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Cerebral microvessel perfusion and pathologic alteration of the brain during drowsiness and coma, caused by brain tumor (A laboratory study in rats)

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Abstract

Background—Deterioration of consciousness and coma, in cerebral compression, is traditionally thought to be caused by compression, shift, hemorrhage, or herniation of the brainstem. This study was done to evaluate the vascular perfusion and pathologic alteration in the entire brain during drowsiness and coma.

Methods—Brain tumors were developed in three newborn rat litters by inoculation of Kirsten Sarcoma Virus (a murine erythroblastosis virus) in the brains. Within several weeks brain tumors developed. When animals became drowsy or comatose, their brains were perfused with microbarium, India ink, or paraformaldehyde solution. In two animals, the brain vasculature was casted by plastic materials. Brains were fixed for magnification radiography, or were prepared for histological examination

Results—The brains of control animals showed an abundance of microvessels and penetrating capillaries, located perpendicular to the cortex and deep within the brain. The latter can not be detected even in the best routine cerebral angiography in man. Microvessels were obstructed, in a patchy and dispersed fashion, in drowsiness especially in ipsilateral hemisphere. Obstruction of microvessels was present not only in the brainstem but also was present in the rest of the brain and in cerebellum of comatose animals; larger vessels appeared markedly narrowed. The study also revealed evidence of diffuse infarcts, cellular ischemia, swelling, and periventricular damage throughout the brain.

Conclusions—During drowsiness and coma, caused by cerebral compression, cerebral capillaries progressively obstruct not only in the brain stem, but also throughout the brain; considerably more severe during coma than drowsiness. These likely causes diffuse neurological disabilities and behavioral changes often seen after recovery from coma caused by cerebral compression.

Keywords

cerebral compression; coma; drowsiness; infarct; microvessels; tumors

Introduction

Deterioration of consciousness in cerebral compression is traditionally thought to be the effect of compression, shift, hemorrhage, or herniation of the brainstem.^{6,18,23} This concept stemmed from the fact that brainstem shift and damage is so frequently noticed in postmortem examination of patients who died after coma and increased intracranial pressure, caused by various space-occupying lesions. Such alterations are now clearly apparent on CT and MRI examinations. The question remains as to what changes may also take place elsewhere in the brain during drowsiness or coma. Because neurological and behavioral disabilities frequently occur in those patients recovering from coma caused by cerebral compression; and because such conditions could well be caused by ischemia, a study of microcirculation in coma appeared relevant and important.

Materials and Methods

Brain tumors were developed in rats through cerebral injections of Kirsten Sarcoma Virus (KSV). With injection of this virus in the brain, Yung²⁹ and coworker developed brain tumors appearing like glioblastoma. Using the same model, we have developed hundreds of brain tumors for various biological studies. When the virus is injected in newborn rats' brain, tumors develop within several weeks and most animals die within 4–8 weeks. The histological characteristics of this tumor will be the subject of a future communication. For this experiment, the model was used to cause gradually increasing cerebral compression as found in tumors or following head injury in chronic subdural hematoma in human.

For this experiment, we developed brain tumors in three rat litters. The virus was obtained in frozen media from the laboratory of Dr. Werner Kirsten at the University of Chicago. At room temperature, as soon as the substance became liquid, 4 µl of the solution were injected in the cerebral hemisphere of 4-day-old Sprague Dawley rats. The injections were made through the coronal suture, 4 mm lateral to midline and at 6 mm depth from the scalp using a 10 µl- Hamilton-syringe.

Eight rats, without tumors, were used as controls; seven rats with tumors died suddenly before any experiments could be done; 13 rats with brain tumors and altered consciousness were used for this experiment. When an animal became drowsy or comatose, it was anesthetized with intra-peritoneal injection of Nembutal (0.1 ml per 100 gm). The chest was opened, a canula was inserted in the aorta through the left ventricle of the heart, the distal aorta was clamped, the right auricle was opened, and the intravascular blood was washed out with saline until clear fluid came from the right auricle. Then one of the four following methods was used on different animals for evaluation of the vascular system:

1. 10 ml of barium sulphate with gelatin (3:1 micro-opaque in water plus 2% gelatin) was injected in the ascending aorta; the animal was refrigerated for 2 hours to allow the gel to set. The brain was removed and fixed in paraformaldehyde. Several days later, when the brain was fixed, magnification radiographs were obtained by placing the entire brain on Kodak Industrial-type M film. It was then exposed by x-ray tube at a distance of 120 cm with exposure factor of 400 mA, one second and 44 KV.
2. 10 ml of India ink were injected in the ascending aorta
3. Perfusion of 150 ml paraformaldehyde in the ascending aorta. Prior to perfusion, the blood was washed away with injection of 400–600 ml saline solution
4. To make a plastic cast of the vascular tree, after washing out the blood from the vessels, we used a modified Mixture of Batson 17 # Anatomical Corrosive

Compound (Paul Valley Industrial Park, Warrington, Pennsylvania, USA.) consisting of 20 ml of Monomer, 10 ml catalyst and 5 drops of accelerator. With some modification, this technique is used for casting of vessels for various organs^{13,16}; dyes can be added for coloring. The brain was then left in a solution of 340 mg per liter of potassium hydroxide to dissolve the tissues. To avoid shadows from various vascular branches, the plastic cast was photographed while immersed in water.

After fixation of the brains, serial sections were made throughout the entire brain; the tissues were stained with Hematoxylin-Eosin, Toluidine, and Gomori Trichrome. Hundreds of slides became available for study. Photomicroscopy was done digitally, using Zeiss Axioscope and Zeiss AxioCam camera/ccd sensor and Zeiss plugin.

Results

Brain tumors produced were essentially similar to those seen in human, causing shifts, ventricular dilation, and edema (Fig. 1). Signs and symptoms were also similar to those seen in human, causing slowness in behavior and activities, hemiparesis and difficulty in walking; drowsiness, coma; and seizures.

Microvessels In the control rats without brain tumor

Normal specimens made through all the four laboratory methods revealed abundant microvessels. In addition, magnification angiograms revealed the architecture of numerous penetrating capillaries in the brain (Fig. 2A). These perforating microvessels penetrate the brain perpendicular to the cortex and are hidden within the brain. Their diameter is too small to be detected by the routine cerebral angiography in man. The India ink injections revealed small and terminal capillaries throughout the brain (Fig. 3A). The brain tissues were compact with little perivascular and pericellular space. The plastic casting of the vessels revealed similar abundance of vasculature, such that they appeared like a cotton mesh with the same shape of the brain (Fig. 7A).

Microvessels In the drowsy and comatose animals

There was reduction of capillary population in all specimens made with the four laboratory methods, more pronounced on the ipsilateral hemisphere (Fig. 2B). The reduction was more severe not only in the brainstem but also was present in the rest of the brain and in cerebellum of comatose animals (Fig. 2C; Figs. 4 C, D, and F; and Fig. 7B). Plastic casting in a rat with coma and with a large tumor showed the large tortuous tumor vessels, but there was marked reduction of capillaries elsewhere in the brain (Fig. 7B).

The histological examination showed in more detailed the alterations of the vessels and the brain tissues. Collapsed vessels, large pericellular and perivascular spaces were noted throughout the brain, including the opposite cortex (Fig. 4C), but it was more severe in the ipsilateral hemisphere (Figs. 4, A and B). The obstruction of microvessels causing infarcts was in a patchy and dispersed fashion. This was appreciated best in animals with paraformaldehyde perfusion. Because the blood was washed out of the vascular tree prior to perfusion of paraformaldehyde, the vessels appear circular on their cross section, open, and are fixed without blood in their lumen (Fig. 5A and Fig. 6A). But the compressed vessels appear without lumen (Fig. 3B) and the occluded vessels are filled with fixed blood clot within their lumen (Fig. 5B).

Infarcts and Ischemic changes

Were present throughout the brain, the samples of which are shown for cortex (Fig. 4C and Fig. 5B); for Ammon's horn area (Fig. 4B); for cerebellum (Fig. 4D); and for brainstem (Figs. E and F). Around the compressed or occluded vessels, there are wide pericapillary and pericellular spaces. The spaces may give the false appearance of open capillaries (Figs. 4, B and C and Fig. 6B); they are more pronounced around the tumor (Fig. 4A).

Periventricular area

Like the area around the tumor, they show paucity of patent vessels, edema and infarcted tissue (Fig. 1 and Figs. 8). The dilated ventricle seems to act like a secondary mass.

Discussion

Plum and Posner¹⁸ described in detail the clinical definitions of various stages of deteriorating consciousness, from decreased alertness to coma. Teasdale and Jennet^{25,26} graded the stages of deteriorating consciousness from 3 to 15, now generally termed Glasgow Coma Scale (GCS). Such distinct staging or grading cannot be applied easily to animals, for lack of language and behavioral communication. Hence, for this experiment we chose drowsiness (the early) and coma (the late) stage of deteriorating consciousness. When an animal appeared subdued, inactive, periodically closing eyes, but could be aroused or walked about, it was considered drowsy. When an animal was poorly responding to touch, not eating, having few spontaneous movements, it was considered comatose.

Tentorial herniation is frequently noticed in comatose patients suffering from intracranial mass lesions or head injury. In 100 intracranial lesions, most of which were brain tumors, Schwarz and Rosner²³ reported 83% herniation; and in 130 brain tumors Finny and Walker⁶ reported 55.4% herniation in postmortem examinations. Thus, because of frequent association of coma with shift or herniation of the brainstem, some seem to associate coma primarily with alteration in a certain location in the brainstem. For example, in referring to thalami as "Sensory Ganglia," Carpenter believed the thalami "constitute the seat of consciousness."² Finney and Walker believed that an "impairment of awareness is undoubtedly one of the earliest and sometimes the only manifestation of transtentorial herniation."⁶ Dandy, through unilateral or bilateral frontal lobectomy or ligation of anterior cerebral arteries for various conditions, concluded that the center for consciousness "is located somewhere in the basal ganglia or thalamus."⁵

Yet deteriorating consciousness is not always associated with uncus herniation and uncus herniation is not always associated with deteriorating consciousness. While 83% of cases in the Schwarz and Rosner series had tentorial herniation, only 25% had preoperative deterioration of consciousness and coma, and 5% with deteriorated consciousness did not have herniation. Also, while 55.4% of patients in the Finny and Walker series had herniation, only 21% of cases were comatose on admission. Ropper²⁰ measured lateral displacement of the pineal from midline and found that a shift of 0 to 3 mm was associated with alertness; 3 to 4 mm with drowsiness; 6 to 8.5 mm with stupor; and 8 to 13 mm with coma. Most patients in his series had conditions arising from spontaneous hemorrhage or traumatic origins; he states that "Contrary to traditional concepts, early depression of the level of alertness corresponded to distortion of the brain by horizontal displacement rather than transtentorial herniation with brain-stem compression."

Here, it is not meant to imply that this study somehow contributes to the highly sought understanding of consciousness and its center(s). For as Francis Crick and Christof Koch put it "The explanation of consciousness is one of the major unresolved problems of modern science"³; nor is it meant to cast any doubt on the involvement of the brainstem in

association with coma as observed by the previous investigators. What it is meant to indicate is that in deteriorating consciousness, caused by cerebral compression, the entire brain is at risk. Microvessels gradually obstruct and cause dispersed areas of infarcts throughout the brain, and the neuronal damage is not limited to the brainstem alone. While the physiologic association of the cortex with the brainstem is well documented⁴, we actually do not know how much conditions such as confusion, lack of alertness, or disturbance of memory that so often are the precursor of the deteriorating consciousness are related to the brainstem. In this study, numerous ischemic neurons in the hippocampus area and in the cerebral cortex were noted which likely contribute to memory loss and confusion.

The number of the animals is not large in this experiment. Yet, the patency of the vessels was studied through four different laboratory methods and all, in one form or another, showed similar findings. The diameter of perforating microvessels studied through microangiography in this experiment is around 20 to 30 microns. These vessels, not yet the smallest, are hidden within the cortex and substance of the brain. Their presence or absence cannot be evaluated through routine cerebral angiography in man; nor can they be seen easily, even under microscopic observation of the cerebral cortex, in pulsating brain. While their presence has been reported by others in brain^{21,28} and in spinal cord^{15,22,24,27}, we are not aware of any previous report in the literature of their study in relation to drowsiness or coma caused by cerebral compression.

The intracranial pressure and the perfusion pressure of the brain were not measured for these experiments since it would have been impractical, without anesthesia, to maintain a suitable recording method for long hours in a drowsy or even in a comatose rat. The study was meant to be qualitative to see the effect of cerebral compression on vasculature during drowsiness and coma; and not quantitative, in relation to intracranial pressure. But we have induced acute cerebral compression by gradual expansion of an extradural balloon in cats, part of which is published^{8,9}. The vital signs were recorded in those experiments, simultaneous with the rise of intracranial pressure while vital dyes were perfused. Although cerebral compression was rather acute, gradual vascular obstruction was similar, and occurred even in early rise of intracranial pressure. But, because the animals were under anesthesia, the stage of their drowsiness or coma could not be determined.

The use of a tumor model (like one used here which causes gradual cerebral compression) together with the use of techniques for three-dimensional analysis of the vascular system^{15,28}, should be very useful for future experiments. Such combination should most likely demonstrate better the state of microvessel circulation (and possibly the location) in relation to behavioral changes such as confusion or memory disturbance which usually occur prior to drowsiness.

The enlarged perivascular and pericellular areas were simply termed as “spaces” here. These are often referred to as swelling on routine light microscopy. The electron microscopic studies, in our earlier work on cerebral contusion¹⁰ and on the effect of cardiac arrest⁷ on the brain, show similar spaces which consist of swollen astrocytic processes, torn membrane, and destroyed cytoplasm. The destruction of the neural tissue, appearing like infarct, around a dilated ventricle observed in this experiment is of note, indicating that a dilated ventricle does not simply contribute to the rise of intracranial pressure; it also becomes like a second space-occupying lesion with its own compressive effect on the surrounding vessels and neuronal tissues.

The relation between the depth of coma and the outcome was studied by Jennett¹² and coworkers in 1000 patients with severe head injury. Their study shows stepwise increase in mortality and disability as the coma became deeper. Somewhat similar stepwise increase

between percentage of mortality and worsening level of GCS was found by Klauber¹⁴ and coworker in 1311 patients with head injury in San Diego County. In relation to minor head injury, Rimel¹⁹ and coworkers studied patients who had a history of unconsciousness for less than 20 minutes and had GCS of 13 to 15. While neurological examination was nearly normal, neuropsychological problems with concentration, memory, and judgment were found in most of 69 patients they tested three months after “seemingly insignificant head injury.”

As in this experiment, we have previously reported *in vivo* blood clot formation in microvessels during acute cerebral compression⁸, cardiac arrest⁷, and cerebral contusion¹⁰. The role of platelet accumulation and deposition in inducing intravascular blood clot formation after acute ischemia is reported by Obrenovitch¹⁷ and Jafari¹¹ and their coworkers. Adhami¹ and their coworkers found the effect of intravascular blood clot formation was enhanced when the ischemia was associated with hypoxia. Hindering recirculation, intravascular clots adds yet another obstacle to others in resuscitation caused by various forms of cerebral ischemia. The ischemic changes and infarcts throughout the cerebrum, brain stem, Ammon’s horn and cerebellum in comatose animals (and in lesser degree in drowsy animals) found in this experiment may explain the disabilities found in patients surviving such conditions.

Conclusions

Brain tumors were developed in rats to cause gradual increase in cerebral compression and to study the resulting pathologic alterations during drowsiness and coma. The findings indicate that drowsiness and coma in cerebral compression are associated with proportional increase in microvessel obstruction and infarcts throughout the brain. The infarcted areas are dispersed with a patchy appearance, worse around the tumor, the cortex, and around a dilated ventricle. The occurrence of such infarcts would likely explain the neurological and behavioral complications found in patients recovering from coma resulting from cerebral compression.

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Abbreviations

GCS	Glasgow Coma Scale
H & E	Hematoxylin-Eosin
Mag	Magnification

Fig. 1.

Cross section of one of the serial cuts of a rat brain with tumor produced through injection of Kirsten Sarcoma Virus with India ink perfusion. Stain Toluidine Blue; mag 20 X. Note edema throughout the entire brain, especially around the tumor and ventricles. The obstruction or the patency of capillaries is demonstrated in the ensuing photographs with much higher magnification. Yet a keen observation of this photograph reveals that the perforating capillaries are proportionally open more in the hemisphere opposite to the tumor (open arrows). Similar open capillaries can hardly be detected in the hemisphere on the side of the tumor and in periventricular regions. The tissues surrounding the tumor are necrotic and soft (closed arrows).

Figs. 2.

Magnification angiography of three rat brains: contrast media barium sulfate, showing a normal brain without tumor (A), a brain with tumor in a drowsy rat (B), and a brain with a large tumor in a comatose rat (C). Note the abundant vasculature, especially numerous penetrating parallel microvessels in the cortex and in the brain in A (arrows), reduced population of penetrating microvessels, especially in the cortex in B, and marked reduction in appearance of these vessels in C. Also note slight narrowing of the larger vessels in B and marked narrowing in C.

Figs. 3.

Photomicrographs of the subcortical area of two rats with intra-arterial injection of India ink. Fig. A is from a comatose rat with brain tumor; the tumor is not shown; the photo is from the cortex opposite to the tumor. Fig. B is from the cortex of a normal rat for control. Note compressed microvessels with no apparent patent lumen and with large perivascular and pericellular spaces in A as compared with those of B (white arrows). Stain H&E; mag 250X.

Figs. 4.

Photomicrographs from the brain of two rats with brain tumor and in coma. The photo in A is from the area around the tumor, and in B is from the Ammon's horn area of the ipsilateral side to the tumor. The photo in C is from the cortex opposite side to the tumor. A portion of the actual tumor tissue is seen in the upper left corner in A. The photos D (cerebellum), E (brainstem) next to 4th ventricle (IVth V) and F (brainstem nuclei) are from the second rat in coma. Twisted arrows point to collapsed vessels. Note that hardly any open vessels can be identified in the sample. The brain tissue is spongy around the tumor, neurons, vessels, and the IVth ventricle, caused by edema. The neurons in the Ammon's horn area (B), cortex (C), cerebellum Purkinje cells (D), and brain stem (F) are shrunken and triangular, suggestive of ischemia (straight arrows). In C, the photo from opposite side cortex shows shrunken neurons (open straight arrows) and wide pericellular and perivascular space (twisted arrows). Stain is Toluidine blue for all 3 photos (in A, B, and C). magnification is 400 for all three. The stain is H&E for Figs A, B, and C and magnification is 100 for D and F and 40 for E.

Figs. 5.

Photomicrograph of cortex in a rat with brain tumor; the tumor is not shown. *In vivo* fixation with paraformaldehyde perfusion was done when the animal was drowsy. Fig A is from the cortex on the ipsilateral and Fig B is from the cortex on the contralateral side of the tumor. Note collapsed lumen of microvessels in A as compared with those of B (arrows). Stain H&E, mag 250 X.

Figs. 6.

Photo micrographs from two separate areas of the brainstem of the same rat presented in Figs 5. Note the cross section of numerous patent microvessels in Fig 6A (arrows). Because of *in vivo* saline perfusion before fixation, the microvessels have no red cell in them. Fig 6 B is from another area of the brain stem; patent microvessels are hardly identifiable; the wide pericellular and perivascular spaces give false appearance of patent microvessels (longer arrows); only a few microvessels can be seen in B, filled with red cells (smaller arrows).

Figs. 7.

Plastic cast of vessels in two rats. Fig 7A is from a normal rat brain without tumor; the abundant, super-imposed microvessels give a dense appearance to the specimen. Fig 7B is from a rat with brain tumor and in coma. Except for the tumor itself, there are far fewer microvessels in the entire brain. Stain H&E, mag 250 X.

Figs. 8.

Photomicrographs of Ammon's horn and periventricular region of a rat with brain tumor (Fig 8A); the tumor is not shown; India ink was injected in the heart when the animal was in coma. Fig 8B is from periventricular region in Fig 8A, marked by a rectangle, magnified 10 times more than that for Fig 8A. Note that hardly any vessels are filled with India ink. Note also severe periventricular swelling and torn cytoplasmic membrane. Stain Toluidine blue, mag 40X for A and 400X for B.