Sustained Release of Bone Morphogenetic Protein-4 in Adult Rabbit Extraocular Muscle Results in Decreased Force and Muscle Size: Potential for Strabismus Treatment

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PURPOSE. To assess the effect of a sustained-release preparation of bone morphogenetic protein-4 (BMP-4) on EOM force generation and muscle size.

METHODS. Sustained-release pellets, releasing 500 nanograms/day of BMP-4 for a maximum of 3 months, were implanted beneath the superior rectus muscle (SR) belly in anesthetized adult rabbits. The contralateral side received a placebo pellet as a control. After 1, 3, and 6 months, SRs were removed, and force generation at twitch and tetanic frequencies as well as fatigue resistance were determined in vitro. Myofiber size, myosin heavy chain isoform expression, and satellite cell density were assessed histologically.

RESULTS. SR force generation was significantly decreased by BMP-4 compared with the contralateral controls. Force generation was decreased by 25–30% by 1 month, 31–50% by 3 months, and at 6 months, after 3 BMP-4-free months, force was still decreased by 20–31%. No change in fatigue was seen. Significant decreases in muscle size were seen, greatest at 3 months. At all time points Pax7- and MyoD-positive satellite cell densities were significantly decreased.

CONCLUSIONS. The decreased force generation and muscle size caused by sustained release of BMP-4 suggests that myogenic signaling factors may provide a more biological method of decreasing muscle strength in vivo than exogenously administered toxins. Treating antagonist-agonist pairs of EOM with titratable, naturally occurring myogenic signaling and growth factors may provide safe, efficacious, nonsurgical treatment options for patients with strabismus. (*Invest Ophthalmol Vis Sci.* 2011;52:4021–4029) DOI:10.1167/iovs.10-6878

Strabismus is a common disorder of children and adults that is characterized by misalignment of the eyes and loss of binocular vision. The goal of treatment is to realign the eyes.

For some children, treatment with glasses is sufficient. For many children and most adults with strabismus, surgical correction is necessary. Traditional incisional surgery has relied on manipulation of the extraocular muscle (EOM) insertional position on the globe or the EOM length to effect realignment of the eyes. However, individual responses to surgery are quite variable, and long-term motor success is poor, with many patients requiring reoperation.¹ The introduction of botulinum toxin type A in the early 1980s² heralded a new era in the treatment for strabismus, making possible the pharmacological manipulation of EOM force generation to adjust the rotational position of the treated eye or eyes. Botulinum toxin injection has proven effective in some adult and pediatric strabismic patients, but reinjection is often required, particularly with large deviations.³ Recently a number of potential new candidate agents for altering EOM strength have been investigated, and one is undergoing limited clinical trials.⁴ These new agents may expand treatment options available to strabismus surgeons and one hopes will improve long-term outcomes.

Our laboratory has been investigating the use of naturally occurring myogenic signaling and growth factors for modulating EOM strength.⁵ The rationale behind this approach is based on properties of myogenic precursor cells within the EOM. These muscle precursor cells are increased in number compared with limb skeletal muscle as well as actively dividing throughout life, allowing for ongoing myofiber remodeling in normal EOM.⁶ Acute studies in animals involving direct injection of growth factors such as insulin growth factors I and II and cardiotrophin into adult or juvenile EOM have demonstrated their ability to increase EOM force generation.^{7–11}

New approaches to pharmacologically weaken the EOM have focused on immunotoxins such as ricin-mAb35.^{12,13} While the immunotoxin has long-lasting weakening effects in animal studies, there are concerns about potential systemic toxicity and therapeutic thresholds prompting study of more biologically acceptable agents. Recent studies from our laboratory have shown that acute treatment with bone morphogenetic protein-4 (BMP-4), transforming growth factor- β 1 (TGF β 1), sonic hedgehog (Shh), or Wnt3A all resulted in decreased EOM force generation.¹⁴ These studies were based on the demonstration that these myogenic signaling factors turn off muscle differentiation in developing head mesoderm.¹⁵ This suggested that when added to mature EOM, they could specifically act on the myogenic precursor cells and decrease the process of myofiber remodeling. Based on this proof-of-principle experiment, BMP-4 appeared to be the most effective of the four myogenic signaling factors tested.¹⁴ However, treatment effect was short lived. Duration of treatment effect is known to be a determinant of successful strabismus therapy. Means of extending the treatment effect of BMP-4, therefore,

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Supported by EY15313 and EY11375 from the National Eye Institute, the Minnesota Medical Foundation, the Minnesota Lions and Lionesses, Research to Prevent Blindness (RPB) Lew Wasserman Mid-Career Development Award (LKM), and an unrestricted grant to the Department of Ophthalmology from RPB.

Submitted for publication November 11, 2010; revised December 15 and 30, 2010; accepted January 4, 2011.

Disclosure: B.C. Anderson, None; M.L. Daniel, None; J.D. Kendall, None; S.P. Christiansen, None; L.K. McLoon, None

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need to be investigated. The current experiments focus on changes in adult EOM muscle twitch characteristics and force generation caused by BMP-4 at 1, 3, and 6 months after implantation of the sustained release pellet.

METHODS

Adult New Zealand White rabbits were obtained from Bakkom Rabbitry (Viroqua, WI) and housed in the AALAC-approved facility at the University of Minnesota. All studies were approved by the Institutional Animal Care and Use Committee at the University of Minnesota and adhered to the ARVO and NIH guidelines for the use of animals in research.

The rabbits were anesthetized with ketamine/xylazine (1:1, 10 mg/kg:2 mg/kg, respectively), and were surgically implanted with sustained released pellets. The pellets were specifically prepared to our specifications by Innovative Research of America (Sarasota, FL). They were prepared to release 500 ng/day for a total of 90 days. They are approximately 4 mm in diameter and easily fit deep to the superior rectus muscle (SR). Under sterile conditions, the conjunctiva was opened directly lateral to the SR. The lateral edge of the SR was raised slightly, and a sustained-release pellet was placed between the sclera and the muscle belly. The placement of the BMP-4 pellet was randomized in each rabbit, so the physiological examination was performed masked relative to treatment side. A placebo pellet was similarly implanted on the contralateral side.

After 1 or 3 months of sustained release, or at 6 months after the start of 3 months of sustained release, the rabbits were euthanized by thoracotomy followed by exsanguination after anesthesia with ketamine and xylazine. The pellets were left in place to minimize surgical manipulation within the orbit. Both SR muscles were carefully dissected from scleral insertion to their origin and prepared for analysis using an *in vitro* force apparatus as previously described.^{10,14} During the removal of the muscles for the *in vitro* muscle physiology, adhesions were routinely noted between one of the pellets and the underlying sclera. These connections were carefully dissected to perform the electrophysiology. After immediate placement in oxygenated Krebs solution at 30°C, the muscles were carefully pinned to their previously determined *in situ* length, and 4-0 silk was tied to each muscle end. The muscles were suspended in the *in vitro* incubation chambers via suture loops attached to a lever arm and force transducer and continuously bathed in 30°C oxygenated Krebs. Force in grams was recorded (1205 Intact Muscle Test System and Dynamic Muscle Control software; Aurora Scientific, Aurora, Ontario, Canada). Muscle length and mass were determined at the termination of the stimulation protocol. Muscle cross-sectional area was determined by dividing muscle mass (g) by the product of muscle length (cm) times a muscle density of 1.056 g/cm³ and converted to mN/cm².

Control and BMP-4-treated SR muscles were tested simultaneously. Supramaximal stimulation intensity was determined by increasing voltage until maximal contraction was achieved using square-wave pulses of 0.5 ms duration (Aurora Scientific 701B biphasic current stimulator) and delivered to the muscles via flanking platinum electrodes. Isometric length-tension curves were determined by stimulating each muscle at supramaximal intensity (500 mA, 0.5 ms) while varying the preload (resting length) over a range of 0.5–10.0 g. Incremental increase in resting muscle length to achieve maximum isometric twitch force, with 60 seconds of rest between stimuli, was used to determine optimal preload. All further tests were performed with supramaximal stimulus intensities at optimal preload. After two stabilizing tetanic stimulations (150 Hz, 500 mA, 0.5 ms) with 2 minutes rest between stimuli and 5 minutes rest after two consecutive stimulations, force development was determined for single, double, and triple pulses (0.5 ms pulse duration) with 2 minutes of rest between stimuli. Muscles were stimulated at frequencies of 10, 20, 40, 100, 150, and 200 Hz at a train duration of 500 ms with an interstimulation rest of 2 minutes. After 2 minutes rest, the muscles were subjected to a fatigue protocol as follows: A tetanic stimulus was delivered every 2 seconds, consisting

of a 1-second train at 150 Hz. The muscles were stimulated for 600 seconds or until there was a 50% reduction in generated muscle force. All muscles were frozen and immunostained, as described in the following section. Data from treated ($N = 6$ rabbits for each postimplant physiological time point) and placebo-treated muscles were pooled at each postinjection interval and compared with the paired *t*-test. $P \leq 0.05$ was considered statistically significant.

In addition to histologically analyzing all 18 pairs of muscles after the *in vitro* physiology, both right and left SR muscles, a second set of rabbits was used for histologic examination only ($N = 4$ rabbits for each posttreatment time point).¹⁴ The animals were anesthetized as above and euthanized by thoracotomy and exsanguination. Both SR muscles were removed, embedded in tragacanth gum, frozen in 2-methylbutane chilled on liquid nitrogen, sectioned at 12 μ m, and stored at 80°C until stained. Serial tissue sections were immunostained for one of the following myosin heavy chain (MyHC) isoforms: pan fast (1:40), slow (1:40), developmental (1:20), neonatal (1:20), myoD (1:100) (all from Vector Laboratories, Burlingame, CA), and pax7 (1:500; Developmental Hybridoma Bank, Ames, IA). All tissue sections were either unfixed (MyHC isoforms) or acetone-fixed (Pax7 or MyoD), rinsed in phosphate buffered saline, and blocked in normal serum, followed by incubation in primary antibody. The sections were rinsed, followed by incubation in the sequential reagents (from the Vectastain kit; Vector), with visualization using diaminobenzidine and heavy metal salts of nickel and cobalt. Mean myofiber cross-sectional area and percent myofibers expressing each of the four MyHC isoforms were quantified (Bioquant Prism Image Analysis System; Bioquant Image Analysis Corp., Nashville, TN). For each antigen, three sections were quantified in the orbital and global layers in both the middle and toward the scleral tendon end of both the right and left SR muscles ($N = 10$ for each treatment duration [six rabbits after *in vitro* physiology and four rabbits where the muscles were directly frozen]; $N = 6$ naive control SR muscles from six rabbits that had no manipulation in either of their orbits). A minimum of 200 myofibers were counted per layer in each tissue section. Pax7- and MyoD-positive muscle precursor cells were quantified as percentage based on the number of positive cells per myofiber number. The percent positive was compared between the orbital and global layers and analyzed for statistical significance using either an unpaired two-tailed *t*-test (if two groups were being compared) or an analysis of variance (ANOVA) and Dunn's multiple comparison tests (aided by Prism and Statmate software; Graphpad, San Diego, CA) for multiple group comparisons. An *F* test was used to verify that the variances were not significantly different. Data were considered statistically significantly different if $P < 0.05$.

RESULTS

After 1 and 3 months of sustained release of BMP-4 there were significant reductions in generated force, in grams, and force normalized for muscle size, in mN/cm², at all stimulation frequencies (Figs. 1 and 2). Muscle force generation after 3 months of BMP-4 treatment decreased between 33% and 41% depending on the stimulation frequency. No statistically significant difference in the force reduction was seen between the 1-month and 3-month treatment groups. At the postimplantation interval of 6 months, after a 3-month period of sustained release of BMP-4 and 3 months delivery-free, force generation was still significantly decreased in the BMP-4-treated muscles (Fig. 3). The percent difference between the treated and untreated sides was not as great, dropping to between 20% and 31% of control levels. No differences were seen in the fatigue levels of the BMP-4-treated muscles (Fig. 4).

Sustained release of BMP-4 resulted in a significant decrease in muscle mass after 1 and 3 months (Fig. 5A). Muscle mass returned to normal control levels at 6 months, after a 3-month period of sustained release of BMP-4 followed by 3 months with no drug delivery (Fig. 5A). Mean myofiber cross-sectional

FIGURE 1. Force generation (A) in grams and (B) in mN/cm² 1 month after sustained release of BMP-4 in one SR muscle. *Statistical difference from control. Error bars, SEM.

area, however, was significantly smaller than control values only after 3 months of BMP-4 continuous exposure (Fig. 5B).

By 1 month after pellet implantation, the fast MyHC-positive myofibers were significantly smaller in mean cross-sectional area compared with naive control SR muscle in all layers and regions but the orbital midregion (Fig. 6). The only other region where myofiber type was decreased in cross-sectional area after 1 month of sustained BMP-4 exposure was the developmental MyHC-positive myofibers in the global middle and tendon end. All the other fiber types were not significantly different from normal control SR muscles. However, at 3 months the mean myofiber cross-sectional areas for populations of myofibers immunostained for fast, slow, developmental, and neonatal MyHC isoforms were all significantly reduced compared with naive control muscles (Figs. 7 and 8). Significant decreases in mean cross-sectional area were seen in both orbital and global layers and in both the midbelly (mid) and tendon end (ten) regions of the muscles examined. There appeared to be an increase in extracellular space between fascicles in the treated muscles, which may explain the differential cross-sectional area losses when the means of single fibers are compared with the entire muscle cross section (Fig. 5). At 6 months, after a 3-month period of sustained release of BMP4, the mean cross-sectional areas were no different from those seen in the naive control muscles (data not shown). The pattern of MyHC isoform expression changes in SR muscles exposed to 3 months of sustained BMP-4 release was assayed, and no significant difference was seen.

Statistically significant increases in the percent expression of fast MyHC isoform were seen in orbital layer of the BMP-4-treated muscles throughout their length, while there was only an increase in fast MyHC expression in the global layer of the

tendon region (Fig. 9A). There was a decrease in the percent expression of slow MyHC isoform in the orbital and global tendon regions (Fig. 9B). Both the global middle and tendon regions had a significant decrease in the percent developmental-positive myofibers (Fig. 9C), and there was a decrease in the percent neonatal-positive myofibers in the orbital and global midbelly region (Fig. 9D). These changes in MyHC expression patterns were retained at the 6-month time point (data not shown).

The effect of sustained release of BMP-4 on myogenic precursor cells was examined, both for percent changes in the overall population of quiescent satellite cells, as identified with Pax7, and for percent changes in the population of activated satellite cells, as identified by MyoD. At all three time points examined, the treated SR muscle had significantly decreased percentages of Pax7-positive and MyoD-positive myogenic precursor cells (Fig. 10). These data correlate very well with the reduction in muscle mass and mean myofiber cross-sectional area.

As some connective tissue adhesions were noted on the treatment side, the eyeballs were examined bilaterally for potential osteogenic effects of BMP-4 using Runx2, a transcription factor that regulates the lineage determination and differentiation of mesenchymal precursors into osteogenic and chondrogenic cells.¹⁵ No sign of Runx2 was seen within the sclera of the sustained-released eyes at 3 months (data not shown), suggesting that the dose was insufficient to induce osteogenesis in the treated tissues.

Because the SR muscle contralateral to the sustained BMP-4 SR muscle might undergo compensatory changes, myofiber cross-sectional area and MyHC isoform expression was exam-

FIGURE 2. Force generation (A) in grams and (B) in mN/cm² 3 months after sustained release of BMP-4 in one SR muscle. *Statistical difference from control.

FIGURE 3. Force generation (A) in grams and (B) in mN/cm² at 6 months, after a 3-month period of sustained release of BMP-4 in one SR muscle. *Statistical difference from control.

ined (Fig. 11), as was density of Pax7- and MyoD-positive myogenic precursor cell populations. At 3 months, only the global layer showed a significant reduction in mean myofiber cross-sectional area of fast-positive myofibers compared with naive control muscles (Fig. 11A). There were no significant differences in mean cross-sectional areas for fibers expressing the other 3 MyHC isoforms examined (data not shown), nor were there significant changes in the percentage of myofibers

FIGURE 4. Fatigue resistance was determined, and no differences were seen at any of the posttreatment intervals.

Effect of BMP4 Sustained Release on Mean Muscle Mass

Effect of BMP4 Sustained Release on Mean Total Muscle Cross-sectional Areas

FIGURE 5. (A) Mean muscle mass (grams) was determined based on weights, after bath removal, of the BMP-4-treated and control SRs after 1, 3, and 6 months, after a 3-month period of sustained release of BMP-4. *Statistical difference from control. (B) Mean myofiber cross-sectional areas were determined morphometrically on the BMP-4-treated and control SRs at 1, 3, and 6 months, after a 3-month period of sustained release of BMP-4. *Statistical difference from control.

expressing each of the 4 MyHC isoforms (data not shown). Although not as ubiquitously spread throughout the muscle length, there was still a significant reduction in the number of Pax7-positive cells in the midbelly region of the SR contralateral to the BMP-4-treated muscle (Fig. 11B) and in the number of MyoD-positive satellite cells in the tendon region of the treated muscles (Fig. 11C).

DISCUSSION

Sustained exposure of adult rabbit EOM to BMP-4 resulted in significant reductions in muscle force generation, muscle mass, and mean myofiber cross-sectional area. This was associated with significant reductions in the number of myogenic precursor cells in the treated muscles. Both MyoD- and Pax7-positive myogenic precursor cells were significantly reduced in density

FIGURE 6. Mean myofiber cross-sectional areas after 1 month of BMP-4 exposure compared with untreated controls for myofibers immunostained for (A) fast MyHC, (B) slow MyHC, (C) developmental MyHC, and (D) neonatal MyHC. *Statistical difference from control. Mid, mid-belly region of the muscle; ten, tendon region of the muscle.

compared with naive controls. The muscle-weakening effects caused by 3 months of continuous exposure were maintained at 6 months, despite the absence of BMP-4 release into the

muscles for this additional 3-month period. Thus, sustained exposure of normal adult rabbit EOM to BMP-4 had a significant and long-lasting muscle weakening effect.

Based on the acute changes in force seen after single injections of BMP-4, TGF β 1, Shh, and Wnt3A,¹⁴ BMP-4 was chosen for the sustained release studies. Of the four myogenic signaling factors, single injections of BMP-4 showed the largest decrement in force, with decreases of 45–50% compared with controls. In addition, 1 week after a single BMP-4 injection, cross-sectional area was most consistently and significantly reduced. BMP-4 resulted in the short-term loss of neonatal and developmental MyHC isoform-positive myofibers. BMP-4 had a significant short-term effect on MyoD-positive satellite cell content. All these supported the potential for long-term muscle-weakening effects of BMP-4, and this is supported by the present study.

The mechanism by which BMP-4 causes these changes in adult rabbit EOM is unclear. However, our data may suggest a possible mechanism. BMP-4 has many effects on skeletal muscle generally, and it is unclear how many of its cell-signaling properties can be applied equally to EOM and limb muscle. For example, during embryonic development, BMP-4 promotes differentiation of somitic and cardiac mesoderm but inhibits differentiation of cranial mesoderm.^{16,17} As normal adult EOM undergo continuous remodeling and contain a population of activated myogenic precursor cells, our data suggest that BMP-4 plays a similar role in adult EOM as in developing cranial mesoderm. In contrast, in adult mice the effect of BMP-4 on limb and masseter muscle satellite cells was to stimulate division and inhibit differentiation.¹⁸ The effects of BMP are tissue specific, and increasing evidence suggests that cell number and cell fate is differentially regulated by BMP-4 concentrations to which individual cell types and tissues are exposed.¹⁹ In the case of the long-term treated SR muscles in the present *in vivo* study, the significant decrease in density of Pax7 and MyoD-positive cells, concomitant with the significant reduction in muscle myofiber cross-sectional area, suggests that in EOM BMP-4 acts to decrease muscle precursor cell division. Ongoing studies will hopefully determine whether this is indeed the case.

FIGURE 7. Cross sections of the global region of BMP-4-treated (A) and control (B) SR muscles of adult rabbits 3 months after treatment immunostained for the fast myosin heavy chain isoform. Bar, 50 μ m. Arrows: fibers negative for fast myosin heavy chain isoform.

FIGURE 8. Mean myofiber cross-sectional areas after 3 months of BMP-4 exposure compared with untreated controls for myofibers immunostained for (A) fast MyHC, (B) slow MyHC, (C) developmental MyHC, and (D) neonatal MyHC. *Statistical difference from control. Mid, midbelly region of the muscle; ten, tendon region of the muscle.

Several hypotheses can be suggested for the difficulty in achieving motor and sensory success after surgical treatment of children with nonparalytic strabismus. First, the etiology of strabismus is not well understood, in part because the control of motor commands for the full complement of normal eye movements is also not well understood. It does appear, however, that neuronal activity within the oculomotor nucleus drives the abnormal cross-axis eye movements, as shown using a nonhuman primate model of strabismus.²⁰ The second is that the eye muscles themselves are highly adaptive. They adapt to visual sensory deprivation²¹ as well as to the surgical manipulations to which they are subjected.^{13,22} Although the control

of these changes is unclear, it does suggest that this adaptability can be manipulated to alter the motive force of the EOM. How can this knowledge of EOM adaptability be used to improve treatment for these patients? One approach to tapping into this adaptability is the use of localized pharmacologic treatments.

The duration of treatment effect for myogenic signaling and growth factors is an important variable to consider in studies of this type of signaling factor manipulation of EOM strength. In contrast to botulinum toxin, which results in paralysis of the neuromuscular junction by proteolytic cleavage of SNAP-25,²³ single injections will not likely be effective because the tissue

FIGURE 9. Percentage of MyHC isoform expression after 3 months of BMP-4 exposure compared with untreated controls for myofibers immunostained for (A) fast MyHC, (B) slow MyHC, (C) developmental MyHC, and (D) neonatal MyHC. Mid, midbelly; ten, tendon. *Statistical difference from control.

FIGURE 10. Effect of sustained release of BMP-4 on satellite cells identified by (A, C, E) Pax7 or (B, D, F) MyoD immunostaining at postimplantation times of (A, B) 1 month, (C, D) 3 months, or (E, F) 6 months, after 3 months of sustained release of BMP-4. All are compared with untreated, naive control SR. Mid, mid-belly; ten, tendon. *Statistical difference from control.

half life is short.²⁴ However, based on the monocular deprivation literature,²⁵ short-term treatments would not permit motor or sensory adaptations that could secure long-term alignment success. On the other hand, excessive duration of treatment effect is also undesirable because it may place the patient at risk for consecutive tropias opposite to the original misalignment. Coupled with duration of treatment is the effect of treatment on the rotational position of the globe. Treatment that results in a large change in alignment and a long duration of effect may be most effective for large-angle strabismus, for example, but would be excessive for smaller angles. In the present study, we found that 3 months of sustained exposure of adult rabbit EOM to BMP-4 resulted in significant decreases in force generation and mass. Even at 6 months, essentially 3 months after treatment had stopped, force generation remained significantly decreased. The return of muscle cross-sectional area and mass to within normal limits suggests that with sufficient time postimplantation the muscle force generation would also return to normal control levels. The clinical effects of sustained treatment with BMP-4 will need to be elucidated with additional studies in nonhuman primates before human trials can begin.

Although there is decreased mean cross-sectional areas of fast myofibers at the 1-month treatment time point, by 3 months of sustained treatment almost all myofibers in all layers are significantly reduced in cross-sectional area. The reason for

this is unclear, as myogenic precursor cells are significantly decreased even at the 1-month time point. The normal rate of myofiber remodeling in adult control EOM is relatively slow, and this may be reflected in the time period needed for the full effect of the continuous BMP-4 exposure to be realized. This would also explain why at 6 months there is still decreased myofiber size. It should be pointed out that there are significant changes in myofiber size and satellite cell numbers 1 week after a single injection of BMP-4, but these changes are not maintained.¹⁴

The minimal changes in MyHC isoform expression suggest that the alteration in force is largely due to overall decreases in muscle mass and fiber size, and not due to altered contractile characteristics due to MyHC isoform switching. This is in contrast with changes that occur after surgical manipulation in rabbit EOM, where either resection or recession resulted in significant MyHC switching within 1 week of surgery.^{22,26} Resection as well as recession and tenotomy in the rabbit EOM demonstrated a tremendous level of adaptation on the cellular level in the treated EOM,^{22,26} so that even within 1 week the muscle has activated its regenerative cell populations. These results could explain why the success rate of strabismus surgery decreases over time in the majority of patients.¹ Future studies will be directed at how long these changes after sustained growth factor treatment are ultimately maintained.

FIGURE 11. Effect of 3 months of sustained BMP-4 delivery on the contralateral, placebo-only treated SR muscle compared with untreated, naive control SR muscles. (A) Mean myofiber cross-sectional areas of fast MyHC isoform positive myofibers. (B) Quantification of Pax7-positive cell density on the contralateral, placebo-only-treated SR muscle. (C) Quantification of MyoD-positive cell density on the contralateral, placebo-only-treated SR muscle. Mid, midbelly; ten, tendon. *Statistical difference from control.

There were compensatory changes in the number of both MyoD- and Pax-7-positive satellite cells in the untreated, contralateral SR, as well as some decrease in mean myofiber cross-sectional areas. None of these changes were as pronounced as on the treated side; however, they were still significant. This is in contrast with the much more pronounced compensatory changes in the ipsilateral antagonist muscle and the contralateral agonist muscle after recession or tenotomy surgery.²² This suggests that the BMP-4 has a significant local effect on the treated muscle, but also causes a compensatory change in the contralateral “yoked” SR similar to that in the treated muscle. Compensatory muscle changes in “yoked” muscles on the contralateral side after a unilateral surgical or drug intervention have been previously described.^{27,28} These compensatory alterations in the untreated EOM mirror similar types of changes seen in limb skeletal muscles after synergist ablation, for example,^{29,30} and those muscle changes were linked to satellite cell proliferation.³¹ It is thought that changes in the antagonist muscle help compensate for the nonlinear muscle properties of the agonist muscle and enables linear scaling of the targeted

forces and accurate movements.³² The explanation for the control of this complex relationship between agonist-antagonist as well as the innervationally yoked muscles is based on the reciprocal innervation between these sets of muscles.³³ Recent studies have provided support for the active and rapid nature of these rapid compensatory changes in both agonist/antagonist as well as in “yoked” muscle pairs.^{22,26} As BMP is known to be retrogradely transported,³⁴ we hypothesize that it has both local and motor neuron effects; we are currently investigating how the sustained exposure to BMP affects the cranial motor neurons.

This study demonstrates that sustained release of BMP-4 results in long-lasting reductions in both muscle mass and muscle force generation. The use of myogenic signaling factors and growth factors to modulate EOM force generation opens up many new potential therapeutic options for treatment of strabismus. These agents could be used alone, in combination with other pharmacological agents for treatment of agonist-antagonist pairs, or in conjunction with strabismus surgery.³⁵ Although substantial work remains to be done to bring these new treatment options into clinical use, the promise exists of future treatment of strabismus that is less invasive and perhaps more effective in delivering long-term motor and sensory success.

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