Physiological Assessment of Muscle Strength In Vitro After Direct Injection of Doxorubicin Into Rabbit Sternocleidomastoid Muscle

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Abstract: Doxorubicin chemomyectomy is a potent method for the permanent removal of a muscle or group of muscles after direct local injection, and has been used successfully to treat blepharospasm and hemifacial spasm patients. The efficacy of doxorubicin chemomyectomy on reducing muscle strength after direct injection of doxorubicin into rabbit sternocleidomastoid muscle was tested. One- and 6-month postinjection force assessment was performed in vitro to measure alterations in peak twitch and tetanic force generation, as well as fatigue responses for the treated muscles compared to control. There were significant reductions of both twitch and tetanic peak amplitudes in the doxorubicin-treated muscles. One month after treatment, the decreases in force were greater after 2 mg doxorubicin injections than after 1 mg doxorubicin. While there was a significant reduction in force generation after doxorubicin treatment, fatigue resistances for the doxorubicin-treated muscles were increased compared to the controls. There were significant reductions in muscle mass after doxorubicin treatment, and by 6 months, the myosin heavy chain isoform distribution was similar to normal sternocleidomastoid, except for an increase in slow myosin-positive fibers. Doxorubicin chemomyectomy resulted in a significant reduction in functional force generation in the treated sternocleidomastoid muscles. These findings suggest a potential clinical use of doxorubicin chemomyectomy to treat cervical dystonia patients. © 2001 Movement Disorder Society.

Key words: doxorubicin; myotoxin; skeletal muscle; cervical muscles; force assessment

Movements of the neck are elicited by a complex set of contractile behaviors in over 20 distinct neck muscles. Studies of normal neck muscle physiology suggest that neck movements are the result of complex motor patterns, with subsets of muscles subserving specific neck movements.17,18 One important muscle involved in neck movements is the sternocleidomastoid, a large muscle located on the ventral side of the neck whose main action is to turn the chin to the side opposite the contracting muscle. Neck muscles, such as the sternocleidomastoid, have a complex anatomy20 and are often involved in the pathological condition of cervical dystonia, or spasmodic torticollis. This condition is characterized by uncontrolled contractions of the neck musculature.6,8 Spasms in these muscles result in abnormal head and neck postures, including lateral rotation (torticollis), flexion (anterocollis), tilting, or extension (retrocollis). The condition can be extremely debilitating and painful.

A recently developed approach for the treatment of focal dystonia in the face involves the direct injection of doxorubicin into the involved muscles.31 Doxorubicin is a myotoxin, which results in permanent skeletal muscle death in both animals21 and patients.25 Doxorubicin chemomyectomy has been used in blepharospasm and hemifacial spasm patients with good results; a full course of treatment results in significant and permanent reduction in muscle spasms for the treated patients.31 Due to the nature of the muscles involved in eyblinks, total removal of the orbicularis oculi muscle does not affect normal function for the patients.31 In contrast, cervical dystonia is a more complex disorder, involving a number

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of large neck muscles. However, to achieve a positive treatment outcome for patients with cervical dystonia may require only sufficient muscle loss to decrease pain and improve posture.

In the present study, the effects of doxorubicin chemomyectomy on muscle contractile properties (e.g., strength) were determined for treated rabbit sternocleidomastoid muscles. These muscles were chosen not only for the aforementioned clinical reason, but also because they are relatively superficial in location and therefore easy to isolate in their entirety for both the in vivo doxorubicin injections and the subsequent tendon-to-tendon dissections for physiological assessment. This is the first examination of the effects of doxorubicin chemomyectomy on skeletal muscle physiology.

**MATERIALS AND METHODS**

All animal studies were approved by the University of Minnesota Animal Care and Use Committee and followed the published guidelines of the NIH for use of animals in research. New Zealand white rabbits were obtained through Birchwood Valley Farms (Red Wing, MN) and housed with Research Animal Resources at the University of Minnesota. The twitch and tetanic peak forces of treated and control sternocleidomastoid muscles were determined 1 month or 6 months after doxorubicin injections. Contractile parameters of the twitch responses were also determined. Muscle force was elicited in vitro by electrical field stimulations of isolated muscles at 30°C. In addition, the effects of fatigue resistance were determined for all treated and control muscles. Histological examination was performed to examine the relative integrity of each muscle and to determine whether there were alterations in individual myofiber size and in myosin heavy chain isoform expression after doxorubicin injury.

Rabbits were anesthetized with an intramuscular injection of 1:1 xylazine:ketamine at doses of 2 mg/kg:10 mg/kg, respectively. All surgical procedures were performed using sterile techniques. The hair on the ventral side of the neck was removed. A skin incision was made, and the sternocleidomastoid muscles were visualized within the neck. Doxorubicin HCl (Adriamycin, Pharmacia, Kalamazoo, MI) was injected directly into one sternocleidomastoid muscle per animal at a dose of either 1 mg (in 0.5 or 1 ml sterile saline) or 2 mg (in 0.5 or 1 ml sterile saline). Muscles from four different rabbits were treated with each experimental dose and injection volume. The data from all eight muscles were combined at identical milligram doses, as no significant differences were seen between identical doxorubicin doses at the two different injection volumes (e.g., twitch tensions of 49.8 ± 14.9 for 1 mg/0.5 ml saline compared to 58.1 ± 10 for 1 mg/ml saline with \( P = 0.9 \)). Seven animals were used to study the long-term treatment efficacy and had a postinjection survival of 6 months. In all long-term studies, the muscles received injections of 1 mg doxorubicin in 1 ml saline. Due to the red color of doxorubicin, the drug could easily be visualized within the muscle epimysial sheath. Care was taken to ensure that the doxorubicin was injected along the full length of the muscle. Any leakage out of the epimysial sheath was immediately irrigated to prevent potential effects on surrounding muscles. Previous studies indicated that doxorubicin does not spread easily within the connective tissue or within the injected muscles. For each animal, the contralateral muscle served as a control, and either received an injection of sterile saline in a comparable volume (sham control) or no injection. A second set of five control animals that did not receive any doxorubicin in either muscle was examined; these muscles were either injected with saline or received no injection in order to test for possible effects of unilateral weakening of one sternocleidomastoid muscle on force generation of the muscle on the opposite side. The animals were checked daily for changes in head position and changes in the skin adjacent to the injection site.

After a minimum of 1 month from the doxorubicin treatment or 6 months for the long-term protocol, the rabbits were deeply anesthetized with ketamine and xylazine. The animals were prepared for surgical isolation of the paired sternocleidomastoid muscles and were given an overdose of barbiturate anesthesia. The sternocleidomastoid muscles were removed completely from tendon end to tendon end. Immediately after removal, the sternocleidomastoid muscles were placed in a dissection dish that was continuously gassed with 95% O₂ and 5% CO₂ at room temperature. The muscles were fixed with small pins to a sylgard lined dish. Connective tissue and fat were carefully dissected away from each muscle. The muscles were mounted in separate experimental chambers by tying loops of suture to each tendon end; one end was fixed to a micromanipulator, which in turn was connected to a force transducer. The other end was attached to a stationary point. The muscle baths were continuously oxygenated in Kreb’s solution at 30°C. The muscles were activated by field stimulation using a pair of platinum electrodes that extended the length of the preparation. Optimal functional muscle lengths were determined when the peak twitch amplitude was observed to be maximal and did not increase after further stretch of the muscle specimen. The muscles were first stimulated with supramaximal pulses of 1 msec duration once every 10 seconds to determine peak twitch amplitude in grams.
The muscles were subsequently stimulated with repeated 1 msec duration pulses for a train duration of 1 second at tetanic frequencies of either 10, 20, or 40 Hz (fused tetanus). Greater tetanic frequency stimulation (e.g., 100 Hz) appeared to cause irreversible muscle damage, such that further analysis of fatigue properties could not be determined, and thus were not included in this study. Single twitch stimuli were applied for 4 minutes between each successive tetanic stimulus to allow the muscle to recover to pretetanic single twitch tensions. A fatigue test was performed based on standard methods, defined as the time it took for the muscle specimen to decrease to 75% of the maximal tetanic amplitude. Stimulations were done continuously every 10 seconds for up to 60 minutes using 1-msec duration pulses for a train duration of 250 msec at a tetanic frequency of 40 Hz. These were used to generate fatigue curves for the treated and control muscles.

At the end of each experiment, wet weights and lengths of the muscles were recorded. The amplitudes of the muscle contractions were measured in grams and were read from both digital meters and a LabVIEW program interfaced with a computer that stored the data. Data were obtained as tension in grams and converted to force, and the force transducers were calibrated immediately prior to each experiment. To be able to compare between muscles with different lengths and masses, the force was normalized to force per cross-sectional area (mN/cm²). The area of the muscle was calculated by dividing the weight of the muscle by the product of the known density of muscle (1.056 g/cm³) times the muscle length. All data were examined for statistical significance using Student’s paired t-tests. Standard error of the means was used for all graphs. Data were considered statistically significant at values $P \leq 0.05$.

Subsequent to the physiological study, the relative anatomical integrity of the muscle was confirmed by histological examination after each experiment; e.g., in untreated muscles, the myofibers were normal in appearance throughout the cross-sectional area of each muscle. Cross-sectional muscle samples were removed from the proximal, middle, and distal regions of the muscles, frozen in methylbutane chilled to a slurry on liquid nitrogen, and sectioned in a cryostat at 12 μm. Sections were stained for immunohistochemical localization of fast, slow, neonatal, and developmental myosin heavy chain isoforms (NovoCastra Labs., Newcastle, U.K.). Primary antibody concentrations were 1:40 for fast and slow myosin, and 1:20 for developmental and neonatal myosin. After incubation in primary antibody, the sections were incubated using the Vectastain Elite ABC kit (Vector Labs., Burlingame, CA) and reacted with diaminobenzidine intensified with heavy metals.

**RESULTS**

Rabbits were checked daily after doxorubicin treatment. No noticeable change in head posture was observed in any of the rabbits, nor were any changes in the skin seen after any of the injections. On removal of the sternocleidomastoid muscles from the animals for physiological analysis, increased connective tissue was apparent around the treated muscles. However, the muscles were still easy to dissect free without injury to the muscle tissue itself.

Injections of either 1 or 2 mg doxorubicin into the sternocleidomastoid muscle resulted in a significant loss of muscle mass compared to the contralateral controls at both 1 month and 6 months after treatment that was obvious by visual inspection (Fig. 1). Peak forces generated by twitch or tetanic stimuli in sternocleidomastoid muscles were significantly reduced in doxorubicin-treated muscles (Fig. 2). One month after an injection of 1 mg or 2 mg doxorubicin, twitch forces of 53.5 mN (±
9.0; n = 9) and 35.4 mN (± 10.1; n = 8), respectively, were seen compared to 159.7 mN (± 13.1; n = 17) for the contralateral saline control muscles. In the long-term treated muscles, 6 months after an injection of 1 mg doxorubicin, twitch forces were reduced to 30.4 mN (± 10.1; n = 7), compared to 207.5 mN (± 15.0; n = 7) for the contralateral saline injected control muscles. This was a slightly greater reduction in force generation 6

FIG. 2. Comparison of force responses from a pair of sternocleidomastoid muscles from the same rabbit. The records on the left were from a saline-injected control muscle and those on the right from a muscle treated with 2 mg/ml doxorubicin 1 month previously. Shown are the relative effects of treatment on force response following either twitch or tetanic stimulation at 10, 20, and 40 Hz (upper set of four records). Note the lower peak forces for the contractions elicited in the treated muscle and also the slower contraction rates. Each trace represents a single twitch or tetanic response. The bottom two records indicate the results from the fatigue test, with fatigue defined as the time for the muscle specimen to decrease to 75% of the maximal tetanic amplitude (see Materials and Methods). The record represents a composite of single tetanic responses, i.e., 360 traces during the 60-minute test. Note the low fatigability in the doxorubicin-treated muscle compared to the control muscle during prolonged tetanization. Note low levels of force generated by the treated muscles compared to the controls.
months after a 1-mg doxorubicin treatment compared to that seen after 1 month at the identical dose, although this difference was not significant. There was no statistical difference in force generated by uninjected control muscles from separate animals compared to contralateral saline-injected control muscles from the doxorubicin-treated animals, 140.2 mN (± 36.3; n = 10) compared to 159.7 mN from the contralateral controls one month after treatment.

Forces were calculated based on a ratio of injected muscle/contralateral control muscle forces at the single twitch stimulation frequency and after 10-, 20-, and 40-Hz stimulations. At a 10-Hz tetanic stimulation frequency, for example, 1 month after injection of either 1 mg or 2 mg doxorubicin, there were approximately 54% and 73% force reductions, respectively, compared to the contralateral control muscles. Six months after a 1-mg doxorubicin treatment, there was an 83% reduction in force compared to the contralateral control muscles (Fig. 3). Thus, all of the treated muscles produced significantly less force for all stimulation paradigms tested when compared to the sham-injected control muscles.

The peak force data were also analyzed by determination of relative twitch force per cross-sectional area of each muscle. After single twitch stimulation by 1 month after a 2-mg doxorubicin treatment, or by 6 months after a 1-mg doxorubicin treatment, there was a significant reduction in force/cross-sectional area (Fig. 4). While the total force produced at all stimulation frequencies was significantly reduced after doxorubicin treatment, the force per cross-sectional area for both the short- or long-term 1 mg doxorubicin-treated muscle was equivalent to that of the contralateral control muscles after 40 Hz stimulation (Fig. 4). The same results were seen after both 10- and 20-Hz stimulation intensities (data not shown). One month after a dose of 2 mg doxorubicin, there was a decrease in force/cross-sectional area compared to the contralateral control muscles at all the stimulation intensities tested.

Multiple contractile properties of individual twitch responses were analyzed for the doxorubicin-treated sternocleidomastoid muscles and compared to the contralateral control sides (Table 1). The doxorubicin-treated muscles had a decreased peak rate of development and an increased peak rate of decay when compared to the control muscles. The contraction times of the 2 mg-treated muscles were significantly longer and had significantly greater variances than those of the other doxorubicin doses or controls. The times to peak development were consistent for the control muscles and the lower doses of doxorubicin, but markedly increased after 2 mg doxorubicin treatments, with large variances between treated muscles. The same trends were observed for the times to peak twitch decay.

Control sternocleidomastoid muscles fatigued the most rapidly, reaching 75% of their maximal amplitude after approximately 300 seconds (Fig. 5). In contrast, doxorubicin treatment resulted in significant increases in the times to fatigue, increasing by 1.7- and 2.2-fold after the 1-mg doxorubicin treatments, 1 month and 6 months, respectively, and by 2.2-fold after the 2-mg doxorubicin treatments.
Doxorubicin significantly reduced the muscle mass of the sternocleidomastoid muscles (Table 2). A previous study demonstrated that 1 month after doxorubicin injection into sternocleidomastoid (SCM) there was an increase in myofibers positive for immature myosin heavy chain isoforms compared to the normal rabbit SCM. Changes in myosin heavy chain isoform expression patterns might account for the alteration in single twitch properties and fatigue rate. Myosin heavy chain isoform composition returned to the normal expression pattern in the long-term treated SCM (Fig. 6a) except for an increase in the number of myofibers positive for the slow myosin isoform. By 6 months after doxorubicin treatment, total muscle cross-sectional area was still significantly decreased compared to controls and not significantly different from the total muscle cross-sectional area 1 month after treatment (Fig. 6b). The cross-sectional areas of single myofibers 1 month after doxorubicin treatment were no different than control myofibers in the sternocleidomastoid; however, 6 months after treatment there was a significant hypertrophy of single myofibers in the treated muscles compared to control myofibers (Fig. 6c).

**DISCUSSION**

The direct injection of doxorubicin into rabbit sternocleidomastoid muscles resulted in significant decreases in muscle strength, following either twitch or tetanic stimulation. The significant reduction in strength in the treated sternocleidomastoid muscles would indicate that the use of direct injection of doxorubicin has therapeutic potential for the permanent treatment of cervical dystonia.

Previous work demonstrated that there was often variability in the amount of muscle killed along the length of the treated muscles. This presumably was due to variations in concentrations of doxorubicin after injection, and was observed after injections of doxorubicin into the eyelids of rabbits and in patients for the treatment of blepharospasm and hemifacial spasm. These studies demonstrated that doxorubicin does not diffuse well after injection. This is in marked contrast to

**TABLE 1. Contractile properties of individual twitch responses**

<table>
<thead>
<tr>
<th></th>
<th>1 mg 1 mo Injected</th>
<th>1 mo Control</th>
<th>1 mg 6 mo Injected</th>
<th>6 mo Control</th>
<th>2 mg 1 mo Injected</th>
<th>1 mo Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak rate development (mN/ms)</td>
<td>1.2 (0.2)*</td>
<td>5.4 (1.2)</td>
<td>0.6 (0.3)*</td>
<td>6.0 (0.6)</td>
<td>0.5 (0.3)*</td>
<td>4.7 (1.1)</td>
</tr>
<tr>
<td>Peak rate decay (mN/ms)</td>
<td>−0.4 (0.1)*</td>
<td>−12.0 (2.0)</td>
<td>−0.2 (0.1)*</td>
<td>−1.4 (0.1)</td>
<td>−0.2 (0.1)*</td>
<td>−1.1 (0.2)</td>
</tr>
<tr>
<td>Time to peak development (ms)</td>
<td>19.9 (2.0)*</td>
<td>12.2 (1.5)</td>
<td>18.1 (1.2)*</td>
<td>13.3 (0.7)</td>
<td>211.6 (206.2)</td>
<td>14.2 (0.7)</td>
</tr>
<tr>
<td>Time to peak decay (ms)</td>
<td>105.0 (17.5)</td>
<td>79.0 (3.4)</td>
<td>427.0 (417.2)</td>
<td>76.7 (5.3)</td>
<td>649.6 (620.1)</td>
<td>84.8 (5.0)</td>
</tr>
<tr>
<td>Contraction time (ms)</td>
<td>68.9 (7.1)*</td>
<td>49.2 (2.1)</td>
<td>69.1 (5.6)*</td>
<td>52.6 (2.9)</td>
<td>265.2 (209.1)</td>
<td>55 (1.7)</td>
</tr>
<tr>
<td>Half-relaxation time (ms)</td>
<td>127.0 (11.5)</td>
<td>104.0 (1.6)</td>
<td>110.3 (2.0)</td>
<td>107.3 (4.1)</td>
<td>139.2 (30.9)</td>
<td>103.2 (3.8)</td>
</tr>
<tr>
<td>Halfmax value time (ms)</td>
<td>171.0 (16.5)</td>
<td>136.0 (2.8)</td>
<td>155.7 (3.8)</td>
<td>142.0 (5.6)</td>
<td>189.6 (31.4)</td>
<td>139.2 (4.8)</td>
</tr>
</tbody>
</table>

Numbers represent means of all values ± SEM.
*Indicates data statistically significantly different from non-injected control sternocleidomastoid muscles.
mg, milligram; mo, month; mN, milliNewtons; ms, milliseconds.
botulinum toxin, which diffuses easily after injection, causing unwanted, albeit short-lived, complications in nearby muscles.² Doxorubicin also has a relatively short half-life within the injected tissue, and essentially no systemic spread was demonstrated after injections into the eyelid of rabbits.²³ In the present study, care was taken to ensure that the injections into the sternocleidomastoid muscles did not leak from the epimysium. However, analyses of possible spread of doxorubicin to neighboring structures within the neck were not performed. While nearby structures appeared normal when the sternocleidomastoid muscles were surgically removed for in vitro analysis, future studies will be needed to ensure that nearby structures were unaffected.

The dose-related effect of doxorubicin chemomyectomy was demonstrated both in terms of greater loss of muscle mass and greater loss of force resulting from the twitch and tetanic stimulations in the 2 mg doxorubicin-treated muscle compared to 1 mg doxorubicin treatment. After 1 month, the force of the injected muscle had decreased to 65% of the contralateral control, and at 6 months the force was 85% of the contralateral control. This would indicate that the effect of doxorubicin on reducing muscle force is long-lived. Previous histological studies demonstrated that the muscle loss was indeed permanent, even 2 years after the last doxorubicin treatment.²⁷ It may also be the case that over time, the decreased activity of the weakened muscle would eventually result in myofiber atrophy, which in turn would further decrease muscle strength.

Interestingly, a difference between force per cross-sectional area of single twitch compared with higher stimulation frequencies was observed following treatment. At single twitch forces, there was a significant reduction of force per cross-sectional area of the injected compared to the contralateral control muscles, while at

![Fatigue Rates of Sternocleidomastoid Muscle](image)

**FIG. 5.** Fatigue rate was defined as the time for the muscle specimen to decrease to 75% of its maximal tetanic amplitude. All doxorubicin-treated muscles at both postinjection intervals showed significantly increased times to fatigue relative to the responses of controls (asterisks).

<table>
<thead>
<tr>
<th>SCM muscle</th>
<th>Weight (g)</th>
<th>Cross-sectional area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Injected</td>
<td>Contralateral control</td>
</tr>
<tr>
<td>1 mg doxorubicin 1 month</td>
<td>0.37 ± 0.05*</td>
<td>1.33 ± 0.10</td>
</tr>
<tr>
<td>2 mg doxorubicin</td>
<td>0.44 ± 0.08*</td>
<td>1.14 ± 0.07</td>
</tr>
<tr>
<td>1 mg doxorubicin 6 months</td>
<td>0.59 ± 0.06*</td>
<td>1.64 ± 0.11</td>
</tr>
<tr>
<td>Non-injected control</td>
<td>1.03 ± 0.15</td>
<td>1.12 ± 0.13</td>
</tr>
</tbody>
</table>

The table shows the weight and cross-sectional area of the 1-month 1 mg, 1-month 2 mg, and 6-month 1 mg doxorubicin-treated sternocleidomastoid muscle, the contralateral control, and non-injected control sternocleidomastoid muscle. Numbers represent means ± S.E.M.

*Indicates significant difference compared to control. Weight is wet weight in grams. CSA is muscle cross-sectional area calculated as muscle wet weight/[muscle density] [muscle length]. Six-month control muscles are somewhat heavier than seen in the 1-month specimens. Rabbits continue to grow throughout life, and this increase is most likely due to the general increase in rabbit weight over the 6-month period.
higher stimulation frequencies, there were only marginal differences in force generation per cross-sectional area comparing injected and the contralateral muscles. Thus, even though there was a dramatic decrease in the total amount of muscle tissue present after treatment, the remaining muscles were able to elicit fairly normal contractile forces at higher tetanic stimulations based on individual myofiber cross-sectional areas. Only under conditions producing high stimulus intensities would the remaining muscle be able to produce contractions with their maximum physiological force. We have observed an apparent increase in nerve fiber networks surrounding the doxorubicin-treated SCM which we are in the process of analyzing. This increase in innervation may explain the more effective use of the remaining muscle when high-frequency contractility is initiated. It should be noted that previous studies have shown that despite an almost complete and permanent doxorubicin-induced chemomyectomy of orbicularis oculi muscle in rabbit eyelid, no facial neuronal loss was evident in the brainstem. This is in contrast to studies where doxorubicin was injected directly into nerves themselves; this increases the dose of doxorubicin directly within the peripheral neurons, and in this case, neuronal loss occurred. The relatively normal muscle forces produced after a twitch stimulus/cross-sectional area (CSA) suggests that the neuronal population was still intact prior to our studies.

The isolated intact sternocleidomastoid muscle preparation was chosen as a convenient way to study the relative differences between a doxorubicin-treated and non-treated neck muscle. While the in vitro preparation has been shown to be a good system for the study of force generation in intact muscle, it should be noted, however, that the absolute values of average strength per cross-sectional area, both for the injected and non-injected sternocleidomastoid muscles, were lower than might be predicted based on muscle size. There are a number of possible explanations for this decrease in overall force compared to predicted values. It is possible that the large size of the sternocleidomastoid and the lower temperatures used in the present study, 30°C compared to 35°C, may alter muscle integrity. However, histological examination of the muscles at the end of each experiment demonstrated that the muscles were relatively normal in appearance. Also, similar force measurements could be obtained with similar stimulation intensities throughout the length of the experiment. This would indicate that the preparation remained healthy throughout the experimental time frame. In situ nerve stimulation studies are currently underway, and preliminary data indicate that greater forces can be obtained in
situ; however the differences between treated and control muscles were in the same range as the present study (Falkenberg et al., unpublished). The goal of this study was to compare the effects of doxorubicin chemomyectomy on SCM force production in relation to that obtained from normal muscle rather than a determination of actual total SCM muscle force capacity at high stimulation frequencies.

Fatigue rates were significantly elevated in the doxorubicin-treated muscles. Four muscles had fatigue resistance greater than 1 hour (i.e., generating greater than 75% of the original 40-Hz tetanic force); three muscles were treated with 1 mg (one short-term, and two long-term), and one muscle was treated with 2 mg doxorubicin. While overall force generation was substantially reduced compared with control muscles, the fibers that remained were much less fatigable. Histological evidence demonstrated that the myofibers that remained 1 month after doxorubicin treatment showed an increased expression of immature myosin heavy chain isoforms, as seen in other injured muscles. In other muscles where myofibers coexpress multiple myosins, the muscles show a continuum in shortening velocity values and stretch activation values. In studies using developing diaphragm muscle as a model, maximum shortening velocity and maximum force generation were inversely proportional to the expression of neonatal myosin. The extraocular muscles also express immature myosin isoforms and have faster twitch contractions, but weaker tetanic tensions. Extraocular muscles are extremely fatigue-resistant, and this may be due partly to their continued expression of immature myosin heavy chain isoforms in the adult. The upregulation of immature myosins in the doxorubicin-treated sternocleidomastoid muscles in the present study may help explain their decreased fatigue rate during in vitro stimulation. However, decreased fatigue rate is still present in muscles 6 months after treatment, when the immature myosin isoform composition has returned to its normal configuration. However, there is a concomitant increase in the number of myofibers positive for the slow myosin isoform. Type I, slow fatigue-resistant fibers are important in postural control. The observations that the treated muscles were more fatigue-resistant and have slower contractile properties may have functional advantages for cervical dystonia patients. These properties might be beneficial for postural support of the head by the remaining muscle. It is also possible that dystonic postures would persist; only with further studies will we be able to predict the possible outcomes in human patients.

The doxorubicin-treated muscles had altered single twitch properties, including a slower rate of decay. This phenomenon has also been observed for immature muscles compared to mature muscles and in mutant mice that lacked the “adult” acetylcholine receptor e subunit. It is possible that the properties of muscle fibers that remain after doxorubicin treatment are affected by regeneration or denervation/reinnervation of the treated muscles. Previous studies have indicated that a small amount of regeneration might occur in muscle where the level of doxorubicin has not attained high enough concentrations; however, tissue culture studies have shown that doxorubicin is a potent inhibitor of satellite cell division in vitro (McLoon, unpublished). Doxorubicin is well known from its use in cancer chemotherapy to have potent anti-mitotic effects. Thus, while some regeneration may have occurred in the treated sternocleidomastoid muscles, this is presumed to be minimal in light of long-term studies that have demonstrated that orbicularis oculi muscle loss, induced by doxorubicin treatment, was permanent.

Few studies have examined the anatomy and physiology of human sternocleidomastoid muscles. These studies indicated that 60–65% of the muscle was type 2 myofibers. This is similar to the composition of rabbit SCM if fiber types are grouped without regard for regional compartmentalization. Innervation of the all the mammalian sternocleidomastoid thus far examined has multiple zones of motor end plates along the muscle length.

Cervical dystonia is difficult to treat effectively due to the variability in the muscles involved in the presentation of this disease in a given patient. It has been shown that when a specific neck muscle has been weakened or removed, other neck muscles will often compensate with increased activity. This was seen in the present study to a small extent, since, although it was not significant, the contralateral control muscles generated more force than unoperated controls from separate animals. It would appear that the contralateral non-injected control sternocleidomastoid muscles were able to increase their force production to at least a small extent in response to decreased force on the treated side. The effect of doxorubicin injection on muscle force generation in the dorsal neck musculature is currently being studied to determine the effective dose for reduction in strength in those muscles as well. Doxorubicin chemomyectomy would appear to have therapeutic potential for the treatment of cervical dystonia. Previous studies have demonstrated that direct injection of the doxorubicin results in a dose-related loss in the amount of muscle, and as demonstrated in the present study, force output after treatment. Preliminary studies in human cervical dystonia patients used an electromyography (EMG)-guided injection pro-
tocot to directly inject doxorubicin into their neck muscles. Lower doses were used in these first patients, and even this conservative treatment resulted in some reduction in muscle strength (Dykstra, unpublished). No side effects were seen in these patients. However, it should be noted that in human blepharospasm patients, skin injury over the injection site in the eyelid was a potential side effect of treatment. Careful injection technique using EMG guidance would be recommended to ensure that the doxorubicin was injected directly into the muscle and not into the connective tissue spaces of the neck.

Doxorubicin chemomyectomy may prove to be a useful treatment for the long-term, or even permanent, reduction in muscle contractile strength in cervical dystonia patients. This in contrast with phenol-induced myectomy, where the effects are not permanent due to regeneration of the muscle. With appropriate clinical use, a patient hopefully would retain sufficient muscle tone for maintenance of normal head postural control, yet show a permanent improvement in posture.

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