Effects of Acetazolamide on Cerebral Ischemia and Infarction After Experimental Occlusion of Middle Cerebral Artery

BY FRANCO REGLI, M.D., TAKENORI YAMAGUCHI, M.D., AND ARTHUR G. WALTZ, M.D.

Abstract: Effects of Acetazolamide on Cerebral Ischemia and Infarction After Experimental Occlusion of Middle Cerebral Artery

Acetazolamide was given to five of ten cats for 48 to 54 hours after extradural occlusion of a middle cerebral artery (MCA). At seven to eight days later, measurements of regional cerebral blood flow (CBF) and estimates of the sizes of the ischemic and infarcted areas of the brains were made. Neurological deficits were more severe and the ischemic and infarcted regions were larger in the cats given acetazolamide. Cerebral edema (brain swelling) was present and reactive hyperemia was common in the treated cats, even one week after MCA occlusion. The hypercapnia and decreases of pH of nonischemic brain tissue that are caused by acetazolamide are harmful for ischemic brain tissue, presumably because of vasodilatation in nonischemic brain tissue with resultant increases of intracranial pressure and decreases of CBF of ischemic regions.

ADDITIONAL KEY WORDS: antipyrine-\textsuperscript{14}C, autoradiography, P\textsubscript{a}CO\textsubscript{2}, hyperemia, CO\textsubscript{2} transport, reactive hyperemia, carbonic anhydrase, cerebral circulation, treatment of cerebral ischemia

In the following article in this issue\textsuperscript{1} it was found that the intravenous injection of acetazolamide could cause an increase of blood flow in ischemic as well as nonischemic cerebral cortex of cats with craniectomies, an occluded middle cerebral artery (MCA), and controlled respiration. The increases of blood flow were, in part, independent of increases of arterial carbon dioxide tension (P\textsubscript{a}CO\textsubscript{2}). However, in animals with craniectomies, vasodilatation and brain swelling could not cause changes of intracranial pressure or secondary changes of cerebral vascular resistance (CVR). Therefore, because of the implications for the treatment of acute cerebral ischemia in patients with cerebrovascular disease, the effects of acetazolamide were studied again. In animals that had intact skulls and survived MCA occlusion, acetazolamide was found to be not beneficial but harmful.

Methods

Production of Cerebral Infarct

Ten adult cats were anesthetized with pentobarbital, approximately 25 mg/kg, injected intraperitoneally. The right MCA of each was approached extradurally and occluded with a small Mayfield clip.\textsuperscript{5,8} The incision was closed and the cat was allowed to recover from anesthesia. Five cats were given 500 mg of acetazolamide intramuscularly within 48 to 54 hours of the occlusion, in five doses of 100 mg. The other five cats received no specific treatment.

Stroke, Vol. 2, September-October 1971

From the Cerebrovascular Clinical Research Center and the Department of Neurology, Mayo Clinic and Mayo Foundation and the Mayo Graduate School of Medicine (University of Minnesota), Rochester, Minnesota, 55901.

This investigation was supported in part by Research Grant NB-6663 from the National Institutes of Health, Public Health Service.

Dr. Regli is the recipient of a Medical-Biological Scholar's Stipend from the Schweizer Akademie der Medizinischen Wissenschaften.
EVALUATION OF NEUROLOGICAL DEFICIT

Each cat was examined after it had recovered from anesthesia and at daily intervals for the presence of a neurological deficit, manifested by weakness of a limb, forced deviation of the head or circling, or defective placing or stepping reactions. The neurological findings were graded on the first or second day after MCA occlusion: 0 = no deficit; 1+ = minimal forced deviation of the head or circling, or defective placing or stepping reactions; 2+ = forced deviation and circling with minimal weakness of the forelimb opposite the occluded MCA; 3+ = moderate to marked weakness of one or both limbs opposite the occluded MCA; and 4+ = hemiplegia.

EVALUATION OF CEREBRAL ISCHEMIA, INFARCTION, AND EDEMA

Eight days after MCA occlusion (seven days for cats 6, 7, and 10), each cat was anesthetized with pentobarbital injected intraperitoneally. Catheters were placed (1) in the abdominal aorta through the right femoral artery for measurement of mean aortic blood pressure (MABP) with a strain gauge and polygraph and for withdrawal of samples of blood for measurement of PaO₂, PaCO₂, pH, and hematocrit value and (2) in the inferior vena cava through the left femoral vein for the injection of drugs and of a diffusible radioactive indicator. An arterial-venous shunt was established by connecting, through a three-way stopcock, a catheter placed in the abdominal aorta through the left femoral artery to a catheter placed in the inferior vena cava through the right femoral vein. Blood pressure in the shunt was monitored with a strain gauge and the polygraph. Small amounts of heparinized saline were used to keep the catheters patent.

Each cat was paralyzed with a minimal dose of d-tubocurarine (injected intravenously) and ventilated mechanically. PaO₂ was adjusted to 32 to 35 torr. Rectal temperature was monitored and kept between 37 and 38 C.

Antipyrine-14C (approximately 375 μCi in 5 ml of isotonic saline) was injected into the inferior vena cava at a constant rate for one minute, for measurement of regional CBF. During the injection, 0.2 ml of blood was withdrawn every ten seconds from the stopcock in the arterial-venous shunt for measurement of the arterial concentration of the diffusible radioactive indicator. The circulation of blood then was stopped by rapid intravenous injection of a saturated solution of potassium chloride.

The brain was removed quickly (within ten minutes) and the location of the clip occluding the MCA was verified. The brain then was frozen at −100 to −150 C in 2-methylbutane cooled with liquid nitrogen. After warming to −20 C in a freezer for two to four days, the frozen brain was cut coronally into five slices. Four serial sections (20 microns thick) were made in a cryostat from the anterior surfaces of each of the three central slices. Two of the sections were used for the autoradiographical measurement of the concentration of antipyrine-14C; the other two were placed on glass slides and stained with hematoxylin and eosin. The remainders of the central slices were fixed in 10% formalin and embedded in paraffin; additional sections were cut and stained with hematoxylin and eosin or Luxol fast blue. Care was taken to obtain the paraffin sections as close to the frozen sections as possible.

The sections of brain used for autoradiography were placed on glass slides in a cassette, facing the emulsion of x-ray film. Plastic disks containing known amounts of antipyrine-14C also were put into the cassette. After exposure for approximately two weeks, the film was developed and the optical densities of the autoradiographical images were measured with a densitometer. The amounts of antipyrine-14C in various regions of the brain were determined from a standard curve prepared from density values for the plastic disks. Values for regional CBF were calculated by a digital computer from the equation:

\[ C_4(T) = \lambda k_1 \int_0^T C_{av} e^{-k_1(t-T)} dt, \]

in which \( C_4(T) \) = concentration of antipyrine-14C in tissue at time T (one minute), \( \lambda \) = blood-tissue partition coefficient (\( \lambda = 1 \) for antipyrine), \( C_{av} \) = arterial concentration of indicator, and \( k_1 \) (with \( \lambda = 1 \)) = CBF value (ml/gm/min).

Measurements of CBF were made from multiple sites in each of the two cerebral hemispheres. A region in the right cerebral hemisphere (on the same side as the occluded MCA) was considered to be ischemic if the CBF value was less than 90% of the value for the analogous region of the left cerebral hemisphere. A region in the right cerebral hemisphere was considered to be hyperemic if the CBF value was (1) more than 115% of the CBF value for the analogous region of the left hemisphere (absolute hyperemia) or (2) more than 115% of the CBF values of adjacent regions of the right cerebral hemisphere with a similar type of cerebral tissue (relative hyperemia).

The autoradiographs were projected onto paper, and the enlarged images were traced directly; regions with ischemia were indicated on the traced reproductions. An index of the extent of ischemia in the right cerebral hemisphere was obtained by summing the areas of the ischemic regions (measured with a planimeter) and dividing the result by the sum of the areas.
Sections stained with hematoxylin and eosin or Luxol fast blue were examined grossly and microscopically for evidence of infarction. An index of the size of the cerebral infarct was obtained by outlining the infarcted regions on enlarged reproductions of the sections and dividing the sum of the areas of infarction by the sum of the areas representing the hemisphere.

An index of edema, or swelling of the right cerebral hemisphere, was obtained by comparing the sum of the areas of the cerebral ventricles of the right hemisphere (measured with the planimeter) with that for the left hemisphere, and by comparing the sum of the areas representing the right hemisphere with that for the left hemisphere. The final estimate of edema was arrived at subjectively, by taking into account the general appearance of the sections of the brain, the measurements of the cerebral ventricles, and the measurements of the cerebral hemispheres; the subjective impression was graded 0 to 4+.

**Results**

**NEUROLOGICAL DEFICITS**

Each cat developed a neurological deficit after MCA occlusion, although the deficit in cat 2 was minimal. Neurological findings were variable, as described previously. Two of the cats given acetazolamide (no. 7 and 8) had a more severe deficit and less improvement with time than any untreated cat. Overall, the cats given acetazolamide appeared to have more severe neurological deficits (table 1).
EFFECTS OF ACETAZOLAMIDE ON CEREBRAL ISCHEMIA AND INFARCTION

CEREBRAL ISCHEMIA AND INFARCTION

The sizes of the areas of infarction and ischemia that developed after MCA occlusion were variable, as reported previously. The sizes of the ischemic and infarcted regions in untreated cats, judged by the ischemic index and the infarct index, were substantially the same as in 11 cats used for studies of hyperventilation. The cerebral infarcts and the areas of ischemia were larger in those cats given acetazolamide: all of the treated cats had a larger infarct than all but one of the untreated cats (table 1). The differences between the two groups did not reach statistical significance at the 95% level of confidence because of the small numbers in each group.

HYPEREMIA AND EDEMA

Gross brain swelling was evident in four of the five cats given acetazolamide (table 1; fig. 1); no untreated cat had obvious edema. Two or more regions of hyperemia were found in each of the treated cats. Histopathological evidence of ischemic neuronal degeneration or of ischemic infarction was found in each region with hyperemia except for the internal capsule of animal 7; thus the hyperemia was of the reactive type. Single regions of hyperemia were found in two untreated cats; pathological evidence of preexisting ischemia was found in each of these regions as well.

In the five cats given acetazolamide, the presence and severity of cerebral edema corresponded to the observed neurological deficits and the sizes of the areas of infarction and ischemia (table 1).

Discussion

In contrast to results from cats with craniectomies, acetazolamide was not beneficial but appeared to be harmful to cats with intact skulls and experimental acute cerebral ischemia. After MCA occlusion, more severe neurological deficits, larger areas of ischemia and infarction, and more brain swelling were found in the cats treated with acetazolamide than in untreated cats. Presumably, increases of CBF of nonischemic cerebral tissue during the first few days after MCA occlusion, produced by acetazolamide, caused decreases of CBF of marginally ischemic tissue because of secondary increases of intracranial pressure and tissue pressure. Nonreactive ischemic arteries could not dilate; thus, CVR of ischemic brain probably increased and CBF decreased. Alternatively, the flow of blood may have been diverted through reactive, dilated arteries to nonischemic tissue.

In the untreated group of cats and in previous studies, cerebral edema, or brain swelling, usually was not found seven to eight days after MCA occlusion. The cerebral edema in the treated cats of the present study may have been a reaction to the more severe ischemia and larger infarcts or may in itself have contributed to the production of increased focal ischemia by causing increases of tissue pressure and, secondarily, of CVR.

Reacting hyperemia likewise is not common seven to eight days after MCA occlusion. An earlier study has shown that hypocapnia (with a presumed secondary increase of tissue pH), induced shortly after MCA occlusion, is associated with hyperemia. In this study the opposite was found: acetazolamide presumably caused decreased pH of nonischemic brain tissue, although hypocapnia may have been compensated for by changes of respiration in the awake, freely ventilating cats. Reactive hyperemia may have contributed to cerebral edema and thus caused increased ischemia in regions normally supplied by the occluded MCA, but it is more likely that both the edema and the reactive hyperemia that were present late after MCA occlusion were caused by the more severe ischemia produced by earlier administration of acetazolamide.

Although the question of whether reactive hyperemia is beneficial or harmful to ischemic brain tissue remains unsettled, there is now ample evidence that vasodilatation and increases of CBF of nonischemic brain tissue, regardless of how produced, are generally of little value or perhaps even harmful in animal models of acute cerebral ischemia.

Acknowledgment

Technical and instrumentation assistance was provided by Robert D. Ostrom, Robert E. Anderson, and the Mayo Computer Facility. Dr. K. E. Holley assisted in preparation of the histological specimens.

References


Effects of Acetazolamide on Cerebral Ischemia and Infarction After Experimental Occlusion of Middle Cerebral Artery
FRANCO REGLI, TAKENORI YAMAGUCHI and ARTHUR G. WALTZ

doi: 10.1161/01.STR.2.5.456

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1971 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/2/5/456

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org/subscriptions/