RESEARCH REVIEW

Resuscitation-Induced Intestinal Edema and Related Dysfunction: State of the Science

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High volume resuscitation and damage control surgical methods, while responsible for significantly decreasing morbidity and mortality from traumatic injuries, are associated with pathophysiologic derangements that lead to subsequent end organ edema and dysfunction. Alterations in hydrostatic and oncotic pressures frequently result in intestinal edema and subsequent dysfunction. The purpose of this review is to examine the principles involved in the development of intestinal edema, current and historical models for the study of edema, effects of edema on intestinal function (particularly ileus), molecular mediators governing edema-induced dysfunction, potential role of mechanotransduction , and therapeutic effects of hypertonic saline. We review the current state of the science as it relates to resuscitation induced intestinal edema and resultant dysfunction. © 2011 Elsevier Inc. All rights reserved.
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BACKGROUND

Survival from trauma has increased considerably over the last several decades, secondary to multiple factors, including early and high volume resuscitation with crystalloid and blood products, advances in operative and nonoperative management, and the widespread adoption and practice of damage control surgery, including abdominal packing. High volume resuscitation and abdominal packing alter hydrostatic and oncotic pressure

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differentials, contributing to the formation of intestinal edema. This may occur even in the absence of intra-abdominal injury [1–7]. One of the major focuses of our research is the pathophysiology associated with resuscitation-induced intestinal edema and associated interventions. In this review, we detail the pre-clinical work that has contributed to our understanding of the mechanisms leading to intestinal edema and subsequent dysfunction as well as the evidence and potential mechanisms governing possible therapeutic intervention.

PRINCIPLES GOVERNING EDEMA DEVELOPMENT

Several key principles play a role in the development and propagation of intestinal edema, including alterations in microvascular permeability, venous and lymphatic pressure and flow, and in serum protein concentration. Key to the mechanism behind edema formation is perturbed microvascular fluid exchange as determined by hydrostatic and colloid oncotic pressures described by the Starling equation (eq 1). Alterations in microvascular and tissue hydrostatic and colloid oncotic pressures result in accumulation of fluid in the interstitium. Factors that govern these pressure variables are a central theme to the pathogenesis of intestinal edema.

Equation 1: Starling equation [8]

$$J_v = K_f[(P_c - P_i) - \sigma(\pi_c - \pi_i)]$$

where J_v – fluid filtration rate; K_f – filtration coefficient; P_c – microvascular (capillary) hydrostatic pressure; P_i – interstitial hydrostatic pressure; π_c – microvascular colloid osmotic pressure; π_i – interstitial colloid osmotic pressure; σ – reflection coefficient to protein

In the multiply injured trauma patient, high volume crystalloid resuscitation and abdominal packing serve to alter these variables (i.e., hydrostatic and osmotic pressures), leading to extravasation of fluid into tissues and subsequent tissue edema, including in the nervous system (brain), respiratory system (lung), and digestive system (gut) [9, 10]. High volume crystalloid resuscitation decreases plasma oncotic pressures (π_c) and abdominal (specifically peri-hepatic) packing, as a component of damage control resuscitation, increases mesenteric venous pressures, leading to increased capillary hydrostatic pressures (P_c). Decreased plasma oncotic pressure and increased capillary pressure both favor movement of fluid from the vasculature into the interstitium.

Increases in capillary permeability (σ), secondary to shock/resuscitation (ischemia/reperfusion mediated injury), also contribute to net fluid flux into the interstitium [8, 11, 12]. As the lymphatics that drain the interstitium flow into the venous system, elevated central venous pressure during resuscitation impedes movement of fluid out of the interstitium [13]. The overall result of these alterations is the development of intestinal edema [14].

With a transcapillary pressure gradient of 12 mmHg, seen with situations of hemodilution (i.e., high volume resuscitation), "filtration secretion" occurs [15]. With the accumulation in interstitial fluid (i.e., edema), there is a subsequent increase in the pressure of mucosal fluid. This leads to an efflux of protein (i.e., albumin) and fluid into the lumen *via* enlarged mucosa interepithelial channels. When studied, the composition of the fluid is similar to lymph; this indicates that the increased intraluminal fluid is likely escape of interstitial fluid [16]. This represents what is seen clinically, i.e., intestinal edema is accompanied by large, fluid filled loops of bowel.

MODELS OF INTESTINAL EDEMA

Ischemia/Reperfusion

Much work has been done on ischemia/reperfusion and the subsequent effect on intestinal tissue water and resultant intestinal dysfunction. It is well accepted that ischemia/reperfusion can lead to the development of intestinal edema, and much of the published literature reflects the mechanisms and potential therapeutic interventions in ischemia/reperfusion injury [3]. This is especially true in cases of hemorrhagic and septic shock, which are components of the early and late course of a significant number of victims of traumatic injuries [17]. Ischemia/reperfusion injury causes edema by various mechanisms, including increased microvascular and mucosal permeability and necrosis, free radical formation and neutrophil activation and infiltration, and release of pro-inflammatory molecules and cytokines [18, 19]. Ischemia/reperfusion and inflammation increase capillary permeability to proteins (σ) and result in a net fluid flux out of the capillary into the interstitium. There are well described animal models examining the role of ischemia/reperfusion injury and effect on intestinal edema, many of which rely on occlusion of the superior mesenteric artery for a period of time (usually 30 min to 1 h) followed by a variable period of reperfusion [20, 21].

Hypoproteinemia/Hemodilution

Hypoproteinemia, frequently resulting from high volume crystalloid resuscitation has been shown, in preclinical and clinical studies, to contribute to the development of intestinal edema. Decreased colloid osmotic pressure associated with crystalloid infusion and decreased serum protein levels leads to increased intestinal edema in patients undergoing gastrointestinal surgery, secondary to an increased net flow of water into tissues [22].

A large body of work regarding tissue edema was initially published in the 1940s by Ravdin and colleagues. Among his many observations was the fact that reduction in oncotic pressure secondary to decreased protein levels ("nutritional edema") is a key factor in the initiation/propagation of tissue edema; this is exacerbated by large volumes of crystalloid solution. The models utilized were predominantly large animal models (i.e., dog) and relied on induced decreases in plasma protein concentration, either through plasmapheresis, dietary restriction, or a combination of both. A correlation was made between hypoproteinemia and decreased gastrointestinal motility, manifested by delayed gastric emptying and small bowel transit [23–26]. These long-term studies (i.e., over several wk) provide much insight into the pathophysiology of edema formation, especially in relation to the role of serum protein concentration. Acute reductions in serum protein have been demonstrated to result in ileus [27]. Large volume crystalloid resuscitation frequently leads to hypoproteinemia, even in patients without preexisting conditions with normal serum albumin levels [28].

Recent animal studies have demonstrated an association between high volume resuscitation and post-surgical complications. High volume crystalloid resuscitation negatively impacts gastrointestinal anastomoses, both functionally and structurally as manifested by bursting pressure and tissue hydroxyproline content [29]. Multiple studies in surgical patients have demonstrated that decreased perioperative administration of fluids leads to improved outcome, including decreased morbidity, hospital length of stay, wound complications, cardiopulmonary complications,

and faster return to normal gastrointestinal function [30–32]. This may be secondary to reduced fluid-related tissue edema formation.

Hydrostatic Factors

One of the initial animal models of edema (nutritional edema) relied on manipulation of oncotic pressures by induced hypoproteinemia (plasmapheresis), as discussed by Ravdin and colleagues. In this model, delayed gastric and intestinal transit was noted [24, 26]. In development of our model, we focused on the manipulation of hydrostatic and oncotic pressure differentials. High volume resuscitation results in hemodilution and alterations in oncotic pressures. Venous hypertension, frequently a consequence of abdominal packing, leads to alterations in hydrostatic pressures [14]. We have developed an edema model that relies on alterations in hydrostatic and oncotic pressures. This has allowed us to study intestinal edema in a setting without the influence of significant ischemic or inflammatory factors.

Adult male Sprague Dawley rats undergo an upper midline laparotomy. Mesenteric venous hypertension is induced by tying a 4-0 silk suture over PE-10 sized tubing around the superior mesenteric vein. The tubing is then removed, creating a reproducible nonocclusive stenosis that causes a sustained elevation in mesenteric venous pressure. An external jugular venous catheter is utilized for administration of high volume crystalloids [33].

This model leads to a sustained elevation in mesenteric venous pressure (20 ± 3 mmHg from a baseline of 11 ± 1 mmHg) and development of significant intestinal edema (as evidenced by increased wet to dry ratios). Intestinal edema is demonstrated (by wet to dry ratios) as early as 30 min following surgery and lasts for at least 12 h after surgery. We also detect no evidence of ischemia (as evidenced by no difference in portal venous lactate), neutrophil recruitment (as evidenced by myeloperoxidase activity), or reperfusion induced mucosal injury (as evidenced by Chiu scoring) in this model [33].

There are several advantages of this model. First, edema develops in a reproducible manner with respect to time of development and amount of edema. Second, the effects of edema can be studied without confounding elements due to ischemia/reperfusion or inflammatory related injury. This model has formed the basis for our work examining the mechanisms behind non-ischemic/inflammatory-related, resuscitation-induced intestinal edema-mediated dysfunction, which we review in detail.

EDEMA AS A MEDIATOR OF INTESTINAL DYSFUNCTION

In addition to changes in permeability, a major functional consequence of edema is ileus, or delay in intestinal transit. Ileus frequently is a consequence of systemic illness, such as sepsis, and is well documented in the postoperative environment [34]. Fluid resuscitation has been shown to induce intestinal edema in a hemorrhage/resuscitation model [35, 36]. In an animal model, intestinal edema induced by high volume crystalloid resuscitation and mesenteric venous hypertension decreases intestinal transit, measured at 6 and 12 h post-surgery [33]. Edema-induced ileus is of significant clinical significance; it results in delayed initiation of enteral feeds, increased hospital morbidity, and increased hospital length of stay, and healthcare costs.

Ileus is mediated by two major factors; mechanical dysfunction secondary to coupling of the muscle cell to surrounding tissue and decreases in intestinal contractility (dysfunction of the intestinal smooth muscle cell). Additionally, ileus may serve to contribute to edema formation. Recent work has begun to define the signal transduction pathways initiated by the onset of interstitial edema. Ultimately, transcription factors/gene products and mediators converge on myosin light chain$_{20}$ (MLC) phosphorylation to regulate intestinal contractility and transit.

Ileus as a Contributor to Edema Formation

While edema functionally leads to ileus, some evidence also suggests that ileus may serve to contribute to, or propagate, edema formation. Lymphatic outflow serves to decrease intestinal edema by providing a conduit for the escape of accumulated interstitial fluid [37, 38]. Unthank and Bohlen demonstrated that lymphatic vessels within the bowel wall are valveless [39]. This implies that flow of lymph from the intestine depends on peristalsis for forward flow. Lack of peristalsis (secondary to ileus) leading to lack of lymphatic outflow may serve to increase the severity of edema, creating a propagating cycle of dysfunction.

Intestinal Contractility

Decreased intestinal contractility is a well-documented consequence of intestinal injury, observed in models of ischemia/reperfusion, hemorrhagic shock, and colitis [40–43]. Signaling pathways governing smooth muscle contractility primarily converge on the MLC phosphorylation/dephosphorylation apparatus (Fig. 1). The decrease in MLC phosphorylation and subsequent decrease in contractility may be mediated by upstream signaling events that converge on MLC mediated smooth muscle contractile apparatus. These include the stress signaling pathways signal transduction and activator of transcription (STAT)-3 and NF-κB, which may lead to increased nitric oxide (NO) production [44] (Uray et al. [111]). Increased concentrations
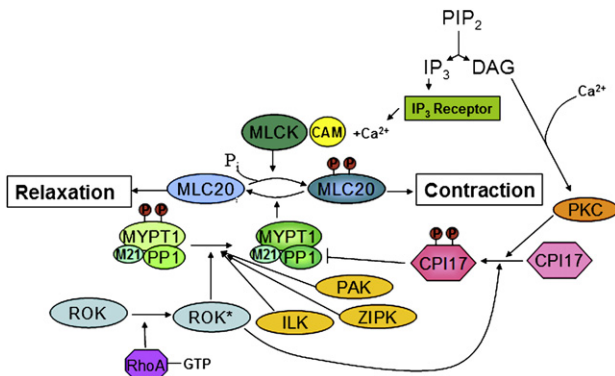
FIG. 1. Regulation of MLC phosphorylation in edema-induced intestinal dysfunction. Intestinal contractility is regulated by phosphorylation of the MLC, which in turn is controlled by the activity of kinases and phosphatases. Preliminary data demonstrate that the control of MLC in edema is likely through the effects on the activity of the phosphatase. (MLC: myosin light chain; MLCK: MLC kinase; MYPT1: myosin phosphatase targeting subunit 1 of MLC phosphatase; CPI17: PKC-potentiated inhibitory protein; ZIPK: zipper interacting protein kinase; ILK: integrin linked kinase; PAK: P21 activated kinase; ROK: Rho kinase; PKC: protein kinase C). (Color version of figure is available online.)

of nitric oxide (NO) may at least partially explain the effect of edema on intestinal smooth muscle contractility. Activation of these pathways may also be important in other situations resulting in ileus, such as post-operative and endotoxin-induced ileus [45–51].

STAT-3

The STAT family of transcription factors is important in activating intracellular signaling pathways and is activated (primarily by phosphorylation by Janus kinases (JAK) and mitogen activated protein (MAP) kinases) in response to stress, cytokines, and various growth factors [52]. Although little is known about the role of STAT-3 activation on intestinal smooth muscle function, there are a few studies that suggest that STAT-3 may play a role in intestinal contractile function. For example, STAT-3 activation has been demonstrated in the intestinal muscularis in response to surgical manipulation and ischemia/reperfusion injury [41, 45]. Both surgical manipulation and ischemia/reperfusion injury have also been shown to decrease intestinal contractile activity. The increase in STAT-3 in these models is believed to be mediated by cytokine production (such as IL-6).

Edema induces increased STAT-3 signaling in the intestinal muscularis compared to nonedematous intestine, including increased STAT-3 phosphorylation, increased nuclear translocation, and increased STAT-3 binding to its DNA consensus site [44]. STAT3 inhibition attenuates edema-induced intestinal dysfunction, including edema-induced intestinal contractile dysfunction, and decreased intestinal smooth

muscle MLC phosphorylation [44]. We conclude from these data that STAT-3 activation is involved in edema-induced intestinal contractile dysfunction.

The mechanism by which STAT-3 is activated in intestinal edema is unclear. As mentioned previously, in both ischemia/reperfusion and surgical manipulation models of intestinal dysfunction of the gut, upregulation of IL-6 in the muscularis and mucosal layers induced STAT-3 activation [41, 45]. Although IL-6 was up-regulated due to surgical stress in our intestinal edema model, we were unable to detect differences in IL-6 levels in the intestinal muscle with edema development compared with nonedematous tissue [44]. Furthermore, AG490, a JAK-2 inhibitor, did not completely block STAT-3 activation in the edematous intestinal smooth muscle suggesting that STAT-3 may be activated through a noncanonical pathway.

Mechanotransduction may be responsible for STAT-3 activation in our model either directly or indirectly [i.e., through a mechanosensitive channel such as the sodium hydrogen exchanger (NHE)]. In cardiomyocytes, stretch has been shown to increase STAT-3 expression [53–57]. Edema alters the mechanical properties of intestinal tissue, representing a potential mechanism.

NF-κB

NF-κB is a transcription factor present in a variety of cells that regulates expression of such factors as cytokines, growth factors, inducible nitric oxide synthase (iNOS), and adhesion molecules. It has been implicated widely in inflammatory processes, and it may play a role in intestinal contractility. [58] In human colonic smooth muscle cells, NF-κB activation by TNFα was shown to suppress cell contractility *via* induction of ICAM-1 [59]. In an animal model of Crohn's disease, NF-κB was shown to decrease colonic circular smooth muscle contractility [60].

We initially became interested in NF-κB for several reasons, including the results of a microarray analysis performed comparing genetic expression in nonedematous and edematous intestinal smooth muscle. Analysis of the microarray data for common function-specific regulatory elements using two different software analyses revealed the importance of NF-κB signaling in edema-induced intestinal dysfunction. (DIRE and oPOSSUM) [61,62,111]. Subsequently, we demonstrated increased NF-κB signaling in edematous tissue compared to nonedematous tissue. Inhibition of NF-κB attenuates edema-induced intestinal contractile dysfunction including decreased contractile activity and decreased intestinal smooth muscle MLC phosphorylation. These data suggest that NF-κB is involved in the signaling pathway leading to decreased intestinal contractile activity.

NF-κB may mediate some of its downstream effects on intestinal contractility by transcription of iNOS. iNOS protein levels are increased in edematous intestine; additionally inhibition of iNOS results in improved intestinal transit [36].

The mechanism of NF-κB activation in intestinal edema is unclear; however, mechanical changes in intestinal tissue may play a role. Edema causes more than a 6-fold increase in intestinal smooth muscle tissue mechanical stress [48]. Stretch has been shown to activate NF-κB in other cell types. Mechanical stretch of rat ventricular myocytes stimulated BNP gene transcription in an NF-κB dependent manner [55]. Biomechanical stress was shown to stimulate IL-6 production via NF-κB in vascular smooth muscle cells [63]. In bladder smooth muscle cells, mechanical stretch activated NF-κB through increased actin polymerization [53]. In our model, we also observed altered actin polymerization that may play a role in edema-induced activation of NF-κB [48, 49, 51] .

NO

iNOS/NO is a known mediator of smooth muscle relaxation (correlating with ileus in the small intestine) in models of ischemia/reperfusion and post-surgical ileus (bowel manipulation models of ileus) [64–67]. Additionally, iNOS has been implicated in barrier dysfunction in burn induced intestinal dysfunction [68]. Although constitutive levels of NO are important, overproduction can lead to pathologic effects [68]. iNOS has been shown to mediate changes in intestinal contractility; inhibition of iNOS has been shown to attenuate decreased intestinal contractility in animal models, including in colitis and decreased ileal contractility induced by peritonitis [43, 69].

iNOS is a constitutively active enzyme that increases production of NO by the conversion of arginine to citrulline and NO. NO binds to soluble guanylyl cyclase (sGC), converting GTP to cGMP. Increased levels of cGMP may mediate decreased phosphorylation of MLC by increasing the activity of MLC phosphatase (MLCP). Additionally, it may lead to hyperpolarization of the cell membrane, leading to an inhibition of calcium release and subsequent decreased contractility. Other potential mechanisms include inhibition of certain neurotransmitters, including acetylcholine and substance P [70, 71].

NF-κB activates transcription of iNOS. We know that NF-κB is increased in edematous intestinal smooth muscle. We have demonstrated that iNOS expression is increased in intestinal edema after initiation of edema [72]. iNOS protein levels also are increased with edema, and inhibition of iNOS with a selective inhibitor (L-NIL) results in marked improvement in

intestinal transit. Increases in iNOS have been observed in other models resulting in ileus, including endotoxin induced ileus, models of mechanical ileus, and ischemia/reperfusion injury [53, 55]. Whether iNOS mediates dysfunction through the classic soluble guanylyl cyclase/cGMP pathway or via a noncanonical pathway is a subject of investigation in our group.

Myosin Light Chain Phosphorylation

We have observed that decreases in MLC phosphorylation in our intestinal edema model correlate well with changes in both intestinal contractile activity and transit. In general, pharmacologic treatments that attenuated edema-induced contractile activity also prevented edema-induced decreases in intestinal smooth muscle MLC phosphorylation. This includes NF-κB inhibition with pyrrolidinedithiocarbamate (PDTC) and STAT-3 inhibition with AG490 [44, 111]. While STAT-3 and NF-κB signaling have been implicated in edema-induced decreases in MLC phosphorylation, the mechanism is unclear. However, preliminary data show that MLC phosphorylation is altered through regulation of MLCP [73].

MLC phosphorylation is an obligatory step in smooth muscle contraction and is highly regulated. The phosphorylation status of MLC depends on the balance between phosphorylation by MLC kinase (MLCK) and dephosphorylation by MLCP. MLCK is dependent on Ca2+-calmodulin; thus, smooth muscle contraction is responsive to intracellular Ca2+. However, both MLC phosphorylation and smooth muscle contractile activity can be modulated via Ca2+-independent pathways. These Ca2+-independent pathways affect contractile activity through direct phosphorylation of MLC or through regulation of MLCP activity. MLC can be directly phosphorylated by a number of kinases other than MLCK, including Rho kinase (ROK), integrin-linked kinase (ILK), and zipper interacting protein kinase (ZIPK). MLCP activity is regulated via phosphorylation of the regulatory subunit of the enzyme, MYPT1, or by phosphorylation of the endogenous inhibitor of MLCP, protein kinase C (PKC)-potentiated inhibitory protein, CPI17 [74]. The regulation of MLC phosphorylation is demonstrated in Fig. 1.

Mechanical Changes as a Contributor to Ileus

As important as dysfunction in the smooth muscle cell is to the pathogenesis of edema-induced ileus, it is important to remember that muscle cells function in the framework of the intestinal tissue matrix. Thus, alterations in tissue properties may serve to alter the behavior of smooth muscle cells. Mechanical changes induced by edema are probably partially responsible

for ileus. There are alterations in cytoskeletal architecture and mechanical properties induced by edema, including changes in calponin and vimentin (intermediate filament) levels and F:G actin ratio and alteration of interstitial and lymphatic pressures [49]. In addition, the mechanical properties of edematous intestine are altered, as manifested by decreased stress and residual stiffness [49, 51]. Certain therapeutic modalities, including hypertonic saline, reverse intestinal dysfunction induced by edema, including ileus, possibly by allowing for more efficient force transmission through intestinal tissue because of the prevention of cytoskeletal changes induced by edema [48–51].

Barrier Dysfunction/Effects on Intestinal Permeability

Intestinal edema, both in the presence or absence of venous hypertension, decreases tissue resistance and increases mucosal permeability [33]. This finding has been seen in other disease processes in which intestinal edema is a component of the manifestation in both clinical and preclinical studies. Patients with chronic heart failure have been shown to have increased intestinal permeability [75]. The increase in permeability is secondary to multiple factors, including alterations in tight junction proteins (notably occludin and claudin), apoptosis of epithelial cells, oxidant stress (by activating certain isoforms of protein kinase C, causing lipid peroxidation in epithelial cells, altering mitochondrial function, and inhibiting ion exchange), nitric oxide (by decreasing epithelial cell viability and/or altering the cytoskeleton), and pro-inflammatory cytokines (IL-1, IL-4, IL-6, TNF-α, and interferon-γ) (possibly, in part by increasing production of nitric oxide) [76–78]. Dysfunctions in intestinal barrier integrity and function are thought to be involved in the pathogenesis of infection and subsequent multi-organ failure [79]. Barrier dysfunction may also contribute to edema secondary to translocation of serum proteins from the intravascular space, notably albumin. This is especially true in the postoperative period and in cases of sepsis, which may complicate the treatment of traumatically injured patients [80].

MECHANOTRANSDUCTION

Basic Principles

Cell and tissue stretch may be an important initiating event of signal cascades known to mediate intestinal dysfunction. In cardiomyocytes, stretch has been shown to activate sodium hydrogen exchange (NHE) receptors [81]. Additionally, stretch has been demonstrated to activate the STAT-3 and NF-κB pathway,

which we have demonstrated to be involved in edema-induced intestinal dysfunction [53, 55, 56, 82, 111].

Our interest in a mechanical explanation for activation of signaling cascades known to result in resuscitation induced intestinal edema is borne out of the observation that edema alters the mechanical properties of intestinal tissue. Intestinal edema is associated with cytoskeletal changes, including changes in intermediate filaments (calponin and vimentin) and in F:G actin ratio. There are alterations in tissue properties, including a decrease in stiffness and residual stress. Therapeutic modalities associated with improvement in edema-induced dysfunction, particularly hypertonic saline, are associated with return of intermediate filaments, F:G actin ratio, interstitial pressures, tissue stiffness and residual stress towards control levels, which may account for at least part of its therapeutic effect [48, 49, 51].

The transcription factors NF-κB and STAT-3 are frequently activated by cytokines and other byproducts of inflammatory processes. In our model, there are no significant inflammatory stimuli present, leading us to explore noncanonical pathways of NF-κB and STAT-3 activation. The evidence for significant, early mechanical changes in intestinal edema indicates that the signals for activation of these cascades in intestinal edema are early and may be mechanical in nature. As others have demonstrated the role of stretch in other organ system related dysfunction, we have pursued development of this potential mechanism in intestinal edema-induced dysfunction.

Sodium Hydrogen Exchanger

Secondary to the early, substantial changes in the mechanical properties of edematous intestinal tissues, we hypothesized that stretch coupled ion exchangers were involved in the pathophysiology of edema-induced intestinal dysfunction. The NHE is a cytoskeletal linked ion exchange protein that has been shown to be activated by stretch. Given its properties as a mechanosensor and the fact that mechanical changes occur early in the course of intestinal edema, we have been interested in NHE as a potential initiator of dysfunctional signaling cascades in edema. NHEs are intimately involved in the regulation of intracellular pH and volume. It is also involved in intestinal absorption. There are nine identified isoforms [83]. The NHE is expressed throughout the intestine, with isoforms 2 and 3 being present primarily on the intestinal epithelial cell brush border. NHE isoform 1 is expressed in both mucosa and muscle [56, 81, 84, 85].

The NHE has been implicated in various intestinal pathologies, including colitis and hemorrhage/resuscitation models [86, 87]. It has been associated with

vascular endothelial cell dysfunction in hemorrhagic shock and consequent hypoperfusion [87]. Administration of the sodium hydrogen exchange inhibitor amiloride has been demonstrated to ameliorate increased intestinal permeability in hemorrhagic shock, and improves lung injury when combined with hypertonic saline resuscitation [88, 89]. Additionally, NHE inhibition in a colitis model has been shown to ameliorate disease activity and decrease IL-8 activation [86]. NHE inhibition has been shown to improve cardiac contractility in ischemia reperfusion injury [90].

NHE may also play a role in edema-induced intestinal dysfunction. NHE inhibition ameliorates myocardial edema after cardioplegic arrest and cardiopulmonary bypass [91]. We have demonstrated that edema induces increases in NHE 1-3 expression [92]. Hypertonic saline, which improves ileus induced by intestinal edema, prevents increased NHE expression. Inhibition of NHE with 5-(N-ethyl-N-isopropyl)-amiloride (EIPA) results in improved contractile activity and intestinal transit, increased MLC phosphorylation, and decreased STAT-3 phosphorylation [92]. The potential mechanism of NHE in edema-induced intestinal dysfunction is demonstrated in Fig. 2.

Mechanical Stretch

Longitudinal stretch of intestinal tissues activates signaling pathways involved in edema. In preliminary data, when the intestine is stretched to correlate to the approximate stress seen in our *in-vivo* model of intestinal edema, there is activation of STAT-3, NF-κB, and decreased phosphorylation of the MLC. Additionally, there is increased expression of NHE [110] (Fig. 3). Preliminary data also indicate that stretch of human intestinal smooth muscle cells activates STAT-3 and NF-κB [73]. This is the first body of work published in the literature examining the effect of mechanical stretch in intestinal tissues, and indicates a potential role for mechanotransduction in edema-induced intestinal dysfunction. This also creates the possibility for an *in-vitro* model to study intestinal edema.

THERAPEUTIC MODALITIES

Hypertonic Saline

Hypertonic saline has been studied and has shown therapeutic benefit in animal models of intestinal ischemia reperfusion. It has been shown to improve intestinal transit, decrease mucosal injury and tissue myeloperoxidase levels, and decrease iNOS protein expression. It has also been shown to reduce injury to lung and liver, presumably secondary to its gut
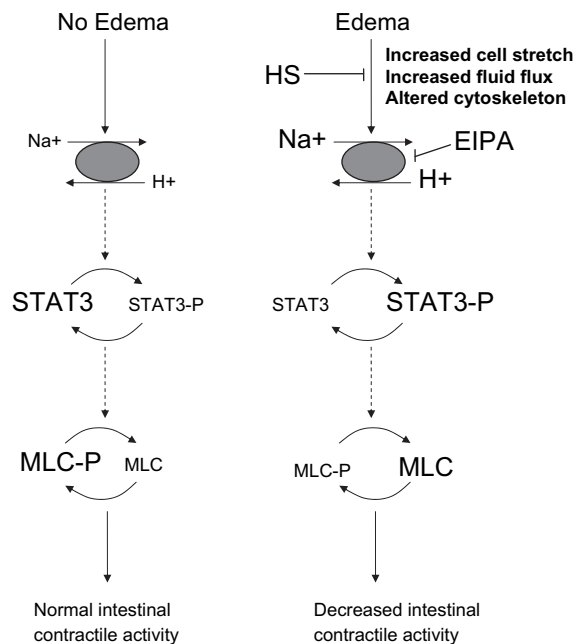

FIG. 2. Role of NHE in edema-induced intestinal dysfunction. Inhibition of NHE by EIPA results in decreased STAT-3 activation, amelioration of the decrease in MLC phosphorylation, and improvements in intestinal contractility and transit. It also suggests that STAT-3 activation in edema is regulated by NHE, as has been demonstrated in other studies, particularly those involving cardiomyocytes HS = hypertonic saline.

protective effects [93–95]. In hemorrhagic shock, administration of hypertonic saline improves intestinal perfusion, reduces small intestinal mucosal apoptosis, and improves barrier function [96–99]. We have studied hypertonic saline in resuscitation-induced intestinal edema, both in an attempt to better understand the pathophysiology behind edema-induced intestinal dysfunction and as a therapeutic intervention.

Administration of hypertonic saline decreases tissue edema, and reduction of tissue edema may be secondary to redistribution of tissue water to the peritoneal, intraluminal, and vascular spaces. This may be due, in part, to up-regulation or prevention of a decrease in expression of water transport proteins, specifically the aquaporin 4 receptor [112]. The decrease in intestinal edema is accompanied by a concurrent improvement in intestinal transit [50].

Edema is associated with alterations in tissue architecture, and hypertonic saline reverses these alterations towards control levels. Hypertonic saline reverses the decrease in tissue stiffness, stress, and strain, and prevents the increase in interstitial pressure due to edema [48, 50]. It prevents the decrease in stress fiber formation induced by edema (as measured by calponin and vimentin) and prevents the alterations in F:G actin ratio induced by edema. Hypertonic saline preserves tissue architecture and possibly allows for
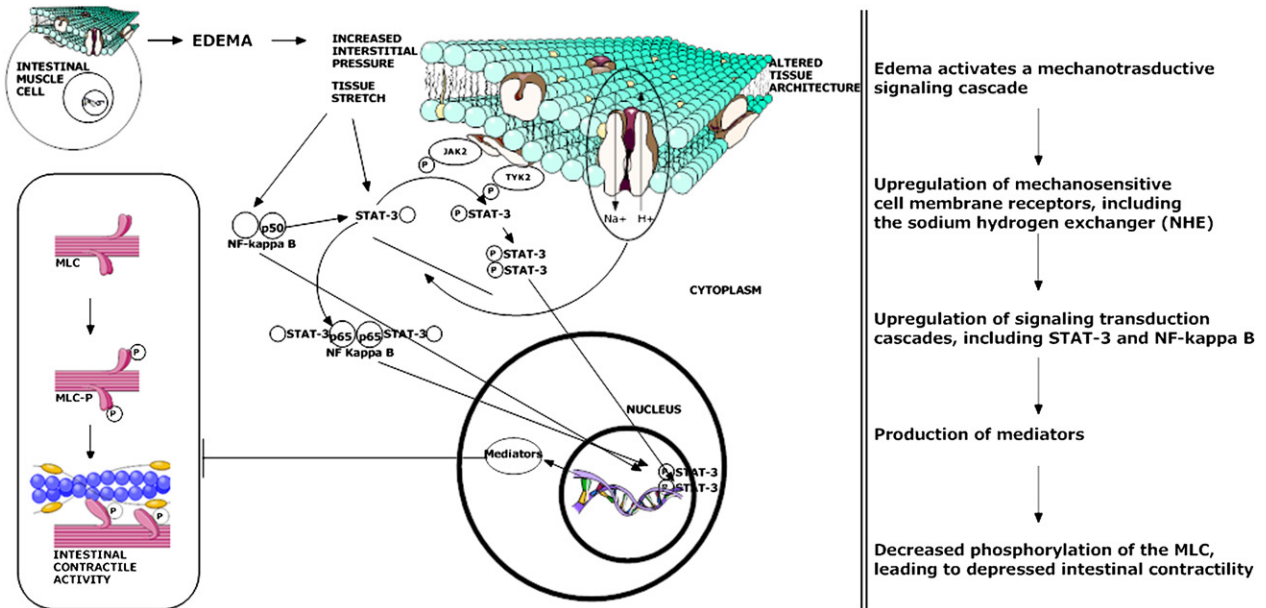
FIG. 3. Mechanotrasduction as a mechanistic explanation for edema-induced intestinal dysfunction. Intestinal edema results in profound changes in the characteristics of intestinal tissue, including increased interstitial pressure. Stretch has been shown to modulate activation of various signaling pathways in cardiomyocytes. Intestinal stretch (in the absence of edema) that mimics similar magnitude forces as measured in edematous intestine results in increased expression of NHE, increased NF-κB activation, increased phosphorylation of STAT-3, and decreased MLC phosphorylation. This indicates that stretch may be the stimulus for activation of dysfunctional signaling pathways in intestinal edema. (Color version of figure is available online.)

more efficient transmission of force through intestinal tissue [49]. Additionally, hypertonic saline prevents the increase in central venous pressure and lymphatic flow seen with intestinal edema [48–50]. Hypertonic saline may modulate some of the early mechanotransductive signals responsible for mediating subsequent intestinal dysfunction, including preventing an increase in NHE expression.

CONCLUSION AND FUTURE DIRECTIONS

Edema is a well accepted component in the pathophysiology of many disease processes involving the intestine, including that of ischemia/reperfusion, hemorrhagic shock, and inflammatory mediated processes. While many of these play a role in traumatic injury and resuscitation (notably hemorrhagic shock and ischemia/reperfusion), edema traditionally has not been viewed as an initiator or amplifier of signal transduction cascades that leads to end organ dysfunction. We have developed a body of literature separating edema from other confounding factors, notably ischemia/reperfusion, and examined it as an isolated disease process. Through our work, we have isolated edema as an initiator and propagator of pathways that lead to end organ dysfunction.

These studies have led to a better understanding of factors involved in the development of intestinal edema, specifically alterations in tissue architecture

and alterations in interstitial pressures. The signal transduction cascades initiated with edema, including STAT-3, NF-κB, and possibly iNOS, mediate decreased MLC phosphorylation, subsequently decreasing intestinal contractility resulting in ileus.

Mathematical modeling may serve as a useful tool in predicting edema development in various organ systems. Factors, such as microvascular filtration, lymphatic resistance, osmotic and interstitial pressures, and interstitial compliance, can be utilized to mathematically predict edema development. This has applied to a variety of models with validation with experimental data [100–109]. Allowing one to predict development of edema as well as factors contributing to edema may allow for initiation of various protective strategies or alternative resuscitation strategies to prevent/ameliorate tissue water accumulation.

Our current work focuses on more specifically delineating the mechanotransductive pathways responsible for activation of signaling cascades known to mediate dysfunction. This includes expanding on the role of mechanosensors, including the NHE family of ion channels. One focus involves examining specific cytoskeletal elements, specifically water transport proteins, which may serve a therapeutic effect by decreasing tissue edema and hence the increase in interstitial pressure. Additionally, we are interested in understanding the mechanism by which activation of specific transcription factors (i.e., STAT-3 and NF-κB) lead to decreased MLC phosphorylation.

ACKNOWLEDGMENTS

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