The effect of a lipid hydroperoxide of arachidonic acid on the canine basilar artery

An experimental study on cerebral vasospasm

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The in vivo spasmogenic capacity of a lipid hydroperoxide (15-hydroperoxy arachidonic acid: 15-HPAA) was studied in a chronic experiment using the dog. The 15-HPAA was injected into the cisterna magna (0.2 or 2 mg emulsified in bovine serum albumin solution). The changes in diameter of the basilar artery were followed by angiography, and the morphological changes were studied by electron microscopy. The cisternal injection of 0.2 mg of 15-HPAA caused a mild constriction of the basilar artery which lasted about 7 hours. The cisternal injection of 2 mg of 15-HPAA caused a biphasic constriction, the initial phase of which was a moderate narrowing lasting about 10 hours. The second phase started on the 2nd or the 3rd day after injection. The intensity of the arterial narrowing was more pronounced in the second phase than in the first. The prolonged secondary constriction of the basilar artery continued until sacrifice on the 7th day after injection.

Electron microscopic study revealed a marked degenerative change in the endothelium and myonecrotic changes in the tunica media. The prolonged arterial constriction in the second phase was invariably associated with remarkable degeneration of the endothelium. On the other hand, myonecrotic changes were limited to a small number of smooth-muscle cells.

The results of the present study are consonant with the hypothesis that lipid peroxidation associated with lysis of the subarachnoid clot is involved in the genesis of chronic vasospasm in subarachnoid hemorrhage.

Key Words • subarachnoid hemorrhage • vasospasm • free radical • peroxide • prostaglandin

Cerebral vasospasm in subarachnoid hemorrhage (SAH) is a problem of great clinical importance because its occurrence in the course of the illness is known to affect the outcome of patients. Yet, its pathophysiology remains unresolved. The frequent association of vasospasm with the presence of subarachnoid clot has been verified by computerized tomography. Based on this fact, a hypothesis has been developed that hemolysates from the subarachnoid clot, especially oxyhemoglobin (oxy-Hb), play an important role in the genesis of chronic vasospasm.

The potent vasoconstricting action of oxyHb has been reported elsewhere. In addition to this direct vasoconstricting activity, oxyHb possesses an action of initiating free radical reactions in the presence of polyunsaturated fatty acids (PUFA's) as follows. It has been shown that oxyHb, in its conversion to methemoglobin, releases activated species of oxygen such as superoxide anion, hydrogen peroxide, and singlet oxygen. These activated oxygens successively initiate free radical reactions in the presence of oxygen, PUFA's, and catalytic metals, producing various lipid peroxides. It is also known that some degradation products of hemoglobin, such as hematin, possess potent catalytic actions of enhancing autoxidation of lipids. In addition to the above nonenzymatic reactions, two distinct pathways of enzymatic reactions involving PUFA's have recently been clarified. The first one involves lipoxygenases of platelets and/or leukocytes which lead to formation of hydroperoxides of PUFA's. The second path-
The dose of 15-HPAA used in this experiment was based on previous experiments. The dose of 15-HPAA (2 mg) was administered to six dogs, which four dogs received 1 ml of 0.5% bovine serum albumin solution intracisternally. In Group 2, six dogs received 0.2 mg of 15-HPAA. In Group 3, 16 dogs received 2 mg of 15-HPAA. In Group 1 dogs, constriction of the basilar artery was not observed on any of the angiograms obtained throughout the period up to the 7th day after injection.

**Materials and Methods**

Adult mongrel dogs, weighing 13 to 20 kg, were anesthetized with intravenous injection of pentobarbital. Arterial blood gases and the body temperature were frequently checked and maintained within the following ranges; pH 7.35 to 7.45, pO2 30 to 35 mm Hg, base excess -3.0 to +3.0, body temperature 37°C to 38°C. The left vertebral artery was catheterized in the neck. The ascending aorta was clamped, and a brief perfusion with heparinized saline was started immediately, followed by perfusion with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The perfusion pressure was always maintained at 150 cm H2O. The basilar artery was excised and immersed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 12 hours. The specimen was then washed for 20 minutes in 10% sucrose in 0.1 M sodium cacodylate buffer. The specimen was then postfixed in 1% OsO4 in 0.1 M sodium cacodylate buffer (pH 7.4) for 90 minutes; it was then dehydrated in graded series of ethanol and embedded in Araldite 502. Ultrathin sections were cut on an LKB Ultrotome V, stained with uranyl acetate and lead citrate, and examined in a JEOL 100 U electron microscope.

**Results**

**Angiographic Changes of the Basilar Artery**

In Group 1 dogs, constriction of the basilar artery was not observed on any of the angiograms obtained throughout the period up to the 7th day after injection.
In Group 2, mild constriction of the basilar artery was seen on the angiogram obtained 1 hour after the injection of 15-HPAA. This constriction continued for 7 hours (Fig. 1). In Group 3, a biphasic constriction of the basilar artery was observed. The initial phase of constriction began 1 hour after the injection and continued for 7 hours. It then began to subside, and at 10 hours after the injection, the basilar artery almost resumed its original caliber. The angiograms obtained on the 2nd or 3rd day, however, revealed recurrence of the basilar artery constriction with a more severe magnitude than that in the initial phase. This second phase of constriction lasted until the 7th day, that is, the day of sacrifice (Fig. 2). Representative angiograms are shown in Fig. 3.

**Ultrastructural Changes of the Basilar Artery**

In Group 1, no abnormal morphological changes were observed. In Groups 2 and 3, the following pathological changes in the arterial wall were revealed.

A degenerative change in the endothelium was invariably found in all the specimens in both groups. There was an increase in the cytoplasmic density and appearance of many small vesicles in endothelial cells. These changes indicate degeneration of the endothelium (Fig. 4). Also, endothelial cells were partially or completely separated from each other, probably at the tight junctions. Separation of the endothelial cells resulted in the denudation of the elastic lamina (Fig. 4). The degenerative changes of the endothelium were essentially similar in Groups 2 and 3. However, the changes were more pronounced in Group 3 than in Group 2.

Interesting changes were also found in the tunica media, consisting of disruption of myofilaments and the appearance of many small vesicles, dense bodies, and vacuoles in the smooth-muscle cells. Pyknotic changes in the nuclei of smooth-muscle cells were also observed (Fig. 5). Many small vesicles and electron-dense granules were observed in the extracellular space between smooth-muscle cells (Fig. 6). These myonecrotic changes were, however, limited to a small number of smooth-muscle cells.
Fig. 3. Angiograms before (left) and after (center and right) intracisternal injection of 2 mg of 15-HPAA. Upper: Angiograms showing the constriction of the basilar artery observed at 1 hour (center) and 3 hours (right) after the injection. Lower: Angiograms at 6 hours (center) and 4 days (right) after injection. Note the marked constriction of the basilar artery on the 4th day.

Discussion

The present experiment revealed that a single intracisternal injection of a hydroperoxide of arachidonic acid, 15-HPAA, is capable of producing a sustained constriction of the basilar artery lasting for days. With the smaller dose (0.2 mg), 15-HPAA caused only a mild and transient constriction. With the larger dose (2 mg), a similar transient constriction occurred initially, and lasted an average of 10 hours, when the basilar artery regained almost its original size. It is of great interest that the angiograms obtained on the 2nd
or 3rd day revealed the recurrence of constriction of the basilar artery, which was even more severe than in the initial phase. This second phase of constriction, which was observed by daily angiograms, continued until the day of sacrifice, that is, the 7th day after the injection of 15-HPAA. Morphological study by electron microscopy revealed various changes in the vessel wall. Most striking was the degenerative change in the endothelium. It was observed as early as several hours after injection and invariably observed through the 7th day. Adhesion of platelets around the degenerated endothelium, which suggests the occurrence of some pathological interactions between the blood and the arterial wall, was occasionally seen in Group 3.
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FIG. 5. Electron micrograph showing myonecrotic changes of the basilar artery. *Left:* Artery from a dog sacrificed 10 hours after intracisternal injection of 2 mg of 15-HPAA. A degenerated smooth-muscle cell surrounded by a basement membrane is seen between two normal ones. Disruption of myofilaments and many vesicles of variable size are observed. The intercellular space contains bundles of collagen fibers (col). × 9000. Bar: 1 μ. *Right:* Artery from a dog sacrificed 5 days after injection of 2 mg of 15-HPAA. The nucleus (N) of a smooth-muscle cell shows a pyknotic change. An electron-lucent vacuole of large size is recognized in the cell. × 6900. Bar: 1 μ. Asterisks indicate central core of cellular organelles of smooth-muscle cell.

Although this endothelial damage appeared to be more severe in Group 3, it was not possible by electron microscopical observation alone to correlate the changes with magnitude of constriction of the basilar artery.

Myonecrotic changes in the tunica media were also observed in most of the specimens examined, but they were limited to a small number of muscle cells and were far less conspicuous than those of the endothelium. These morphological changes produced by cisternal injection of 15-HPAA bear a striking similarity to those induced by whole-blood injection as reported by others.¹ ¹ ¹ ¹,¹ ² ²,¹ ³ ³ However, much remains to be clarified with regard to the mechanism of action of 15-HPAA in the present study.

We have already reported that 15-HPAA and the hydroperoxide of linoleic acid possess a mild vasocontractile action to the canine basilar artery in vitro.¹ ² Similar results have been reported using the rabbit abdominal aorta.² Oxidation of sulfhydrl groups of the cellular membrane by peroxides was suggested as a possible mechanism of vasoconstriction by these authors. Also, in the present in vivo experiment, a mild and transient vasoconstriction was observed following the intracisternal injection of 15-HPAA. This initial phase of vasoconstriction may be explained by the direct action of 15-HPAA. However, the occurrence of the second, more severe phase of vasoconstriction in Group 3 is difficult to explain by the direct action of 15-HPAA, because its concentration in the CSF should have much declined in this period. Therefore, some other effect of 15-HPAA should be sought.

Tani, et al.,¹ ³,¹ ⁴ suggested that the most characteristic change of myonecrosis was the appearance of aggregated granules and vesicles in the widened extra-
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Fig. 6. Electron micrograph showing myonecrotic changes of the basilar artery from the same specimen as in Fig. 5 left. Left: A large number of small vesicles and many dense bodies are seen in a smooth-muscle cell. × 18,000. Bar: 1 μ. Right: Many small vesicles and multivesicular bodies are observed scattering in the intercellular space between collagen fibers (col). × 18,600. Bar: 1 μ. sm: normal smooth-muscle cell.

Cellular space between smooth-muscle cells, and that these granules and vesicles might represent a vasoactive substance that affects the adjacent normal muscle cells, causing them to constrict. Their findings are of particular interest because they recorded granules and vesicles similar to those we found. The chemical natures or the actions on the smooth muscles of these substances are unknown, but the concept that the cause of vasospasm resides in the vessel wall itself may help to interpret the present results. In a similar context, the cause of prolonged vasospasm might be related to the pathological changes in the endothelium. The severe damage of the endothelium that was invariably associated with prolonged vasoconstriction in the present study would suggest that some pathological interactions took place between the luminal surface of the arterial wall and the blood components, such as platelets. The damaged endothelium provides a suitable condition for adhesion and aggregation of platelets. It has already been suggested by some investigators that the release of thromboxane A2 (TXA2) from aggregated platelets and production of other vasocontractile prostaglandins may be the cause of cerebral vasospasm.\(^1,11,38\)

On the other hand, it has been shown that normal cerebral vessels are protected from the action of TXA2 by the simultaneous synthesis of prostaglandin \(\text{I}_2\) (PGI\(_2\)) in their walls, particularly in the endothelium.\(^{18,22}\) Although not yet proved experimentally, this protective mechanism may not be functioning in the basilar artery, in which the endothelium has undergone such a severe degenerative change as seen in the present model. In this regard, Boullin, \textit{et al.},\(^8\) have recently suggested that vasospasm may be due to diminished synthesis of PGI\(_2\) in the cerebral artery. Since \(15\)-HPAA and other lipid peroxides are known to act as specific inhibitors of PGI\(_2\) synthetase,\(^{21,27}\) it seems possible that reduction of PGI\(_2\) synthesis in the basilar artery participated in the occurrence of prolonged vasoconstriction in the present model. Thus, the endothelial injury might be expected to exert dual influences on the vessel wall as follows. One is the accumulation and aggregation of platelets causing continuous supply of vasoconstrictors such as throm-
boxane A2, serotonin, and norepinephrine. The other is diminution of PGI2 synthesis in the arterial wall, which means that the artery is continuously exposed to unopposed actions of vasoconstrictive agents resulting in prolonged vasospasm. The actual determination of PGI2 synthesis capacity of the cerebral artery following SAH will be mandatory to confirm the above hypothesis.

What, then, would be the cause of the endothelial damage in the present model? The first possibility is that this is the result of prolonged vasoconstriction. So far as the present model is concerned, this suggestion seems unlikely, since the endothelial change was observed as early as several hours after injection of 15-HPAA, thus far preceding the occurrence of the second phase of vasoconstriction. It is also hard to conceive that endothelial nutritional disturbances would occur associated with moderate constriction of the artery, because the endothelium is directly fed by the blood stream.

The second possibility, which seems more likely to us than the first, is that 15-HPAA caused free radical injury in the cerebral artery. As a species of lipid peroxides, 15-HPAA is expected to induce free radical chain reactions. It has been reported that cerebral vessels are liable to undergo free radical damage because they are particularly rich in PUFA's as compared to vessels of other organs. The histotoxic influence of free radicals has been well established. It has also been shown by Fischer and Nelson, that tocopherol depletion coupled with augmented intake of linoleic acid in chicks led to the occurrence of encephalomalacia which was due to the pathological changes in the cerebral vasculature, especially in the endothelium. Thus, free radical injury by 15-HPAA seems to be a possible mechanism of the endothelial damage. However, it may be necessary to consider some other effects of 15-HPAA, such as its detergent action. As to the mechanism of action of 15-HPAA in relation to the production of the endothelial injury, the present study does not permit us to draw any definite conclusions. This will remain an interesting subject for further investigation.

The cause of vasospasm following SAH is presently considered as multifactorial; the present study suggests that free radical reactions associated with lysis of the subarachnoid clot may also play an important role in the genesis of vasospasm.

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