Effects of Dexamethasone on Distributions of Water and Pertechnetate in Brains of Cats After Middle Cerebral Artery Occlusion

BY WAYNE E. HOPPE, M.D., ARTHUR G. WALTZ, M.D., MARGARET M. JORDAN, B.CHEM., AND RONALD L. JACOBSON, M.S.

Abstract:
Large parenteral doses of dexamethasone were given to five of ten cats after transorbital occlusion of one middle cerebral artery and to two of four cats after sham operations. Two days later the water content and brain/blood ratios of pertechnetate were measured in samples of cerebral tissue that were categorized as nonischemic, ischemic, or infarcted. Values for infarcted tissue were greater than those for ischemic tissue in untreated cats, but not in cats that received dexamethasone. The drug had no apparent effect in ischemic tissue that was not necrotic. These results help explain inconsistencies in previous studies and help define the potential usefulness of dexamethasone for treatment of cerebrovascular disease.

Additional Key Words
cerebral infarction
technetium-99m

Cerebral ischemia and infarction are accompanied by increases of the water content of the brain (cerebral edema) and the brain/blood ratios of small and large molecules (alterations of the "blood-brain barrier"). Although these changes begin shortly after the onset of ischemia, water content reaches a maximum at approximately two days while brain/blood ratios of pertechnetate, albumin, and sodium continue to increase and remain increased for many days. Adrenal corticosteroids, particularly dexamethasone, may influence the development of certain types of cerebral edema, but the results of studies of ischemic cerebral edema have been variable, depending on the experimental model, the type and dose of steroid used, the duration of ischemia, and the criteria used to determine effectiveness. In the present study, large doses of dexamethasone were given parenterally to cats with occlusion of one middle cerebral artery (MCA) to determine the effects of the drug on ischemic cerebral edema and on the extravascular distribution of sodium pertechnetate containing technetium-99m.

Methods
Fourteen unselected adult cats were anesthetized with phenytoin sodium, hydrochloride, 1 mg per kilogram injected intramuscularly, and sodium pentobarbital, 25 mg per kilogram injected intraperitoneally. The left MCA was approached transorbitally and exposed by enlargement of the optic foramen, without additional craniectomy. In ten cats the MCA was occluded at its origin by bipolar coagulation; the other four cats had sham operations, in which the artery was grasped gently with a forceps but not coagulated.

After closure of the surgical incision (approximately 20 to 30 minutes after coagulation or manipulation of the MCA) five cats with occluded arteries and two cats with sham operations were given dexamethasone, 4 mg per kilogram injected intramuscularly. Similar doses of the drug were given twice the day following the operative procedure and once the morning of the second day. After recovery from anesthesia each cat was examined for the presence of neurological deficits. Food and water were available but no fluids were given parenterally.

The second day after MCA occlusion or sham operation each cat was anesthetized and 2 mCi of sodium pertechnetate containing technetium-99m were injected intravenously. One hour later a sample of arterial blood was taken for measurement of pertechnetate content and the circulation was stopped by the intravenous injection of a saturated solution of potassium chloride. The brain was removed quickly (within ten minutes) and frozen at ap-
proximately −140°C in 2-methyl butane cooled with liquid nitrogen. After warming to approximately −20°C a coronal section about 3 mm thick was made at the level of the tips of the temporal lobes. Sixteen samples of cerebral tissue weighing from 10 to 50 mg were taken from each hemisphere, from vertical and horizontal strips that included the centers of the hemispheres.

Each sample of brain was placed in a separate sealed container while still frozen and weighed for determination of wet mass. The count rate for pertechnetate in each sample of brain and blood was measured in a well counter and corrected for background activity and natural decay of radioactivity. The samples of brain then were heated in an oven at 60°C for 48 hours, exposed to a vacuum reaching 0.1 μ Hg for at least four hours, and weighed again for determination of dry mass. The difference between the weight of a sample before drying and the weight after drying was considered to be the water content, which was expressed as percent of wet mass. The relative concentration of pertechnetate in a sample of brain was expressed as a brain/blood ratio, that is, the ratio of the count rate per unit wet weight of the sample of brain to the count rate per unit weight of the sample of blood from the same cat.

A histological section was prepared from the cut surface of the brain posterior to the section used for measurements of water and pertechnetate content, after fixation in 10% formalin. The section was stained with hematoxylin and eosin for microscopic examination of regions of tissue adjacent to the samples used for analysis. On the basis of this examination each sample was categorized as "nonischemic," "ischemic," or "infarcted." Samples from the right cerebral hemispheres of cats with an occluded left MCA and samples from both hemispheres of cats with sham operations were categorized as "nonischemic" after examination of the appropriate histological sections to be certain that there were no signs of pathological change. Data from an additional five cats with sham operations were not used because pathological changes were observed, presumably caused by damage to small perforating branches of the MCA. Samples from the hemispheres on the side of an occluded MCA were categorized as "ischemic" unless examination of the adjacent sections revealed that more than 25% of the region of tissue corresponding to a sample was necrotic, in which case the sample was categorized as "infarcted." No correction was made for varying amounts of gray matter and white matter.

For analysis of the data each cat was considered as an experimental unit. Experimental units were assigned without selection to one of four different groups: sham operation without dexamethasone; sham operation with dexamethasone; left MCA occlusion without dexamethasone; or left MCA occlusion with dexamethasone. Within each experimental unit either one (nonischemic) or all three categories of samples were represented. The numbers of samples in each category varied within and among experimental units, and were dependent in part on the assigned group. Therefore, the method of analysis of components of variance was used. The two variables that were measured in each sample (percent water and the brain/blood ratio of pertechnetate) were analyzed separately. Effects related to the position of a sample in a hemisphere were not considered. The observed variability among categories was not homogeneous, particularly for the brain/blood ratios of pertechnetate in samples from hemispheres on the side of an occluded MCA. For these ratios the sample variances appeared to be proportional to the squares of the sample means; thus, logarithms to the base 10 were used for analysis. The antilogarithms of the mean values of the transformed variable are equivalent to the geometric means of the original variable. No transformation of the values for percent water was made. The original data used for analysis are available on request.

**Table 1**

**Water Content, in Percent**

<table>
<thead>
<tr>
<th>Surgical procedure</th>
<th>Tissue</th>
<th>Without dexamethasone</th>
<th>With dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N(cats)</td>
<td>Arithmetic mean</td>
<td>SE</td>
</tr>
<tr>
<td>Sham operation, left side</td>
<td>2</td>
<td>75.9</td>
<td>1.68</td>
</tr>
<tr>
<td>Right hemisphere, nonischemic</td>
<td>76.1</td>
<td>0.79</td>
<td>32</td>
</tr>
<tr>
<td>Left hemisphere, nonischemic</td>
<td>77.5</td>
<td>0.79</td>
<td>32</td>
</tr>
<tr>
<td>Occlusion left MCA</td>
<td>5</td>
<td>76.2</td>
<td>0.50</td>
</tr>
<tr>
<td>Right hemisphere, nonischemic</td>
<td>77.8</td>
<td>0.86</td>
<td>54</td>
</tr>
<tr>
<td>Left hemisphere, ischemic</td>
<td>80.4</td>
<td>1.04</td>
<td>33</td>
</tr>
<tr>
<td>Left hemisphere, infarcted</td>
<td>82.7</td>
<td>1.08</td>
<td>26</td>
</tr>
</tbody>
</table>

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TABLE 2

<table>
<thead>
<tr>
<th>Brain/Blood Ratios of Pertechnetate</th>
<th>Mean ± SE</th>
<th>Median ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonischemic, right hemisphere, nonischemic</td>
<td>0.030 ± 0.003</td>
<td>0.030</td>
</tr>
<tr>
<td>Ischemic, right hemisphere, ischemic</td>
<td>0.036 ± 0.005</td>
<td>0.035</td>
</tr>
<tr>
<td>Infarcted, left hemisphere, infarcted</td>
<td>0.040 ± 0.006</td>
<td>0.040</td>
</tr>
</tbody>
</table>

In cats with an occluded left MCA the means of the values for water content and for the brain/blood ratios of pertechnetate were greater for samples categorized as ischemic than for nonischemic samples from the right hemispheres, whether or not dexamethasone was given (P < 0.01) (tables 1 and 2; figs. 1 and 2). However, in cats that received dexamethasone...
amethasone the mean values for infarcted samples were no greater than those for ischemic samples (P > 0.09), while in untreated cats the mean values for both water and pertechnetate were greater in infarcted tissue than in ischemic tissue (P < 0.01). The mean value for the pertechnetate ratios of infarcted tissue from untreated cats was significantly greater than that for infarcted tissue from cats that received dexamethasone (P < 0.05) (tables 1 and 2; figs. 1 and 2); the mean value for water content likewise was greater in untreated cats, but the difference did not reach statistical significance (P > 0.10).

The numbers of samples of cerebral tissue that were categorized as infarcted varied among cats. Twenty-six of 80 samples (32.5%) obtained from cats with left MCA occlusion that were not given dexamethasone, and 47 of 80 samples (58.8%) from treated cats, were categorized as infarcted. This difference was not statistically significant (P > 0.05).

Cats with sham operations did not develop neurological deficits other than transient lethargy related to anesthesia and the surgical procedure. Each cat with occlusion of the left MCA developed a neurological deficit related specifically to cerebral ischemia and infarction.\textsuperscript{5, 14} The deficits in the cats that received dexamethasone were not different from those in the treated cats.

\textbf{Discussion}

\textbf{EFFECTS OF DEXAMETHASONE}

The results of the present study indicate that large parenteral doses of dexamethasone lessen the extravascular distribution of pertechnetate in cerebral tissue made necrotic by two days of arterial occlusion, even when administration of the drug is begun after the onset of ischemia. The extravascular distribution of water also is lessened by dexamethasone: although the difference between the water content of infarcted tissue in treated and in untreated cats did not reach statistical significance in the present study, the lack of a greater water content in infarcted tissue than in ischemic tissue in treated cats appeared meaningful when compared to the results from untreated cats. However, dexamethasone has little or no influence on the water content or brain/blood ratio of pertechnetate in ischemic cerebral tissue that does not become necrotic.

These results help explain the apparent inconsistencies in previous studies of the effects of adrenal corticosteroids on ischemic cerebral edema, in which distinctions between ischemic and infarcted (necrotic) tissue were not made. If necrotic tissue makes up a large part of a sample of ischemic brain used for analysis, as in gerbils with one carotid artery occluded,\textsuperscript{11, 12} dexamethasone may be shown to protect against the development of edema; when samples contain relatively smaller amounts of necrotic tissue, protective effects may not be demonstrable.\textsuperscript{7–10, 14} The duration of ischemia also is important; there is evidence that pretreatment with dexamethasone influences the development of edema early after the onset of ischemia\textsuperscript{1} in tissue that is not necrotic but that may become necrotic if ischemia persists.

\textbf{MECHANISMS OF THE EFFECTS OF DEXAMETHASONE}

Cerebral edema has been classified as "cytotoxic" or "vasogenic," depending on such features as the site of accumulation of water and the associated passage of albumin, protein-bound dyes, and other large molecules into the brain.\textsuperscript{18} This classification does not appear to be applicable to ischemic cerebral edema. With ischemia, increases of water content occur both extracellularly and intracellularly,\textsuperscript{19} and in both the gray matter and the white matter of the brain.\textsuperscript{18} The increases are at least in part independent of the extravascular distributions of larger molecules. Moreover, cerebral edema is present both early after the onset of ischemia, when the junctions between endothelial cells are tight\textsuperscript{20} and when larger molecules are not found in abundance in the parenchyma,\textsuperscript{6, 18} and later when endothelial junctions have separated and extravascular accumulations of larger molecules occur.

It is likely that the increased extravascular distributions of water and of pertechnetate in ischemic cerebral tissue are due both to increased transendothelial passage and to sequestration ("trapping") in the tissue.\textsuperscript{5, 6} Although water appears to cross the cerebral vascular endothelium freely,\textsuperscript{18} an increased transendothelial passage of pertechnetate indicates "breakdown of the blood-brain barrier" for pertechnetate: in normal brain only small amounts of pertechnetate cross the endothelium to distribute extravascularly.\textsuperscript{6} An increased transendothelial passage of pertechnetate may be related to the fenestration of endothelial junctions that is caused by ischemia.\textsuperscript{20} The sites and mechanisms of the accumulation of pertechnetate in ischemic cerebral tissue are unknown although chloromerodrin appears to accumulate around cells.\textsuperscript{21} The sequestration of water may be osmotic, related to the concentrations of solutes as well as to the presence of larger molecules.

In humans with metastatic brain tumors, there is evidence that dexamethasone is more effective against cerebral edema when inflammation, rather than necrosis, is the predominant pathological process.\textsuperscript{2} With ischemic cerebral edema, inflammation is rare; prominent, and the results of the present study indicate that dexamethasone has an effect in region with necrosis. Dexamethasone also has been shown to influence the extravascular distribution of pertechnetate in humans with cerebral infarcts long after the onset of ischemia, when there is little or no inflammation.\textsuperscript{29} Perhaps undefined steroid-sensitive components of inflammation, related to the ex
travascular sequestration of solutes, larger molecules, and accompanying water, exist in or near ischemic and necrotic cerebral tissue. These components may influence the movement of solutes and larger molecules back into the blood from necrotic tissue. However, the exact mechanisms underlying the effects of dexamethasone remain unknown.

WATER CONTENT OF THE NONISCHEMIC HEMISPHERES

In a previous study, the water content of samples of brain from the nonischemic hemispheres of cats with one MCA occluded was found to increase transiently; bilateral cerebral edema developed with unilateral ischemia. In the present study, using the same experimental model of cerebral ischemia, the water content of samples from the nonischemic cerebral hemispheres was not greater in cats with one MCA occluded than in cats with sham operations. The differences in the results of the two studies may be related to the sizes of the infarcts that occurred: in the earlier study, 22 of 32 samples (68.8%) taken from the ischemic hemispheres were categorized as infarcted, but in the present study only 32.5% of samples from cats that did not receive dexamethasone were categorized as infarcted. Forty-seven of 80 samples (58.8%) taken from cats that received dexamethasone were categorized as infarcted. It is possible that the smaller infarcts in the present study may have contributed to the differences observed.

THERAPEUTIC IMPLICATIONS

Dexamethasone affects ischemic cerebral edema two hours after MCA occlusion and two days later (present study). However, the drug does not influence the neurological deficits or the sizes of the cerebral infarcts that result from experimental ischemia, perhaps because it is effective against ischemic edema only in regions of brain that are already necrotic or progressing to inevitable infarction. Although dexamethasone appears to have little or no therapeutic effect when cerebral ischemia results in small or moderately sized infarcts, when infarction and edema are massive, as in gerbils with one carotid artery ligated, or when intracranial pressure gradients are adequate to cause displacement of intracranial structures, dexamethasone in large doses and in conjunction with other measures may have potential therapeutic value.

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