The Effect of Dexamethasone on the Edema of Focal Cerebral Ischemia

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Abstract:
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The effect of large dosages of dexamethasone on the edema of cerebral ischemia and infarction in the squirrel monkey after temporary occlusion of the middle cerebral artery is reported. There was no difference between the ten monkeys treated with dexamethasone and the 11 control animals in clinical course, mortality, morbidity, histological appearance, or amount of edema associated with this ischemic lesion. The differences in the electron microscopic and pathophysiological findings of the edema from ischemia secondary to single major vessel occlusion and in the edema from an intracranial mass lesion are reviewed. The possible therapeutic or pharmacological effects of steroids are considered along with available data on lysosomes in the brain. At the present time, there is no justification for the use of large doses of dexamethasone in the treatment of cerebral infarction.

Additional Key Words
- energy-dependent cell membranes
- blood-brain barrier
- lactic acidosis

The rather dramatic beneficial effects of steroids on the cerebral edema associated with gliomas have led to their application in other disease states associated with cerebral edema: spinal cord injury, head trauma, subarachnoid hemorrhage, and focal cerebral ischemia with infarction. The usefulness of steroids in these other conditions has been questioned, although recent studies have shown some apparent application for steroids in the management of spinal cord injuries. This salutary effect may be a special situation because of the unique chemical environment after trauma to the spinal cord that includes high levels of free norepinephrine. Therefore, these findings cannot be extrapolated to other types of injuries involving the cerebral vascular system.

Langfitt et al. have presented evidence that cerebral swelling from head injury is partly related to a dramatic early increase in cerebral blood flow after paralysis of autoregulation. In turn, this is associated with subsequent cerebrovascular congestion. Obviously, in states of severe head trauma, there are many other additional factors to be considered, including focal areas of hemorrhage and necrosis, subarachnoid hemorrhage with delayed vasospasm, and mass lesions. Therefore, if a beneficial effect of steroids in this particular illness can be demonstrated, this effect cannot be extrapolated to other situations.

In primary subarachnoid hemorrhage from a ruptured aneurysm, there is diffuse damage to the conducting vessels in the subarachnoid space which produces an end-organ sympathectomy. There is progressive spasm of these conducting vessels related to the release of vasoconstrictive agents from the breakdown of blood in the subarachnoid space but also, in part, probably the result of a sensitization of these conducting vessels. Cerebral blood flow is reduced as a result of this vasospasm, and when it is reduced to critical levels, cerebral infarction occurs. Cerebral edema routinely is found in patients with subarachnoid hemorrhage, with or without infarction, and may be related to the irritation from the blood in the subarachnoid space, impairment of spinal fluid flow and absorption, or reduction of cerebral blood flow from vasospasm. Therefore, an effect of steroids on edema in this illness, to date unproved, cannot be extrapolated to other conditions with cerebral edema.

Focal cerebral ischemia from single major vessel occlusion represents an entirely different entity from those previously discussed. Our purpose was to study the effect of dexamethasone on the edema associated with the occlusion of a single major conducting vessel. Results of this investigation cannot be translated fully to other disease states associated with cerebral edema.
EFFECT OF DEXAMETHASONE

Techniques

TECHNIQUE OF MIDDLE CEREBRAL ARTERY OCCLUSION
Squirrel monkeys (Saimiri sciureus), weighing 600 to 1,000 gm, were anesthetized with sodium pentobarbital (15 mg/kg intraperitoneally). The monkeys were then fixed in a head rest and placed in the prone position on a heating blanket. The cutting current of an electrosurgical unit was utilized to make a skin incision just behind the eyebrow and lateral orbital ridge. The periorbital contents were reflected anteriorly with a subperiosteal dissection, after the communicating vessel from the orbit supplying the middle meningeal artery had been divided with the bipolar coagulator. The retro-orbital contents were not exenterated but were swept forward after aspiration of the globe. This avoided dissection of any vascular structures, and blood loss was further minimized. With the orbital contents held anteriorly by a specially adapted retractor, the operating microscope was used for the remainder of the surgery. A small craniectomy was carried out over the optic nerve using a small air drill. The dura overlying the middle cerebral artery was incised, and with the tip of a no. 11 blade knife the arachnoid surrounding the middle cerebral artery was divided. A miniaturized Mayfield clip, manufactured for this purpose, was applied to the middle cerebral artery. The time was noted, and the wound was filled with a saline solution. After four hours of middle cerebral artery occlusion, the clip was removed, the artery was inspected for patency, and the degree of edema at this stage of ischemia was determined by the relative herniation of cerebral contents through the dural incision.

SEQUENCE OF ANIMAL PREPARATIONS
All surgery was performed by one of us (R.F.D.) after experience with the microscopic operative procedure on cats. The control and treatment groups were subsequently created by alternating sequentially the preparations to avoid an alteration from improved surgical technique that might prejudice the results.

TREATMENT SCHEDULE WITH DEXAMETHASONE
The dose of dexamethasone was proportional by weight to the standard treatment schedule employed at our institution for the edema of gliomas. In humans, this amounts to 10 mg as an initial loading dose, followed by 4 to 6 mg every six to eight hours, with a tapering dosage after the first 48 hours. Conversion of these dosages on a weight basis represents 0.14 mg/kg and 0.075 mg/kg, respectively. The type of monkey that we used usually weighs slightly less than 1 kg. At the time that the clip was applied, each monkey received 0.14 mg intramuscularly of dexamethasone and then 0.075 mg every eight hours for 48 hours or until death, whichever was the longer period. The agent was discontinued at 48 hours empirically; notably, no deaths occurred from the time he began to arouse from the anesthesia until the time of spontaneous death or sacrifice.

Notations were made regarding lateralizing signs and the level of consciousness.

PATHOLOGICAL EXAMINATION
After death, each brain was removed, examined carefully for evidence of gross hemispheric swelling and softness, and placed in formaldehyde for fixation. After fixation, each brain was sectioned in a standard mortar box to produce uniform sections for examination. These gross sections were photographed, and, through the area of maximal swelling, block hemispheric sections were stained with hematoxylin and eosin and luxol-fast blue. The slides were then examined microscopically, and judgment on the amount of edema was made, based on the gross appearance of the brain at removal, the gross sections, and the histological appearance of the brain in the area of maximal swelling.

Results

STABILITY AND RELIABILITY OF PREPARATION
No monkeys died from surgery, and blood loss never exceeded 3 ml. No monkey was in a deep plane of anesthesia, as determined from the respiratory rate and tendency for spontaneous movements. Although blood pressures were not monitored with intraarterial catheters, blood pressure was judged from the femoral artery pulsations, which were excellent in all animals. Our previous extensive experience with this preparation revealed that blood pressures correlated closely with femoral pulses. All monkeys showed evidence of awakening from anesthesia by increasing spontaneous movements and attempts at ambulation.

EFFECT OF TREATMENT
Results of treatment are summarized in table 1. There was no statistical difference between the group treated with dexamethasone and the control group. The mortality and morbidity for the control group and the group treated with dexamethasone were similar to that found in previous series of this preparation, although the mortality in these animals was slightly less than that previously reported. This may be related to a modification in the approach to the middle cerebral artery that eliminates even minimal retraction of the brain.

PATHOLOGICAL EXAMINATIONS
Brains of the few monkeys who survived without gross infarcts or edema (some of which had histological areas of focal necrosis) had symmetric and normal-appearing hemispheres, with no evidence of surgical trauma (fig. 1). Most monkeys had areas of edema and infarction in the regions previously described for this preparation. Edema involved primarily the white matter and, when severe, caused early death, spreading to involve a large measure of the hemisphere (fig. 2). In monkeys that lived with infarctions, the infarcts were demarcated by the time the monkeys were killed after the surgery and were typical of the infarcts described previously (fig. 3).
TABLE 1

<table>
<thead>
<tr>
<th>Clinical course</th>
<th>Autopsy results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control (11 monkeys)</strong></td>
<td></td>
</tr>
<tr>
<td>0 died, 0-8 hours</td>
<td>. . .</td>
</tr>
<tr>
<td>5 died, 8-24 hours</td>
<td>Severe edema in all five</td>
</tr>
<tr>
<td>1 lived, severe hemiplegia</td>
<td>Deep infarct (putamen, caudate, internal capsule), moderate edema</td>
</tr>
<tr>
<td>2 lived, transient hemiparesis</td>
<td>Small infarct (insula, superior temporal gyrus) in one; no infarct in one</td>
</tr>
<tr>
<td>3 lived, no deficit</td>
<td>Small infarct (subcortical, caudate) in two; no infarct in one</td>
</tr>
<tr>
<td><strong>Dexamethasone-treated (10 monkeys)</strong></td>
<td></td>
</tr>
<tr>
<td>2 died, 0-8 hours</td>
<td>Massive edema in both</td>
</tr>
<tr>
<td>3 died, 8-24 hours</td>
<td>Severe edema in all three</td>
</tr>
<tr>
<td>1 died, 24-48 hours, severe hemiplegia</td>
<td>Deep infarct (putamen, caudate, internal capsule), severe edema</td>
</tr>
<tr>
<td>1 lived, severe hemiplegia</td>
<td>Deep infarct (putamen, caudate, internal capsule)</td>
</tr>
<tr>
<td>2 lived, 3-day transient hemiparesis</td>
<td>Large infarct with hemorrhage (insula, putamen, frontal operculum, superior temporal gyrus) in one; moderate infarct (head of caudate, putamen, but not of internal capsule) in one</td>
</tr>
<tr>
<td>1 lived, no deficit</td>
<td>Moderate infarct (putamen, head of caudate)</td>
</tr>
</tbody>
</table>

There was a small variation in the location of the infarcts, and although individual infarcts did not involve each of the following structures, the major changes were found in the head of the caudate nucleus, internal capsule, putamen and globus pallidus, external capsule, insula, frontal operculum, and superior temporal gyrus.

**Histological Appearance of Infarcts**

Although three of the infarcts had small areas of perivascular hemorrhage, generally they were pale and typical of five-day-old to seven-day-old ischemic infarcts. Ischemic necrosis was clearly delineated; polymorphonuclear cells were present in the parenchyma, and there was extensive glial proliferation. Swollen macrophages were identified. Neurons had lost their identity, and the laminated cyto-architecture of the cortex was obscured. Fluid spaces surrounded degenerating ganglion cells. There was some mitotic activity in the small blood vessels, with definite endothelial proliferation. Perivascular edema was consistently present. In the white matter, the myelin failed to stain, definite fiber tracts could not be identified, and the general appearance was that of “spongy degeneration.”

**Histological Appearance in Areas of Edema**

The most striking change in areas of edema was the increased vacuolization, with fluid spaces surrounding ganglion cells and astrocytes in the cortex. The neuropil appeared less dense, with separation of the fibrils. There was an apparent reduction in the number of identifiable ganglion cells. Myelin stained poorly, and there was separation of fiber tracts, with apparent fluid spaces in the white matter. Small vessels were full of polymorphonuclear cells that had not yet migrated outside of the vessels. Examination of the fluid spaces surrounding the nuclei of neurons and glial cells suggested a fluid replacement of the cytoplasm, perhaps as a result of the rupture of cell membranes. In monkeys that died of more severe edema and within eight hours after restoration of flow, the neurons still could be identified. The histological findings in monkeys that died of edema were no different, whether in the control group or in the group treated with dexamethasone.

**Discussion**

**BIOLOGICAL VARIATION**

Because of biological variations, conclusions must be drawn from analysis of the group and interpretations must not be based on the findings in single animals. Previous studies have demonstrated that cerebral blood flow in the core area of ischemia in these preparations decreases from 20% to 50% of normal during occlusion of the middle cerebral artery. There are similar variations in the individual levels of ATP and lactate in the central zone of ischemia after two hours of such occlusion. However, least-square curves of cerebral ATP and lactate levels developed from the composite group show a slow fall in ATP and a progressive rise in lactate during the ischemia—changes that are highly significant. With individual variations in regional cerebral blood flow, animals will differ in tolerance.
EFFECT OF DEXAMETHASONE

FIGURE 1
Animal surviving without infarct. Note lack of surgical trauma.

FIGURE 2
Animal treated with dexamethasone. Death was caused by edema involving frontal lobe and basal ganglia.

to ischemia and edema formation from occlusion of the middle cerebral artery.

TYPES OF CEREBRAL EDEMA
Experimental edema has been created by cold injury, local masses (psyllium seeds, balloons), tissue toxins, and a combination of anoxia and ischemia. Unfortunately, primary ischemic edema as seen in the common stroke from occlusive disease has not been studied experimentally to any great extent. Many determinations based on other types of edema probably cannot be extrapolated to the edema of focal ischemia and cerebral infarction.

Klatzo classified brain edema into two major categories but stated that this was primarily a framework for analysis and that there would be considerable overlapping. These two major categories were cytotoxic edema and vasogenic edema. The main feature of cytotoxic edema was the intracellular swelling noted on electron microscopy. In the vasogenic type, the swelling was located primarily in the white matter, with increased permeability in the blood-brain barrier and leakage of protein and extravasation of conventional barrier indicators. This resulted in enlargement of the extracellular spaces in the white matter, but some cellular swelling also affected astrocytes. Ischemic edema probably is a combination of the two forms of edema. Tissue perfusion with blood of reduced oxygen content (pure anoxia) produces the cytotoxic type of cerebral edema. In focal cerebral ischemia, perfusion is decreased or even absent. Thus, the tissue not only is not receiving oxygen but also is not having acid metabolites and other waste products removed.

NORMAL ULTRASTRUCTURE OF THE BRAIN
In evaluating the results of electron microscopy, physiologists have objected to the estimates of brain extracellular space from this technique. The 5% volume of extracellular space derived from such
FIGURE 3A

A: Typical seven-day-old infarct in control animal involves putamen, head of caudate, internal capsule, insula, frontal operculum, and superior temporal gyrus. B: Hemorrhagic infarction that did not cause death.

studies is considerably different from values of the functional extracellular space. These physiological studies have yielded values ranging from 10% or 15% to as high as 20% or 30%. To fully appreciate this disparity, one need only examine a gross specimen before and after fixation for electron microscopy and observe the marked diminution in volume. This dehydration could explain some artificial reduction in the extracellular space, but cellular swelling after death also must be considered. These problems are well recognized by workers in the field, and newer techniques are in continuous evolution.

Perhaps one of the most significant contributions from electron microscopy has been the structural delineation of the cerebral spaces: extracellular, glial, and neuronal. The neuropil, that area under light microscopy existing between cell nuclei and stained nerve fibers, is completely filled with cell processes. The blood-brain barrier is composed of capillary endothelium, basement membrane, and glial cell processes, forming a complete sheet around the capillary. The pathway for the transfer of fluid, oxygen, electrolytes, and glucose is likely through the astrocytic space and not the extracellular space as in other tissues. These facts are important when the source and management of cerebral edema are considered.

ELECTRON MICROSCOPY OF VARIOUS FORMS OF EXPERIMENTAL CEREBRAL EDEMA

The various methods of producing experimental cerebral edema have in large measure relied on the effects induced by an expanding mass lesion. In the work by Long et al., evaluating the response of experimental cerebral edema to glucocorticoid administration, edema was produced by implantation of psyllium seeds. The electron microscopic findings were similar to those previously described for the...
edema secondary to an expanding mass lesion. There was enlargement of the pericapillary astrocytic processes and considerable increase in the size of the astrocytic cell body without nuclear change. In the neuropil, there was also generalized swelling and increased clarity of astrocytic processes. The extracellular space in the white matter increased greatly, along with disruption of myelin lamination and axonal compression. The authors identified a "pseudoextracellular space," which they believed was secondary to rupture of plasma membranes. Areas of more severe involvement had signs of tissue damage, with ischemic cell changes and tissue necrosis.

**Electron Microscopy of Cerebral Infarction and Ischemic Edema**

The results of the study by Garcia et al. of the electron microscopic findings of cerebral infarction in the squirrel monkey should be reviewed. These findings are somewhat different from those of various investigators, who used expanding mass lesions to produce experimental cerebral edema. Garcia et al. used a modification of the model originally reported and one similar to that of our study.

Early after clipping of the middle cerebral artery Garcia et al. found extensive swelling of astrocytic processes, particularly in the pericapillary areas, with relatively good preservation of the mitochondria. The endothelial cells were essentially unchanged. The good preservation of the mitochondria and other cell organelles is in striking contrast to that found in vitro by Bakay and Lee in which swelling of neuronal mitochondria was observed after hypoxia of 20 minutes. This supports previous investigations that indicated the difference between cellular paralysis and cellular death and the difficulty in extrapolating data from one type of clinical problem to another.

By four hours, astrocytic swelling had extended from the perivascular location to regions more distant from capillaries and involved the neuropil in general. An important finding in the judgment of Garcia et al. was the increase in volume of the pericytes. Notably, the endothelial cells at this stage of evolution did not show hyperplasia of their pinocytic vesicles, separation of endothelial junctions, or any other significant structural abnormalities. This is in contrast to the findings of Chiang et al. with the so-called no-reflow phenomenon described in models of total circulatory arrest. This is additional evidence that results from studies of total and complete cerebral ischemia, as seen in cardiac arrest, cannot be applied to the focal and incomplete cerebral ischemia typical of cerebral infarction.

Occasional collections of polymorphonuclear leukocytes were encountered in the lumen of some capillaries by four hours and were numerous and generalized by 12 hours, at which time there was also extensive swelling of endothelial cells and some early necrosis. These findings are consistent with our observations under the light microscope.

**Pathophysiology of Ischemic Edema**

In previous communications, we have indicated the complex nature of ischemic edema and suggested that the primary cause was a significant decrease in the level of available ATP necessary to maintain the complicated cell membrane transport system and particularly the sodium pump. Other investigators have arrived at similar conclusions. A disproportionate amount of energy in the nervous system is normally expended for this purpose.

In our laboratory preparation, the level of available ATP decreased to 25% of normal after two hours of occlusion of the middle cerebral artery. This coincides with an eightfold increase in the level of tissue lactic acid. Thus, tissue lactic acidosis may produce a tissue acidemia and alteration in the oncotic environment of ischemic cells, which further leads to intracellular swelling.

The blood-brain barrier consisting of capillary endothelium, basement membrane, and perivascular glial cell processes is also an energy-dependent system. With the loss of available energy for maintenance of this barrier, there is a leakage of those particles normally excluded.

The possibility exists that the progression of edema after restoration of flow is partly related to a reactive hyperemia. This has been documented and discussed in previous communications.

**Rationale for Steroids in Edema**

The consistent good clinical results of large doses of dexamethasone in the treatment of cerebral edema for gliomas have led to the assumption by some that steroids would be effective for other forms of cerebral edema. Clinical studies have failed to provide convincing evidence that steroids are effective in the treatment of cerebral infarction, head trauma, or subarachnoid hemorrhage. For the purposes of this discussion we wish to compare only the differences in the edema from a glioma and the edema from cerebral infarction.

One must only look at an angiogram of a glioblastoma to be impressed with the high vascularity and supernormal flow values that are present. This should be contrasted with focal cerebral ischemia in which cerebral blood flow is low. In addition to this major difference, a malignant glioma may produce cerebral edema not only by compression and alteration in blood flow but also by release of irritative substances secondary to the altered
metabolism of these malignant cells and the products of necrosis in the tumor itself. Therefore, steroids may be effective in the edema of gliomas by acting as an anti-inflammatory agent and secondarily by protecting the cells from the effects of compression and irritation.

It is presumed that cortisol and other anti-inflammatory steroids exert their action in pharmacological concentrations by stabilizing the membrane of lysosomes against the disruptive influences of hypoxia and chemical toxins. It has been speculated that the glucocorticoids suppress inflammation by restraining the hydrolases to the lysosomes.

In this regard, a recent important work by Clendenon et al. and a subject review of cerebral metabolism by Allen should be considered. Lysosomes are contained in the central nervous system primarily in the neurons and not in the glial cells. This does not coincide with the early swelling of the glial cells in the electron microscopy studies of standard models for experimental edema and the edema of ischemia. Clendenon et al. indicated in their model of anoxic-ischemic edema that early release of lysosomal enzymes was minimal and occurred after three hours at a time when it was not a decisive factor in the pathogenesis of cell injury in the nervous system. In a compressive lesion, the situation is different and lysosomes could be an early factor.

There is little clinical evidence and no laboratory data from our study or from previous investigations to support the use of steroids for the edema associated with cerebral ischemia or infarction from single major vessel occlusion. Large dosages of these drugs cannot be considered innocuous, and the unproved potential benefit is overbalanced by the known risk of the drugs in the dosages commonly employed.

References


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