INTRODUCTION:
Although muscle contusions are capable of healing, incomplete functional recovery often occurs. We have previously reported that when a safe human dose of losartan (10mg/kg/day), one of the FDA approved Angiotensin II Receptor Blockers (ARBs) and anti-fibrotic agent, that block TGF-β1, was administrated 3 days after injury it can promote functional improvement, muscle regeneration and decrease fibrosis at 4 weeks after injury [1]. Moreover, some reports have shown that Platelet-Rich Plasma (PRP), which includes many kinds of growth factors, including TGF-β1, can accelerate muscle healing after injury [2]. Our hypothesis that losartan treatment along with PRP can further accelerate the muscle healing process compared to the use of losartan or PRP treatment alone. The purpose of the current study is to investigate the potential functional improvement of contusion injured skeletal muscle in mice using both losartan and PRP in combination.

METHODS:
The contusion injury was created on the tibialis anterior (TA) muscle of C57BL/6 wild-type mice. All injured mice were randomly assigned to 1 of 4 groups: (1) fed plain drinking water (control group, n=14); (2) administered 10mg/kg/day of oral losartan starting 3 days (losartan group, n=14), (3) injected with 20 µl of PRP in the injured TA muscle 1 day after injury (PRP group, n=14), (4) combined treatment as (2) and (3) (PRP/losartan group, n=14). PRP was isolated from the rat whole blood via a double centrifuge technique. The concentration of the platelelets obtained in the PRP was 5.5 times higher than that of the whole blood. All animals were sacrificed at 1, 2 and 4 weeks post-injury to evaluate, histologically and physiologically, muscle healing (Fig.1).

Histological evaluation was performed using hematoxin and eosin staining to monitor the number of regenerating myofibers, and Masson’s trichrome staining was used to measure areas of fibrotic tissue within the injury sites. Immunohistochemistry was performed to evaluate angiogenesis in the injured site. Statistical analysis was performed with Scheffe’s F test as a post hoc test. Statistical significance was defined as p < 0.05.

RESULTS:
Injection of PRP enhanced muscle regeneration in injured muscle:
After hematoxin and eosin staining, the centronucleated regenerating myofibers in the injured muscle were counted and compared among the groups at 2 weeks post-injury. PRP treated groups showed significantly higher numbers of regenerating myofibers (PRP group, 124.9±20.7; PRP/losartan groups, 140.4±19.1/hpf) compared with control group at 2 weeks post-injury (54.9±14.5/hpf) (Fig. 2A).

Administration of losartan decreased fibrosis formation in injured muscle:
After Masson’s trichrome staining, the area of fibrotic scar tissue was evaluated and compared among the groups at 4 weeks post-injury. Losartan treated groups showed significantly less fibrotic area ((losartan group: 4.05±2.35% and PRP/losartan group: 2.05±1.30%) compared with control and PRP groups (21.4±6.14 and 9.78±3.40%, respectively) (Fig. 2B).

Injection of PRP and the administration of losartan showed rapid and greater improvement of muscle strength:
At 2 weeks post-injury, PRP treated groups (PRP and PRP/losartan groups) showed significantly greater specific peak twitch and tetanic forces (twitch; 42.5±12.5 and 46.5±10.2, tetanic; 77.9±11.5 and 87.0±26.5g/cm²) than the control group (twitch; 24.1±8.9, tetanic; 47.6±7.26 and g/cm², respectively). Interestingly, PRP/losartan group showed significantly greater specific peak twitch and tetanic forces (twitch; 60.2±11.2, tetanic; 112.2±25.9g/cm², respectively) than the other groups at 4 weeks after the injury (control group: twitch; 24.4±5.1, tetanic; 59.1±13.3, losartan group: twitch; 39.2±3.0, tetanic; 81.9±23.2, and PRP group: 39.9±13.8, tetanic; 84.2±20.5g/cm², respectively). Moreover, there was no significant difference between the PRP/losartan group and non-injected group (twitch; 59.5±18.7, tetanic; 125.4±30.4g/cm², respectively) (Fig. 3).

Injection of PRP and the administration of losartan enhanced angiogenesis and reduced p-Smad2/3 in injured muscle:
CD31 expressing areas in the injured TA muscles were measured at 1 week post-injury. The PRP injected groups (PRP, PRP/losartan groups) showed significantly greater angiogenesis areas (4.17±1% and 5.18±1.61%, respectively) when compared with control group and losartan group (0.94±0.82 and 1.38±1.29 %, respectively) (Fig. 4A). pSmad2/3 areas in the injured TA muscles measured at 2 weeks post-injury. Losartan treated groups (losartan group: 0.82±1.15%, PRP/losartan group: 0.92±0.54%) showed significantly less pSmad2/3 positive area than control and PRP groups (4.22±1.57 % and 3.22±0.59 %, respectively) (Fig. 4B).

DISCUSSION:
The combination treatment using losartan and PRP following a contusion injury can accelerate skeletal muscle healing. We observed a larger number of regenerating myofibers, greater angiogenesis, less fibrosis, and better functional recovery in the PRP/losartan group. These results suggest that the combination treatment of PRP and losartan after skeletal muscle injury could be more effective than the individual treatments alone and the beneficial effect of combining PRP and losartan is likely related to the inactivation of TGF-β1 within the PRP through losartan treatment.

Significance:
Our study will be helpful to contribute to the development of biological treatments to accelerate muscle healing.

ACKNOWLEDGEMENTS:
The authors are grateful for technical assistance from Jessica Tebbets, Michelle Witt, Oyster Nicholas and James H. Cummins. Funding support was provided by the Department of Defense (W81XWH-06-1-0406) and AFIRM.

REFERENCES: