Dichloroacetate Increases Skeletal Muscle Pyruvate Dehydrogenase Activity During Acute Limb Ischemia

Jeffrey S. Wilson, MD, Greg Rushing, MD, Brad L. Johnson, MD, Jeffrey A. Kline, MD, Martin R. Back, MD, and Dennis F. Bandyk, MD, Tampa, FL

The purpose of this study was to evaluate the effects of dichloroacetate sodium (DCA), a drug that inactivates pyruvate dehydrogenase kinase (PDH-K), on pyruvate dehydrogenase (PDH) activity, lactate level, and function of skeletal muscle in an experimental model of acute limb ischemia. Thirty-two male Sprague-Dawley rats underwent right iliac artery ligation to produce hindlimb ischemia. After 2 hours of ischemia, 16 animals received intravenous DCA (15 mg/100 g body weight) and 16 control animals received an equivalent volume of normal saline. After an additional 1 hour of ischemia (total 3 hours) tibialis anterior muscle from the ischemic limb and contralateral nonischemic limb was excised, rapidly freeze-clamped with Wallenberg tongs cooled in liquid nitrogen, and stored at -70°C. Muscles specimens were subsequently assayed for PDH activity and lactate level by use of spectrophotometric techniques. An additional 16 animals (DCA-treated, n=8; control, n=8) underwent ex-vivo gastrocnemius muscle fatigue testing with a 10 g tension preload after 3 hours of limb ischemia. In ischemic hind limbs, DCA treatment significantly (p=0.025) increased PDH activity (19.6 ±1.6 μmol/min/g dry weight) compared to controls (13.1 ±1.3 μmol/min/g dry weight). DCA treatment did not increase (p=0.13) skeletal muscle PDH activity in the nonischemic limbs (9.6 ±1.1 μmol/min/g dry weight, controls; 13.2 ±1.3 μmol/min/g dry weight, DCA group). In DCA-treated animals, hind limb ischemia resulted in no significant increase in muscle lactate levels compared to the nonischemic limb, while control animals demonstrated a significant (p=0.005) elevation in lactate level in ischemic limbs compared to contralateral nonischemic limb. Ischemia induced a significant decrease in time to muscle fatigue in both DCA-treated and control animals (p=0.002 and 0.001, respectively). Time to muscle fatigue in DCA-treated animals was increased compared to controls (2.6 ±0.3 versus 2 ±0.6 minutes; p<0.05) in ischemic limbs but was not significantly different in nonischemic limbs (DCA = 3.3 ±0.5 minutes; control = 3.1 ±0.6 minutes). Treatment with DCA during acute limb ischemia reduced the depression of PDH activity and lactate level of skeletal muscle. Ischemic muscle function was also improved by DCA treatment. Further investigation of the potential beneficial effects of DCA treatment on muscle injury during ischemia and reperfusion is warranted.
Introduction

Acute lower extremity ischemia from thrombosis, embolism, or injury results in significant morbidity and mortality from limb loss and remote organ (cardiac, renal, pulmonary) dysfunction. The reported incidence of acute limb ischemia is 2 per 10,000 population per year, and despite treatment a 20% limb loss in surviving patients has been reported. Amputation is the result of irreversible tissue injury caused by a multifactorial process occurring both during ischemia and following reperfusion. Tissue injury is induced by ATP depletion that occurs as result of insufficient blood flow, and is hastened by abnormalities of pH, temperature, and enzyme function. Irreversible injury from failure of ATP-dependent ionic pumps produces a loss of osmotic gradients, cell swelling, and lysis. During ischemia, low tissue oxygen tension results in the activation of pyruvate dehydrogenase kinase (PDH-K), a cell enzyme that inactivates pyruvate dehydrogenase (PDH) and thus alters the metabolism of pyruvate produced during glycolysis. The resulting decrease in PDH activity interferes with acetyl Co-A production from pyruvate and shifts pyruvate metabolism toward the production of lactate with subsequent tissue acidosis. This produces a depression of oxidative phosphorylation, and a concomitant depletion of high-energy phosphate molecules causes the formation of reactive oxygen species contributing to the process of cell injury.

The drug dichloroacetate sodium (DCA) may be able to prevent the ischemia-induced reduction in PDH activity by the inhibition of PDH-K. In an animal model of acute myocardial ischemia, DCA administration during the ischemic event was shown to improve muscle contractility and reduce cardiac muscle lactate levels. The effect of DCA on ischemic skeletal muscle function has not been studied. We hypothesize that peripheral intravenous DCA administration will increase PDH activity, decrease muscle lactate levels, and improve skeletal muscle function during acute ischemia. Therefore treatment with DCA at the onset of limb ischemia may delay the occurrence of irreversible tissue injury and thus provide additional time for restoration of limb perfusion.

Materials and Methods

Experimental Animal Model

Thirty-two male Sprague-Dawley rats were anesthetized with ketamine and xylazine. After a surgical plane of anesthesia was confirmed, a venous catheter was placed in the right internal jugular vein via surgical cut-down. The right iliac arteries were exposed and ligated with silk suture to produce acute hind limb ischemia for a 3-hour duration. This technique was chosen over tourniquet-induced ischemia because it better simulates acute ischemia seen in man, and allows for drug delivery via collateral blood flow to the ischemic limb. The duration of acute skeletal muscle ischemia was selected based on prior studies in a rat model. After 2 hours of hind-limb ischemia, DCA (15 mg/100 g body weight) was adminis-

![Figure 1](image_url)

**Figure 1.** PDH activity (μmol/min/g dry weight) in control versus DCA-treated animals was similar to that in nonischemic limbs, but in ischemic limbs, PDH activity was significantly increased (p = 0.025) with DCA administration.
tered via the jugular vein catheter to 16 animals, while an additional 16 animals received an equivalent volume of normal saline. After an additional 1 hour of hind limb ischemia (total of 3 hours), the tibialis anterior muscles were surgically exposed in both the ischemic and contralateral nonischemic hind limb. Skeletal muscle tissue was excised and freeze-clamped with Wallenberg tongs dipped in liquid nitrogen and stored at -70°C. By use of spectrophotometric techniques previously described, muscle samples were assayed for PDH activity and lactate level. Results obtained from ischemic and nonischemic limbs were compared between DCA treated and control animals.

Sixteen additional animals had acute limb ischemia induced through use of an identical procedure for a total of 3 hours, at which time the hind limbs were attached to a myograph transducer with a 10 g preload. The gastrocnemius muscle was then stimulated with 5 pulses per second at 40 volts with a 0.2 msec duration until fatigued (loss of sustained contraction). The time to muscle fatigue for DCA-treated (n = 8) and control animals (n = 8) was compared in ischemic and nonischemic hind limbs.

Data Analysis

Data were expressed as mean ± standard deviation. Skeletal muscle PDH activity (μmol/min/gram dry weight), lactate level (μmol/gram), and fatigue times (min) were compared between control and DCA-treated animals, and ischemic and nonischemic limbs by use of the unpaired Student’s t test.

Results

PDH Activity

In nonischemic hind limbs, DCA administration resulted in a similar measured PDH activity of 13.2 ± 1.3 compared to 9.6 ± 1.1 μmol/min/g dry weight in controls, p = 0.13 (Figure 1). However, in ischemic hind limbs, skeletal muscle PDH activity was significantly higher (p = 0.025) in DCA-treated animals (19.6 ± 1.6 μmol/min/g dry weight) than in controls (13.1 ± 1.3).

Lactate Level

With DCA treatment (Figure 2), there was no significant difference (p = 0.41) between muscle lactate levels measured in ischemic limbs (83.3 ± 37.3 μmol/g) compared to the contralateral nonischemic limb (75.1 ± 41.5 μmol/g), indicating a similar level of tissue acidosis. In the absence of DCA, control animals’ muscle lactate level was significantly higher (p = 0.005) in ischemic (121.9 ± 55.7 μmol/g) compared to nonischemic (53.3 ± 23.8 μmol/g) limbs. Lactate levels of ischemic limbs treated with DCA (83.3 ± 37.3 μmol/g) were lower than those measured in the control group (121.9 ± 55.7 μmol/g), but the difference was not statistically significant (p = 0.09).

Muscle Contraction

Ischemia significantly decreased the time to muscle fatigue in both DCA-treated animals (p = 0.002) and saline-treated control animals.

Figure 2. Lactate levels (μmol/g dry weight of muscle) were similar in the ischemic and nonischemic limbs of DCA-treated animals. In control animals, lactate levels were significantly increased (p = 0.005) in ischemic compared to those in nonischemic limbs.
(p = 0.001). DCA treatment improved muscle function in ischemic hindlimbs. Following 3 hours of muscle ischemia, the gastrocnemius muscle fatigue time of DCA-treated animals (2.6 ± 0.3 min) was significantly increased (p < 0.05) compared to controls (2.0 ± 0.6 min). Gastrocnemius muscle fatigue time in nonischemic limbs was not significantly (p = 0.51) different between DCA-treated (3.3 ± 0.5 min) and control (3.1 ± 0.6) animals.

**Discussion**

In this experimental model of acute limb ischemia, the ligation of the iliac artery for 3 hours produced significant skeletal muscle ischemia as evident by measured decreases in PDH activity in tibialis anterior muscle and an increase in muscle lactate levels. Treatment with DCA, a drug that inactivates PDH-K, during the ischemic time interval appeared to provide metabolic protection to the skeletal muscle. The muscle PDH activity was increased, indicating DCA was able to reach the ischemic muscle tissue via collateral flow and inactivate PDH-K. Also, lactate levels were reduced corresponding to a less severe cellular acidosis. No differences in lactate levels were measured in DCA-treated animals in the ischemic and contralateral nonischemic limbs. Beneficial effect of DCA administration during limb ischemia was also confirmed by physiologic studies of muscle function that demonstrated an increase in time to muscle fatigue.

The drug DCA is essentially a pyruvate molecule with 2 chloride ions attached to the methyl group. It is known to stimulate PDH activity in myocardial tissue under conditions of hypoxia by direct inhibition of PDH-K and despite structural similarity to pyruvate, does not appear to serve as a substrate for PDH leading to acetyl Co-A production. The enhancement of PDH activity has correlated with improvement in cardiac muscle functional recovery after myocardial ischemia in animal models. Although DCA administration has not shown clear benefit in subsequent clinical trials in the treatment of acute myocardial ischemia syndromes, it was found to be safe for administration to humans, with little or no side effects, in doses found to be effective in maintaining PDH activity in animal and organ culture models of ischemia. The application of DCA in the treatment of skeletal muscle ischemia has not been evaluated. Despite the failure of DCA to provide clinical physiologic benefit in reperfused myocardial tissue after ischemia in man, we theorized that in skeletal muscle, which has less flow-dependent oxygen extraction and better tolerates intermediate periods (hours) of ischemia, DCA may demonstrate better efficacy. Enhancement of PDH activity with corresponding sustained production of acetyl-CoA, the substrate for the tricarboxylic acid cycle, and resulting decrease in tissue lactate levels, can reduce irreversible muscle injury.

During short periods (minutes) of ischemia, energy stores, most notably in the form of creatine phosphate, which is hydrolyzed by CPK to convert ADP to ATP, are adequate to meet the metabolic demands of the cell, although accumulation of lactate will still occur. Minimizing the time between the onset of ischemia and the restoration of tissue perfusion remains the best way to improve outcome and thus minimize irreversible tissue damage in acute ischemia. It seems reasonable that there might be a protective benefit of maintaining PDH activity that normally decreases during ischemia. During hypoxia, energy stores are depleted and ATP-dependent cellular processes are suppressed, eventually resulting in physiologic failure and cell death. During early hypoxia, these metabolic processes are further inhibited by local tissue acidosis from a shift in pyruvate metabolism from acetyl-CoA production toward lactate as a result of PDH inhibition. This acidosis hastens the failure of ATP-dependent ionic pumps and other cellular machinery, all of which function best at a normal tissue pH.

In our study, we demonstrated that DCA administration during ischemia would maintain PDH activity at levels similar to that in nonischemic limbs, and also result in similar levels of muscle lactate. While limb ischemia did not change muscle lactate levels in DCA-treated animals, control animals demonstrated significantly higher lactate levels in ischemic versus nonischemic limbs, consistent with the development of tissue acidosis. The difference in lactate level between ischemic DCA-treated and control ischemic limbs was not statistically significant (p = 0.09), which may reflect an inadequate ischemia time or a Type II statistical error. The 3-hour ischemia time used in this study has correlated with histologic evidence of cellular injury in other experimental models using rats. It may be appropriate in future studies to examine longer ischemia times and their effect on muscle lactate level with and without DCA treatment. Additionally, the effects of DCA after ischemia and reperfusion are
not known and were not examined in this study. Future studies should include evaluation of the effects of DCA on skeletal muscle after reperfusion from ischemic insult.

The increase in muscle PDH activity associated with DCA treatment was associated with an improvement in function of the ischemic hindlimb. Time to muscle fatigue was increased in the animals that received DCA after 2 hours of ischemia. The mechanism(s) for improved muscle function was not directly studied in our protocol. Improved muscle contraction may have been due to a more favorable tissue pH or higher levels of high-energy phosphate molecules such as creatine phosphate or ATP—parameters not measured in this study. It is possible that DCA treatment may allow the limited production of high-energy phosphate molecules, such as ATP, in ischemic skeletal muscle. It is known that mitochondria, when provided with proper substrate, can continue to produce ATP even at very low oxygen tensions. However, when PDH activity is inhibited during hypoxia, there is a failure of substrate production in the form of acetyl-CoA, which in turn reduces ATP production in the mitochondria. We plan to evaluate this potential beneficial effect of DCA in future studies. The effect of DCA treatment on altering irreversible tissue injury also needs to be studied. We plan to employ a rabbit model of hindlimb ischemia and reperfusion to investigate differences in ischemia-induced muscle necrosis with and without DCA treatment after the onset of critical limb ischemia. These studies should help quantify potential protective affects of DCA in the treatment of acute limb ischemia.

Conclusions

Treatment with DCA reversed the ischemia-induced depression of PDH activity with a corresponding reduction in tissue lactate levels in a rat model of acute hind limb ischemia. This effect was accompanied by a statistically significant increase in time to muscle fatigue in animals treated with DCA as compared to controls. Further study of the effects of DCA on ischemia and reperfusion-induced tissue injury is warranted. In the future there may be a role for DCA treatment to increase the time interval before irreversible tissue injury occurs, which may lead to improved outcomes after limb revascularization.

REFERENCES