Microvascular alterations following cerebral contusion in rats

Light, scanning, and electron microscope study

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✓ Cerebral contusion was caused in 18 rats by dropping various weights on an exposed dura of one hemisphere. One or 3 hours after the injury the animals were sacrificed by perfusion with paraformaldehyde-glutaraldehyde solution. The traumatic microvascular and neural tissue alterations were studied with light, scanning, and electron microscopic techniques. The microvascular obstructions and damage were revealed in this study as major histological alterations, causing secondary neural damage. The obstruction of the vessels appeared to have been caused 1) by extravascular pressure from destroyed and swollen tissue, petechial hemorrhage, and dissecting extraluminal clots; and 2) by intravascular clots. Besides the tearing and shearing effects causing petechial hemorrhages, the capillary walls were often thinned and irregular.

KEY WORDS • cerebral contusion • microvasculature • histology • electron microscopy • nerve injury • ultrastructural study • capillary

Cerebral contusion is a frequent and serious problem in man. While a large body of literature is available on the pathological alterations of the brain after cerebral contusion, the associated microvascular alterations of the brain have been inadequately studied, since most investigators mainly focused on alterations in the neural tissue. Some have reported on the gross appearance of the brain without performing histological examinations. Few experiments have been designed specifically to study microvascular alterations following cerebral contusion.

In previous experiments, the extremely abundant and elaborate capillary bed of the brain was shown by special techniques, such as in vivo injection of vital dyes, india ink, and microbarium, and in vivo perfusion fixation of the tissue with glutaraldehyde. Brains studied without these techniques do not show the vast extent of the capillary bed (see Fig. 2). The lumina of the capillaries in the brain are wide enough to allow the passage of a few red cells; in some capillaries only one red cell can pass at a time. We have studied the histopathological alteration of these vessels after cerebral contusion in rats, and our findings are presented in this paper.

Materials and Methods

Eighteen adult Sprague-Dawley rats, weighing 270 to 390 gm each, were anesthetized by intraperitoneal injection of 40% chloral hydrate solution (1 cc/kg body weight). The animal’s head was secured in a stereotaxic apparatus and a craniectomy was performed in the right parietal region, producing a burr hole approximately 9 mm in diameter. The dura was left intact. The burr hole was made with a small fine drill to avoid trauma to the dura and the cerebral cortex.

Solid glass rods with a base diameter of 5 mm, but of various lengths and weighing 1.2, 2.4, or 4.8 gm, were dropped on the brain from a height of 50 cm. The glass rods were dropped like pistons on the exposed dura through vertically positioned glass tubing of a slightly wider diameter than the glass rods. The size of the burr hole was made slightly larger than that of the piston end to prevent the piston from striking the edge of the calvaria. The glass tubing was mounted with the lower end positioned about 2 mm above the dura so that the air in the tube could escape easily as the piston fell. A piston with a diameter of 5 mm was selected so that the area of impact would be confined within one
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Fig. 1. Cross sections of brains from nine rats with cerebral contusions, three each from Groups A, B, and C. The contusion was caused by dropping on the exposed dura from a height of 50 cm weights of 1.2 gm in Group A, 2.4 gm in Group B, and 4.8 gm in Group C. In general, the extent of the injury was deeper with heavier weights.

hemisphere of the rat, but so that the end of the piston would not be small enough to penetrate the dura and brain. The 1.2-, 2.4-, and 4.8-gm weights were chosen after many trials because they were found to cause various degrees of brain contusion sufficient to be observed with the naked eye, yet they did not cause death within 3 hours. Each weight was used in six animals to obtain three levels of injury intensity: Group A animals received the least severe injury and Group C the most severe injury (Fig. 1).

In each group, three animals were kept alive for 1 hour and three others were kept alive for 3 hours before

Fig. 2. Photomicrographs of specimens from a normal rat brain without in vivo perfusion of formaldehyde-glutaraldehyde (A: H & E, × 77; B: H & E, × 308). The animal was killed by injection of pentobarbital into the heart. The brain was removed immediately and fixed in formaldehyde-glutaraldehyde solution. This brain was used as the control for comparison with other normal brains and with contused brains from animals that underwent in vivo perfusion of fixative. Note that the capillaries are filled with blood, without which they cannot be easily identified. The extent of the capillary bed also cannot be visualized well in these specimens in comparison with other normal brains in which in vivo perfusion was performed (see Fig. 3).
Fig. 3. Specimens from brains of normal control rats that underwent in vivo perfusion of formaldehyde-glutaraldehyde. A: Photomicrograph showing the abundance of capillaries. H & E, × 83. B: Photomicrograph at a higher magnification showing the normal appearance of the nerve cells. H & E, × 332. C and D: Scanning electron micrograph (C, × 830) and transmission electron micrograph (D, × 43,990) of normal brain revealing abundant vessels that are clearly open and without perivascular spaces. The neural tissues are compact and continuous. In the scanning electron micrograph (C), the size of the vessels can be estimated as compared with the size of a red cell (arrows).

being killed with in vivo perfusion fixation. Perfusion was carried out with 100 ml of 0.9% saline solution to remove the red cells from the vessels, followed by 250 ml of modified 4°C Karnovsky’s paraformaldehyde-glutaraldehyde fixative (1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2). The perfusion materials were injected via a cannula inserted through the left ventricle into the aorta. Insertion of the cannula into the aorta was performed rapidly, with the entire procedure for opening the chest and insertion of the cannula taking less than 1 minute.

Immediately after injection of perfusion material, the cranium was opened and the brain was removed and immersed in Karnovsky’s fixative for at least 48 hours. Cross sections of the brain were dehydrated in graded alcohol solutions, and sections were cut and stained with hematoxylin and eosin or toluidine blue. Sections were obtained from the area of contusion, from its periphery, and from the opposite hemisphere. These tissues were postfixed with 2% osmium tetroxide solution, dehydrated in the graded alcohol, dried in a Polaron critical point dryer, coated with a mixture of gold and platinum, and viewed through a Wikscan 100 scanning electron microscope.* Samples from the same areas were also stained with toluidine blue for evaluation by electron microscopy.

* Polaron critical point dryer manufactured by Polaron Instruments, Inc., 2293 Amber Drive, Hatfield, Pennsylvania; Wikscan 100 scanning electron microscope made by Coates and Welter Instrument Corp., 777 North Pastoria Avenue, Sunnyvale, California.
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Four animals without brain contusion were used as controls: intravascular perfusion was performed in vivo in three, and one was not perfused. This latter animal was killed with an intravenous injection of pentobarbital, so as to demonstrate the difference between the appearance of the vessels without perfusion (Fig. 2) compared to those with in vivo perfusion (Fig. 3). The histological appearance of each contused hemisphere was compared with that of the opposite hemisphere and with that of normal controls.

Results

In all three normal brains from control rats with in vivo fixation, the microvessels were so abundant that a cross section of a vessel could be found between every few nerve cells (Fig. 3). Furthermore, since the blood was washed out of the vessels prior to fixation in vivo, only occasional or sporadic red cells could be detected in the vessels. The fixed vessels could be clearly seen, with their lumina open and without any perivascular spaces. There was no space around the nerve cells. The neural tissues were perfectly fixed, compact, and continuous.

In the brains with contusions, there were various degrees of vessel obstruction, vascular and perivascular alterations, and neural tissue damage depending on the severity of the impact. These findings are summarized below.

Vascular Obstructions

Blood vessel obstruction was conspicuous and was present in three distinct forms: collapsed vessels, intravascular clotting, and extravascular clotting.
Collapsed Vessels. Light microscopy demonstrated a marked paucity of capillaries, and some could not be seen because their walls were collapsed. Unless capillaries were filled with red cells, identifying them was difficult. Under low magnification (Fig. 4A) and with a superficial glance, this paucity of vessels may not be appreciated because the enlarged perivascular and pericellular spaces may be mistaken for vessels. However, when a higher magnification (Fig. 4B) is compared with normal tissue (Fig. 3A and B) and with tissues from the opposite hemisphere (Fig. 4C and D) the difference is easily discerned.

Intra- and Extravascular Clotting. The lumina of a considerable number of vessels were blocked by intravascular clots (Fig. 5A, B, and C). The obstruction was specifically present in the capillaries, and the red cells were noted to be packed in the vessels like rows of coins. Some vessels were partially or totally blocked by blood extravasated along the outside wall of the vessel (Fig. 5D).

Perivascular Alterations

With light microscopic study, large perivascular and pericellular spaces were visible (Fig. 6A and B). Electron microscopic studies revealed that the spaces were caused by swollen or torn astrocytic processes and sheared cell membranes (Fig. 6C and D), and by extravasation of fluid into the tissues. The torn vascular walls caused petechial hemorrhages which dissected through the neural tissues causing further nerve damage remote from the site of the vascular tear (Fig. 7). Occasionally, a vessel was sheared and separated from its bed (Fig. 7B).
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FIG. 6. A and B: Photomicrographs showing the perivascular (arrows) and pericellular (arrowheads) spaces that were conspicuous features following cerebral contusion. H & E, × 320. The perivascular space formation was most severe in and around the area of contusion (A), but extended even to the opposite hemisphere (B) when the trauma was severe. C and D: Transmission electron micrographs (× 53,000) reveal that these spaces were created by swollen (C) and torn (D) astrocytic processes (AP): arrows indicate the thinned wall of the capillaries (Cap).

Neural Damage

The nerve cells lost their usual plump and healthy appearance, becoming elongated, irregular, and triangular in shape (Fig. 8A), the configuration commonly seen in hypoxia. The membranes of some nerve cells were torn (Fig. 8B and C). This, together with the shrunken appearance, contributed further to what appeared as wide spaces on light microscopy. The nuclei of some of the cells appeared naked and were practically denuded of cytoplasm. The mitochondria and other cytoplasmic organelles appeared dispersed (Fig. 8B and C). These alterations were quite severe when compared with normal brains (Fig. 3D and Fig. 8D). The white matter fibers were torn or separated by the extravasation of blood or exudate, as shown in Fig. 7C and D.

Comparison of Experimental Groups

In all three experimental groups, the vascular and neural alterations were most prominent in the center of the contused area, becoming gradually reduced in the tissues farther from the site of injury. What appeared as a contused area on gross examination was mainly related to that part of the brain which is associated with petechial hemorrhages. However, the microscopic vascular and neural tissue alterations mentioned above extended considerably beyond the grossly contused area. In Group A animals with the lightest injury (1.2 gm), these areas extended into the white matter. In Group C, with the most severe injury (4.8 gm), the alterations extended across the midline into the opposite hemisphere. The extent of the damage in Group B (2.4-gm impact) was somewhere between that seen in the other two groups.

Besides shearing and tearing of the vessels, which caused petechial hemorrhage, the walls of the capillaries were often thinned and denuded of surrounding tissues (Fig. 6C and D). Finally, comparison of brains from...
FIG. 7. A and B: Scanning electron micrographs showing the torn vascular walls (A: × 500, arrows) and sometimes sheared vessels (B: × 207). C: Photomicrograph showing the petechial hemorrhage and extravasated fluid dissected through the neural tissues, sometimes across the hemisphere. Arrows indicate direction of flow. H & E, × 83. D: Transmission electron micrograph. The extravasated fluid has torn and destroyed the cell membrane, dispersing mitochondria (M) and other cytoplasmic organelles, × 43,990.

animals sacrificed at 1 and 3 hours following injury revealed that, at 3 hours after the injury (Fig. 9B and D), the vascular and neural damage was more severe than at 1 hour (Fig. 9A and C).

Discussion

These studies reveal that, in addition to neural tissue damage, microvascular obstruction is a major pathological alteration following cerebral contusion. Actually, a major portion of the neural damage may be a secondary result caused by vascular obstruction. This obstruction appears to be caused 1) by extravascular pressure from destroyed and swollen tissue, petechial hemorrhage, and dissecting extraluminal clots; and 2) by intravascular clots. These alterations cannot be detected well unless the circulating blood is washed out and the brain is fixed in vivo. When the brain is perfused with fixatives, the normal vessels stand out as patent and are easily detected. Conversely, the vessels obstructed by in vivo intravascular clots or by external tissue pressure would not receive the perfused fixative. Removal of the red cells prior to perfusion is, therefore, essential so that the intravascular clots occurring before death can be differentiated from postmortem clots.

Our studies also show that the microvascular obstruction develops within 1 hour after cerebral contusion and is more extensive at 3 hours. These intervals were chosen because it is usually within these periods that head-injured patients are brought to the hospital for treatment. Assuming that alterations similar to those in our study occur in the brain of man after cerebral contusion, it seems that irreversible neural damage often takes place before patients are brought to the hospital. Because the tissue damage after contusion is more extensive at 3 hours than at 1 hour, one can assume that, if an effective treatment were available, its early application would reduce the progression of brain damage.

Courville defined cerebral contusion as a “bruise, a localized [sic] and circumscribed” alteration of the
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FIG. 8. Photomicrograph (A: toluidine blue, × 320) and transmission electron micrographs (B: × 42,400; C: × 31,200) of contused brain. The nerve cells appear shrunken, distorted, and triangular in shape (A, arrowheads). This, together with torn cytoplasmic membrane (B and C) created pericellular spaces. The torn membrane has destroyed the entire cytoplasm of the nerve cells, dispersing the mitochondria (M) and other cytoplasmic organelles. The nerve cell nuclei (N) are practically naked. A comparison with the transmission electron micrograph of normal nerve cells (D: × 42,400) reveals the degree of cytoplasmic destruction in the injured brain.

Scheinker and Evans were among the few investigators who paid special attention to the vascular alteration of the central nervous system after trauma. They noted the dilation and stasis of smaller veins and capillaries with dilation of the perivascular spaces, and attributed these changes to “vasoparalysis.” Although Scheinker was not certain of the physiological basis, he believed that, in some “unknown manner,” the smaller veins and capillaries dilated “to such an extent that actual slowing of the blood stream and partial or complete stasis” resulted. According to Courville, it was Fischer who, in 1871, “introduced the idea” of paralysis of small blood vessels following concussion. However, Fischer’s idea, like that of Scheinker, was only a hypothesis; neither investigator was certain nor had any laboratory proof to support his idea. Our studies, however, demonstrate that the cessation of circulation in these vessels could simply be caused by intravascular clotting and by increased tissue pressure

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FIG. 9. Photomicrographs of specimens from four animals comparing the alterations that took place at 1 hour (A and C: H & E, × 74) with those at 3 hours (B and D: H & E, × 74) after contusion. A 1.2-gm weight was used in two animals (A and B) and a 4.8-gm weight in the other two (C and D). Note that the alterations are less severe at 1 hour after contusion (A and C) than at 3 hours after contusion (B and D). Severe tissue destruction and necrosis (N) are particularly evident at 3 hours after contusion in the animal with the 4.8-gm injury (D).

outside the lumina. A neurophysiological explanation for a phenomenon such as vasoparalysis should at least wait for more definitive proof.

Like most neuropathologists, Scheinker obtained his material from postmortem human brain and spinal cord tissue obtained from victims of trauma in which no in vivo intravascular fixation could be used. Hence, it would have been impossible for him, as it has been for others, to appreciate the vast degree of vascular obstruction noted in our material. Nor could he have differentiated well the circulating red blood cells from the noncirculating clots in the capillaries. We believe that the extent of capillary obstruction cannot be detected if the tissues are studied mainly under high magnification with the electron microscope since only a very small segment of the tissue can be studied. A combination of both methods is essential.

The formation of pericellular and perivascular spaces is commonly referred to as "cerebral swelling." Our material revealed that, besides swelling of the astrocytic processes, a major portion of these spaces is caused by shearing of the membrane and by destruction of the cytoplasm of the cells (Fig. 8B and C). Swelling, then, while a partial cause, does not explain the entire process. A term such as "swelling," taken literally, may also convey the expectation that it should subside. In view of the marked cellular destruction after cerebral contusion, such an expectation is, of course, unrealistic.

Since the fixative material cannot reach the brain tissue in the areas with obstructed blood vessels, we cannot presume that, even in material fixed in vivo, all the histological alterations are due to events that have taken place before death. Still, a small amount of tissue alteration will occur following death. Hence, when the tissue is removed quickly for fixation and when the fixation takes place rapidly in the entire brain, the degree of postmortem changes will be reduced. We believe that the immediate removal of the brain after in vivo perfusion and the small size of the brain used in these experiments (allowing rapid fixation of the entire brain) permitted us to observe those tissue alterations that most closely represent the last in vivo condition.

Because we exposed the dura, our model is not
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entirely similar to the cerebral contusion that occurs commonly in man. Of course, the presence of the scalp and skull, the angle of impact, and the mobility of the head and neck in man have considerable effect on the extent of the injury. Our study was not performed to evaluate the biomechanics of cerebral contusion. Instead, our hope was that our model would offer a reasonably reproducible degree of injury to the brain that could be studied within the period stated.

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