Elucidation of the Detrimental Synergy of Intestinal Surgery Followed by an Abdominal Sepsis – the Septic Surgical Patient.

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Introduction: Clinically, surgery is unfortunately commonly followed by an iatrogenic-induced or nosocomial-associated bacterial infection. We have shown that surgical manipulation of the intestine triggers a complex molecular cascade of transcription factor activation (MAPK p38, Egr-1, NF-κB, etc.) and the subsequence induction of multiple pro-inflammatory mediators (MCP-1, IL-6, TNF-α, iNOS) that cause the recruitment/extravasation of numerous circulating leukocytes into the intestinal muscularis externa, which together result in the development of clinical postoperative ileus (POI). Previously, we have also shown that sustained pre-exposure to bacterial lipopolysaccharide (LPS) dramatically "prevents" the development of POI. And, additionally, we published that mild hemorrhagic shock "beneficially" pre-conditions the gut to a subsequent polymicrobial sepsis. Our study is designed to elucidate the intestinal molecular, cellular and functional alterations that occur during the detrimental clinical scenario of a surgical patient acquiring an infection using a rodent model.

Methods: A standardized POI rodent model utilizing mild non-traumatic intestinal manipulation (IM) followed by a 24hr delayed intraperitoneal injection of low dose LPS (0.5mg/kg) will be used and compared to controls, IM alone and LPS alone at specified time points. *In vivo* gastrointestinal transit (GIT) will be measured using non-digestible, orally administered FITC-labeled dextran (70kD MW) and jejunal circular muscle strips will be functionally evaluated using organ bath recordings after IM (48hrs), LPS (24hrs) and combined IM+LPS (48hrs). Myeloperoxidase (MPO^+) staining of jejunal muscularis whole-mounts will assess leukocyte infiltration, qPCR will be used to quantify induction of pro-inflammatory genes, and Luminex analysis will measure tissue and serum cytokines/chemokines.

Results: Individually, mild IM or low dose LPS (0.5 mg/kg) treatment minimally suppressed *in vivo* GIT compared to control (calculated transit geometric center measurements: control=10.6±0.3, IM=9.2±2.00, LPS=7.3±0.09 alone vs. IM+LPS=5.6±0.37 (N=2-3 each). Similar synergistic results of IM+LPS on jejunal circular muscle contractility has been observed using *in vitro* muscle strip organ bath recording experiments. The synergistically delayed transit was accompanied by a significant recruitment of leukocytes into the intestinal muscularis (control=2±0.3, IM=7±1.6, LPS=3±0.6 alone vs. IM+LPS=25±2.4 (MPO^+ PMNs/200X field N=4 each). The induction of pro-inflammatory genes by qPCR and cytokine/chemokine Luminex analysis is pending.

Conclusion: We conclude that mild IM followed by a simulated infection potentiates the inflammatory response within the intestinal muscularis leading to a synergistic aggravation of POI. Thus, instead of being a protective pre-conditioning response – intestinal responses to surgery are potentiated by a subsequent septic event, which explains the detrimental clinical morbidity and mortality associated with the septic surgical patient.