A Novel Trauma Induced Mouse Heterotopic Ossification Model

INTRODUCTION:
Traumatic injury of soft tissues with or without skeletal fractures is often accompanied by heterotopic ossification (HO), abnormal deposition of bone in soft tissues and in particular muscle. Battle field injuries including severe muscle damage, spinal cord injury, head trauma, neurological trauma and joint arthroplasty are frequently accompanied by HO. Heterotopic ossification has proven difficult to prevent and once developed is often extremely difficult to treat. A critical barrier to the development of more effective prevention and treatments is the lack of clinically relevant animal models. Although mechanical injury is believed to be key factor for the development of HO, previous attempts to create a murine HO model solely based on muscle injury were not successful. Bone morphogenetic protein-2 (BMP-2) plays a critical role in bone development and mineral deposition. Our objective in this project is to develop a new murine model of HO with intramuscular controlled released BMP-2 that incorporates muscle trauma as a critical component.

METHODS:
 Controlled BMP-2 release. In vivo controlled release of BMP-2 was achieved by mixing various doses of rhBMP-2 (Medtronic Sofamor Danek USA Inc. Memphis, TN) with a heparin-chitosan, ionic complex, in the form of a hydro gel (ExThera Inc. Stockholm, Sweden) (1). 20ug of hydro gel with 0, 0.25, 0.5, 0.75, 1, 1.5 and 2 ug BMP-2 was injected in the quadriceps muscles of 12 weeks old adult male C57/BL6 mice (JAX, Sacramento, CA) with a micro-syringe. The mice were allowed to move free in the cage after injection.

Muscle impaction injury: Six animals in the groups of 0 and 0.5 ug BMP-2 injection received a onetime muscle impaction injury after injection. This injury was achieved by dropping a metal ball of 16.3g from 100 cm high to the quadriceps muscles. The mice were allowed to move free in the cage after injection. All procedures were approved by SFVAMC Institutional Animal Care and Use Committee.

MicroCT scanning. HO formation was monitored using in vivo microCT scanning for the thighs of the animals at 1, 2 and 4 weeks after treatment. Bone volume (BV) for HO in each leg was quantified. Statistical analysis. Analysis of Variance (ANOVA) was used to compare the BV in each leg between animals receiving different doses of BMP-2. Student T-test was used to compare the BV in the legs from animals receiving 0.5 ug BMP-2 with and without mechanical injury. Statistical significance was considered when P<0.05.

RESULTS:
HO formation was observed as soon as two weeks after BMP-2 injection. Significant HO formation was observed in mice receiving 1, 1.5 and 2 ug BMP-2 injection (Figure 1). Mice receiving 0 ug BMP-2 had no notable HO after impaction at any time points in microCT scanning. However, mice receiving 0.5 ug BMP-2 injection with muscle injury showed significantly more HO than those received the same amount of BMP-2 injection without muscle injury (Figure 2). MicroCT scanning analysis showed that the mice received muscle injury has nearly 10 times more heterotopic bone volume compared to the mice without injury (Figure 3).

DISCUSSION:
Most previous models of HO rely on the use of excessive bone morphogenetic protein-2 (BMP-2), various genetic models such as fibrodyplasia ossificans progressiva (FOP), and growth factor-doped matrices or carriers such as collagen gels, tricalcium phosphate mixtures and hydrogels in the form of implants. However, the development of HO in these models is not trauma-dependent. On the other hand, muscle trauma alone has not been shown to induce HO in normal animals, as proven once again by our own result in this study. In our new mouse model, we show that muscle trauma combined with a sub-threshold dose of BMP-2, can induce HO. This data suggests that BMP-2 signaling may play a critical role in the development of HO. This novel model will serve as a powerful tool to study the mechanisms responsible for HO and to devise preventative measures and effective treatments for HO.

SIGNIFICANCE:
Trauma induced HO has a great impact on the orthopedic patients. However, the mechanisms of HO development remain unknown, partially due to the lack of appropriate animal models. In this study, we have generated a novel mouse HO model, which will facilitate future investigations for the mechanisms and possible treatments for HO.

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