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Research

Tissue inhibitor of metalloproteinase-2 inhibits burn-induced derangements and hyperpermeability in microvascular endothelial cells

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Abstract

BACKGROUND: Burns induce microvascular hyperpermeability. We hypothesize that this occurs partly through an imbalance between matrix metalloproteinases (MMPs) and endogenous MMP inhibitors such as tissue inhibitors of metalloproteinases (TIMPs), and that such derangements can be attenuated with the use of TIMP-2.

METHOD: Rats underwent either sham or burn: serum and tissue were collected. Western blot was used to examine MMP-9 and TIMP-2 levels and MMP activity was assayed from lung tissue. Rat lung microvascular endothelial cells were used to assess monolayer permeability and evaluate the adherens junction proteins β-catenin, vascular endothelial cadherin and filamentous actin after exposure to burn serum ± TIMP-2.

RESULTS: Lung tissue from burn animals showed increased MMP activity, decreased levels of TIMP-2, and no difference in levels of active MMP-9 in burn vs control groups. Burn serum increased monolayer permeability, damaged adherens junction proteins, and incited actin stress fiber formation; TIMP-2 attenuated these derangements.

CONCLUSIONS: Burns may lower TIMP-2 levels and increase MMP activity and that TIMP-2 application in vitro may attenuate burn-induced hyperpermeability and decreases damage to endothelial structural proteins. These links warrant further investigation.

Burn patients and their management represent extremely common, but very complex, clinical challenges. Aside from the morbidity and mortality directly related to thermal injury, the physiological derangements caused by burns are equally deleterious.[1] In the acute phase, adequate resuscitation is paramount to minimize further injury from such derangements; however, its importance is often equal to the difficulty of striking the appropriate balance between enough and too little fluid.

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Delineated protocols outline resuscitation and include the Parkland, modified Parkland, Brooke, modified Brooke, Evan's, and Monafo's formulas.[2] Despite these guides, patients in burn units often display laboratory or clinical signs of under-resuscitation such as lactic acidosis or acute kidney injury; over-resuscitation is equally problematic and can result in abdominal compartment syndrome or intense tissue swelling.[3–5] The challenges of fluid management in burn patients stem from dysfunction on multiple fronts; however, dysregulation of the vascular system is thought to be paramount in the increased vascular permeability and subsequent edema that commonly accompany severe thermal injuries.[4,6]

The reasons for this dysregulation are multiple, but integral to the pathophysiologic process are the vascular endothelial (VE) cells, connections between these cells such as adherens junctions, and the proteins that comprise those connections, including β-catenin and VE-cadherin[4,6–9] and filamentous (F) actin of the cytoskeletal assembly. Our laboratory has shown that, among the numerous mechanisms that act on endothelial cells and their adherens junctions, proteases such as matrix metalloproteinases (MMPs) may be extremely important regulators of permeability associated with burn injury.[7,10] Our focus thus far has been on MMP-9 and we have demonstrated that burns directly damage adherens junctions and increase microvascular permeability both in vitro and in vivo. Additionally, we have illustrated that such damage can be mitigated with anti-MMP-9 agents such as doxycycline, MMP-9 inhibitor-1, or melatonin.[7,10]

MMP-9 is a zinc-dependent endopeptidase, also referred to as gelatinase B, and has a broad range of actions in addition to cleavage of adherens junction proteins, cleavage of extracellular matrix proteins, cytokines, and chemokines, illuminating importance in angiogenesis, tissue remodeling, embryogenesis, and both innate and adaptive immunity.[11–14] Proteolysis by gelatinase B of both secreted and membrane-bound growth factor receptors, tyrosine kinase receptors, and cell adhesion molecules has been demonstrated.[14,15]

With actions so diverse, expression and activity of MMP-9 are tightly structured.[11,16,17] This control is multifactorial: important among these various avenues is regulation by a group of endogenous compounds known as tissue inhibitors of metalloproteinases (TIMPs).[18] TIMPs are a family of 4 distinct proteins that bind to and inhibit MMPs[16,19]: the balance between MMPs and TIMPs is paramount in maintenance of normal homeostasis.[19–22] Aside from their powerful anti-MMP activities, TIMPs also have recently been shown to have MMP-independent roles in cell signaling and growth, angiogenesis, metastasis, and tumor progression[20,23] hinting at much broader importance than initially thought. Additionally, the balance between TIMPs and MMPs has profound physiologic roles, and unchecked increase in either MMPs or TIMPs has been implicated in numerous disease processes including multiple sclerosis, Alzheimer's disease, central nervous system infections, many types of cancers, and ischemic brain injury.[18] Although it is known that to some degree all TIMPs have at least minor interactions with MMP-9, TIMP-1 and TIMP-2 may have increased affinity for gelatinase B.[18] As such, both may play a role in MMP-9-mediated postburn vascular permeability and consequently warrant investigation. There is evidence that TIMP-2 may play a role in endothelial cell stabilization[24] and may play a larger role in the balance between MMPs and TIMPs in such cells.

This relationship between TIMPs and MMPs in endothelial cells has not been explored in the setting of burn. Therefore, as we have demonstrated that MMPs are important in burn-induced pathophysiology, we hypothesize that thermal injury disrupts the balance between MMPs and TIMPs, and that burn-induced junctional damage and hyperpermeability could be attenuated with the use of TIMP-2 in vitro.

Patients and Methods

Chemicals and reagents

Rat lung microvascular endothelial cells (RLMEC) from VEC technologies (Rensselaer, NY) were used for all in vitro experiments. Cell culture utilized dishes coated with fibronectin from bovine serum (.1% solution; Sigma-Aldrich, St. Louis, MO), MCDB-131 complete media (VEC Technologies), and Trypsin-Ethylenediaminetetraacetic acid solution (.25%; Invitrogen-Gibco, Grand Island, NY). Fluorescein isothiocyanate-bovine serum albumin (FITC-albumin; Sigma-Aldrich) was used to measure permeability for monolayer experiments and was prepared by dissolving 5 mg/mL FITC-albumin in phenol red-free media (Dulbecco's Modified Eagle Medium FluoroBrite; Life Technologies, Grand Island, NY). β-Catenin and VE-cadherin antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and rhodamine phalloidin (Life Technologies) was used for F-actin staining. VECTASHIELD mounting medium with the nuclear stain 4′,6-diamidino-2-phenylindole was obtained from Vector Laboratories (Burlingame, CA). TIMP-2 obtained from R&D (Minneapolis, MN) was used for in vitro experiments and a dose of 10 ng/mL was utilized based on previous work from Hayakawa et al.[25]

Animals

After Institutional Animal Care and Use Committee approval was obtained for all experiments, male Sprague-Dawley rats (175 to 300 g, aged 5.5 to 9.0 weeks) were acquired (Charles River Laboratories, Wilmington, MA). Animals were housed in compliance with the National Institutes of Health guidelines at the institutional animal facility, which is approved by the Association for Assessment and Accreditation of Laboratory Animal Care International. Rats had free access to food and water, and were