The Effect of Ritanserin, a 5-HT2 Receptor Antagonist, on Ischemic Cerebral Blood Flow and Infarct Volume in Rat Middle Cerebral Artery Occlusion

Kiyoshi Takagi, MD; Myron D. Ginsberg, MD; Mordecai Y.-T. Globus, MD; Raul Busto, BS; W. Dalton Dietrich, PhD

Background and Purpose In a previous study from our laboratory, ritanserin, a specific 5-HT2 serotonin receptor antagonist, reduced ischemic damage in the setting of transient global ischemia. In this study, we examined the effect of ritanserin on ischemic cerebral blood flow, systemic blood pressure, and infarct volume in the model of permanent focal ischemia with brain temperature controlled at 35.0°C to 36.0°C.

Methods Thirty-seven male Sprague-Dawley rats were used. The right middle cerebral artery was permanently occluded. Ritanserin (8 mg/kg) or vehicle was continuously administered intravenously for 90 minutes starting 10 minutes after middle cerebral artery occlusion. Cerebral blood flow was monitored by laser Doppler flowmetry in the ischemic cortex before and for 2 hours after arterial occlusion. Brains were perfusion-fixed 3 days later, and infarct volumes were measured.

Results Mean arterial blood pressure was not affected by treatment. In the vehicle and ritanserin groups, mean ischemic cerebral blood flow (percent of preischemic values) was 34.6±14.7% (mean±SD) and 26.6±15.0%, respectively. Hemispheric infarct volumes were 119.3±49.4 mm3 and 136.6±49.6 mm3, respectively. No significant differences were recognized.

Conclusions Intravenous administration of ritanserin did not affect mean arterial blood pressure or cerebral blood flow in the ischemic region during the acute phase of ischemia. No protective effect of ritanserin was apparent in the setting of permanent focal ischemia when treatment was begun shortly after the onset of ischemia. (Stroke. 1994;25:481-486.)

Key Words • cerebral ischemia, focal • ritanserin • serotonin • rats

Many studies have disclosed the involvement of excitatory amino acids in the development of ischemic brain damage.1-6 It has also been demonstrated that glutamate receptor antagonists have a protective effect against cerebral infarction.7-17 Nevertheless, other neurotransmitters have also been shown to be involved in ischemic brain damage.18-24 Destruction of substantia nigra leading to dopaminergic denervation protects striatal neurons from global ischemia18,19 and reduces infarct volume following focal ischemia.25 A participatory role for serotonin has been suspected because of its possible neurotoxic effects mediated directly or indirectly.23,26-30 Anatomic studies have disclosed serotonin 5-HT2 receptors in cerebral cortex and striatum of the rat. 5-HT2 receptors are also recognized on cerebral vessels, and immunohistochemical studies show that pial arteries are innervated by serotonergic fibers.31,32 Furthermore, serotonergic denervation partially protects the striatum from kainic acid–mediated damage.26 In a previous report from our laboratory, Globus et al23 reported the involvement of serotonin in ischemic neuronal damage in the setting of transient global forebrain ischemia. Therefore, it is possible that a selective 5-HT2 receptor antagonist might have a protective effect against ischemic damage in focal ischemia.

The aim of this study was to assess the effect of ritanserin, a highly selective 5-HT2 antagonist,25,26 in focal ischemia. Serotonin may be involved in the hemodynamic changes in ischemic or periischemic regions37 since it has vasocostrictive properties. In a model of thrombotic cortical infarction, Dietrich and colleagues37 showed that the 5-HT2 antagonist, ketanserin, inhibited some of the remote hemodynamic consequences of this focal injury. In that study, it was suggested that 5-HT2 receptor antagonist might therefore prove useful in reducing infarct size by improving collateral circulation. Previous studies from our laboratory have examined the effects of preischemic administration of 5-HT2 antagonist on ischemic outcome.23 In this study, ritanserin was administered after middle cerebral artery occlusion, and systemic blood pressure and cerebral blood flow (CBF) were measured during the ischemic period.

Because temperature has a marked influence on the ischemic damage in both global and focal ischemia,38-40 we monitored ipsilateral cortical brain temperature and, by that means, regulated brain temperature.
Materials and Methods

We used 37 male Sprague-Dawley rats (Charles River Laboratories, Inc, Wilmington, Mass), weighing 300 to 400 g. Animals were fasted overnight but were allowed free access to water. Anesthesia was induced with 4% halothane, 70% nitrous oxide, and a balance of oxygen and was maintained with 2% halothane and 70% nitrous oxide during the surgical procedures. Atropine sulfate (0.04 mg/kg) was injected. The right femoral artery and vein were cannulated with PE-50 polyethylene catheters for monitoring of arterial blood pressure and blood gases and for the administration of drugs. Rats were then intubated endotracheally, immobilized with pancuronium bromide (initial dose, 0.6 mg/kg; additional dose, 0.2 mg/kg), and were mechanically ventilated. The animals were fixed in a stereotaxic frame (Stoelting, Ill). Blood gases (ABL 30 system, Radiometer, Copenhagen, Denmark), plasma glucose, and lactate (Glucose/Lactate Analyzer Model 2300 STAT, YSI, Ohio) were monitored before and during ischemia (70 minutes after middle cerebral artery (MCA) occlusion, i.e., 60 minutes after drug administration). Physiological variables were kept within normal limits (mean arterial blood pressure [MABP], 100 to 150 mm Hg; PaCO2, 100 to 150 mm Hg; PaO2, 30 to 45 mm Hg; pH, 7.30 to 7.45). Rectal temperature was maintained between 37.0°C and 38.0°C during the experiment by means of a heating lamp placed above the body.

The right MCA was exposed by the method of Tamura et al. In brief, the cranial vault and the right lateral surface of the skull were exposed via a longitudinal skin incision between the eye and the ear. The zygomatic arch was removed. A burr hole (1.5 mm in diameter) for the brain temperature probe was made above the right parietal cortex by means of a high-speed mini-drill (Nihon-Seimitsu Kikai K.K., Japan) under an operating microscope (Carl Zeiss, Germany); the field was irrigated frequently with cooled saline to avoid thermal damage. Brain temperature was monitored with a thermocouple probe (CN 9000, Omega), which was inserted into the cerebral cortex (approximate coordinates: 4 mm lateral to the bregma, 2-mm depth from brain surface). Brain temperature was maintained between 35.0°C to 36.0°C by means of a small heating lamp placed 20 cm over the head. Another burr hole (2 mm lateral to the burr hole for the temperature probe, 2 mm in diameter) was made in the lateral surface of the temporal bone to permit continuous CBF measurement by laser Doppler flowmetry (P433-3, Vasamedics). This probe was connected to a perfusion monitor (LaserFlo BPM 403A, Vasamedics). This position of the probe was determined based on previous studies from our laboratory31 and by others5255 using the same proximal MCA occlusion model in Sprague-Dawley rats; we anticipated that in this “penumbral” cortical site, the effect of the pharmaceutical would be most evident in that this cortical area is not consistently involved in infarction.5152 A temporal burr hole was then made in the retro-orbital region to occlude the MCA.50 The dura mater covering the MCA was opened.

After these surgical procedures, the inspired halothane was discontinued to avoid the effect of halothane on systemic blood pressure and CBF. Anesthesia was maintained with 70% nitrous oxide and 30% oxygen. Thirty minutes after discontinuation of halothane, measurement of the preschismic physiological variables, CBF, MABP, and pulse rate was begun. Steady-state baseline values were recorded before MCA occlusion, and CBF was expressed as a percentage of the average of six baseline measurements taken every 5 minutes before MCA occlusion.56 Because ambient light interferes with the flow reading, the heating lamp was turned off for 30 seconds at the time of each CBF recording. Brain temperature did not decrease below 35.0°C during this period. To detect the effect of ritanserin on CBF and MABP, these variables were measured every 10 minutes during a 2-hour observation period after MCA occlusion.

After 30 minutes of preschismic data sampling, the proximal portion of the right MCA was electrocoagulated and cut.
TABLE 1. Physiological Variables

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<th>Glucose, mg/dL</th>
<th>Lactate, nmol/L</th>
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Values are mean±SD.
*P<.05 vs preischemic value.
†P<.05 vs same category of vehicle group.

Discussion

The present results indicate that postischemic ritanserin fails to reduce infarct volume in a permanent focal ischemia model of the rat produced by electrocoagulation and surgical sectioning of the right MCA. Similarly, intravenous ritanserin administration has no acute effect on CBF during ischemia or on MABP. The ineffectiveness of ritanserin in reducing infarct volume cannot be attributed to the dose used in this study in that we used the same dose as that used by Globus et al in their study of transient global forebrain ischemia in the rat, in which a highly beneficial effect of ritanserin was demonstrated in protecting hippocampal CA1 neurons from ischemic damage. The discrepancies in the results from the two studies can be explained by the use of different experimental protocols (focal versus global ischemia) or by the differences in treatment paradigm (preischemic versus postischemic administration of the 5-HT2 antagonist). The rationale for postischemic treatment with ritanserin is based on our study in which ritanserin, administered after ischemia, improved postischemia.

We anticipated, in designing this permanent focal ischemia study, that ritanserin would reduce infarct volume and would increase CBF when administered during ischemia. This expectation was based on several previous observations: (1) Both the rat and human brain are rich in 5-HT2 receptors. (2) Brain blood vessels are well endowed with 5-HT2 receptors, which may be involved in vasoconstriction. (3) Moderate ischemia induces an elevation of serotonin in the extracellular space of the rat striatum. (4) Ischemia has been shown to induce the release of serotonin in the caudate putamen. (5) There is evidence from the literature that 5-HT itself may be neurotoxic. (6) Serotonin antagonists have been shown to be effective in certain forms of central nervous system ischemia. As noted above, Globus et al found the administration of ritanserin to protect hippocampal neurons from morphological damage following global ischemia. (7) Ketanserin, another 5-HT2 receptor antagonist, has been reported to increase the CBF in remote cortical brain regions after cortical thrombotic infarction.

In addition to the above evidence, there are other, more indirect, reasons to have expected a beneficial effect of ritanserin in focal ischemia: (1) It has been suggested that 5-HT selectively enhances voltage- and Ca²⁺-dependent NMDA responses. (2) (S)-Emopamil, a calcium channel blocker having 5-HT2 receptor-blocking properties, has been shown in our laboratory to have a marked beneficial effect in reducing infarct volume in the same rat model of permanent MCA occlusion as that used in the present study. We have also shown (S)-Emopamil to be effective in global ischemia. (3) Ohno and coworkers have shown that ritanserin, administered after ischemia, improved postischemic working memory. Although that study lacked histopathology, it showed functional improvement of treated animals.

Despite the evidence cited above, the present study was unable to demonstrate a protective effect of ritanserin in focal ischemia. We interpret these negative findings in the context of the specific experimental design and methodology employed.

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results as follows: 5-HT may not be involved in the pathophysiology of ischemic injury in this model. In part, this may relate to the fact that the model involves mechanical occlusion of the MCA and that platelet thrombi, the main source of intravascular 5-HT in ischemia, may not be formed in this model. In this regard, Wester and colleagues have recently demonstrated a 5-fold increase in plasma serotonin levels after common carotid artery thrombosis.

Although this study failed to demonstrate a therapeutic effect of ritanserin when administered postschemically, we feel that it may nonetheless be important to consider the role of the serotonergic system in human cerebral ischemia; the human brain is richly endowed with 5-HT receptors, and the most frequent causes of human cerebral ischemia are thrombosis and embolism, which may well involve the release of serotonin from aggregated platelets.

Acknowledgments

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References

27. Felder CC, Kanterman RY, Ma AL, Axelrod J. Serotonin stimulates phospholipase C and the release of arachidonic acid in hippocampal neurons by a 2 serotonin receptor that is independent of inositol phospholipid hydrolysis. Proc Natl Acad Sci U S A. 1990;87:2187-2191.
Clarification of the early events as well as identification of interventions that alter the events and the occlusion test outcome. The main objective of this study was to evaluate a drug intervention applied in an attempt to protect tissue from infarction and thus improve the MCA occlusion test outcome.

After focal ischemia by permanent occlusion of the MCA, neocortical infarct volumes were of similar size in MCA, neocortical infarct volumes were of similar size in rats. Stroke. 1990;21:1318-1325.


54. Reynolds JN, Baskys A, Carlen PL. The effects of serotonin on CA1 neurons by ritanserin is unknown. The authors argue that ritanserin protects hippocampal CA1 neurons against ischemic damage in a model of focal ischemia. J Neurochem. 1992;59:1056-1061.


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