The Effect of Dichloroacetate on Brain Lactate Levels Following Incomplete Ischemia in the Hyperglycemic Rat

A. R. T. Colohan, M.D.,* F. A. Welsh, Ph.D.,† E. D. Miller, M.D.,‡ and N. F. Kassell, M.D.*

SUMMARY Dichloroacetate (DCA) is known to prevent the phosphorylation of the pyruvate dehydrogenase complex (PDHC) by blocking the action of PDH kinase. This action allows the active PDHC to exert its effect on the metabolism of glucose, lactate and alanine to acetyl CoA. DCA has been shown to reduce serum lactate levels in humans and animals in such conditions as diabetes, phenformin-induced hepatic failure, exercise, and endotoxin-induced shock. Lactic acidosis in the brain has often been postulated as a cause of neuronal damage following ischemia and hypoxia. Therefore, we examined the effect of intravenously administered DCA (100 mg/kg) in rats that were rendered hyperglycemic by intravenous glucose (2 g/kg), and then made to undergo 15 minutes of incomplete cerebral ischemia by bilateral carotid ligation and systemic hypotension (mean arterial pressure of 50 mm Hg). DCA significantly reduced serum lactate levels pre-ischemia, but had no effect on serum lactate levels after ischemia induction. Brain levels of lactate, ATP and PCr after 15 minutes of incomplete ischemia were unaffected by DCA. We conclude that in this in-vivo model the control of PDHC activity in the brain may be different than that in the periphery, and that DCA was not effective in reducing brain tissue lactate levels.

Stoke Vol 17, No 3, 1986

DICHLOROACETATE (DCA) has been shown to reduce serum lactate levels in pathological conditions such as diabetes,1,2 phenformin-induced hepatic failure,3-5 and endotoxin induced shock.6-8 Its mechanism of action appears to be as an inhibitor of the PDHC kinase which phosphorylates PDHC into an inactive form.9,10 PDHC is a rate-limiting enzyme in the conversion of glucose, lactate and alanine to acetyl CoA.9,10 DCA has been shown to reduce tissue lactate levels in heart,9 diaphragm,11 muscle and liver.12 Little work has been done on its effect on brain metabolism.

Rehncrona et al have postulated that brain damage following ischemia may be due in part to excess tissue lactate build-up and intracellular changes in pH.13,14 Hyperglycemia in the setting of cerebral ischemia in the rat,15 cat16 and monkey17 has been shown to be deleterious and brain tissue lactate levels have been higher. The present study was designed to see if DCA administered before the induction of cerebral ischemia could reduce the brain tissue lactate elevations found by previous workers. In order to maximize the brain lactate levels so as to increase any possible effect that DCA might have, rats were rendered hyperglycemic prior to ischemia induction. Since Eklof18 has shown inhomogeneity in cerebral blood flow reduction in incomplete ischemia in the rat under normotensive conditions, rats were rendered hypotensive (mean arterial blood pressure of 50 mm Hg) by withdrawal of blood at the induction of complete ischemia which was accomplished by bilateral carotid ligation; a model in which severe reduction of cerebral blood flow in forebrain cortical structures has been shown.19,20

Materials and Methods

Wistar rats of either sex weighing 350-450 gms were fed ad libitum until the time of surgery. Anesthesia was induced and maintained with 0.6% halothane and 30% O2. Mechanical ventilation was maintained after intubation and administration of pancuronium (2 mg/kg IV), supplemented hourly (1 mg/kg). Catheters were placed in both femoral arteries and one femoral vein. Arterial pressure was monitored continuously (P23 ID Statham) and displayed on a strip chart recorder (Gould Brush 2400). Serial arterial blood gases were performed (Radiometer Mark II) and ventilator adjustments made to maintain PO2, PCO2 and pH in a physiological range. Temperature was measured via rectal probe and maintained at 37°C by heat lamp. Both carotid arteries were dissected in the neck for later ligation. An incision was made in the scalp to facilitate subsequent freezing of the brain.

Two groups of rats were then studied. In the first group (n = 7) rats received 100 mg/kg DCA as an intravenous bolus at time 0 and at 75 minutes an intra-
venous infusion of 50% glucose (2 g/kg) was given over 15 minutes. At 90 minutes both carotid arteries were doubly ligated and cut and systemic hypotension, to achieve a mean arterial blood pressure of 50 mm Hg, was produced by withdrawal of blood from a femoral artery catheter as described by Nordstrom and Seisjo. DCA was obtained as the acid from Fischer Scientific Company (Fair Lawn, NJ 07470) and prepared fresh daily with titration to a pH of 7.4 immediately before infusion. Blood samples for glucose and lactate determination (200 μl) were drawn at 0, 30, 60, 75, 90, 100 and 105 minutes and centrifuged for 1 minute before storing at −70° C for later analysis. Fifteen minutes after the induction of ischemia brains were frozen in situ by the method of Ponten and Ratcheson.

In the control group (n = 8) all above described procedures were carried out with the exception of DCA infusion. Those animals received a volume of saline equal to the volume of DCA infused at the beginning of the experiment. Statistical analysis was performed using Student’s t test for unpaired data, p < 0.05 was considered significant.

The frozen brains were sectioned in the coronal plane at the level of the striatum, and four samples of cerebral cortex were dissected and weighed (0.5-0.8 mg) in a glove-box maintained at −25° C. The samples were extracted in perchloric acid, and the extracts were analyzed for ATP, phosphocreatine and lactate using enzymatic, fluorometric methods. For internal control, samples from normoxic mouse brain were included in each batch of assays. Aliquots of plasma were analyzed for glucose and lactate, also using enzymatic methods.

**Results**

Intravenous DCA in the dosage used (100 mg/kg) significantly lowered serum lactate levels as compared to controls at 30, 75 and 90 minutes, but not after the induction of incomplete cerebral ischemia and systemic hypotension (fig. 1). Serum glucose levels in the two groups were not significantly different (fig. 2). There were no significant differences in the mean arterial blood pressure, heart rate, PO2, PCO2, pH, or temperature between the DCA and saline treated groups (table 1). The mean weights in the two groups were 409 ± 14 gms in the DCA group and 420 ± 11 gms in the saline treated group. The amount of blood withdrawn to produce hypotension was 7.2 ± 0.8 mls in the DCA group versus 7.9 ± 0.8 mls in the saline treated group (all mean values are ± SEM).

Analysis of the brain levels of ATP and PCr revealed significant decrease from normoxic brain and the brain lactate levels significantly increased (table 2). However, there was no statistically significant difference in the ischemic levels of lactate, ATP and PCr in the brains of the saline-infused rats versus those of the DCA-infused rats.

**Discussion**

The present study failed to show any significant reduction in cerebral tissue lactate levels by pre-treatment with DCA in rats undergoing incomplete ischemia. The model of incomplete ischemia and systemic hypotension in the rat has been well-worked out by Nordstrom and Seisjo. The protocol in the present study was designed to parallel theirs. DCA did significantly lower serum lactate levels in both the hyperglycemic and normoglycemic rats before ischemia induction, but had no effect on serum levels once ischemia had been induced. The levels of brain tissue lactate achieved by incomplete ischemia in the present study are comparable to those reported in similar models in rats and cats.

DCA has been shown by Evans to cross the blood-brain barrier in rats when given by the gastric route (100 mg/kg every 6 hours for 24 hours). Brain tissue concentrations of DCA were found to be similar to those found in muscle and liver by the same investigator (88.6 ± 9.4 μg/g). Therefore, it would not appear that the failure of DCA to reduce lactate levels in the brain in the present study was due to its inability to cross the blood-brain barrier.

Rather, it is possible that during incomplete ischemia, the supply of oxygen is not sufficient to re-oxidize the NADH generated by PDHC and the citric acid cycle. Thus, even if brain PDHC were activated by
TABLE 1  Physiological Parameters

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MAP mm/Hg</th>
<th>H.R.</th>
<th>PO₂ mm/Hg</th>
<th>PCO₂ mm/Hg</th>
<th>pH</th>
<th>Temp °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCA</td>
<td>0</td>
<td>103 ±3</td>
<td>362 ±22</td>
<td>114 ±6</td>
<td>40 ±5</td>
<td>7.36 ±0.04</td>
</tr>
<tr>
<td>Saline</td>
<td>110 ±4</td>
<td>414 ±18</td>
<td>120 ±9</td>
<td>31 ±2</td>
<td>4.1 ±2</td>
<td>7.41 ±0.01</td>
</tr>
<tr>
<td>DCA</td>
<td>30</td>
<td>115 ±6</td>
<td>386 ±24</td>
<td>107 ±9</td>
<td>45 ±4</td>
<td>7.31 ±0.03</td>
</tr>
<tr>
<td>Saline</td>
<td>109 ±3</td>
<td>404 ±70</td>
<td>120 ±10</td>
<td>38 ±3</td>
<td>7.37 ±0.02</td>
<td>37.7 ±0.2</td>
</tr>
<tr>
<td>DCA</td>
<td>60</td>
<td>110 ±10</td>
<td>372 ±26</td>
<td>109 ±9</td>
<td>40 ±4</td>
<td>7.34 ±0.02</td>
</tr>
<tr>
<td>Saline</td>
<td>105 ±8</td>
<td>384 ±14</td>
<td>124 ±10</td>
<td>40 ±3</td>
<td>7.37 ±0.02</td>
<td>37.2 ±0.2</td>
</tr>
<tr>
<td>DCA</td>
<td>75</td>
<td>94 ±9</td>
<td>346 ±20</td>
<td>119 ±10</td>
<td>38 ±4</td>
<td>7.34 ±0.02</td>
</tr>
<tr>
<td>Saline</td>
<td>102 ±8</td>
<td>374 ±22</td>
<td>122 ±9</td>
<td>36 ±4</td>
<td>7.38 ±0.02</td>
<td>37.1 ±0.2</td>
</tr>
<tr>
<td>DCA</td>
<td>90</td>
<td>121 ±8</td>
<td>376 ±20</td>
<td>107 ±5</td>
<td>41 ±4</td>
<td>7.34 ±0.03</td>
</tr>
<tr>
<td>Saline</td>
<td>118 ±4</td>
<td>388 ±12</td>
<td>126 ±11</td>
<td>45 ±2</td>
<td>7.29 ±0.02</td>
<td>36.9 ±0.1</td>
</tr>
<tr>
<td>DCA</td>
<td>95</td>
<td>46 ±1</td>
<td>322 ±34</td>
<td>110 ±8</td>
<td>39 ±1</td>
<td>7.30 ±0.02</td>
</tr>
<tr>
<td>Saline</td>
<td>46 ±7</td>
<td>330 ±22</td>
<td>138 ±14</td>
<td>38 ±2</td>
<td>7.30 ±0.02</td>
<td>36.6 ±0.1</td>
</tr>
<tr>
<td>DCA</td>
<td>105</td>
<td>50 ±0</td>
<td>360 ±28</td>
<td>108 ±7</td>
<td>41 ±2</td>
<td>7.28 ±0.03</td>
</tr>
<tr>
<td>Saline</td>
<td>50 ±2</td>
<td>340 ±14</td>
<td>138 ±12</td>
<td>38 ±3</td>
<td>7.32 ±0.03</td>
<td>36.5 ±0.3</td>
</tr>
</tbody>
</table>

DCA, entry of pyruvate (and lactate) into the citric acid cycle may be limited by oxygen availability.

Although there has been considerable work done on the effects of DCA in the periphery there has been little work on its effect on neural tissue. The metabolic effects of DCA were extensively reviewed by Crabb in 1981.* Schaffer in 1980 observed no change in the activation of PDHC by the addition of DCA to isolated rat brain synaptosomes during membrane depolarization.26 However, Baudry et al found that the addition of DCA to rat hippocampal slices resulted in an increase in the active portion of PDHC; a response that was dose-related and maximal after only 5 minutes incubation.27 Browning et al also showed concentration-dependent inhibition by DCA on the phosphorylation of intra-mitochondrial PDHCα sub-unit, with accompanying stimulation of PDHC activity.28 In the study by Evans cited previously there was no significant change in PDHC activation in the rat brain by DCA and in the same paper DCA had no effect on PDHC activation in isolated rat brain mitochondria.24 Since the brain tissue concentrations of DCA were comparable to that of muscle and liver, this led him to conclude that the regulation of PDHC activity in the brain was different from that in other tissues. The present study would tend to support this conclusion.

The effect of DCA as a single 100 mg/kg bolus on reducing serum lactate levels in normal rats has been shown by Evans and Stacpoole to last as long as 6 hours.12 Therefore the time-frame of the present study in which 115 minutes were used should have been adequate to demonstrate the effect of DCA on reducing lactate levels. At 90 minutes the DCA animals did indeed have significantly lower serum lactate levels than the controls, but this effect was lost once ischemia was induced.

The percentage of PDHC in the activated form in the brain has been a source of some dispute. Evans found that the percent of PDHC in the active form in the brain was 60–80%, and only 30% in the liver.24 This figure is in close agreement with earlier work on brain homogenates,25 mitochondria,26 and synaptosomes.26 Therefore, one might expect that DCA would have a much smaller effect on reducing tissue lactate levels in the brain than in the liver, since so little of PDHC in the

TABLE 2  Selected Brain Metabolites* (mM/kg)

<table>
<thead>
<tr>
<th></th>
<th>Lactate</th>
<th>ATP</th>
<th>PCr</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCA</td>
<td>32.0 ±1.8</td>
<td>0.47 ±0.21</td>
<td>0.31 ±0.22</td>
</tr>
<tr>
<td>Saline</td>
<td>29.5 ±2.2</td>
<td>0.87 ±0.30</td>
<td>0.49 ±0.24</td>
</tr>
<tr>
<td>Normoxic control</td>
<td>2.4 ±0.4</td>
<td>2.75 ±0.03</td>
<td>3.60 ±0.24</td>
</tr>
</tbody>
</table>

*All values are mean ± SEM.
brain is present in the unactivated form to be converted by DCA. However, Baudry et al place the activated portion of PDHC in brain at 50%, and Ksiezk-Reding et al at 30% in mouse brain frozen in situ.

**Conclusion**

DCA, which is known to reduce lactate levels in the peripheral tissues, was unable to so in the brain when given as a single 100 mg/kg intravenous bolus to hyperglycemic rats undergoing 15 minutes of incomplete ischemia produced by bilateral carotid occlusion and systemic hypotension. This in-vivo model would tend to support the conclusion by Evans that the control of PDHC activity in the brain may be different from that of PDHC in other tissues and that DCA would not be an effective drug for the treatment of cerebral ischemia.

**Acknowledgments**

The authors wish to thank Dr. Andrew Clark for providing assistance in the design of the experimental model, Judy Beckman and Margaret Oppenheimer for expert technical assistance and Mrs. L. Staiger for typing the manuscript.

**References**


The effect of dichloroacetate on brain lactate levels following incomplete ischemia in the hyperglycemic rat.
A R Colohan, F A Welsh, E D Miller and N F Kassell

*Stroke.* 1986;17:525-528
doi: 10.1161/01.STR.17.3.525

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 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/17/3/525

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/