Muscle Regeneration is Improved in Pregnant Mice: A Potential Form of Parabiotic Pairing

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Introduction: Muscle regenerative potential declines with age, and this decline has been attributed to a diminished responsiveness of tissue-specific stem and progenitor cells [1]. Heterochronic parabiosis for the study of the effects of aging on stem cells and their niches has been widely investigated [2] and these studies have demonstrated that aged progenitor cells can be rejuvenated by exposure to a young systemic environment. One interesting idea is that pregnancy could represent a natural form of parabiosis. To test this hypothesis we evaluated the muscle regeneration potential of pregnant mice via cardiotoxin (CTX) injury. Interestingly, our results indicate that the pregnant mice showed an acceleration in muscle healing when compared to the non-pregnant control mice at the same time points following muscle injury, including improvements in muscle histology, superior muscle regeneration, and a reduction in inflammation and necrosis. These beneficial effects could be attributed to both elevations in female hormones and heterochronic parabiosis between the mother mice and their fetuses.

Methods: Animals and muscle injury:
The CD1 pregnant and non-pregnant mice were purchased from Jackson Laboratories. All animal protocols used for these experiments were approved by the University of Pittsburgh’s Animal Care and Use Committee. 4uM of CTX was injected intramuscularly into the gastrocnemius muscles of the recipient mice. Seven days after injury the mice were sacrificed, and the muscles were harvested and flash frozen in liquid nitrogen-cooled 2-methylbutane and cryosectioned (10um sections).

Histochemistry:
H&E staining was performed according to the manufacturer’s instructions.

Immunohistocytochemistry:
The cryosections from the gastrocnemius were fixed with 5% formalin, blocked with 5% Donkey serum, and then incubated with an antibody against mouse IgG (Biotinilated) to determine the extent of muscle fiber necrosis. An antibody against embryonic myosin heavy chain (e-MyHC) was used to evaluate myogenic regeneration and an antibody against F4/80 (macrophage marker) was used to analyze the extent of inflammation in the muscle tissues. Streptavidin Cy3 conjugate, Alexafluor 594 conjugated anti-mouse IgG and Alexafluor 488 conjugated anti-rat IgG were used as secondary antibodies.

Results: 1. Muscle histology of pregnant mice improved faster than non-pregnant mice
H&E showed histology improvement in the muscles of the pregnant mice at the 7 day time point following the muscle injury. We observed many centrally nucleated muscle fibers in the muscles of the pregnant mice, while the non-pregnant muscle revealed large areas of necrotic fibers and massive cellular infiltration (Figure 1).

2. There was less necrosis in the injured muscles of pregnant mice than non-pregnant mice
Mouse IgG staining showed that there were less necrotic muscle fibers in the muscles of the pregnant mice compared to the non-pregnant muscles (Figure 2).

3. There was reduced necrosis and increased regeneration in the injured muscles of the pregnant mice
Less macrophage infiltration and more newly regenerated muscle fibers were found in the muscles of pregnant mice in contrast to the non-pregnant mice (Figure 3).

Discussion: The decline of tissue regenerative potential is a hallmark of aging and may be due to both age-related changes in tissue-specific stem cells and the systemic environment. Many investigators have used parabiotic pairings between young and old mice to study the role of cells and microenvironment. In parabiosis, animals share their circulatory systems; similar to what occurs between a mother and fetuses. In this study, we compared the muscle healing processes of pregnant and non-pregnant mice following CTX injury. We found that the muscles of the pregnant mice regenerated faster than non-pregnant mice, suggesting that this beneficial effect could due to either circulating factors from the fetuses or an increase in female hormones, since female hormones have been reported to promote skeletal muscle regeneration [3]. Future studies will focus on using pseudopregnant mice as controls instead of non-pregnant mice to rule out the role that female hormones play in promoting muscle regeneration.

Significance: The use of pregnant mice as a form of natural parabiosis could give us insights into the study of aging and aging
related diseases.

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Figure 3

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