Peripheral Neuropathy in Rats Exposed to Dichloroacetate

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Abstract
The use of dichloroacetate (DCA) for treating patients with mitochondrial diseases is limited by the induction of peripheral neuropathy. The mechanisms of DCA-induced neuropathy are not known. Oral DCA treatment (50–500 mg/kg per day for up to 16 weeks) induced tactile allodynia in both juvenile and adult rats; concurrent thermal hypoalgesia developed at higher doses. Both juvenile and adult rats treated with DCA developed nerve conduction slowing that was more pronounced in adult rats. No overt axonal or glial cell abnormalities were identified in peripheral nerves or spinal cord of any DCA-treated rat, but morphometric analysis identified a reduction of mean axonal caliber of peripheral nerve myelinated fibers. Dichloroacetate treatment also caused accumulation of oxidative stress markers in the nerves. These data indicate that behavioral, functional, and structural indices of peripheral neuropathy may be induced in both juvenile and adult rats treated with DCA at doses similar to those in clinical use. Dichloroacetate-induced peripheral neuropathy primarily afflicts axons and involves both metabolic and structural disorders. The DCA-treated rat may provide insight into the pathogenesis of this peripheral neuropathy and facilitate development of adjuvant therapeutics to prevent this disorder that currently restricts the clinical use of DCA.

Key Words: Axons, Dichloroacetate, Mitochondria, Neurotoxicity, Peripheral neuropathy.

INTRODUCTION
The halogenated acetic acid dichloroacetate (DCA) is an investigational drug used to treat metabolic disorders that involve mitochondrial dysfunction (1). The mechanism of action of DCA includes both inhibition of pyruvate dehydrogenase kinase, an enzyme that deactivates the pyruvate dehydrogenase complex, and stabilization of the pyruvate dehydrogenase complex. The resulting relative increase in pyruvate dehydrogenase complex activity promotes enhanced decarboxylation of pyruvate to acetyl CoA, thereby reducing the amount of pyruvate substrate available for metabolism to lactate and increasing the amount of acetyl CoA for mitochondrial oxidative phosphorylation. Dichloroacetate treatment lowers plasma lactate levels in patients with diabetes (2), acquired lactic acidosis (3), and inherited mitochondrial disorders (4). Recently, DCA has also been proposed to inhibit tumor growth by shifting cell metabolism toward oxidative phosphorylation with subsequent increased formation of reactive oxygen species (5) and is being considered for off-label use by cancer patients (6).

The major dose-limiting side effect of DCA in clinical use is peripheral neuropathy that presents as tingling or painful sensations and numbness in the extremities; it is accompanied by nerve conduction slowing (7). Peripheral neuropathy has recently been shown to be particularly severe in adults receiving DCA as a treatment of the inherited syndrome of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) (8); it has been suggested that this may reflect differences in DCA metabolism between children and adults (9). The mechanism by which DCA induces peripheral neuropathy is not known; it is also not clear which cell types within the nervous system are targeted. Several studies have investigated DCA-induced toxicity in animals (10, 11), but observations have highlighted lesions in the CNS rather than in peripheral nerves (12–14).

In the present study, we characterized a rat model of DCA-induced peripheral neuropathy to investigate the impact of DCA on different cellular components of the peripheral nervous system (PNS) and to model the potential age-dependent peripheral neurotoxicity that has been inferred from recent clinical experience.

MATERIALS AND METHODS

Animals and Treatments
All studies were performed in female Sprague-Dawley rats (Harlan, San Diego, CA). Dichloroacetate was delivered by daily oral gavage using tap water as the vehicle to mimic the oral bolus dosing used in clinical studies; animals were treated for 2 to 4 months to encompass the time over which onset of neuropathy has been observed in DCA-treated MELAS patients (8). Rats were maintained 3 per cage under a 12-hour light-dark cycle with free access to food (Purina 5001) and municipal water in a vivarium approved by the Association for the Assessment and Accreditation of Laboratory Animal Care.
### TABLE 1. Behavioral, Physiological, and Structural Indices of Peripheral Neuropathy in Immature Sprague-Dawley Rats Treated With Dichloroacetate for 16 Weeks

<table>
<thead>
<tr>
<th>Location</th>
<th>Paw</th>
<th>Sciatic</th>
<th>Teral</th>
<th>Sural</th>
<th>Tibial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
<td></td>
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<tr>
<td><strong>Weight, g</strong></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>146 ± 2</td>
<td>268 ± 5</td>
<td>758 ± 0.45</td>
<td>6.8 ± 1.5</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>DCAs (50)</td>
<td>180 ± 2</td>
<td>260 ± 5</td>
<td>723 ± 0.62</td>
<td>8.6 ± 1.5</td>
<td>6.5 ± 1.6</td>
</tr>
<tr>
<td>Unpaired</td>
<td>129 ± 2</td>
<td>267 ± 5</td>
<td>644 ± 0.39</td>
<td>16 ± 4</td>
<td>18 ± 4</td>
</tr>
<tr>
<td><strong>50% Response Latency, g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DCAs (50)</td>
<td>6.47 ± 0.06</td>
<td>5.53 ± 0.15</td>
<td>5.33 ± 0.14</td>
<td>5.10 ± 0.11</td>
<td>5.01 ± 0.11</td>
</tr>
<tr>
<td>Unpaired</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Mean Axonal Diameter, m</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
</tr>
<tr>
<td>DCAs (50)</td>
<td>5.39 ± 0.08</td>
<td>5.98 ± 0.18</td>
<td>5.27 ± 0.10</td>
<td>5.28 ± 0.13</td>
<td>5.21 ± 0.11</td>
</tr>
<tr>
<td>Unpaired</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Note:** Values are mean ± standard error of the mean (n = 8/group).

Animal research protocols were approved by the Institutional Animal Care Use Committee of the University of California San Diego.

#### Paw Thermal Response Latency

Rats were placed in an observation chamber on top of the thermal testing apparatus (UARD, San Diego, CA) and allowed to acclimate to the warmed glass surface (30°C) and surroundings for 30 minutes. The mobile heat source was then maneuvered to below the center of the right hind paw and turned on, activating a timer and locally warming the glass surface at a rate of approximately 1°C per second for a maximum of 20 seconds to selectively activate C fibers (15). Limb withdrawal activated movement sensors that stopped the timer and turned off the heat source. Both hind paws were measured 3 times, with repeated measurements on the same paw made at least 5 minutes apart. The median of 3 measurements on each paw was calculated, and the mean of the values for both paws was used to represent thermal response latency (16).

#### Paw Tactile Response Threshold

Von Frey filaments (Stoelting, Wood Dale, IL) were used to determine the 50% threshold for foot withdrawal. Rats were transferred to a testing cage with a wire mesh bottom and allowed to acclimate. A series of filaments, starting with one that possessed a buckling weight of 2.0 g, were applied in sequence to the plantar surface of the hind paw for 5 seconds with a pressure that caused the filament to buckle. Lifting of the paw was recorded as a positive response, and the next lightest filament was chosen for the subsequent measurement. Absence of a response after 5 seconds prompted use of the next filament of increasing weight. This paradigm was continued until 4 measurements had been made after an initial change in the behavior or 5 consecutive negative or 3 positive responses had been obtained. The sequence of positive and negative scores was used to interpolate the 50% response threshold; group mean values less than 5 g were considered to represent allodynia (17).

#### Nerve Conduction Velocity

Rats were anesthetized with isoflurane, and both motor and sensory nerve conduction velocities (MNCV and SNCV, respectively) were measured in the left sciatic nerve, as described in detail elsewhere (18). Briefly, stimulating electrodes were placed at the sciatic notch and Achilles tendon, and recording electrodes were placed in the ipsilateral interosseous muscles. Nerve temperature was maintained at 37°C using a heating lamp. Nerves were stimulated (5–10 V, 0.05 millisecond pulse width), and the resulting M and H waves were recorded on a digital storage oscilloscope. H waves were verified by their appearance at lower stimulating voltages than M waves, and F waves were avoided. Conduction velocity was calculated as the distance between the stimulation sites divided by the time difference between the M and H wave peaks obtained from each stimulation site and expressed as meters per second. Measurements were made in triplicate for each
nerve, and the median was used to represent conduction for that particular nerve.

**Nerve Morphometry**

Rats were anesthetized with an intraperitoneal injection (2 mL/kg) of a solution containing pentobarbital (12.5 mg/mL) and diazepam (1.25 mg/mL) in 0.9% sterile saline. Rats were killed by transcardiac perfusion with saline followed by 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer. The lumbar spinal cord and right sciatic nerve were removed and fixed for 24 hours at 4°C in 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer. Tissue was postfixed in 2% aqueous osmium tetroxide for 1.5 hours before dehydration using a series of graded alcohols and propylene oxide. After infiltration with a 1:1 mixture of propylene oxide and araldite for 2 hours, nerves were placed in 100% araldite overnight before embedding in fresh araldite resin. Sections (1 μm thick) were cut and stained with p-phenylenediamine before light microscopic examination and image capture. Computer-assisted analyses of myelinated fibers in the sciatic nerves were performed using either National Institutes of Health Image software or HistoQuant, a custom-written program that also allows measurement of both axon and myelin thickness (written by Dr. Jared Goor, Bioengineering, University of California San Diego) and subsequent derivation of the axon diameter–fiber diameter ratio (g ratio). In both cases, nonoverlapping fields were sampled by a systematic serpentine progression across the whole fascicle with artifacts and nonideal axonal cross sections excluded from the analysis (18).

**Paw Skin Epidermal Innervation**

Foot skin was dissected from the region where thermal withdrawal latency measurements were made and immersion fixed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer for 24 hours before embedding in paraffin. Sections were cut at a thickness of 6 μm, collected onto glass slides, and incubated with an antibody against the pan-neuronal marker PGP9.5 (1:1000, Biogenesis Ltd, Poole, UK) to visualize innervation of the skin using a light microscope. The total numbers of intraepidermal PGP9.5-immunoreactive axonal profiles were counted, and the length of the dermal-epidermal interface for each section was determined using a grid reticle and point-counting methods. Care was taken to exclude Langerhans cells, which are also PGP9.5 immunopositive but which can be discriminated by the greater thickness of their projections compared with axons of sensory nerves (19, 20).

**Nerve Oxidative Stress**

Oxidative damage to sciatic nerve lipids was quantified by both spectrophotometric and chromatographic methods using separate nerve samples taken from the same animals. For spectrophotometric assay, segments (~2 cm) of sciatic

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**TABLE 2.** Indices of Peripheral Neuropathy in Mature Sprague-Dawley Rats Treated With 500 mg/kg per day Dichloroacetate for 8 Weeks

<table>
<thead>
<tr>
<th>Location</th>
<th>Initial Body Weight, g</th>
<th>Final Body Weight, g</th>
<th>Thermal Response Latency, seconds</th>
<th>50% Tactile Response Threshold, g</th>
<th>MNCV, meters per second</th>
<th>SNCV, meters per second</th>
<th>MDA + HAE, nmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paw</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>266 ± 3</td>
<td>292 ± 2</td>
<td>8.74 ± 0.50</td>
<td>14.6 ± 0.7</td>
<td>12.0 ± 1.6</td>
<td>52.7 ± 0.8</td>
<td>52.9 ± 1.0</td>
</tr>
<tr>
<td>DCA</td>
<td>265 ± 3</td>
<td>262 ± 5</td>
<td>10.58 ± 0.60</td>
<td>18.5 ± 1.3</td>
<td>1.9 ± 0.1</td>
<td>44.8 ± 1.5</td>
<td>Absent</td>
</tr>
<tr>
<td>Unpaired</td>
<td>nsd</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.01</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are mean ± SEM of n = 9 to 18 per group.

DCA, dichloroacetate; HAE, 4-hydroxynalkanals; IENF, intraepidermal nerve fibers; MDA, total malondialdehyde; MNCV, motor nerve conduction velocity; SNCV, sensory nerve conduction velocity.
nerve were weighed and homogenized in 400 μL of PBS buffer, pH 7.4, containing 5 mmol/L butylated hydroxytoluene and 0.4% protease inhibitor cocktail (Sigma, St Louis, MO). Samples were centrifuged (10,000 g, 15 minutes, 4°C), and supernatants were collected for subsequent assay of protein concentration (BCA Protein Assay Kit; Pierce, Rockford, IL) and total malondialdehyde (MDA) and 4-hydroxyalkanals (HAE) by spectrophotometric assay (LPO-586 assay, Oxis International Inc, Portland, OR). The total MDA + HAE concentration was expressed as nanomoles per milligram protein (21).

Separate nerve samples were prepared for measurement of the biomarkers MDA and 4-hydroxynonenal (4-HNE), which are products of the oxidation of arachidonic and linoleic acid (22). Sciatic nerve segments (~2 cm) were weighed, 800 pmol of the internal standard (benzaldehyde-2,3,4,5,6-d5; Cambridge Isotopes, Andover, MA) was added, and the samples were homogenized in 800 μL deionized water containing 400 μm EDTA and 20 μm butylated hydroxytoluene for 10 minutes by conical pestle. Samples were centrifuged (10,000 × g, 15 minutes, 4°C), and 200 μL of 0.95 mol/L O-(2,3,4,4,6-pentafluoro-benzyl)hydroxylamine hydrochloride was added and allowed to react at room temperature for 1 hour. Tissue proteins were precipitated with 1 mL of added ethanol. The derivatized aldehydes were extracted with 2 mL hexane by vortex-mixing for 2 minutes and centrifugation for 10 minutes at 3,000 revolutions per minute to allow the layers to separate. The upper hexane layer was carefully transferred to another centrifuge tube and approximately 0.5 g of sodium sulfate was added to remove residual water. The sample was extracted with an additional 2 mL of hexane as previously described, and the hexane was combined with the previous extract and dried over sodium sulfate. The hexane was transferred to a centrifuge tube and gently evaporated to dryness under nitrogen. The residue was further derivatized by the addition of 50 μL of N,O-bis(trimethylsilyl)trifluoroacetamide in 1% trimethylchlorosilane, and the tube was tightly capped and placed on a heating mantle for 15 minutes at 80°C. Tubes were removed and allowed to cool to room temperature, 50 μL of hexane was added, and the samples were vortex-mixed and transferred to gas chromatograph (GC) auto sampler vials. Sample extracts (1 μL) were injected into a GC mass spectrometer ([GC-MS] 5973N, Agilent Technologies, Palo Alto, CA) operating in the negative-ion-chemical-ionization configuration (23, 24). 4-Hydroxynonenal and MDA concentrations were quantified by comparing their response area ratio and the internal standards to calibration curves produced by injection of known amounts of each compound. 4-Hydroxynonenal and MDA tissue concentrations were expressed as nanograms per milligram protein.

Analysis

All animals, tissues, and slides were coded to preclude observer bias. Statistical analysis was performed by unpaired t-test or 1-way analysis of variance, with Dunnett post hoc test used to identify differences between a reference group and all other groups.

RESULTS

DCA-Induced Neuropathy in Juvenile Rats

We initially investigated the impact of DCA treatment on peripheral nerve function and structure in immature rats to model the use of DCA in children with mitochondrial diseases. Immature (~4- to 6-week old) female rats were treated daily for 16 weeks with 50 mg/kg per day DCA, a dose used in clinical practice. Dichloroacetate treatment did not markedly inhibit weight gain during the treatment period (Table 1); all rats completed the study period. At the end of the study, DCA-treated rats showed paw tactile allodynia and slowing of MNCV and SNCV in large myelinated fibers of the sciatic nerve, whereas response times to thermal...
stimulation were normal (Table 1). Morphological inspection of the sural and tibial nerves by light microscopy did not reveal any overt axonal or Schwann cell pathology or cellular infiltrates. Morphometric analysis of these nerves showed a normal mean axonal diameter of myelinated fibers (Table 1).

In a follow-up study, we exposed immature female rats to increased doses of DCA (100–500 mg/kg per day) to reflect the rat equivalent of clinical doses of DCA (25) and measured nerve conduction at regular intervals to establish the time course of onset of neuropathy. No behavioral indices of paralysis or motor dysfunction were noted during daily observation of the rats, but 1 of 11 rats in the 500–mg/kg per day DCA treatment group died of unknown causes during the 16th week of the study. This highest dose of DCA also significantly restricted body weight gain in the group during the course of the study (p < 0.01 vs control, Table 1). At the conclusion of the study, DCA induced a dose-dependent thermal hypoalgesia in the hind paw that was not accompanied by any loss of intraepidermal nerve fibers (IENF) in the skin of the same paw (Table 1). Both MNCV and SNCV were reduced by DCA (Table 1), with a particularly marked and dose-dependent effect on SNCV appearing only after 12 weeks of treatment (Fig. 1). Nerve conduction slowing at the conclusion of the study was accompanied by a dose-dependent

FIGURE 3. Representative spinal dorsal horns with associated spinal nerve (A, B) and ventral spinal cords (C, D) from control rats (A, C) and rats treated with 500 mg/kg per day dichloroacetate for 8 weeks (B, D). No overt pathological abnormalities are identified in the peripheral nerves or spinal cords despite the failure to record the H wave during electrophysiological testing (see text). Images are light micrographs of 1-μm sections cut from araldite blocks and stained with p-phenylenediamine. Scale bar = 100 μm.
reduction in mean axonal caliber of the sciatic nerve and its distal sural and tibial branches (Table 1). There was no obvious demyelination, Wallerian degeneration, or other gross pathology in any nerve when inspected by light microscopy and no change in myelinated fiber density of the sural nerves (Table 1).

DCA-Induced Neuropathy in Adult Rats

Recent reports of severe peripheral neuropathy in adults with MELAS who were treated with DCA prompted us to examine the PNS of adult (12- to 14-week-old) female rats treated with 500 mg/kg per day DCA, a dose that induced marked conduction slowing and reduced axonal caliber in juvenile rats after 12 to 16 weeks (Table 1). Dichloroacetate treatment attenuated body weight gain during the 8 weeks of treatment (Table 2). No obvious signs of paralysis or motor dysfunction were evident in DCA-treated rats, although 1 of 18 DCA-treated rats died at Week 7 from unknown causes. Behavioral studies of sensory-motor function indicated both tactile allodynia and thermal hypoalgesia in DCA-treated rats (Table 2). Thermal hypoalgesia was not accompanied by any IENF loss in paw skin, and indeed there was a significant increase in IENF profile number per unit length of DCA-treated animals (Table 2). Electrophysiological testing indicated slowing of large myelinated fiber MNCV and SNCV that was established within 4 weeks of DCA treatment (Fig. 2). The MNCV deficit persisted at 8 weeks of DCA treatment, whereas we were unable to obtain H waves in DCA-treated rats at this time point so SNCV values could not be calculated. No pathological abnormalities were identified in the peripheral nerves or the white and gray matter of DCA-treated rats by light microscopy (Fig. 3).

To provide a more detailed analysis of the impact of DCA treatment on nerve fibers, we performed morphometric analysis of axonal and myelin dimensions in myelinated fibers of the tibial and sural nerves. Both nerves showed a DCA-induced reduction in mean axonal diameter that was not associated with myelinated fiber loss but reflected a leftward shift in the fiber frequency distribution (Table 3; Fig. 4). In the tibial nerve, there was no change in myelin thickness, so that the myelin-axon size ratio was significantly increased and the g ratio was significantly decreased. A similar pattern was noted in the sural nerve, although values did not attain statistical significance.

Analysis of portions of sciatic nerve for markers of lipid peroxidation indicated that there was significantly increased oxidative stress to lipids of peripheral nerve from DCA-treated rats, as indicated by total MDA + HAE levels (Table 2).

### TABLE 3. Quantification of Myelinated Fiber Axon and Myelin Dimensions in Peripheral Nerves of Adult Sprague-Dawley Rats Treated With 500 mg/kg per day DCA for 8 Weeks

<table>
<thead>
<tr>
<th>Nerve</th>
<th>Fibers/Nerve, n</th>
<th>Fiber Density, no. per μm²</th>
<th>Mean Axonal Diameter, μm</th>
<th>Large (&gt;8 μm) Fibers, %</th>
<th>Myelin Thickness, μm</th>
<th>Myelin-Axon Ratio</th>
<th>g ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1925 ± 99</td>
<td>11.2 ± 0.1</td>
<td>7.2 ± 0.2</td>
<td>57.0 ± 3.2</td>
<td>3.8 ± 0.1</td>
<td>1.34 ± 0.03</td>
<td>0.66 ± 0.01</td>
</tr>
<tr>
<td>DCA</td>
<td>1810 ± 60</td>
<td>10.3 ± 1.2</td>
<td>6.6 ± 0.3</td>
<td>41.8 ± 6.0</td>
<td>4.0 ± 0.1</td>
<td>p &lt; 0.05</td>
<td>nsd</td>
</tr>
<tr>
<td>Unpaired t-test</td>
<td>nsd</td>
<td>nsd</td>
<td>p &lt; 0.05</td>
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<td>nsd</td>
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<td>nsd</td>
</tr>
<tr>
<td>Sural</td>
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</tr>
<tr>
<td>Control</td>
<td>884 ± 46</td>
<td>16.2 ± 0.1</td>
<td>7.2 ± 0.2</td>
<td>52.9 ± 3.8</td>
<td>3.3 ± 0.2</td>
<td>1.15 ± 0.05</td>
<td>0.69 ± 0.01</td>
</tr>
<tr>
<td>DCA</td>
<td>875 ± 69</td>
<td>16.3 ± 0.1</td>
<td>6.2 ± 0.2</td>
<td>34.6 ± 2.1</td>
<td>3.2 ± 0.1</td>
<td>1.28 ± 0.03</td>
<td>0.66 ± 0.01</td>
</tr>
<tr>
<td>Unpaired t-test</td>
<td>nsd</td>
<td>nsd</td>
<td>p &lt; 0.01</td>
<td>nsd</td>
<td>nsd</td>
<td>nsd</td>
<td>nsd</td>
</tr>
</tbody>
</table>

Data are mean ± SEM of no. 5 to 8/group.
DCA, dichloroacetate; nsd, no significant difference.

FIGURE 4. Axonal size-frequency distribution in the tibial (A) and sural (B) nerves of adult control rats (black circles) and rats treated with 500 mg/kg per day dichloroacetate (gray bars). Each data point represents group mean ± SEM. See Table 3 for statistical analysis of axonal and myelin parameters.
or by individual measurement of MDA or 4-HNE content by GC/MS (Fig. 5).

DISCUSSION

Several investigations of DCA toxicity in animals have included comment on damage to the nervous system. Although species, sex, dose, route, and duration of treatment have varied, a common finding has been that high doses of DCA, particularly when given in drinking water or diet, induced limb weakness or paralysis that might indicate CNS, PNS, or muscle damage (10–12). Prolonged exposure to high doses (up to 2,000 mg/kg per day) of DCA also induced edema and demyelination in the brain and spinal cord (12). In contrast, superficial examination of the PNS did not reveal demyelination or other pathological features (12, 26). One study found DCA-induced conduction slowing in both motor and sensory nerves, but again did not identify PNS pathology other than a reduction in fascicular diameter that was attributed to impaired nerve maturation accompanying reduced food intake and weight gain (11). The phenotype described in prior studies of DCA-intoxicated rodents differs from the clinical description of DCA-induced peripheral neuropathy, which is not associated with evidence of CNS lesions. This discord between the animal models and human condition has limited the investigation of pathogenic mechanisms associated with DCA-induced peripheral neuropathy.

The lack of an animal model that approximates DCA-induced peripheral neuropathy prompted us to perform a detailed characterization of PNS function and structure in rats exposed to a range of DCA doses, extending from replication of the human dose upward to established rat-equivalent doses (25). Dichloroacetate was given by oral gavage to mimic oral bolus drug delivery approaches in patients. Dichloroacetate, at a dose of up to 500 mg/kg per day for 4 months, did not induce paralysis in rats that were either immature or mature at the start of treatment and, along with the survival of most DCA-treated rats, suggests a less toxic regimen than those previously described. Unlike a previous report in which high doses of DCA induced touch-evoked allodynia (12), our DCA-treated rats were not sensitive to handling; however, rats treated with as low as 50 mg/kg per day DCA for 4 months developed tactile allodynia of the paw in response to von Frey filaments. Similar tactile allodynia occurs in other animal models of neuropathic pain, including physical nerve injury and diabetes (16, 17), and may provide a viable behavioral marker for aspects of pain associated with DCA treatment in humans. Interestingly, tactile allodynia in adult rats treated with 500 mg/kg per day of DCA was associated with concurrent loss of a separate sensory modality, as illustrated by paw thermal hypoalgesia. The coincidence of both positive and negative sensory disorders reassures that the thermal hypoalgesia is not a consequence of general limb weakness, and no behavioral manifestations of weakness or paralysis were noted. Thermal hypoalgesia was not accompanied by loss of the predominantly heat-sensitive intraepidermal fibers of the paw, implying DCA-induced disruption of primary afferent function, spinal and higher CNS processing, or effector function, rather than loss of the thermal transducer. A similar peripheral neuropathy, where thermal hypoalgesia precedes loss of intraepidermal fibers, occurs in diabetic mice (19). Daily oral doses of 50 to 500 mg/kg per day DCA therefore seem to model aspects of both pain and sensory loss described in the DCA-treated humans.

Although impaired behavioral responses to sensory stimuli may reflect impaired PNS function or CNS processing, the decreased MNCV seen at daily oral DCA doses as low as 50 mg/kg per day is a clear manifestation of peripheral neuropathy that parallels the effect of DCA in humans (7). The MNCV slowing induced by low doses of DCA in immature rats was not accompanied by significant structural pathology in the peripheral nerve, implying a metabolic component to the disorder. At higher doses, however, MNCV slowing coexisted with a reduction in mean axonal diameter of the sciatic nerve and its distal branches. Axonal caliber is a major determinant of conduction velocity in myelinated
fibers (27), and reductions of axonal diameter seen in DCA-treated rats are comparable to those associated with conduction slowing in other models of peripheral neuropathy (28). Reduced axonal caliber has not been previously described in DCA intoxication and is direct evidence of peripheral neuropathy, despite the absence of overt pathological changes when nerves are viewed by light microscopy (12, 26). As no axonal loss occurred in any nerve branch studied, reduced mean axonal diameter reflects a decrease in the proportion of larger axons indicative of impaired radial growth or atrophy, rather than any selective degeneration of large axons. Whether reduced axonal caliber is somatofugal or distal in origin remains to be determined, and examination of the impact of DCA on the production of cytoskeletal proteins in neuronal cell bodies of the dorsal root ganglia and spinal ventral horn and their subsequent axonal transport may provide valuable insights into the pathogenic mechanism of DCA neuropathy.

Damage to myelin has been recorded in the CNS of rats exposed to high doses of DCA (12, 26), but neither overt demyelination nor any quantitative change in myelin thickness was noted in the present study. These findings seem to contradict recent studies showing that Schwann cell cultures exposed to DCA have an impaired capacity to remyelinate axons (29, 30). Schwann cells in vivo seem to tolerate DCA to the extent that they maintain myelination, although we have not yet determined whether the ability to promote remyelination is compromised. Our current structural evidence suggests that DCA induces a primarily axonal disorder. Nevertheless, it is possible that DCA impedes axonal support via disrupting metabolic functions of other cells, including Schwann cells, as occurs in animal models of diabetic neuropathy (31–33).

In the present study, mature rats treated with DCA showed a decline in conduction from onset values, suggesting failure of established determinants of conduction velocity rather than impairment of maturation. Another pertinent electrophysiological feature in DCA-treated adult rats was the failure to obtain H waves after 8 weeks of treatment despite clear detection of M waves. As only the H wave includes a spinal reflex, disruption of the spinal cord could contribute to this electrophysiological failure. We did not find any overt vacuolation or other damage to either white or gray matter of the spinal cord, which contrasts with other studies using higher doses of DCA (12), but cannot yet exclude the possibility that subtle structural damage or neurochemical changes contribute to loss of the H reflex. Loss of H waves was seen only in rats that were adult at onset of treatment, indicating differential effects of DCA on the nervous system in young and older animals. This is consistent with our recent finding that DCA metabolism varies with age in rats, and that neurotoxic metabolites of DCA were present only in plasma of older animals (34). The rat model therefore seems to replicate recent clinical reports of more severe DCA neuropathy in adults than children (8, 9) and offers an opportunity to explore the causes of exaggerated neuropathy in DCA-treated adults.

The mechanisms by which DCA induces peripheral neuropathy are not known. Dichloroacetate-induced activation of thiamine-dependent enzymes leading to thiamine deficiency and subsequent peripheral neuropathy has been proposed (26), but thiamine did not prevent conduction slowing in patients treated with DCA (7–9). Dichloroacetate is metabolized in the liver by glutathione transferase Z1 to form glyoxylate, which then enters the general carbon pool (1), and a number of consequences of DCA metabolism that may lead to neurotoxicity have been suggested. For example, the heme precursor δ-aminolevulinate (δ-ALA) appears in the urine of DCA-treated patients (35), and we have recently demonstrated that δ-ALA has disruptive effects when applied to peripheral neurons and Schwann cells in culture (29). One interesting feature of the impact of δ-ALA on cultured nerve cells is the increase in markers of oxidative stress, an event that has been implicated in the pathogenesis of a number of neurological diseases (36). We measured products of lipid oxidation by both spectrophotometric and GC/MS methods and found that whereas the former slightly underestimated nerve MDA content, the relative increase seen in the nerve of DCA-treated rats was similar (49% and 40%, respectively). Levels of 4-HNE were an order of magnitude lower than MDA when measured independently, but the DCA-induced increase was proportionately much greater (4-fold) than that seen for MDA. Whether this increase in oxidative damage is mediated via formation of δ-ALA (29) or a direct consequence of DCA actions such as activation of pyruvate dehydrogenase (1) and subsequent mitochondrial overdrive (5) remains to be determined. Nevertheless, the increased lipid peroxidation in peripheral nerve may implicate production of excess reactive oxidative species and subsequent oxidative damage to lipids in the pathogenesis of DCA-induced peripheral neuropathy and suggests plausible adjuvant therapy approaches that could extend clinical use of DCA by restricting the occurrence and severity of the associated neuropathy.

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