem angiography for defining the site of rupture<sup>10</sup>. Despite this, in at least one previous case report, such lesions are visible in photographic representation of histological changes of a traumatized vertebral artery.<sup>5</sup> The present study was performed to 1) characterize the histologic change, and 2) to test the proposed pathogenesis of these stereotypical lesions.

In agreement with previous experimental work, physical distortion of cadaveric vertebral arteries by either longitudinal stretching or uniform distension by perfusion, failed to reproduce the histologic changes of the tunica media observed in naturally-occurring cases of traumatic vertebral artery rupture.<sup>9</sup> This result indicates that physical distortion of arterial constituents alone is not sufficient to cause the medial lesions, and an ante mortem event associated with disruption of vessel integrity must be involved. Since cell injury includes disruption of cell membranes and decompartmentalization of cytosolic and organelle catabolic enzymes, we speculated that this may have a role in the pathogenesis of the localized changes of the arterial media which we have observed.

Incubation of unfixed cryostat sections of histologically normal vertebral arteries with a crude smooth muscle cytosol extract resulted in similar histologic alteration of the tunica media as observed in traumatized vertebral arteries. This cytosol-induced change of the media was inhibited by heating of the cytosol fraction. Abolishing degradative activity by heating strongly suggests involvement of heat labile catabolic enzyme(s) in the pathogenesis of the medial lesions.

Although degradation of the tunica media of traumatized vessels appears to be an important development, it is not likely involved in the rupture of the vertebral artery for two reasons. First, the time course involved in enzymatic catabolism is inconsistent with the rapid time course of vessel rupture and fatal hemorrhage. Consistent with this, we have observed cases of traumatic rupture of the vertebral artery with near instantaneous death that do not show histologic changes of the tunica media.

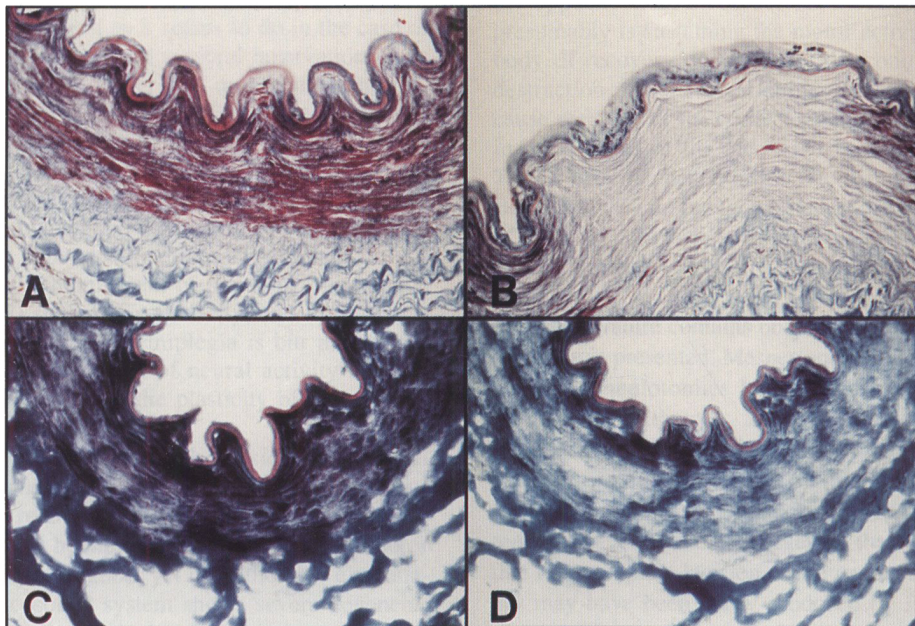


Figure 2 — (A) Normal tunica media of a healthy vertebral artery showing characteristic tinctorial properties of smooth muscle cytoplasm (400X, Masson's Trichrome). (B) Discontinuous traumatic medial lesion of the ruptured vertebral artery of case 3. This lesion was not associated with the site of rupture (400X, Masson's Trichrome). (C) Normal tunica media of a cytosol section of a healthy vertebral artery (400X, Masson's Trichrome). (D) Traumatic medial lesion-like change induced by incubation of a normal vertebral artery with smooth muscle cytosol (400X, Masson's Trichrome).

Second, rupture can be achieved *in vitro* using cadaveric arteries perfused at systolic pressure under fluid dynamical situations which model transient occlusion of the vertebral artery which is presumed to occur during mechanical displacement of the head during trauma. We believe that recognition of the enzyme-induced changes in the tunica media of the traumatized vertebral artery is of diagnostic importance when faced with cases of traumatic subarachnoid hemorrhage. However, the fate of traumatic medial lesions in cases of non-fatal traumatic subarachnoid hemorrhage requires clarification but may lead to later complications such as spontaneous dissection.

In summary, we have presented evidence that characteristic lesions of the tunica media in traumatized vertebral arteries are due to liberation of catabolic enzymes following damage to vascular smooth muscle. Although degradation of the tunica media is not a necessary condition for arterial rupture which can be achieved on a fluid dynamical basis alone, histologic alterations of the tunica media cannot be explained by physical distortion of vessel constituents alone. Recognition of this histologic change will help in the post mortem diagnosis of traumatic rupture of the vertebral artery.

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