Neurological protection by dichloroacetate depending on the severity of injury in the paraplegic rat

DANIEL R. LE MARY, B.S., GERALD B. ZELENOCK, M.D., and LOUIS G. D'ALECY, D.M.D., Ph.D.

Departments of Physiology and Surgery, University of Michigan Medical School, Ann Arbor, Michigan

Hyperglycemia has been shown to exacerbate neurological deficit associated with central nervous system ischemia. Iodoacetate or dichloroacetate was administered intraperitoneally to rats in a study to examine the role of glycolysis in hyperglycemic exacerbation of neurological deficit. Sprague-Dawley rats were injected with saline, iodoacetate, or dichloroacetate and then made paraplegic by temporary occlusion for 10, 12, 13, or 15 minutes of the right and left subclavian arteries and the aorta distal to the left subclavian artery. Glycolytic blockade by iodoacetate was lethal in doses of 15 mg/kg or more, whereas rats receiving 10 mg/kg survived but showed no significant neurological improvement compared to the saline-treated control group. Dichloroacetate, 500 mg/kg, protected neurological function, which suggests a possible detrimental role for lactate accumulation and the benefit of maintaining tricarboxylic acid cycle activity by stimulating pyruvate dehydrogenase. The protection seen with dichloroacetate depended on the severity of ischemic injury. Dichloroacetate administration had a minimal effect on neurological outcome with occlusion periods of 13 and 15 minutes, mild improvement with 12 minutes of occlusion, and a significant protective effect with a 10-minute occlusion period. The dose-response nature of ischemic injury and neurological outcome in this rat model of paraplegia therefore appears to play an important role in determining the effect observed with a specific intervention.

Key Words: spinal cord • ischemia • hyperglycemia • dichloroacetate • iodoacetate • metabolism • rat

It has been shown by both clinical and experimental studies that central nervous system (CNS) ischemia in the presence of hyperglycemia results in worse neurological outcome than in conditions of normal or mildly reduced blood sugar levels. In a previous study using the rat paraplegia model, we demonstrated that elevated glucose availability alone is not damaging but instead the metabolic events responsible for increased damage are subsequent to the first step of glucose metabolism by hexokinase. Several factors may play an important role in hyperglycemic exacerbation of neurological deficit, and tissue lactate accumulation has been suggested as a major contributor. In the present study, we examined the role of glucose metabolism occurring after the hexokinase step of glycolysis and the tricarboxylic acid (TCA) cycle on hyperglycemic exacerbation of neurological deficit. Iodoacetate was used to block an intermediate step of glycolysis and dichloroacetate was used to increase the flux of glycolysis metabolites into the TCA cycle. An additional goal of this study was to examine the influence of the severity of ischemic injury on the effectiveness of an intervention. Therefore, iodoacetate and dichloroacetate were tested in paraplegic rats with various degrees of severity of ischemic injury.

Materials and Methods

Male Sprague-Dawley rats, aged 2 to 3 months and weighing 200 to 300 gm each, were housed individually in metal cages with free access to food and water. Animal care complied with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals," 1985. The rats received intraperitoneal injections of either 500 mg/kg dichloroacetate up to 30 minutes before occlusion, 10 to 100 mg/kg iodoacetate 15 minutes to 24 hours before occlusion, or an equivalent volume of 0.9% saline at corresponding times. The pH of dichloroacetate was buffered to approximately 7.0 with 10N NAOH.

As previously reported, rats were subjected to experimental spinal cord ischemia. Each rat was anesthetized by placing them in a chamber containing 2% halothane. Tracheal intubation with a tube, 8 cm long with a 2.5-mm outer diameter, was assisted by a neonatal laryngoscope reduced to a blade width of 7 mm.
Dichloroacetate protection and severity of ischemic injury

![Graph showing dose-response curve for neurological deficit scores](image)

**Fig. 1.** Dose-response curve showing the total neurological deficit scores evaluated at four times (1, 4, 18, and 24 hours) following occlusion for four different occlusion duration periods in saline-treated rats. Rats have a maximum deficit at 13 and 15 minutes of occlusion. At 10- and 12-minute occlusions, the neurological deficit is less severe and decreases with the time of occlusion up to the final evaluation made at 24 hours postocclusion.

The rat was then ventilated with an open-circuit volume ventilator* at 100 cycles/min with 1.0% to 1.5% halothane. Body temperature was continuously monitored with a thermistor inserted 3.5 cm into the rectum, and was maintained between 35.5°C and 36.5°C with a heating pad. The upper extremities and tail were fixed to the operating surface with adhesive tape. A longitudinal incision was made through the skin in the sternal region. The chest wall was incised from the apex of the manubrium caudad along the left sternal border, through the second rib, to the top of the third rib, carefully avoiding (staying medial to) the left internal thoracic artery. The thymus was excised and the aortic arch was isolated distal to the left subclavian artery. A 15-cm segment of polyethylene (PE) 10 tubing was placed around the aorta, avoiding the left vagus and recurrent laryngeal nerves, and the free ends were passed through a 4-cm segment of PE 160 tubing, creating a vessel snare. The origins of the right and left subclavian arteries were isolated and snared placed in a similar manner.

The three snares were pulled and secured with a clip, thus occluding each vessel. Occlusion of each vessel was verified by inspection of the snare site and the vessel distal to the snare. A positive end-expiratory pressure (PEEP) of 12 cm H₂O was started and maintained throughout the occlusion period. The snares exited the incision cephalad to the manubrium, and the chest was closed in three layers up to the snares with 4.0 silk suture. At the end of the occlusion period, the snares were released and withdrawn and the closure was completed. When the rat maintained voluntary ventilation on disconnection from the ventilator, anesthesia and PEEP maintenance were discontinued and extubation was performed. A neurological deficit score was assigned at 1, 4, 18, and 24 hours postocclusion to quantify hindlimb neurological deficit in five categories of deficit with a range as follows: 0–4 walking with slower extremities, 0–4; grasping onto a horizontal rope platform: 0–3; grasping onto a rotating screen: 0–3; grasping onto a wooden bar 1 in. in diameter at 45°: 0–3; and pain sensation: 0–2. A total score of zero indicated no deficit detected, with increasing numbers corresponding to an increasing deficit up to a maximum deficit score of 15.

Data analysis was performed using the Student unpaired two-tailed t-test for neurological deficit score with Bonferroni multiple-comparison correction. Values are significantly different from control values at p<0.05 (calculated on an Apple Macintosh computer with Statview 512+ software). All average values are expressed as mean ± standard error of the mean.

**Results**

Figure 1 shows neurological deficit in saline-treated rats at 1, 4, 18, and 24 hours following a single occlusion for four different occlusion durations of 10, 12, 13, and 15 minutes. For all occlusion periods, the neurological deficit that resulted 1 hour following the ischemic event was very similar. With 10- and 12-minute ischemic periods, there was substantial improvement in neurological function over 24 hours. For 13-minute occlusions, there was little change over 24 hours and, with 15-minute occlusions, rats had a slight increase in deficit over the following 24 hours resulting from the loss of pain sensation. One aspect of the deficit not included in the score is the spastic versus flaccid nature of the hindlimbs. With 15-minute occlusions, virtually all saline-treated rats had flaccid limb tone whereas, following 13-minute occlusions, most rats had spastic limb tone at 24 hours following occlusion.

Figure 2 contains the same data for saline-treated rats 24 hours following occlusion as shown in Fig. 1 as a control for the data from dichloroacetate-treated rats 24 hours following occlusion. There was a convergence of neurological deficit toward a maximum as the duration of occlusion increased. The ability of dichloroacetate to protect neurological function decreased as the severity of the injury increased. Figures 1 and 2 illustrate the dose-response nature of ischemic duration and neurological outcome in this model.

Based on results shown in Figs. 1 and 2, neurological deficit was compared for saline- and dichloroacetate-treated rats at 1, 4, 18, and 24 hours following a 10-minute occlusion period (Fig. 3). Dichloroacetate-treated rats showed significant improvement in neurological outcome over saline-treated rats at 18 and 24 hours following the ischemic event. At 24 hours following occlusion, dichloroacetate-treated rats had greater

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*Ventilator, Model 683, manufactured by Harvard Apparatus, South Natick, Massachusetts.
grasping strength and a substantially less uncoordinated gait in the hindlimbs than saline-treated control rats.

Iodoacetate was tested with occlusions of 10- and 13-minute duration at doses from 10 to 80 mg/kg administered from 10 minutes to 4 hours before ischemia. At iodoacetate doses of 20 mg/kg or greater, all rats died in the posts ischemic period. With a 10-minute occlusion period one of six rats survived with an iodoacetate dose of 15 mg/kg; however, there was no neurological improvement. At an iodoacetate dose of 10 mg/kg, most rats survived but neurological deficit was not significantly different from that of saline-treated rats. Normal nonoperated rats tolerated iodoacetate doses of 100 mg/kg. Ischemic rats that died following treatment with iodoacetate, 15 to 20 mg/kg, often died within 4 hours following the ischemic period due to respiratory and apparent cardiac depression. Iodoacetate doses of 40 to 80 mg/kg often resulted in death during the ischemic period apparently due to cardiac arrest.

**Discussion**

This study demonstrates in the rat paraplegia model that hyperglycemic exacerbation of neurological deficit can be reduced by dichloroacetate and that the degree of severity of ischemic injury can significantly influence the effectiveness of a given treatment. The protective effect of dichloroacetate is supported by another study which showed that neurological deficit can be reduced by treatment with this agent following CNS ischemic injury.¹

The damage that results from an ischemic injury is most probably due to many potentially detrimental processes including (in addition to the damaging effect of depletion of energy stores) factors such as lactate accumulation,⁷,¹⁵,²⁰,²⁴,²⁵ and oxygen radical formation,²¹,²⁹ each of which may have a variable contribution in different tissues. Dichloroacetate, which increases the activity of pyruvate dehydrogenase, could help decrease the extent to which adenosine triphosphate depletion occurs in an ischemic tissue by reactivation (or reducing the depression) of the TCA cycle during or following ischemia. This would occur by stimulating the flux of carbon atoms from lactate through pyruvate into the TCA cycle. Dichloroacetate also has the effect of decreasing lactate accumulation following ischemia,²¹ which would tend to remove the inhibition of lactate on phosphofructokinase. Although lactate levels were not measured in this study, the results are consistent with the hypothesis that protection by dichloroacetate may be due to reduced lactate concentration in spinal cord ischemic tissue as well as restoration of glycolytic flux into the TCA cycle.

The other compound tested was iodoacetate, which is an irreversible inhibitor of the glycolysis enzyme glyceraldehyde-3-phosphate dehydrogenase.²⁵ Iodoacetate was lethal in the rat paraplegia model in doses of 15 mg/kg or greater, while rats that did survive iodoacetate, 10 mg/kg, showed no improvement over the saline-treated control group. Based on these data it is evident that the lethal dose of iodoacetate is lower than...
any dose which might be protective and, as a result, no conclusion can be made about its effectiveness related to hyperglycemic exacerbation of neurological deficit.

Results in a previous study using 2-deoxyglucose (2-DG) and 3-O-methylglucose as inhibitors of glucose uptake (glucose transporter) and initial metabolism (hexokinase) suggested that the activity of the glucose transporter by itself does not significantly contribute to hyperglycemic exacerbation of neurological deficit. In contrast, the hexokinase step, at least in combination with the transporter and possibly alone, does play a significant role in hyperglycemic exacerbation of lower-extremity neurological deficit in the paraplegic rat. The role that hexokinase plays may be as a gate into glycolysis at a crucial bottleneck which has no alternative bypass step, implying that steps subsequent to hexokinase may be responsible for the actual damage in hyperglycemic ischemia. Protection resulted from the inhibition of hexokinase by 2-DG (1 gm/kg), which should decrease flux into the TCA cycle, while dichloroacetate afforded protection by increasing flux into the TCA cycle. These apparently opposing actions have the common effect of decreasing the accumulation of any metabolite between the hexokinase step and the pyruvate dehydrogenase step, which involves the metabolite lactate. With a higher dose of 2-DG (2 gm/kg) there is presumably more complete inhibition of hexokinase and a significantly increased mortality rate. This result and the finding that the irreversible inhibition by io-dichloroacetate is lethal strongly suggest that partial activity of glycolysis is essential to maintain a minimal activity of the TCA cycle. The protection by dichloroacetate may therefore be due to maintenance of TCA cycle activity in addition to reduced tissue lactate accumulation.

Both dichloroacetate and iodoacetate were tested using different degrees of severity of ischemic injury. This was important because there appears to be a dose-response nature to the degree of ischemic injury and neurological outcome which can influence the effectiveness of a treatment. With a severe ischemic insult (13 to 15 minutes of occlusion), paraplegic rats showed little change or even an increase in deficit compared to paraplegic rats with moderate injury (10 to 12 minutes of occlusion); rats with moderate injury showed significant improvement in neurological function over 24 hours with both saline treatment (Fig. 1) and dichloroacetate treatment (Fig. 2).

Although an intervention may not demonstrate protection in the setting of severe injury because the damage is too extensive in the treated group, or show a detrimental effect because damage is too extensive in the control group, a significant effect may result when the degree of injury is reduced. If the degree of injury was too mild, then it would also be difficult to observe a protective or detrimental effect for a treatment. Figure 2 shows that the protective nature of dichloroacetate was only apparent with a moderate degree of ischemic injury and, as the severity of injury increased to that obtained with a 15-minute occlusion period, the effectiveness of protection was eliminated. While a dose-response relationship is familiar in considering the action of drugs, these data highlight a similar dose-response relationship of the duration of ischemia and the extent of neurological deficit. The interaction of these relationships intensifies the need for conservative conclusions about interventions for CNS ischemia.

References

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Address reprint requests to: Louis G. D’Alecy, D.M.D., Ph.D., Department of Physiology, 7799 Medical Science II Building, University of Michigan, Ann Arbor, Michigan 48109.