Summer 8-1-2013

Post-Injury Calcium Chelation Rescues Skeletal Muscle Regeneration in Mice

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Post-Injury Calcium Chelation Rescues Skeletal Muscle Regeneration in Mice

Honors Thesis

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August 2013
Abstract:

Antibiotics, surgery and organ transplants have pushed average lifespans towards the upper limits of the human body. Drastically reduced morbidity from infection, toxins and traumatic injury have allowed ever greater portions of the populace can reach eighty or ninety years old before dying of old age. Despite the increased role of aging as a source of morbidity, many aspects of aging are poorly characterized.

Sarcopenia, progressive muscle loss, and loss of adult myogenic potential, the ability to produce new muscle tissue from adult stem cell sources, are key causes of decreased mobility and strength in aged individuals. If more youthful muscle quality could be restored in old patients they would experience greatly improved quality of life and perhaps even longer lifespans. Satellite cell populations are known to decline sharply by 6-7th decade of life but traditional treatments for sarcopenia, namely exercise intervention, have been shown to exacerbate the degeneration in aged such patients.

Previous studies have identified key regulators of the myogenic repair pathway and implicated two regulators in aged sarcopenia: Notch and TGF-β. In vitro expression studies have found that these genes act on the satellite cell, the resident stem cell of adult muscle tissue, to regulate its ability to break quiescence to begin regeneration and also show that genes show expression defects during aging. Exogenous stimulation of the Notch receptor by calcium chelators or protein ligands can be used to rescue/restore young regenerative potential in old mouse cells and demonstrate that the defect is not cell-
autonomous. This paper reports an *in vivo* pilot study to investigate whether a calcium chelation method of Notch receptor stimulation and enhanced pathway activation will rescue efficient regeneration in a mouse model of skeletal muscle repair. Several candidate chelators are screened to test for toxicity as well as pro-regenerative function.
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Acknowledgements:

This work was carried out under the guidance of researchers from the UConn Health Center, specifically from the Center on Aging and the Carlson Lab. I would like to personally thank Dr. Morgan Carlson for accepting me to this project, assisting during the injury and harvest of the muscle samples, guiding me through the writing process, as well as invaluable mentorship throughout the design of this project. Further, I wish to thank Victoria Greenwood for her assistance during the selection of chelating agents and compiling of data. I also extend my gratitude to Dr. Anu Maharjan for her guidance during the data parsing and writing portions of this project. Additionally I would like to thank Dr. Kenneth Noll, my honors advisor, for his assistance in the revision process.
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Introduction

Skeletal Muscle and Satellite Cell Overview:

Skeletal muscle fibers (myofibers) are very unusual cells: not only are they a rare example of terminally-differentiated, multi-nucleated cells but muscle fibers also contain repeated actin-myosin units called sarcomeres and a small nuclei to cytoplasm ratio[1]. Myofibers contain large numbers of peripherally located nuclei which reside along the length of the fiber just under the myofiber plasma membrane (sarcolemma) and beyond the architecture of the sarcomeres. Myofibers do not originate as single cells; myofibers result for the fusion of many muscle progenitors whose fused plasma membranes give rise to the sarcolemma[2]. Once incorporated into the muscle fiber these nuclei loose the ability to divide. When muscle fibers are damaged myonuclei from other regions of the fiber cannot proliferate to replace those losses nor can division occur at the fiber level to replace fibers which are destroyed entirely[3].

Their inability to divide makes myofibers dependent on resident adult stem cell populations for tissue homeostasis and repair. The resident stem cell of skeletal muscle tissue is known as the satellite cell and although other cell types have been shown to be able to contribute to muscle repair, the large majority of regeneration results from satellite cells and their progeny [1]. Satellite cells make up only ~4% of the nuclei within adult muscle yet maintain the integrity of the entire tissue [4]. However in aged patients satellite cell numbers drop to .6%-3.4% of nuclei present within the muscle [3]. These cells can be characterized by a larger nuclear to cytoplasmic ratio, residence between the sarcolemma and
basal lamina and a number of marker proteins such as M-cadherin, Pax7 or NCAM [1]. These small, largely quiescent cells play a pivotal role in muscle tissue maintenance and regeneration. When actively dividing, satellite cells are capable of producing large numbers of muscle progenitors as well as renewing the satellite cell pool in neighboring muscle tissues. Satellite cell-derived muscle progenitors are the only means by which myonuclei can be replaced and can even give rise to entirely new fibers [3].

Like many stem cells, satellite cell division leads to two distinct populations: additional satellite stem cells, also known as renewal cells, and transit-amplifying cells [6]. Transit-amplifying cells are the transitory progenitor cells which proliferate and mature into the new differentiated muscle tissue needed immediately after injury. Renewal cells, on the other hand, are vital for long term muscle maintenance because these cells can replenish satellite cell numbers and ensure that regenerative potential is not consumed by successive rounds of regeneration [6]. The expenditure model posited that myogenesis was dependent on a progenitor cell with a finite number within an adult, such as oocytes, and that as progenitors were expended after each injury the myogenic potential of the tissue would decrease until even tissue homeostasis could no longer be maintained. The identification of renewal cells falsified this model and led to investigations of signaling changing in aged stem cells.

Satellite cell-mediated myogenesis follows a well characterized path of differentiation which starts with activation of the satellite cells near the site of injury [7]. Activation then leads to the formation of a transit-amplifying cell: the
myoblast. Myoblasts are partially differentiated cells that undergo successive rounds of division to proliferate and form myocytes. In parallel with this proliferation the area of cell death becomes inflamed and dead tissue is removed by macrophages and other immune cells that infiltrate from the circulatory system. After proliferation is complete myocytes can either fuse with damaged fibers to replace lost nuclei or fuse with each other to form nascent myotubes. Myotubes show many of the characteristics of myofibers but can be distinguished by prominent, centrally located nuclei that migrate the outer edges of the plasma cell membrane upon maturation [5].

Transit-amplifying cells, in contrast, move out of the satellite cell niche, partially differentiate and undergo vigorous proliferation to produce large numbers of muscle precursors: myoblasts [7]. These myoblasts further mature either by fusion with damaged muscle fibers or by synchronous membrane fusion with other myoblasts. Fusion with existing fibers promotes repair while mass fusion with other myoblasts allows de novo formation of myotubes. Myotubes undergo a final round of differentiation as they assemble actin-myosin contractile machinery and move their centrally located nuclei to the periphery [6]. Once these changes are complete myotubes become terminally differentiated myofibers and begin to take part in normal muscle contractions. Upon complete differentiation these newly formed myofibers establish new satellite cell niches which can be colonized by satellite cells, such as those from the renewal population [2].
Satellite Cell Niche and Breaking Quiescence:

Later studies instead upheld a niche model of age-based degeneration which posited that the muscle environment undergoes changes during the aging process that inhibit proper regeneration despite ample supply of relevant repair cells. One critical change uncovered during the aging of the muscle niche is the imbalance of Notch and TGF-β [7]. As muscle ages TGF-β levels rise until muscle regeneration becomes compromised and degeneration begins [8]. Studies also found that exogenous stimulation of Notch receptors could restore regenerative function despite high levels of TGF-β signal [6,10]. This phenomenon was eventually linked to the ability for satellite cells to break quiescence despite defective signaling in the niche [9]. High TGF-β induces rapid up-regulation of Cyclin-Dependent Kinase inhibitor (CDKi) genes, preventing the satellite cell from properly activating and engaging in productive repair [10]. Activated Notch was discovered to physically outcompete downstream TGF-β signaling effectors for their affinities to CDKi promoter regions[10].

Satellite cells are normally quiescent, only actively proliferating in response to external signals. As resident stem cells, satellite cells particularly benefit from the lower mutation load and resistance to damage that result from long periods of quiescence. It is therefore unsurprising that breaking quiescence requires successful transduction of pro-regenerative signals or that there are endogenous signals (such a TGF-β) which maintain the quiescent state. It is
possible that together Notch and TGF-β allow satellite cell sensitivity to be
adjusted so that mass activation and the production of transit-amplifying cells
only occurs during injury when the production of such cells is more valuable than
maintaining a low mutational load within the satellite cell population.

Studies of satellite cell activation revealed that if old satellite cells were
able to break quiescence despite high levels of TGF-β, their transit-amplifying
progeny were able to complete myogenesis efficiently[8]. These results
suggested that this singular defect in satellite cell regulation was largely, if not
completely, responsible for the drop in myogenic potential observed in aged
patients.

**The Notch Receptor:**

The Notch receptor is a single pass trans-membrane receptor (see Figure
2) which undergoes proteolytic cleavage in the Golgi network and is trafficked to
the plasma membrane as a hetero-dimeric complex [9]. It has been
experimentally demonstrated that calcium depletion by treatment with EDTA is
sufficient to induce dissociation of the Notch extra cellular domain (N^{EC}) [13].
Without N^{EC} the remaining Notch protein undergoes a conformational change
which leads to release of the intracellular domain (also called active notch or
Notch*) from its membrane anchor [13]. Once released from the membrane,
Notch* is free to diffuse throughout the cytoplasm where it is known to traffic into
the nucleus [9].
Once within the nucleus the Notch* fragment can act as a transcription factor and activate the expression of genes such as CBF-1[16]. It has been suggested that the calcium dependent stability of the Notch complex reflects direct coordination of calcium ions by the amino acids of one or more subunits but further experimentation is needed to confirm this possibility[12]. The low stability of Notch* remains an obstacle to more thorough characterization of the Notch signaling pathway.

**Figure 1- Histological Artifacts**
The newly formed fibers can be easily distinguished from both resting muscle and non-muscle cellular materials. (Left) This field shows normal resting muscle. Resting myofibers can be recognized by their large eosin-retaining cytoplasms and their peripherally located nuclei. The white river-like gaps between some of the myofibers of this field are stretching artifacts which result from the sectioning process and do not reflect actual gaps that existed in the muscle before removal from the animal. (Right) This field also shows resting muscle. The jagged white shapes within the cytoplasm of these cells are also an artifact, resulting from the cyro-freezing process. If jagged ice crystals form during freezing they can damage the cell membrane and prevent proper staining, resulting in unstained sections of cytoplasm or nucleus.
Investigations into structure and activation of Notch revealed that Notch is endogenously activated by membrane-bound protein ligands such as Jagged or Delta [12,14]. These proteins can act on Notch receptors during cell-cell contact and induce changes in Notch to produce the active Notch (Notch*) fragment after proteolytic cleavage. Binding of the correct ligand to Notch’s extracellular domain results in a trans-membrane signal that targets the Notch intracellular domain for cleavage[12]. The resulting cleavage frees the Notch* fragment of the intracellular domain for its membrane tether. Once able to diffuse freely throughout the cell Notch* is trafficked to the nucleus where it acts as a transcription factor and establishes expression levels favorable for satellite cell activation [14]. Notch characterization studies also revealed that soluble, recombinant proteins of these ligands had an antagonistic effect on Notch signaling and blocked positive signaling [15]. Recombinant ligands would work
like their endogenous counterparts only if anchored to ECM, cell membranes or other semi-rigid structures [15].

While immobilized proteins are easy to introduce in a culture dish it presents a substantial barrier to clinical applications of Notch agonists. Muscle tissue is deep and incurs damage with each needle insertion; a soluble therapeutic would be able to be delivered by fewer injections, reducing needle damage, and distribute more evenly throughout the injury. Structural characterizations of the Notch protein revealed calcium ion interactions within the protein structure and it was found that calcium depletion could lead to Notch activation, although by a different mechanism than Jagged or Delta signaling [13]. Without ample calcium supply the Notch receptor’s extracellular domain undergoes structural changes which induce activation of the intracellular domain. In this manner calcium depletion mimics ligand binding at the regulatory domain and transmits it to the active domain within the protein.

**Calcium Chelators:**

Soluble calcium chelators, especially EDTA, are well characterized small molecules which can dramatically decrease the concentration of free calcium ions in solution [13]. The electron rich hetero-atoms of the EDTA and similar compounds have a high affinity for the empty orbitals of the calcium ion and the reversible reaction equilibrium strongly favors the chelate product (Figure 4). Formation of this chelate depletes free calcium which is a reactant for the Notch-calcium equilibrium. Depletion of a reactant favors the reverse reaction and thus Notch-calcium complexes destabilize as free calcium levels drop. Ultimately the
affinity of EDTA for calcium is high enough to destabilize a large number of Notch-calcium complexes which in turn produces Notch* and positive Notch signaling [13].

**Figure 3 – Histology of Injury and Regeneration**

(A) A field of newly regenerated fibers. Regenerated fibers could be newly formed fibers or existing fibers that recently underwent repair. In both cases these fibers can be distinguished from their resting counterparts by their central nuclei and increased interstitial space. As these fibers mature the spaces between them will shrink while their nuclei move to just below the surface of the plasma membrane. (B) An area of injury without subsequent regeneration. There are a large number of cell nuclei in the center of the field, but these cells do not possess large cytoplasm nor strong eosin-staining. These cells might be immune cells, fibroblasts, adipocytes or incompletely differentiated muscle precursors.
Specific Aims:

The ultimate purpose of this pilot study was translate previous work on calcium-depletion Notch activation into an *in vivo* model. The experiments were designed to reveal any off-target effects or efficacy concerns which might render chelation a poor clinical tool. It was hypothesized that calcium chelation would lead to local Notch activation and greatly increase successful regeneration with

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**Figure 4 – Calcium Chelators and Coordination Structure**

A) The structure of EDTA. B) The structure of EGTA. C) The structure of BAPTA, note this chelator contains two aromatic rings. D) The coordination confirmation of soluble EDTA while chelating a divalent cation (reproduced from ). Although the structures are shown in unionized form, in aqueous solution the acidic hydrogen atoms within each molecule’s carboxyl moieties would ionize to yield carboxyl-anions. It is the anionic species which is responsible for the hexa-dentate
minor side effects, such as transient inability to form blood clots, but no side effects which might disqualify for further testing. To test this old, and therefore regeneration impaired, mice were injured and later injected with chelators. Additional mice were injected only with a chelating agent and used to screen for possible off-target effects that might be obscured by the cardio-toxin injury. Finally control mice were injected with solvent solutions ensure any effects observed could be confidently ascribed to the chelators.
Materials and Methods

In this pilot study, cardiotoxin (CTX-1) obtained from Sigma-Aldrich was used to produce local cytotoxicity and acute muscle injury in old mice via intramuscular injections of CTX-1 into the tibialis anterior and gastrocnemius. Mice were handled and sacrificed in accordance with the UConn Health Center animal care policies. Injections of CTX-1 produce well characterized,
reproducible injuries [16]. The mice received 10 microliter injections of 0.1 microgram per microliter cardiotoxin in phospho-buffered saline for a total injection of 1 microgram of toxin. This injection creates a pocket of cell death in the center of the muscle belly emanating from the needle track. During recovery this pocket is cleared of dead muscle fibers and colonized by new tissue; in regeneration-competent mice the injection pocket becomes filled with newly formed and repaired muscle fibers, while in regeneration-impaired mice the area of fiber death becomes infiltrated by neighboring tissues, immune cells or acellular fibrosis.

Three days following initial injury mice were injected at same site within the muscle belly with a calcium chelating agent: EDTA, EGTA, BAPTA or a control saline solution. The chelators were injected at 2mM and 5mM concentration into left or right legs respectively. These concentrations were chosen based on previously conducted titration studies (data not shown) which indicated that these concentrations were capable of inducing Notch activation following injury in 10 month old mice. Chelating agents were introduced on the third day following injury because that time point coincides with the time of maximal satellite cell activation in regeneration-competent young mice.

Three days post-injury was also chosen for injection of the chelator to avoid any confounding interactions between the calcium chelators and the calcium-dependent toxic mechanism of the cardiotoxin had they been co-injected. After injection with a chelator, the mice were allowed to recover until seven days post injury when they were sacrificed and their muscles harvested for
analysis. At seven days post injury newly regenerated fibers can still be distinguished from resting fibers by their centrally located nuclei.

Once harvested, the injected muscles were imbedded into cutting media and then processed into tissue micro-sections sections of the muscle cross-sections. These tissue sections were stained with Eosin and Hematoxylin so that regeneration could be examined and quantified histologically. Newly formed myotubes retain centrally located nuclei within their syncytium while matured fibers possess nuclei pushed to the boarder of the plasma membrane. Fibers were identified or excluded as regenerated by observing the position of their nuclei in cross-section using bright-field microscopy. These images were processed using two ImageJ features: manual cell counting and pixel area measurement. Regenerated fiber counts, normalized for area of regeneration, were used as the metric of regenerative efficiency. Regenerative efficiency for each condition was determined using three replicate sections from the same muscle while error bars were created using standard deviation.

**Statistical Analysis:**

For the quantitative data presented all conditions were based on three replicate sections from each muscle but each condition was tested in a single mouse. Statistical significance was determined by the results of a two-tailed T-test. Differences with $P < .02$ were considered statistically significant.
Results

Rescue of Regeneration:

Figure 6- Post-Injury Calcium Chelation Increases Regeneration

To determine the effects of calcium chelation on regeneration, mice were injected with cardiotoxin (CTX-1) to induce acute injury in the gastrocnemius muscle. At three days post injury the mice were injected with a calcium chelating agent, or phosphor-buffered saline (PBS) control solution, to induce Notch activation and rescue regenerative function. Regenerative function was determined by regenerated fiber count per unit area within histology fields. Error bars represent 1 standard deviation from the mean. All three calcium chelators (EDTA, EGTA and BAPTA) show statistically significant increased regeneration (indicated by *) at both 2 mM and 5 mM as compared to the two control solutions with P < .02. These results are consistent with calcium-depletion mediated Notch activation and Notch-mediated rescue of regenerative function.

All of the tested concentrations of calcium chelator resulted in statistically significant increases in regeneration (Figure 5). These results are exactly what was expected based upon previous in vitro experiments and present histology akin to younger mice. There is an unusual decrease in regeneration in the higher
concentration of EGTA as compared to the lower concentration of EGTA. This difference was not statistically significant, but additional toxicity screens should to be conducted to quantify any myotoxic characteristics in the chelators.

Though there were no statistically significant differences in regenerative efficiency between the three chelators or either concentration, there was a noticeable increase in spacing between the fibers at 5mM of any chelator as compared to 2mM of the chelator (Figure 6). This phenomenon was not observed in titration studies but inter-fiber spacing could not be reliably quantified in those studies either. Representative samples of each condition have been included below.
Figure 7 – Representative Histology Fields
(A) Saline Control (B) CTX Control (C) EDTA 2mM (D) EDTA 5mM (E) BAPTA 2mM (F) BAPTA 5mM (G) EGTA 2mM (H) EGTA 5mM
Note: All fields in this figure are at the same scale.
Chelation-Induced Injury:

Those mice injected with only the calcium chelator at day 3 show the histological hallmarks of injury with no clear signs of regeneration and bled for longer from these wounds. The presence of the chelator is the only difference in the injection received by these mice and the control mice it can be inferred that the injected concentration of chelator is toxic to resting fibers (Figure 3 and 8). It should be noted that samples were harvested at day 7 which is only four days after injury is believed to have taken place. Therefore the regeneration, or lack there of, observed in these fields cannot be directly compared to the muscles injured with cardio-toxin at day 0. It is unclear if these muscles might have shown any regeneration had they been harvested at three day.

Figure 8 – Cytotoxicity of Chelators
A) Muscle injected with control solutions at both day 0 and day 3 show no evidence of injury.  B) Muscle injected with a control solution at day 0 and a calcium chelator at day 3 show evidence of fiber death, increased clustering of nuclei but very little regeneration. Field B superficially resembles the failed regeneration histology found in old injured mice. This cytotoxicity was not found in the case of BAPTA.
Discussion

Potential Application for Acute Muscle Injury:

The results of this pilot study suggest that calcium chelators represent a viable treatment to promote productive muscle regeneration in old injured patients and that more robust testing should be conducted. The statistically significant increase of regeneration in calcium chelator-treated muscle injured is consistent with the model that calcium chelation leads to widespread activation of Notch signaling in previously quiescent satellite cells. Once assisted to break quiescence these old satellite cells are able to follow the myogenic pathway from amplification to complete myotube development without further intervention.

Chelation therapy could be extremely useful in areas with poor access to healthcare. Because therapy is administered three days post-injury patients who must travel or wait to seek medical assistance, especially in cases where injury has rendered the patient unable to walk, could still receive the chelator at the optimum time. Aqueous chelator solutions are also relatively stable and could be stored for short periods without refrigeration, making them easier to deploy in areas with poor medical infrastructure than recombinant proteins or other pharmaceuticals.

Toxicity to Resting Fibers without Regenerative Induction:

Despite the pro-regenerative effects of these chelators, it also appears that calcium chelation is toxic to resting muscle fibers and does not promote subsequent regeneration.
Several mechanisms might explain these results; the chelating agents could be toxic at the injected concentration but as the agent diffuses towards the edge of an injured area the concentration drops below the cell death threshold but remains above the threshold to activate Notch. Since the area of injection is already largely clear of cells at day 3 post injury, there is no significant loss near the injection site but the neighboring satellite cells are still activated to begin regeneration. Alternatively calcium chelators are generally toxic to resting myofibers but only induce activation in satellite cells that have been sensitized by other injury signals such as inflammation or have moved into the wound where they are more exposed than when between the sarcolemma and basal lamina. In this case injection of the chelator caused injury but not regeneration because the Notch stimulation occurred before satellite cells were sensitized by other niche factors. Most likely, the observed histology reflects the fact that these injuries have not had enough time to clearly show regeneration and that chelator injection at day 0 instead of day 3 might have yielded opposite results.

Additional experiments are needed establish whether chelators alone can induce both injury and regeneration, specifically assays of regeneration seven days post-injury in mice injected with chelators at day 0, day 3, and both days along all necessary controls. Such an experiment would confirm or deny whether the lack of regeneration observed was an artifact resulting from harvesting the muscle too early after injury or reveals a dangerous side-effect of therapy.

If chelators are toxic to healthy tissue but only induce regeneration in already injured tissue, chelation therapy is only beneficial when a patient has
undergone an acute injury, such as a surgery, where both the time and area of injury are well defined. In such cases chelation could be injected in a confined area to bolster regeneration to the injured area and minimize damage to surrounding tissues.

If chelation is toxic to resting muscle but also induces regeneration without prior injury then chelation therapy could be used in cases where injury is present but the area of injury is poorly defined. In this case any off target use of chelators would result in unnecessary but ultimately successful regeneration with minimal risk to long term tissue function.

Regardless of the need for prior injury, chelation’s toxicity to resting muscle suggest chelation therapy is a poor treatment for chronic degeneration such as sarcopenia which does not produce a contiguous area of injury which could be targeted for chelation [17]. Sarcopenia treatment might be better addressed by a non-toxic means of inducing global, prolonged, low-level activation of Notch to allow for slight global regeneration to counterbalance the sarcopenia-mediated global degeneration [18]. Calcium chelators do not appear to fit these criteria.

**Future Directions:**

The results of this paper present several opportunities for additional research. First and foremost these experiments are ultimately a pilot study and this work should be reproduced in a larger sample size. A larger pool of mice, perhaps multiple mouse lines, could reveal any differences between lines and confirm that these results do not reflect a small sample size. Some of the
chelating agents tested, specifically EDTA, have already received FDA approval for treatment for heavy metal poisoning and therefore would be ideal candidates for eventual human clinical testing.

However a major caveat of these results is that Notch activation has only been inferred by increased regeneration in this study. A followup study using muscle harvested at four days post injury (one day post chelation) or even sooner, and subsequent immuno-histochemical detection of the Notch* fragment are needed to confirm that Notch activation actually occurred while experiments comparing chelation with or without co-injection of a Notch antagonist, such as soluble Delta, are needed to test if Notch activation shares a casual link with regeneration rescue [15]. Calcium is known to regulate a large host of biological processes; it is possible that the rescue observed is due to calcium-mediated changes in other signaling pathways [19]. The prominence of Notch in many stem cell regulatory networks [20] requires that off target effects be extensively characterized.

Given these results, optimization studies should also be conducted to test if the chelator toxicity can be eliminated without compromising Notch activation. Such alterations would allow calcium chelation to be serious considered for long term use as treatment for chronic conditions like sarcopenia. If such optimization fails future researchers could consider a more selective molecule that specifically strips the Notch receptor of calcium ions, rather than pull all such ions out of solution. Even so, the Notch receptor plays a role in many other pathways relevant to the injury-adjacent environment, especially angiogenesis [20].
Neighboring neurons, vessel endothelial cells and osteocyte progenitors which express Notch could be effected in significant ways even by a Notch-specific stimulant [20]. As with all injury inflammation can occur, but injury macrophages are known to play a role in proper regeneration of skeletal muscle and therefore any effects on these cells must also be studied in the context of chelation therapy [21].

During this study it was also observed that mice bled more from injections of calcium chelators. It is suspected that transient inability to clot results from the inability to activate a calcium-dependent clotting factor. Future studies should be conducted to quantify this observation with proper controls and ensure that poor clotting post-treatment does not pose a significant threat to patients. The depletion of free calcium almost certainly effects the ability of local neurons to generate action potentials. Neural excitotoxicity, for example, would both negatively impact the niche and possibly effect regeneration: de novo fibers might have a critical window for forming neuromuscular junctions post-injury.

Although chelation treatment is meant for older patients, future longitudinal studies of repeated injury, activation and regeneration should be conducted. It is unclear how the self-renewal pathway of satellite cells may be effected by strong Notch activation. If chelation suppresses the formation of the renewal cell population then long-term use of this treatment could deplete the muscle of satellite cells entirely.

In conclusion, the results of this study demonstrate that calcium chelators can rescue skeletal muscle regeneration in mice with age-based regenerative
defects. These results show promise as means to translate Notch-mediated regenerative rescue into the geriatric clinic. If these results hold up under more robust testing conditions and replication, then testing in an alternative animal model and eventual human testing can begin.
Works Referenced


