ABSTRACT: The sternocleidomastoid muscle (SCM) is one of the major muscles involved in producing abnormal head position in cervical dystonia patients. This study tested whether doxorubicin chemomyectomy, direct injection of doxorubicin into the SCM to permanently remove muscle fibers, has the potential to be a nonsurgical, permanent treatment for cervical dystonia. The right SCM of rabbits was injected with either 1 or 2 mg doxorubicin. Animals were sacrificed 1–2 months postinjection. The SCM was prepared for histological examination of muscle fiber loss and fiber type composition. In all cases, direct injection of doxorubicin resulted in significant decreases in total muscle cross-sectional areas ranging from 75% up to 98%. Individual myofiber cross-sectional areas were smaller than normal after 2 mg doxorubicin treatment, but similar to normal fiber size after 1 mg doxorubicin. There were increased numbers of myofibers that expressed slow and neonatal myosin heavy chain isoforms in these remaining muscle fibers compared to the untreated SCM on the contralateral side. Developmental myosin heavy chain (MHC) was also present in 53% of the remaining myofibers of the treated muscles. The fiber type composition of muscles contralateral to the doxorubicin injections was compared to the fiber type composition of SCM from normal, untreated controls; no difference was seen in the proportions of fast, slow, and neonatal MHC fiber types in these SCM muscles. In summary, the direct injection of doxorubicin into the SCM resulted in significant muscle loss. This supports the use of doxorubicin chemomyectomy as a potential permanent, nonsurgical treatment for cervical dystonia.

DOXORUBICIN CHEMOMYECTOMY AS A TREATMENT FOR CERVICAL DYSTONIA: HISTOLOGICAL ASSESSMENT AFTER DIRECT INJECTION INTO THE STERNOCLEIDOMASTOID MUSCLE

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Cervical dystonia, or spasmodic torticollis, is a muscle disorder characterized by forceful, involuntary contractions of the cervical musculature. While not life-threatening, cervical dystonia is functionally disabling and often painful. Disease onset is most common beginning at age 50.11 Cervical dystonia has an incidence rate of 1.2 per 100,000, and a female to male ratio of 3.6 to 1.8 There are three common treatments for this condition: (1) oral medications, such as anticholinergic agents;9 (2) surgical intervention, including thalamotomy or selective peripheral denervation1 and (3) the most common treatment in current use, injection of botulinum...
toxin. Both the use of oral medications and surgical interventions can be ineffective or have unacceptable complications. While botulinum toxin injections are of significant benefit to the majority of treated patients, as with any medical treatment, botulinum toxin injections are not perfect. The botulinum injections result in only temporary paralysis, so patients require reinjection every few months. When published studies of botulinum toxin use in cervical dystonia patients are examined, all gave high, temporary success rates, that is, the percent of patients for whom the botulinum toxin injections were effective out of the total number of treated patients. Success rates in these studies were determined both by subjective and objective measures. The results from all these studies were added together for the present analysis and resulted in an overall short-term success rate of 64.8%. This indicates that there is a sizable population of cervical dystonia patients who are not effectively treated, even transiently, with botulinum toxin. Additionally, recent studies indicate that with prolonged use patients can develop antibodies to botulinum toxin, and the development of antibodies has been linked to decreased therapeutic effectiveness.

The most commonly involved muscles in cervical dystonia, and those usually treated with botulinum toxin, are the sternocleidomastoid, the splenius, and the semispinalis. The sternocleidomastoid muscles are large paired muscles in the neck which extend from the manubrium and medial end of the clavicle to the mastoid process of the temporal bone. The main action of these muscles is to turn the head toward the side opposite the contracting muscle. Mammalian sternocleidomastoid muscle is composed largely of type 2 myofibers, which have fast contractile properties. Rabbit sternocleidomastoid has an outer region composed almost completely of fast myosin positive myofibers, and an internal region with a more mosaic expression of both fast and slow myosin heavy chain (MHC) isoforms. There is also continued expression of a neonatal MHC, which is extremely rare in adult mammalian skeletal muscle. The rabbit sternocleidomastoid has multiple end-plate regions located between 0.7 and 1.0 cm apart that extend from tendon end to tendon end along the length of the muscle. It is not known whether there are changes in these complex anatomical patterns within the muscle in cervical dystonia patients.

Doxorubicin chemomyectomy has been successfully employed for the permanent, nonsurgical treatment of blepharospasm and hemifacial spasm. This treatment involves the direct injection of doxorubicin into the eyelids of these patients and results in permanent muscle loss. Doxorubicin is a myotoxic drug, particularly when the muscle is injected locally. Doxorubicin myotoxicity is dose-dependent. The drug works rapidly, resulting in myofibrillar dissolution with 5 min of treatment, and there is little evidence of regeneration after treatment, presumably due to the antimitotic nature of doxorubicin.

In order to assess whether doxorubicin chemomyectomy would have a similar level of efficacy as seen in its use as a treatment for blepharospasm and hemifacial spasm, rabbit sternocleidomastoid muscles on one side of the neck were injected with one of several doses of doxorubicin using visual guidance. After 1–2 months, the muscles were examined histologically for amount of muscle loss and for alterations in fiber type configuration.

**MATERIALS AND METHODS**

All animal studies were approved by the University of Minnesota Institution Animal Care and Use Committee, and conformed with the published guidelines of the National Institutes of Health on the use of animals in research. New Zealand white rabbits (Birchwood Farms, Red Wing, MN) were anesthetized with an intramuscular injection of ketamine:xylazine (1:1, at doses of 10 mg/kg:2 mg/kg, respectively). A midline neck incision was made, and under sterile conditions the sternocleidomastoid muscle was dissected from the overlying fat and connective tissue. After visualization of the muscles, the right sternocleidomastoid muscles were injected with doxorubicin (Adriamycin, Pharmacia, Kalamazoo, MI) at doses of either 1 mg in either 0.5 or 1 mL sterile isotonic saline, or 2 mg in either 0.5 or 1 mL saline. Doxorubicin was injected so as to distribute the injection volume uniformly throughout the length of the muscle from origin to insertion by careful needle insertion under visual guidance and slow withdrawal along the muscle length during the injection. Doxorubicin is red. Thus, it can be seen within the muscle during the injection procedure, which helps ensure relatively even distribution. Four muscles were used for each injection parameter, for a total of 16 rabbits. The necks of the rabbits were examined daily for changes to the skin overlying the injection site. Sternocleidomastoid muscles were also dissected from two uninjected, normal rabbits as controls.

After 1 or 2 months, the animals were euthanized with an overdose of barbiturate anesthesia. The sternocleidomastoid muscles were dissected from the...
neck from tendon end to tendon end. Since previous studies indicated that there can be regional differences in the amount of muscle loss after local doxorubicin injection, \(^\text{27,28}\) samples through the full cross-sectional thickness of both the control and doxorubicin-injected muscles were taken from the proximal, middle, and distal regions of the muscle. Tissue samples were frozen on methylbutane chilled to a slurry in liquid nitrogen, and cross sections were cut on a cryostat at 12 µm. Tissue sections were stained either using myosin adenosine triphosphatase histochemistry at pH 10.5 or using immunohistochemistry with antibodies to fast, slow, neonatal, or developmental MHC isoforms (NovoCastra Labs., Newcastle, U.K.). For immunohistochemical processing, the sections were blocked in normal horse serum, followed immediately by incubation in primary antibody, at a concentration of 1:40 for fast and slow MHC and 1:20 for neonatal and developmental MHC. The slides were rinsed in phosphate-buffered saline, and incubated in biotin–avidin horseradish peroxidase secondary antibodies (Vectastain Elite kit, Vector Labs., Burlingame, CA). The slides were reacted with diaminobenzidine and heavy metals. Total muscle cross-sectional areas and myofiber number and fiber composition were determined using the Bioquant image analysis software (R & D Biometrics, Nashville, TN). All statistics were done using the Prism and Statmate software (Graphpad, San Diego, CA), and results were compared using an unpaired, two-tailed t-test. An F-test indicated that the variances were not significantly different. Results were considered significantly different at \(P < 0.005\).

**RESULTS**

In contrast to injections into the eyelid, where 100% of the treated animals developed short-term skin injury over the injection site, \(^\text{31,33}\) none of the rabbits developed short-term visible injury of the skin overlying the injection site, nor did they show a decreased ability for the skin incisions over the injection site to heal. Since the injections were made with direct visualization of the muscle, it was easier to ensure that the injection stayed within the confines of the muscle epimysium. Nevertheless, it was noted that 4 of the first rabbits injected developed small sores over the sternum between 1 and 2 weeks after injection. This may be explained based on the location of the insertion of the superficial fascial layer of the neck. Any doxorubicin that entered this connective tissue space would presumably flow, via gravity, to this location. These sores all healed within 3–7 days. Care was taken in all the remaining rabbits to ensure that the injection did not leak from the epimysium of the injected muscle; none of these rabbits developed skin sores.

Direct injection of the sternocleidomastoid resulted in significant reduction in the total muscle cross-sectional area (Fig. 1). When the muscles were dissected out, often there was no apparent muscle tissue within the connective tissue bundle except at the most proximal and/or distal segments near the muscles’ origins and insertions. Muscle loss was quantified by measuring total muscle cross-sectional area (Fig. 2). There was a significant reduction in cross-sectional area at each of the doses of doxorubicin employed, with a range of reduction of 75–

**FIGURE 1.** Cross section through a sternocleidomastoid muscle that had been injected 1 month previously with 2 mg doxorubicin in 2 mL saline. The arrow indicates muscle fibers that remained, stained for the fast MHC isoform. Bar is 100 µm.
98% of total muscle cross-sectional area compared to the normal sternocleidomastoid muscle (SCM). Individual myofiber cross-sectional areas were unchanged from control myofibers after 1 mg doxorubicin treatment (Fig. 3), but were significantly smaller after 2 mg doxorubicin treatment.

The doxorubicin-treated SCM was examined for MHC isoform expression (Fig. 4). The doxorubicin-treated muscle still consisted largely of fast MHC-positive myofibers; however, there was a significant increase in the numbers of slow MHC-positive myofibers after doxorubicin treatment. There was also significant increase in the numbers of fibers that expressed two immature MHC isoforms, neonatal MHC and developmental MHC (Fig. 5). While normal rabbit sternocleidomastoid muscle expresses neonatal MHC, it does not express developmental MHC. There was significant coexpression of multiple MHC isoforms in single myofibers; individual myofibers could be found that expressed all four of these MHC isoforms (Fig. 4). These changes were quantified (Fig. 5). There were significant increases in the numbers of myofibers expressing slow, neonatal, and developmental MHC at each of the injected doses of doxorubicin. It should be noted that concurrent physiological assessments have demonstrated a significant, but not proportional, reduction in muscle force after doxorubicin injections into this muscle. There was also an increase in the time to fatigue after repeated stimulation.

In order to ensure that the sternocleidomastoid muscle on the side contralateral to the doxorubicin injection was normal, the number of fibers that expressed fast, slow, and neonatal MHC was examined in all the muscles contralateral to the doxorubicin injections and compared to normal SCM from untreated control rabbits (Fig. 6). There were no significant differences in MHC expression between control muscles from totally untreated animals and the SCM contralateral to a doxorubicin treatment.

**DISCUSSION**

Direct injections of doxorubicin into rabbit sternocleidomastoid muscle resulted in a significant decrease in muscle mass in the treated muscles 1–2 months after treatment. This is similar to the effect of local injection of doxorubicin into the eyelid region. One pronounced difference between these studies was the lack of skin injury directly over the injection site after sternocleidomastoid muscle injection compared to injection into the eyelid. Two reasons can be postulated to account for this difference. First, the sternocleidomastoid muscle is much less superficial than the orbicularis oculi, which actually inserts directly into the eyelid skin. This necessitates a more superficial injection protocol to treat the orbicularis oculi. Second, the sternocleidomastoid muscle is surrounded by an epimysial covering, and injections made into the muscle can be contained more completely within its epimysium without leakage into the surrounding connective tissue spaces. This latter hypothesis was supported by the occasional formation of skin sores over the suprasternal space at the sternal junction in those rabbits.
where there was some doxorubicin leakage into the surrounding connective tissue spaces during the injection procedure; any doxorubicin that had leaked from the muscle presumably drained into this location. Thus, careful injection technique should minimize the most common side effect due to the injection of doxorubicin into the eyelid, which is localized inflammation and skin injury over the injection site.

FIGURE 4. Sternocleidomastoid muscle from the uninjected, control side (A, B) and from a muscle injected with 2 mg doxorubicin in 1 mL saline. (A) is normal SCM stained for fast MHC. (B) is normal SCM stained for neonatal MHC. (C–F) is doxorubicin-treated SCM stained for the presence of fast MHC (C), neonatal MHC (D), slow MHC (E), and developmental MHC (F). Note fibers positive for all four myosin heavy chain isoforms. While there is a great reduction in muscle cross-sectional area, there is a marked increase in the numbers of fibers positive for neonatal myosin. There is also an increase in numbers of fibers positive for slow myosin and developmental myosin compared to normal (not shown). Bar is 100 µm.
The expression of immature myosin heavy chain isoforms is commonly seen during normal development and during the regenerative period after a muscle injury. The presence of neonatal MHC in the sternocleidomastoid in normal adult rabbits is considered unusual; however, other muscles which retain expression of neonatal MHC in adult mammals have been described, including the masseter muscle and the extraocular muscles. It is interesting that after doxorubicin treatment, the muscle fibers that remained in the treated sternocleidomastoid muscle showed increased coexpression of multiple MHC isoforms in individual myofibers, particularly developmental and neonatal MHC. Coexpression of multiple MHC in single fibers resulted in greater variances in shortening velocity and maximal force generation. In the treated sternocleidomastoid muscles, a large percentage of the remaining myofibers expressed neonatal and/or developmental MHC isoforms. Studies have demonstrated that myofibers containing these “immature” MHC isoforms have decreased contractile force.

The signal for the increased expression of these unusual “immature” myosin isoforms in the doxorubicin-treated SCM is unknown. In other studies, high levels of expression of these embryonic MHC isoforms have been correlated at least in part to denervation of muscle fibers and is seen in patients with neurogenic disease. Whether this indicates that the spinoaccessory nerve innervation of the doxorubicin-treated sternocleidomastoid muscle remains intact is unknown at this time. This is an issue that will require additional studies to clarify. However, after doxorubicin treatment of the orbicularis oculi muscle in the eyelid, retrograde tracing techniques demonstrated that there was no identifiable loss of facial motor neurons, even after removal of the majority of the target orbicularis oculi muscle. Histological sections of eyelids which were relatively devoid of muscle fibers still showed intact nerves within them. Also, if all the muscle was not removed at the time of doxorubicin injection, the eyelids of blepharospasm patients, some muscle twitching remained, indicating intact facial nerve innervation in these eyelids despite extensive muscle loss. Stretch also has been shown to induce expression of embryonic MHC isoforms in adult muscle. Since the majority of muscle fibers were killed as a result of the local injection of doxorubicin into the SCM, what remained was a thin connective tissue filled tube, usually with small amounts of muscle proximally and/or distally, or thin longitudinal strips of muscle extending for variable distances within the epimysial tube. With neck movements, this residual muscle may be subjected to greater stretch forces than when the muscle is intact. In addition, it is not known if neuromuscular function is altered; hence, we have initiated in situ force measurements utilizing direct nerve activation.

The neck musculature is extremely complex, with over 20 muscles described as participating in head movements. While there is apparent redundancy possible in the subset of muscles used during specific neck movements, each neck muscle usually participates in a given head movement in a characteristic manner. When a specific neck muscle is weakened or removed, other neck muscles will often compensate with increased activity. Even patients who received no clinical benefit from injection of botulinum toxin injections into their neck muscles show changes in the pattern of muscle activity. This would suggest that these changes in muscle activity reflect a general motor program for head position, rather than specific muscle activity patterns. In an in vitro preparation, chronic increased muscle activity was reflected in alterations in MHC isoform expression within the stimulated muscles. In the present study, even when the majority of the right SCM muscle was destroyed, the pattern of MHC isoform expression in the muscle fibers within the contralateral SCM resembled that seen in uninjected control muscles from normal rabbits. In electromyographic studies of neck muscles in naturally behaving cats, the sternocleidomastoid and trapezius muscles show changes in the pattern of muscle activity. This suggests that these changes in muscle activity reflect a general motor program for head position, rather than specific muscle activity patterns. In an in vitro preparation, chronic increased muscle activity was reflected in alterations in MHC isoform expression within the stimulated muscles. In the present study, even when the majority of the right SCM muscle was destroyed, the pattern of MHC isoform expression in the muscle fibers within the contralateral SCM resembled that seen in uninjected control muscles from normal rabbits. In electromyographic studies of neck muscles in naturally behaving cats, the sternocleidomastoid and trapezius muscles show changes in the pattern of muscle activity.

FIGURE 5. Quantification of MHC expression in myofibers from uninjected control SCM from both the outer and inner compartments of the muscle compared with SCM injected with 1 or 2 mg doxorubicin in either 0.5 or 1 mL saline. There was a statistically significant increase in the number of myofibers positive for slow and neonatal MHC compared to either compartment of the uninjected control muscles. Normal SCM does not express developmental MHC, yet all the doxorubicin-treated SCM muscles examined had myofibers positive for developmental MHC. Stars indicate statistical significance.
were only recruited during forceful or ballistic head movements. It is possible that in normal, behaving rabbits the SCM muscle may not be vigorously recruited. If this were true, compensatory changes in the contralateral muscle would not be expected. However, after muscle weakening caused by botulinum injections in cervical dystonia patients, there are often compensatory increases in activity of other neck muscles, and this is correlated to a lack of clinical effectiveness for these patients. It will be important to monitor changes in agonist and antagonist neck muscles after doxorubicin chemomyectomy in the SCM in human patients.

In summary, doxorubicin chemomyectomy causes permanent muscle loss in orbicularis oculi and would appear to act similarly in the treated sternocleidomastoid muscles; thus its effect can be considered as similar to surgery, where denervation results in improved head position, although often with a decreased range of motion. The potential advantage in the use of doxorubicin chemomyectomy for the treatment of cervical dystonia is that the same effect can be produced in a more graded, dose-dependent manner and can be realized without surgical intervention. Analysis for the use of this protocol in blepharospasm and hemifacial spasm patients demonstrated that chemomyectomy saved both time needed for successful treatment and money for the patients.

REFERENCES


