Heterotopic Ossification Following Blast Amputation in Forelimbs and Hindlimbs of a Sprague-Dawley Rat

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Introduction:
Heterotopic ossification (HO) commonly complicates extremity blast amputations in soldiers in the contemporary war theater. This injury has become more prevalent due to the increased use of improvised explosive devices in modern warfare and the use of body armor that increases victim survival. High rates of heterotopic ossification are clinically important since ectopic bone growth represents a painful and devastating problem for the wounded soldier; complications related to heterotopic bone in blast amputation stumps include pain, overlying skin and muscle breakdown, poor prosthetic fitting and function, a need for surgical stump revision, and delayed rehabilitation. The objective of this project is to compare the frequency, quantity, and quality of heterotopic bone following blast amputation of the hindlimb and forelimb in a rat model.

Methods:
On an IACUC-approved protocol, twenty-five 12-week old, anesthetized male Sprague-Dawley (SD) rats, underwent controlled extremity blast amputation with a column of propelled water after detonation of a submerged explosive. The blast-injured limb was manually irrigated with a 40:1 saline/chlorhexidine solution prior to fascial closure. Minimal skin debridement was performed to facilitate primary wound healing. Thirteen rats underwent forelimb and 12 underwent hindlimb amputation. The rats were followed clinically and with serial radiographs until euthanasia at 24 weeks. Half of the specimens underwent post-mortem CT imaging of the residual limb. Three independent observers quantified and classified HO on radiographs and CTs. HO severity was defined as absent, mild, moderate, or severe and given a corresponding numerical score (0, 1, 2, or 3 respectively). Each stump was classified as one of three types. Type I was defined as new bone growth within the normal bony distribution; Type II was defined as heterotopic bone outside of the normal bony envelope, but still contiguous with the stump; and Type III was defined as ectopic bony islands developing within the surrounding soft tissue. The radiographic system for quantifying and classifying HO was validated using a kappa statistic. The severity and type of HO was compared amongst forelimbs and hindlimbs.

Results:
One rat did not survive forelimb amputation due to an anesthetic overdose. The remaining 24 rats survived blast amputation and closure until euthanasia at 24 weeks. All animals developed HO; one experienced a hindlimb wound dehiscence with bone protrusion at 4 weeks post blast requiring bone resection and stump closure revision. This animal subsequently developed severe HO. High interobserver reliability was seen on radiograph assessments of HO quantity and type (kappa=0.852 and 0.671, respectively). On average, hindlimbs’ HO severity was 2.08 (moderate), compared to 1.08 (mild) in forelimbs (p=0.0029). Moderate to severe HO was found in 8/12 hindlimbs vs. 4/12 forelimbs (p=0.009). Additionally, 10/12 hindlimb amputations developed Type III HO compared to 4/12 forelimbs (p=0.036). CT evaluation of HO severity was highly correlated with radiographic grade (Spearman’s r = 0.9045, p<0.001), but type correlation was weak (Spearman’s p = 0.243, p = 0.4458). Figures 1 and 2 demonstrate 24 week radiographic and computed tomography images of a hindlimb and forelimb, respectively.

Conclusions:
This simulated blast model produced a high prevalence of heterotopic ossification in the amputated limb stump without addition of any exogenous osteogenic agent. The radiographic grading scale developed for this animal model was a reliable means of assessing HO severity and type. CT assessment of severity was highly correlated to the radiographic severity. CT may have a higher sensitivity for detection of small bony islands, but this cannot be definitively concluded without the use of immediate post-injury CT scans. Hindlimb blast amputation developed more severe HO than forelimbs and more likely involved the surrounding soft tissues than the forelimb. The unexplained protective influence of the forelimb parallels the human clinical experience.

Significance:
Further understanding the pathophysiology of heterotopic ossification following blast injury is imperative to developing potential preventive and therapeutic modalities for wounded soldiers who sustain blast amputations in the field of battle.

Figure 1: 24 week images of a hindlimb amputation stump with severe heterotopic bone, type 3, noted to be present on both plain radiographs (a, c) and computed tomography 3D reconstruction images (b,d).

Figure 2: 24 week images of a forelimb amputation stump graded as mild heterotopic ossification on both radiographs (a,c) and 3D computed tomography (b,d), classified as type 1 on radiographs and type 2 on computed tomography.

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