Sequential Morphological Changes of the Constrictive Basilar Artery in a Canine Model of Experimental Cerebral Vasospasm by Talc Injection

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ABSTRACT. To demonstrate the possible role of foreign-body reaction to extravasated blood in provoking chronic cerebral vasospasm, talc (crystallized hydrous magnesium silicate) was injected as a non-biologic foreign material into the canine cisterna cerebellomedullaris, and pathologic changes were followed. Anglegraphically, this cisternal talc injection induced delayed and prolonged constriction of the basilar artery, without any evidence of so-called early phase cerebral vasospasm that should occur shortly after an insult. Pathologically, around the spastic artery in the subarachnoid space with talc injection, the appearance of a moderate cellular migration coincided with cerebral vasocostriction, which took place 2 days after talc injection. In the spastic basilar arterial wall, marked constrictive and degenerative changes including myonecrosis and subintimal proliferation were induced by cisternal talc injection as early as on day 2, and the changes were progressive with time. These pathologic changes were extremely analogous to those of the human autopsy cases with chronic cerebral vasospasm (chronic VS) but more prominent than those observed in the experimental autologous blood-induced model. The present study demonstrated that a foreign-body reaction to talc alone could induce chronic VS in the absence of extravasated blood. Thus, it is possible to consider that inflammatory reactions to extravasated autologous blood in subarachnoid hemorrhage may give rise to chronic VS.—KEY WORDS: basilar artery, canine, cerebral vasospasm, talc.


Chronic cerebral vasospasm (chronic VS) following subarachnoid hemorrhage (SAH) is considered to be responsible for mortality and functional failure of patients with ruptured intracranial aneurysms [4, 6, 12–17, 20]. Despite extensive experimental and clinical research, the etiology of chronic VS remains to be clarified.

Inflammatory reactions seen in the spastic vascular wall and its surrounding tissue have been implicated in the pathogenesis of cerebral vasospasm [11–13, 17], and it is generally accepted that cerebral vasospasm initially occurs when the vessel is in contact with autologous blood clot in the subarachnoid space [4, 6, 12–17, 20]. Since extravasated autologous blood is known to be processed by macrophages as a foreign material [22], we hypothesized that extravasated autologous blood clot in SAH could induce the foreign-body reaction in the subarachnoid space. We have previously reported that injection of either autologous blood or a non-biologic foreign material into the cisterna cerebellomedullaris could induce delayed and prolonged vasoconstriction [17–19]. The foreign material we have selected was t alc (crystallized hydrous magnesium silicate), which is known to promote a strong chemical inflammation with granuloma formation [3, 7, 21]. Its inflammatory properties are well documented both clinically and experimentally in achieving pleurodesis [1, 7].

In the present study, the sequential microscopic and ultrastructural changes in the talc-induced constrictive vessels are followed. Furthermore, it is also aimed to compare the nature of the inflammatory lesions in this talc-induced model and the usual autologous blood-induced model in an attempt to elucidate the pathogenesis of chronic VS in relation to the inflammatory processes.

MATERIALS AND METHODS

Animal model and surgical procedures: Fourteen adult mongrel dogs of either sex, each weighing 10 to 15 kg, were used in this study. All animals were anesthetized with intravenous sodium pentobarbital (30 mg/kg), with an intratracheal tube installed for a patent airway, and fixed in a prone position with the neck flexed 30 degrees down by using a stereotaxic frame. They were maintained with spontaneous breathing and blood gas was frequently monitored during the course of the following procedure. To demonstrate baseline angiogram of the basilar artery, the right vertebral artery was aseptically exposed, and a polyethylene catheter (0.86 mm in diameter) was advanced, through which vertebral angiography was performed using 8 ml of meglumine diatrizoate at a rate of approximately 3 ml/sec. Subsequently, the cisterna cerebellomedullaris of the talc-induced group (n=11) was punctured aseptically with a spinal needle (23 gauge). One gram of sterile talc powder was suspended in 7.5 ml of physiological saline, and was injected into the cisterna cerebellomedullaris over a period of 2 min after removing approximately 8 ml of cerebrospinal fluid. The animals were maintained at the same angle with the stereotaxic
frame to distribute the injected material within the preoptin cistern. The day when the above procedure was carried out was designed as day 0. Furthermore, vertebral angiography was individually performed to monitor the vascular constriction by the above mentioned protocol with follow-up on day 0 (1 hr), day 2 and 7. A sham-operated group of dogs (n = 3) served as the control with the identical amount of the physiological saline injected into the cisterna cerebellomedullaris.

The care of the animals and the present protocols complied with the "Principle of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals" (DHHS publication No. [NIH] 85-23, revised 1985) and had been approved by the Animal Care Committee of the Nippon Medical School.

Pathologic procedures: The animals were sacrificed at 1 hr, day 2 or 7 with sodium pentobarbital (30 mg/kg) followed by exsanguination after each angiography. Following infusion of 300 ml heparinized physiological saline into the vertebral artery, intravital perfusion-fixation of the cerebral vasculature was performed with 200 ml of phosphate buffered 4% paraformaldehyde under a perfusion pressure of 150 cm H2O. The craniotomy was performed, and the brain and cervical cord were dissected free. The part of the basilar artery was separated from the brain stem, and was immediately cut into rings (about 1 mm thick) for the microscopic and ultrastructural studies. Small pieces of the basilar artery were fixed in 2.5% glutaraldehyde with postfixation in 1% osmium tetroxide, dehydrated through a graded series of ethanol solutions, and embedded in Epon 812. Serial semithin sections (1 μm) from resin-embedded blocks were stained with 1% safranine-0.5% toluidine blue, and examined by light microscopy for orientation or observation of the spastic artery. Ultrathin sections (70 nm) cut with an LKB V ultramicrotome were double stained with uranyl acetate and lead citrate, and observed with a Hitachi H-7000 transmission electron microscope. In addition, to observe the subarachnoid space around the spastic basilar artery, other parts of the basilar artery including brain stem were also simultaneously fixed in 10% neutral buffered formalin, routinely embedded in paraffin, sections (3 μm) were cut, and stained with hematoxylin-eosin.

RESULTS

Angiographic findings: The cisternal talc injection yielded delayed and prolonged constriction of the basilar artery without any evidence of so-called early phase VS that should occur shortly after an insult. The arterial constriction occurred at 2 days after the cisternal injection, and lasted till day 7. Furthermore, the basilar artery showed uniform vasoconstriction in the entire length. On the other hand, sham-operated group did not show angiographic narrowing. All the animals but sacrificed one survived during the course of 7 days.

Gross findings: Talc suspensions were mostly distributed in the ventral surface of the brain stem. Both 1 hr and 2 days after cisternal talc injection, the basilar artery was embedded in the milky talc suspension clots.

On day 7, the talc suspension clots were also present in the same area (Fig. 1). Sham-operated group had no abnormal findings.

Microscopic findings: At 1 hr and on day 2, the subarachnoid space was moderately expanded due to abundant talc crystals. Furthermore, on day 2, a moderate inflammatory reaction consisting of macrophages, neutrophils and lymphocytes was diffusely present around the basilar artery in close association with talc crystals. No granulomatous reaction was present in any place examined.

On day 7, the subarachnoid space was still expanded, and inflammatory reactions showed an increase in cellularity. Furthermore, granulomas containing a large number of talc crystals were seen and they were surrounded by fibroblasts, histiocytes and foreign-body giant cells with strands of reticulin and some collagen. The granulomas were relatively adherent to the brain tissue in the subarachnoid space. Talc crystals were occasionally seen in macrophages and in foreign-body giant cells within the granuloma (Fig. 2 A, B). The sham-operated group showed no abnormal histologic findings. The basilar artery appeared almost unaltered at 1 hr. Marked spastic changes were observed as early as day 2 and lasted to day 7. On day 2, the endothelial cells with round nuclei were swollen and round in shape. These cells were partly desquamated from the severely folded elastic lamina. The

Fig. 1. Ventral surface of the brain on day 7 after cisternal talc injection. The basilar artery is embedded in injected talc suspension encasing the vessels' cistern.
smooth muscle cells in the basilar artery were often shortened in its major axis, and contained intracytoplasmic vacuoles of various size. Some smooth muscle cells had homogeneously dark-stained cytoplasmas with pyknotic nuclei. Fibrocytes containing intracytoplasmic vacuoles were also seen occasionally in the adventitia. On day 7, the same type of microscopic lesions were seen in the basilar artery, but they were more severe than those on day 2. Moderate subintimal proliferation and mild interstitial edema were observed in the subintima. The elastic lamina was often broken up, and was partly torn off. Focal fibrosis was observed in the widened extracellular space between smooth muscle cells. The above mentioned cytoplasmic alternations of smooth muscle cells increased in frequency at the luminal side of the tunica media (Fig. 3A, B). In addition, mild spongy degeneration, prominent astrocytes, and ischemic nerve cells were diffusely observed within the tissue of the brain stem from day 2. The sham-operated group did not show abnormal findings in any specimens.

Ultrastructural findings: There were no abnormal changes in the basilar artery at 1 hr. On day 2, in addition to the above microscopic findings, endothelial cells had an increased number of various cytoplasmic organelles, such as mitochondria, lysosome and rough endoplasmic reticulum. The elastic lamina remained dense along its luminal edge but contained vacuoles with a few smooth muscle cells in the elastic lamina or in the subintima (Fig. 4). The smooth muscle cells had irregular cell membranes, deeply clefted nuclear membranes, and numerous cytoplasmic dense bodies. Occasional smooth muscle cells contained intracytoplasmic vacuoles of various sizes and content, some appearing as "extended lysosomes" containing fine granules, myelin figures or more amorphous materials. Some smooth muscle cells that microscopically had condensed cytoplasm with pyknotic nuclei were observed disruption of the sarcolemma and the dissolution of myofibrils in cytoplasm, and the condensation of high electron density chromatin in nuclei.

On day 7, these ultrastructural changes became more conspicuous. In the most drastic sections, endothelial cells had irregular cell membranes with cell processes, whose cytoplasm had a honeycomb appearance with numerous intracytoplasmic vacuoles and severely degenerated organelles. These included destruction of internal lamella (crista) of mitochondria and expansion or fusion of lysosome. A prominent loss of tight junctions between endothelial cells was also often observed, and the gaps contained membranous materials and migrated macrophages. The luminal aspect of the elastic lamina lost 'uniformity in the width of the electron dense region. The elastic lamina often appeared thin or double-layered. Numerous proliferated smooth muscle cells in intima appeared migrating through the fenestra or the fissure of
Fig. 4. Spastic basilar artery on day 2 after cisternal talc injection. Note round form of endothelial cells, and corrugated elastic lamina which remained dense along its luminal edge but contained vacuolar formation and a few smooth muscle cells (arrow). Bar represents 3.5 μm.

the elastic lamina. The proliferating smooth muscle cells were distinguishable from normal ones since the former were changed its form to an amoeboid shape with abundant organelles, including rough endoplasmic reticulum, lysosome, mitochondria, and scanty myofibrils. The subintimal space between proliferated smooth muscle cells were severely edematous with aggregation of fine granules or cytoplasmic fragments, fibrin-like materials and degenerated collagen fibers (Fig. 5, 6). The smooth muscle cells closest to the severely corrugated elastic lamina were particularly irregular in shape and had numerous cytoplasmic fragments outside the cells as if the intercellular attachments were torn off. They had deeply grooved or folded nuclei, and abundant myofibrils occupied most of the cytoplasm running waved in parallel to the major axis of these cells and moderately increased pinocytic vesicles beneath or away from the cell membrane. Increased collagen fibrils were also occasionally present in the widened interstitial space between smooth muscle cells corresponding to the focal fibrosis observed by the light microscopy (Fig. 7).

Despite the vertebral angiogram showed uniform arterial luminal narrowing, these pathologic changes in the spastic vascular walls varied in severity among specimens or individuals. The sham-operated group showed no abnormal findings in electron microscopy.

Fig. 5. Most severely affected spastic basilar artery on day 7 after cisternal talc injection. Note the proliferation of numerous smooth muscle cells (*) passing through the fissure of the thin or double elastic lamina (arrow), and subintimal edema included plenty of cell debris. Bar represents 3.5 μm.

Fig. 6. Most severely affected spastic basilar artery on day 7 after cisternal talc injection. Note desquamated endothelial cells (*) containing numerous intracytoplasmic vacuoles, and degenerated and myonecrotic smooth muscle cells (arrow). Bar represents 3.5 μm.
Fig. 7. Most severely affected spastic basilar artery on day 7 after cisternal talc injection. Note irregular cell membranes, detached intercellular attachments, several separations of cytoplasmic parts, deeply grooved nuclei, numerous dense bodies, intracytoplasmic vacuoles (arrow) in the smooth muscle cells, and widened interstitial space between the smooth muscle cells (*). Bar represents 3.5 μm.

DISCUSSION

The present study showed sequential microscopic and ultrastructural changes in the talc-induced constrictive vessels. Around the spastic basilar artery in the subarachnoid space with talc injection, the occurrence of a moderate cellular migration coincided with development of cerebral vasoconstriction, which took place 2 days after talc injection. This cellular reaction may be a host defense mechanism against the injected talc recognized as a foreign material. The involvement of the inflammatory reaction have been implicated in the pathogenesis of chronic VS [11, 12], and it is also supported by our previous findings that interleukin-1β increased in the cerebrospinal fluid of patients with vasospasm after SAH [18]. It is, therefore, interesting that chronic vasoconstriction appeared in close association with the cellular infiltration with a foreign material in the absence of autologous blood clot. It is, however, necessary to further demonstrate if the nature of inflammatory processes against autologous blood and against talc are completely identical by measuring chemical mediators and cytokines.

A variety of morphologic changes including myonecrosis and subintimal proliferation were induced by talc, and the changes were progressive with time after cisternal talc injection. It is considered, however, that these changes are largely a consequence of sustained vasoconstriction, and are likely to cause a loss of elasticity in the vascular walls. Furthermore, the microscopic and ultrastructural changes were extremely analogous to those reported as specific lesions of naturally-occurring cerebral vasospasm [4, 6, 13, 14]. The pathologic changes in the talc-induced constrictive vessels were also similar to but more prominent than those observed in the experimental cerebral vasospasm with autologous blood injection [6, 8, 14–17, 20].

The morphologic variations in severity among specimens or individuals were related to difference in the degree of smooth muscle cell proliferation. In the vascular wall, the repair of the injured endothelium is commonly accomplished by the proliferation of smooth muscle cells from the intima. The proliferated smooth muscle cells, so-called synthesized type, were distinguishable from the spastic type since the former were rich in cell organelles and collagen synthesis was active [2]. Then, these cells often looked penetrating the intima and elastic lamina. Under chronic contraction of the muscular artery, this phenomenon would not only be responsible for the luminal narrowing but also for destruction of the vascular wall by migrating cells. In fact, there were a correlation between number of proliferated smooth muscle cells and the degree of degenerative changes in the talc induced-constrictive vessels.

In addition, an increased number of pinocytic vesicles was noted beneath or away from its cell membrane, probably due to the adaptation of the smooth muscle cells to the contraction. These organelles are considered as an intracellular calcium pool [5], mechanically resembling the T-system of the striated muscle [9, 10]. It is suggested, therefore, that the multiplied pinocytic vesicles seen in the smooth muscle in the lesions of chronic VS would be due to an increased uptake of calcium from the extracellular space, and that it further promoted vasoconstriction.

From these results, it is possible to conclude that a foreign-body reaction alone is capable of producing chronic VS in the absence of hemorrhage. Thus, the present study further supports the inflammatory pathogenesis of chronic VS in which extravasated autologous blood in SAH may play a role in provoking the inflammation.

REFERENCES


