Paper - Basic Science

Paper Session: Quantifying Fracture Healing

Hemorrhagic Shock Affects Specific Immune Cell Populations in Early Fracture Callus

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Purpose: Multiply injured patients (MIPs) are at risk for fracture nonunion. MIPs with hemorrhagic shock (HS) have a systemic immunologic response that has been associated with poor acute and long-term outcomes. It is possible that HS affects the early immunologic response in fracture callus. The purpose of this study was to determine how HS affected cell populations in early fracture callus.

Methods: 64 Sprague Dawley rats were subjected to an open tibia fracture and a crush injury to the adjacent anterior compartment muscle. Fractures were stabilized with an intramedullary pin. One-half of the rats were also subjected to pressure controlled HS with a mean arterial pressure of 40 mm Hg for 60 minutes. 32 rats were treated with extracorporeal blood purification that reduced circulating cytokines (Cytosorb; Cytosorbents) and the other 32 rats had identical extracorporeal circulation but no blood purification. This established 4 experimental groups including sham and treatment rats with or without HS. Eight rats from each group were sacrificed 3 days (3D) after injury and 8 rats were sacrificed 7D after injury. Tissue was harvested from the early fracture callus and prepared for flow cytometry (FC). FC quantified tissue concentrations (cells/mg) of lymphocytes, macrophages, and polymorphonuclear leukocytes (PMNs). Lymphocytes and macrophages were also subtyped. Cell populations were compared by t tests between groups with or without HS.

Results: In sham and treatment animals, HS reduced lymphocytes and macrophages in callus at both 3D and 7D. PMNs were less affected. At 3D, HS reduced lymphocytes, macrophages, and PMNs by 85% ($P < 0.01$), 96% ($P < 0.01$), and 88% ($P < 0.01$) in treatment animals. Likewise, at 7D, HS reduced lymphocytes, macrophages, and PMNs in callus by 65% ($P < 0.01$), 84% ($P = 0.08$), and 85% ($P = 0.07$) in treatment animals. In sham-treated animals, HS reduced lymphocytes and macrophages by 61% ($P = 0.10$) and 70% ($P = 0.10$) at the 7D time point. CD4+/CD8- lymphocytes ($P < 0.01$ all groups at both time points) were the most consistently reduced cell type by HS in both sham and treatment animals. In addition, HS reduced reparative macrophages including resident and intermediate macrophage cell types in sham animals by 73% ($P = 0.09$) and 18% (not significant) at 7D and reduced these cell types by 89% (not significant) and 81% ($P = 0.02$) in treatment animals.

Conclusion: In recent work at our institution, HS has been shown to be an independent risk factor for developing a nonunion. This study supports that HS affects the cellular constitution of early fracture callus in a rat mangled limb experiment. In both sham and treatment rats, lymphocytic and macrophage cell populations were consistently reduced in animals subjected to HS. The most significant reductions involved lymphocytes, specifically CD4+/CD8- cells which are adaptive and reparative macrophages. These data indicate how HS affects early fracture healing.

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