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Plasma ameliorates endothelial dysfunction in burn injury

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ABSTRACT

Background: A complex inflammatory response mediates the systemic effects of burn shock. Disruption of the endothelial glycocalyx causes shedding of structural glycoproteins, primarily syndecan-1 (SDC-1), leading to endothelial dysfunction. These effects may be mitigated by resuscitative interventions.

Materials and methods: Sprague–Dawley rats were used to create small, medium, and large burns and uninjured controls. Three different intravenous resuscitation protocols were applied within each group: Lactated Ringer's (LR) alone, LR plus fresh frozen plasma (FFP), or LR plus albumin. Blood was serially collected, and plasma SDC-1 was quantified with enzyme-linked immunosorbent assay. In one cohort, Evan's Blue Dye (EBD) was administered and quantified in lung by spectrophotometry as a functional assay of vascular permeability. In a second cohort, intact SCD-1 was quantified by immunohistochemistry in lung tissue. Statistical analysis employed two-way analysis of variance with multiple comparisons and Student's *t*-test.

Results: EBD extraction from lung was significantly greater with higher injury severity *versus* controls. Extraction decreased significantly in large-burn animals with addition of FFP to LR *versus* LR-only; addition of albumin to LR did not decrease EBD extraction. Plasma SCD-1 increased in injured animals compared with controls, and changes correlated with injury severity in all resuscitation groups (significance, $P < 0.05$). Lung SCD-1 staining reflected the results in the EBD assay.

Conclusions: Addition of FFP, not of albumin, to post-burn resuscitation diminishes vascular leakage associated with large burns. Addition of colloid does not affect SDC-1 shedding as measured in plasma. Ongoing work will further define pathophysiologic mechanisms and potential therapeutic interventions to mitigate injury and promote repair of the endothelial glycocalyx.

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Introduction

For decades, many resuscitation regimens have been proposed in an attempt to prevent and counteract the systemic response caused by burn injury. A known catalyst in the initiation of this inflammatory cascade lies in the disruption in endothelial cell function, which begins with damage to the vascular endothelial surface layer, or the endothelial glycocalyx (EGL). The EGL is a protective and highly dynamic layer lining the luminal surface of vascular endothelial cells, composed of carbohydrate and protein moieties. It functions as the primary interface between the circulating blood and the endothelial cell layer and regulates pathways involved in cell adhesion, migration and signaling, as well as inflammation, coagulation, vascular tone, and endothelial permeability.[1] Its glycosaminoglycans and proteoglycans (the most abundant of which are syndecans) normally allow for ligand specificity and dynamic plasma protein interactions and can be shed into the circulation in certain states of physiologic stress.[2,3] This shedding causes exposure of vulnerable endothelial cells, as well as systemic interactions by the shed glycocalyx fragments, ultimately leading to enhanced circulating leukocyte attachment, increased vascular permeability, and propagation of the inflammatory response.[4]

Assessing the physical integrity of the EGL can be difficult even with advanced imaging techniques such as electron microscopy. For this reason, indirect methods of quantification of EGL disruption have historically been employed, either measuring circulating levels of specific EGL components or utilizing assays analyzing its dysfunction, primarily focusing on vascular permeability.[5-7] Furthermore, quantitative data have previously supported the use of plasma syndecan-1 (SDC-1) specifically as a reliable clinical biomarker of glycocalyx shedding.[8]

Glycocalyx damage has been examined in numerous pathologic states including hemorrhage and traumatic injury, sepsis, ischemia/reperfusion, atherosclerosis, and diabetes, but its behavior specifically following burn injury is largely understudied.[6] Particularly in hemorrhagic shock, increased shedding of SDC-1 has been associated with inappropriate inflammation, higher severity of pathology, abnormal endothelial cell permeability,[1] coagulopathy related to endogenous heparinization, low plasma oncotic pressure, and increased mortality in both animal and human studies.[6,9]

An early mouse model by these authors demonstrated a correlation between loss of pulmonary syndecan and increase in plasma SDC-1, prompting selection of lung tissue as a focus in this study.[10] Previous studies by other institutions utilizing radioactively tagged albumin in a small-animal model have also demonstrated pulmonary microvascular dysfunction as a result of swelling of pulmonary capillary endothelial cells and widened gap between endothelial cells following traumatic injury.[11]

Optimization of resuscitation strategies to address inflammation, coagulopathy, and endothelial permeability during the physiological response to burn injury has been a significant focus of research. Comparative effectiveness studies of various crystalloid and colloid formulations and combinations thereof have yielded important advances in resuscitation practices and improved survival.[12] However, there remains a high degree of interinstitutional discordance in protocols regarding the use of adjunctive therapies, including fresh frozen plasma (FFP) and albumin, undoubtedly due to an evolving understanding of their specific biomolecular mechanisms in this setting.

Methods

Animals

The MedStar Health Research Institute Institutional Animal Care and Use Committee reviewed and approved all procedures and animal work described. Male Sprague–Dawley rats weighing approximately 300 g with preinserted tunneled jugular venous catheters (Envigo, Frederick, MD) were used in all experiments. Animals were maintained and housed per facility standard operating procedures.

Injury

Animals were fully anesthetized before all procedures and were subsequently monitored for signs of pain or distress. Rats were thermally injured with a 10%, 30%, or 40% total body surface area (TBSA) scald burn using a previously described model.[13-15] Briefly, animals were anesthetized using isoflurane gas induction and transitioned to a nose cone for the duration of the procedure. Upon confirming a sufficient plane of anesthesia, the fur on the torso was clipped and skin was depilated using Veet Gel Cream Hair Remover (Reckitt Benckiser, Parsippany, NJ). After rinsing the skin, the animals were then secured supine within a plastic vessel with a window exposing the animal's dorsum. The dimensions of the exposure window were determined by applying the Meeh equation[16] to estimate burn areas equivalent to 10%, 30%, and 40% TBSA for the animals. Thermal injury was induced by submerging the vessel in 100°C water for 10 s with the animal secured and fully anesthetized. For animals assigned to the larger injury groups, the burns were extended from the dorsum to the flanks; flank burns were exposed for 7 s instead of 10 s to account for the thinner nature of this skin. Injury depth was confirmed by histological examination of biopsied sections of the wounds. Control animals were depilated and treated in identical fashion, with the exception of submersion into the scalding water.

Resuscitation

Animals in all groups were placed on a heating pad until recovery and were given an intraperitoneal (IP) buprenorphine injection (0.05 mg/kg) for analgesia. Animals were resuscitated by one of three different methods: lactated Ringer's (LR) only, LR plus FFP, or LR plus albumin. In the LR-only group, the control and 10% TBSA groups were resuscitated with 6.0 mL of LR administered intraperitoneally immediately after injury, and at hour 8 after burn. For the 30% and 40% injury groups, a volume of 18 mL of LR was administered intraperitoneally on the same schedule as controls. The crystalloid dosing in this