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Syndecan-1 plays a novel role in enteral glutamine's gut protective effects of the post ischemic gut:

Role of syndecan-1 in gut protection by glutamine

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Abstract

Syndecan-1 is the predominant heparan sulfate proteoglycan found on the surface of epithelial cells, and like glutamine, is essential in maintaining the intestinal epithelial barrier. We therefore hypothesized that loss of epithelial syndecan-1 would abrogate the gut protective effects of enteral glutamine. Both an in vitro and in vivo model of gut ischemia/reperfusion (I/R) was utilized. In vitro, intestinal epithelial cells underwent hypoxia/reoxygenation (H/R) to mimic gut I/R with 2mM (physiologic) or 10 mM glutamine supplementation. Permeability, caspase activity, cell growth, and cell surface and shed syndecan-1 were assessed. In vivo, wild type (WT) and syndecan-1 knockout (KO) mice received $\pm$ enteral glutamine followed by gut I/R. Intestinal injury was assessed by fluorescent dye clearance and histopathology, permeability as mucosal to serosal clearance ex vivo in everted sacs, and inflammation by myeloperoxidase (MPO) activity. In an in vitro model of gut I/R, glutamine supplementation reduced epithelial cell permeability and apoptosis and enhanced cell growth. Shed syndecan-1 was reduced by glutamine without an increase in syndecan-1 mRNA. In vivo, intestinal permeability, inflammation, and injury were increased after gut I/R in WT mice and further increased in syndecan-1 KO mice. Glutamine's attenuation of I/R-induced intestinal hyperpermeability, inflammation, and injury were abolished in syndecan-1 KO mice. These results suggest that syndecan-1 plays a novel role in the protective effects of enteral glutamine in the post ischemic gut.

Keywords

heparan sulfate; epithelial cells; gut ischemia/reperfusion; hypoxia/reoxygenation; permeability

INTRODUCTION

Multiple organ dysfunction syndrome (MODS) remains the most common cause of death in severely injured patients in shock who do not die from early uncontrolled hemorrhage or brain injury.(1) The institution of early enteral nutrition decreases septic morbidity and therefore lessens the incidence of post-injury MODS.(2) However, standard practice is to begin enteral nutrition once resuscitation is complete, well after the localized (gut) and systemic production and release of multiple pro-inflammatory mediators has occurred. The separation of nutritional support from the provision of key nutrients has led to a further

appreciation of the immunomodulatory and anti-inflammatory benefits of isolated nutrients, such as glutamine.(3) We have shown in a pilot study that the use of enteral glutamine as a gut specific pharmaconutrient administered at the time of injury and initiation of resuscitation is safe and well tolerated in trauma patients in shock.(4) The mortality of MODs remains high, in part due to the lack of early therapeutic interventions to prevent shock-induced gut dysfunction.(5) The early use of enteral glutamine during shock may be one such intervention. Glutamine is the preferred substrate of enterocytes and is conditionally essential during times of stress. We have demonstrated in a rodent model of mesenteric ischemia/ reperfusion that enterally delivered glutamine preserved mucosal integrity, absorptive capacity, and ATP levels, as well as maintained gut barrier function. (6,7)

The endothelial glycocalyx is a complex network of components that projects from the cell surface of the endothelium into the vessel lumen and protects against inflammatory cell adhesion and infiltration. It consists of proteoglycans and glycoproteins attached to the cell membrane. The proteoglycans provide the structural support for the glycocalyx and consist of a core protein, either syndecans or glypicans, to which the glycosaminoglycans are attached. The syndecan family is the major source of heparan sulfates for all cell types. Although the majority of glycocalyx work has been focused in endothelial syndecan-1, emerging data on epithelial syndecan-1 demonstrate that it plays an important role in protecting the underlying epithelium. We and others have shown that intestinal epithelial syndecan-1, like enteral glutamine, is essential in maintaining intestinal epithelial barrier function.(8,9) We therefore hypothesized that loss of epithelial syndecan-1 would abrogate the gut protective effects of enteral glutamine.

MATERIALS AND METHODS

In vitro studies

Cell culture model—Rat intestinal epithelial cells (IEC-6) were purchased from the American Type Culture Collection (ATCC, cell line CRL-1592). IEC-6 cells are small bowel in origin, and the small bowel is the site for glutamine absorption. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 2 mM glutamine. Because glutamine is necessary for normal cell growth, we considered the physiologic concentration found in the media as our control group. Glutamine was added to cells at a concentration of 10 mM, as we have determined this to provide maximal protection. (10)

In vitro model of intestinal ischemia/reperfusion—To model intestinal ischemia/reperfusion (IR) in vitro, we utilized hypoxia/reoxygenation (H/R). Cells were placed in a humidified incubator (HERAcell 150i CO₂ incubator, Thermo Scientific) supplied with 1% O₂-5% CO₂-94% N₂ at 37°C for 24 hours then returned to a regular incubator supplied with 5%CO₂-95% air at 37°C for an additional 4 hours of reoxygenation. We realize that H/R may not perfectly mimic gut IR, as no in vitro model perfectly reflects the in vivo setting. However, we have been able to replicate in vivo findings in vitro using this model (11).

In vitro permeability—Permeability was assayed using in vitro Vascular Permeability Assay Kit (Millipore, ECM640). Twenty-four well transwell inserts were coated with 1 µg/ml of collagen/ insert and incubated at room temperature for 1 hour, 500ul DMEM was added to each well of a 24-well plate, then IEC-6 cells were seeded at 2×10^5 cells /insert and cultured until formation of integrated cell monolayers. Cells were treated as described above. At the end of reoxygenation, inserts were transferred to the permeability detection plates with 500ul basal medium/well. Media was removed then FITC-Dextran (150 µl)

added into the inserts and incubated for 5 minutes. Fluorescent measurements were determined using excitation/emission wavelengths of 485nm/530nm.

Caspase 3/7—As an index of injury, the Apo-ONE homogeneous caspase 3/7 assay kit (Promega, Madison, WI) was used to measure the activities of caspase-3/7 according to the manufacture's protocol. IEC-6 cells were seeded at 10^4 cells per well into 96-well plates. The caspase-3/7 substrate rhodamine 110 bis-(N-CBZ-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide; Z-DEVD-R110) is acted upon by caspase-3 and -7. The amount of fluorescent product generated is proportional to the amount of caspase-3/7 cleavage activity present in the sample.

Cell growth—Cell growth was indirectly measured utilizing the cleavage of the yellow tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (Sigma) to purple formazan crystals by metabolically active cells. IEC-6 cells were seeded in 24-well plates at a density of 2×10^5 cells /well. Cells were cultured and treated as described above. At the end of reoxygenation, 50 μ l of 5 mg/ml MTT was added to each well, including one set of wells with MTT but no cells (control). DMSO (250ul) was added to dissolve purple formazan crystals then transferred to the formazan dye to 96-well plates to be quantified by measuring absorbance at 540 nm with a reference filter of 590 using TECAN spectrophotometer.

Quantitative real-time reverse-transcription polymerase chain reaction—IEC-6 cells were cultured and treated as described as above and total RNA was extracted from the cells using TRIzol-Reagent (Sigma, St. Louis). One μ g of RNA was reversed transcribed to cDNA using Bio-rad iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) and 50ng of cDNA was performed quantitative real-time PCR to check mRNA expression of syndecan-1 using TaqMan® Gene Expression Assays with syndecan-1 probe (Mm00448920_g1, Applied Biosystems) as target and actin probe (Mm00607939_s1, Applied Biosystems) as the internal reference. The relative expression level of syndecan-1 was normalized to that of the internal reference gene and to a calibrator sample that was run on the same plate. The normalized relative expression levels were automatically calculated using commercial software (StepOne Software V2.1, Applied Biosystems).

Western blot analysis—To perform Western blot analysis, whole cell lysates were prepared by lysing cells with RIPA buffer (Sigma, Milwaukee, WI) containing protease inhibitor cocktail (Sigma, Milwaukee, WI). The whole cell lysate proteins were denaturalized, loaded on 4% to 12% linear gradient NuPAGE gel and transferred to Immobilon PVDF blotting membrane (Millipore, Billerico, Massachusetts). The membranes were blocked for 1 hour at room temperature with 5% non-fat milk in PBS-T, incubated with rabbit anti-syndecan-1 antibody (1:1000, Invitrogen, cat: 36–2900) overnight at 4°C, followed by washing and incubation with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (1:5000, GE, Healthcare UK limited) for 1 hour at room temperature. The membranes were washed and treated with chemiluminescence (ECL) for film developing. The films were scanned and the syndecan-1 bands were semi-quantified with ImageJ software.

Cell surface syndecan-1 expression—IEC-6 cells were seeded into 8 well-CultureSlide (BD Falcon™). Cells were cultured and treatments were as described as above. At the end of the experiment, cells were washed with phosphate buffered saline (PBS), then incubated on ice for 1 hour with 1:50 rat monoclonal antibody against mouse syndecan-1 (281–2; BD Pharmingen). Unbound antibody was washed with PBS. Cells were fixed with 2% formaldehyde for 20 minutes, blocked with 2% BSA and 2.5% goat serum in PBS for 1

hour at room temperature, and incubated with 1:500 secondary antibodies (Alexa Fluor goat anti-rat IgG, Invitrogen) for 1 hour at room temperature. Cell nucleolus was stained with DAPI for 5 minutes. Images were obtained using an Olympus 1X71 microscope with SimplePCI6 software. The relative fluorescence intensity was quantified using Image J software (NIH) and reported as relative fluorescence units (RFU). (12) Four views per well and four wells/group were used for quantification. A negative control was used as a technical control for staining without using primary antibody.

Shed syndecan-1—Media was collected and the level of syndecan-1 ectodomain assessed by ELISA. 120ul of media was diluted in 200ul of ELISA coating buffer (eBiosciences, 00-0044-59) containing 0.5mM EDTA, 0.1mM PMSF and proteinase inhibitor cocktail and used to coat wells of the Nunc Maxisorp 96-well plates (eBiosciences) overnight at 4 °C (2 wells per each sample). After incubation, the plate was washed 3 times with 200ul per well of PBS, 0.1% Tween-20 and blocked for 1 hour at 4 °C with 200ul per well of PBS plus 1% BSA, then incubated in 100ul per well of fresh blocking solution plus 1:1000 dilution of rat anti-mouse syndecan-1 antibody 281–2 (BD Pharmingen) for 2 hours at 4 °C, washed 5 times with 200ul per well of PBS, 0.1% tween-20, and finally incubated with 100ul per well of anti-rat HRP-conjugated secondary antibody (abcam ab6734) diluted 1:5000 with blocking solution at room temperature for 1 hour. After incubation, plate wells were washed 5 times with 300ul per well of PBS, 0.3% tween-20 and developed using 100ul per well of tetramethyl benzidine reagent (sigma, T4444) and incubated at RT for 30 minutes, absorbance was detected at 450/570 nm with TECAN plate reader. The syndecan-1 expression for each sample was calculated by subtracting the sample absorbance minus the absorbance of the blank control wells without media.

Animals

All procedures performed were protocols approved by the University of Texas Houston Medical School Animal Welfare Committee. The experiments were conducted in compliance with the National Institutes of Health guidelines on the use of laboratory animals. All animals were housed at constant room temperature with a 12:12-h light-dark cycle with access to food and water ad libitum. *Sdc1*^{-/-} mice on the C57BL/6J background and littermate WT mice were used at an age of 8 to 10 weeks.(13)

Superior mesenteric artery occlusion model

As we have previously described, small bowel enteral sacs $\pm$ 60 mM glutamine were fashioned in the jejunum of wild type and *Sdc1*^{-/-} mice.(14) The superior mesenteric artery was clamped for one hour followed by six hours of reperfusion. We have shown this time to result in maximal activation of pro-inflammatory mediators.(15) There were no mortalities in either the wild type or knock out mice.

Intestinal permeability—To evaluate the small intestinal mucosal barrier function, the ex-vivo isolated everted ileum sac method was used.(16) Briefly, the everted intestinal sac was prepared in ice-cold modified Krebs-Henseleit bicarbonate buffer (KHBB, pH 7.4). Fluorescein-isothiocyanate dextran with a molecular weight of 4000 Da (FD4) was used as a permeability probe. One end of the intestinal segment was ligated with a 3-0 silk ligature. The sac was then everted onto a thin plastic rod, and the resulting everted sac was connected to a 3-ml plastic syringe containing KHBB. The everted sac was gently distended by injecting 1.5 ml of KHBB. The everted sac was then suspended in a 50-ml beaker containing 40 ml of KHBB with added FD4 (20 μ g/ml). The solution in the beaker was maintained at 37°C and was continuously bubbled with a gas mixture containing 95% O₂ and 5% CO₂. A 1.0-ml sample was taken from the beaker before placing the everted sac to determine the initial FD4 concentration of the mucosal side. After incubation for 30 minutes, the length of

the gut sac was measured. The fluid was aspirated from the inside of the sac to determine FD4 concentration of the serosal side. The serosal and mucosal samples were centrifuged for 10 minutes at 1000 g at 4°C. One hundred microliters of supernatant was diluted with 900 μ l of phosphate-buffered saline (PBS). Fluorescence measurements were made with a fluorescence plate reader at an excitation wavelength of 492 nm and an emission wavelength of 515 nm. Gut permeability was expressed as the mucosal-to-serosal clearance of FD4 calculated as described by Tawadrous *et al.* (17)

Myeloperoxidase Activity—Intestinal MPO activity is as an indicator of the extent of neutrophil infiltration into the mucosa. MPO activity in the intestine was measured using Myeloperoxidase (MPO) Colorimetric Activity Assay Kit (BioVision Research Products, Mountain View, CA) normalized to milligrams of protein determined by Bio-Rad Protein Assay (Bio-Rad Laboratories, Inc. Hercules, CA).

Histopathologic injury—Intestinal tissues from within the intestinal sac were harvested and fixed for 24 hours in buffered formaldehyde solution (10% in PBS) at room temperature, then imbedded in paraffin wax, cut into 5 μ m sections, deparaffinized, and stained with hematoxylin and eosin for villus injury determinations. Five random fields with 200–250 villi from each animal were then analyzed in a blinded fashion using light microscopy at 100x magnification. Villus injury was deemed present if part of the villus tip had sloughed or necrosis, or if submucosal edema was present. The overall percentage of jejunal villous damage was determined by dividing the number of injured villi by the total number of villi examined.(18)

Ex Vivo Assessment of Clearance in the Gut—The Caliper Instruments In Vivo Imaging System (IVIS) Lumina XR animal imaging platform was used to image the gut ex vivo with visual and quantitative analysis of injury. Coimbra et al previously validated this technique to measure dye clearance as an indices of gut injury in a rodent burn model.(19) They stated that the uninjured bowel can clear the dye while the injured bowel, due to either decreased villous height or decreased perfusion, would exhibit reduced clearance. One microgram of Alexa Fluor 680 dye (3kDA, Introgen, Carlsbad, CA) was injected into gut sacs 30 minutes prior to harvest. Sacs were excised then images obtained with identical camera settings from field of view. Quantification of fluorescence was performed using Living Images 3.0 software (Caliper LifeSciences) by measuring and comparing fluorescence intensity within a standardized region of interest applied to all experimental samples.

Statistical analysis

All data are presented as mean $\pm$ SEM, with significance at $p < 0.05$. Results were analyzed by one-way ANOVA with Tukey post hoc tests. For cell culture experiments, results are mean $\pm$ SEM of 3 independent experiments/group. For animal experiments, $n = 8$ /group.

RESULTS

In vitro

Glutamine has beneficial effects after ischemia/reperfusion in vitro—As shown in Table 1, cell permeability and caspase 3/7 activity significantly increased while cell growth decreased after in vitro ischemia/reperfusion. Glutamine supplementation reversed these adverse effects down to normoxic levels.

Effect of glutamine on syndecan-1 mRNA and ectodomain protein expression

—There was no change in syndecan-1 transcription with increasing doses of glutamine

(Figure 1A). There was a slight increase in syndecan-1 protein expression with glutamine supplementation beyond the physiologic range (Figure 1B).

Effect of glutamine on syndecan-1 cell surface expression and shedding—

Immunostaining revealed that syndecan-1 cell surface expression was significantly reduced by in vitro ischemia/reperfusion (598 ± 81) compared to normoxic controls (1001 ± 76) but partially restored to normoxic levels (899 ± 116) by glutamine supplementation (Figure 2A). These changes correlated with a significant increase in shed syndecan-1 after in vitro ischemia/reperfusion (0.408 ± 0.006) and a reduction to normoxic levels (0.234 ± 0.16) after glutamine supplementation (0.231 ± 0.002) (Figure 2B).

In vivo

Glutamine's reduction in intestinal permeability, inflammation, and histopathologic injury was abolished in syndecan-1 KO mice—

As shown in figure 3, there were no significant differences between sham WT (28.3 ± 1.6 ng/cm²/min) and sham KO mice (33.8 ± 3.4 ng/cm²/min) mice in intestinal permeability. WT IR mice had a significant increase in permeability (87.8 ± 2.5 ng/cm²/min), which was further increased in KO IR mice (119.8 ± 9.0 ng/cm²/min). Glutamine, however, reduced permeability after IR in WT (59.2 ± 9.03 ng/cm²/min) but not KO mice (116.8 ± 9.92 ng/cm²/min). Similarly, there was a significant increase in inflammation and histopathologic injury in WT IR mice and inflammation was further increased in KO IR mice (Table 2). Glutamine WT IR mice demonstrated a significant decrease in injury and inflammation compared to WT IR or KO IR mice. Glutamine had no effect on either parameter in KO IR animals.

Glutamine's reduction in ex vivo intestinal fluorescence is mitigated in syndecan-1 KO mice—

We used the novel technique described by Coimbra et al to assess clearance of a fluorescent dye in an excised gut sac, using the Caliber Instrument IVIS Lumina XR which allows both visual and quantitative analysis.⁽¹⁸⁾ Images of the excised gut sacs are shown in Figure 4A and 4B and show fluorescence intensity remaining in the gut sacs at the time of harvest. Fluorescence remaining after IR in WT mice ($3.2 \times 10^8 \pm 1.4 \times 10^7$) compared to shams ($1.9 \times 10^8 \pm 2.2 \times 10^7$ WT and $1.7 \times 10^8 \pm 3.0 \times 10^7$ KO) was significantly increased. Fluorescence was further increased in IR KO mice ($4.8 \times 10^8 \pm 1.8 \times 10^7$). Glutamine, however, significantly decreased fluorescence intensity after IR in WT ($2.6 \times 10^8 \pm 1.8 \times 10^7$) but not in KO mice ($4.6 \times 10^8 \pm 2.1 \times 10^7$).

DISCUSSION

In an in vitro model of ischemia/reperfusion, glutamine supplementation reduced intestinal cell permeability and caspase activity and enhanced cell growth which was associated with a decrease in shed syndecan-1. In a mouse model of gut ischemia/reperfusion, intestinal permeability, inflammation, and functional injury were all increased in WT mice and further increased in syndecan-1 KO mice. Glutamine's reduction in intestinal hyperpermeability, inflammation, and histopathologic and clearance after I/R were abolished in syndecan-1 KO mice. These results indicate that syndecan-1 plays a novel role in the protective effects of enteral glutamine in the post ischemic gut.

The precise mechanism by which syndecan-1 regulates glutamine's protective effects is unknown. One potential mechanism would be for glutamine to reconstitute cell surface syndecan-1 by mobilizing an intracellular pool of syndecan-1. Syndecan-1 mobilized to the surface could then reconstruct the protective network of proteoglycans to re-establish an effective epithelial barrier. Consistent with these data, glutamine increased expression of

syndecan-1 on the surface, suggesting that mobilized syndecan-1 repairs hyperpermeability. Alternatively, glutamine may stimulate syndecan-1 gene expression, but our real time PCR data argue against this mechanism. How glutamine increased cell surface expression of syndecan-1 in a post-transcriptional manner is incompletely understood, but several agonists have been shown to increase cell surface syndecan-1 in this manner, possibly by recruiting pre-formed syndecan-1 in intracellular compartments.(20,21)

A second potential mechanism by which glutamine could increase syndecan-1 levels is either by inhibiting sheddases or regulating inhibitors of shedding. Ectodomain shedding is regulated by multiple signaling pathways converging on a diverse group of proteases, referred to as sheddases.(22) Several enzymes belonging to the family of matrix metalloproteinases (MMPs) and A Disintegrin And Metalloproteinases (ADAMs) have been found to shed syndecan-1. ADAM-17, in particular, is an effective sheddase in epithelial tissues.(23) Recent data by Pruessmeyer et al demonstrated that ADAM17 mediated inflammation-induced shedding of syndecan-1 in lung epithelial cells which was inhibited by the MMP inhibitor, GW280264.(24) Shedding releases ectodomains as soluble molecules which reduce the concentration of syndecan-1 on the cell surface. Shed syndecan-1 ectodomains have been assigned both beneficial and deleterious roles in tissue injury. (25,26) We hypothesize that syndecan-1 shedding produces a pathologic phenotype by exposing the underlying epithelium to pro-inflammatory and injurious mediators. We have shown that metalloproteinases (MMPs) are important in IR-induced gut injury and that glutamine inhibits MMP2 and MT1-MMP. (27) Interestingly, MT1-MMP is a syndecan-1 sheddase, (28) suggesting that glutamine can modulate shedding via this mechanism.

Available data suggests that epithelial syndecan-1 is essential in barrier function. Loss of renal epithelial syndecan-1 is associated with proteinuria in patients with native kidneys and with worsened graft survival in kidney transplant patients.(29) Loss of intestinal epithelial syndecan-1 leads to protein leakage in a model of protein losing enteropathy.(8) Lastly, loss of epithelial syndecan-1 is associated with more aggressive and advanced stages of colorectal cancer.(30) Our study suggests that syndecan-1 plays an important role in barrier function after gut I/R. Loss of syndecan-1 on the cell surface further enhanced permeability and glutamine lost its ability to restore permeability in the absence of syndecan-1. Further studies are required to determine if glutamine broadly protects against epithelial tissue injury in vivo by influencing cell surface levels of syndecan-1.

In conclusion, we have shown that the gut protective effects of enteral glutamine are abolished with the loss of intestinal epithelial syndecan-1. The novel findings of the current study shed more light onto the complex mechanisms by which glutamine exerts its gut protective effects of the post ischemic gut and further support the early use of enteral glutamine.

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References

1. Pfeifer R, Tarkin IS, Rocos B, Pape HC. Patterns of mortality and causes of death in polytrauma patients-has anything changed? *Injury*. 2009; 40(9):907–911. [PubMed: 19540488]
2. Moore FA, Feliciano DV, Andrassy RJ, McArdle AH, Booth FV, Morgenstein-Wagner TB, Kellum JM Jr, Welling RE, Moore EE. Early enteral feeding, compared with parenteral, reduces postoperative septic complications - the results of a meta-analysis. *Ann Surg*. 1992; 216:172–183. [PubMed: 1386982]

3. Santora R, Kozar RA. Molecular Mechanisms of Pharmaconutrients. *J Surg Res.* 2010; 15;161(2): 288–94. Epub 2009 Jul 17.
4. McQuigan M, Kozar RA, Moore FA. Enteral glutamine during active shock resuscitation is safe and enhances tolerance. *JPEN.* 2008; 32(1):28–35.
5. Rupani B, Caputo FJ, Watkins AC, Vega D, Magnotti LJ, Lu Q, Xu da Z, Deitch EA. Relationship between disruption of the unstirred mucus layer and intestinal restitution in loss of gut barrier function after trauma hemorrhagic shock. *Surgery.* 2007 Apr; 141(4):481–489. [PubMed: 17383525]
6. Kozar RA, Schultz SG, Hassoun HT, DeSoignie R, Weisbrodt NW, Haber MH, Moore FA. The type of sodium-coupled solute modulates small bowel mucosal injury, transport function and ATP after Ischemia/reperfusion injury in rats. *Gastroenterology.* 2002; 123(3):810–816. [PubMed: 12198707]
7. Kozar RA, Schultz SG, Bick RJ, Poindexter BJ, DeSoignie R, Moore FA. Enteral glutamine but not alanine maintains small bowel barrier function after ischemia/reperfusion injury in rats. *Shock.* 2004; 21(5):433–437. [PubMed: 15087819]
8. Bode L, Salvestrini C, Park PW, Li JP, Esko JD, Yamaguchi Y, Murch S, Freeze HH. Heparan sulfate and syndecan-1 are essential in maintaining murine and human intestinal epithelial barrier function. *J Clin Invest.* 2008; 118:229–238. [PubMed: 18064305]
9. Day R, Forbes A. Heparin, cell adhesion, and pathogenesis of inflammatory bowel disease. *Lancet.* 354:62–65. [PubMed: 10406379]
10. Ban K, Kozar RA. Glutamine protects against apoptosis via downregulation of Sp3 in intestinal epithelial cells. *Am J Physiol Liver Physiol.* 2010; 209:G1344–G1353.
11. Ban K, Santora R, Kozar RA. Enteral arginine modulates inhibition of AP-1/c-Jun by SP600125 in the postischemic gut. *Mol Cell Biochem.* 2010 Oct 29;347:191–9. 2011. [Epub ahead of print]. [PubMed: 21046201]
12. Kozar RA, Peng Z, Zhang R, Holcomb JB, Pati S, Park P, Ko TC, Paredes A. Plasma restoration of endothelial glycocalyx in a rodent model of hemorrhagic shock. *Anesth Analg.* 2011; 112:1289–1295. [PubMed: 21346161]
13. Park PW, Pier GB, Hinkes MT, Bernfield M. Exploitation of syndecan-1 shedding by *Pseudomonas aeruginosa* enhances virulence. *Nature.* 2001; 411:98–102. [PubMed: 11333985]
14. Sato N, Moore FA, Smith MA, Zou L, Olufemi-Moore S, Schultz SG, Kozar RA. Differential induction of PPAR{gamma} by luminal glutamine and iNOS by luminal arginine in the rodent post ischemic small bowel. *Am J Physiol Gastrointest Liver Physiol.* 2006; 290(4):G616–623. Epub 2005 Oct 27. [PubMed: 16257923]
15. Hassoun H, Zou L, Moore FA, Kozar RA, Weisbrodt NW, Kone B. -Melanocyte-stimulating hormone protects against mesenteric ischemia-reperfusion injury. *Am J Physiol Cell Physiol.* 2002; 282:C1059–C1068.
16. Wattanasirichaigoon S, Menconi MJ, Delude RL, Fink MP. Lisofylline ameliorates intestinal mucosal barrier dysfunction caused by ischemia and ischemia/reperfusion. *Shock.* Apr; 1999 11(4):269–75. [PubMed: 10220304]
17. Tawadrous ZS, Delude RL, Fink MP. Resuscitation from hemorrhagic shock with Ringer's ethyl pyruvate solution improves survival and ameliorates intestinal mucosal hyperpermeability in rats. *Shock.* 2002; 17(6):473–7. [PubMed: 12069183]
18. Deitch EA, Shi HP, Lu Q, Feketeova E, Xu DZ. Serine proteases are involved in the pathogenesis of trauma-hemorrhagic shock-induced gut and lung injury. *Shock.* 2003; 19:452–456. [PubMed: 12744489]
19. Costantini TW, Eliceiri E, Peterson CY, Loomis WH, Putnam JG, Baird A, Wolf P, Bansal V, Coimbra R. Quantitative assessment of intestinal injury using a novel in vivo, near-infrared imaging technique. *Molecular Imaging.* 2010; 9:30–39. [PubMed: 20128996]
20. Hayashida K, Johnston DR, Goldberger O, Park PW. Syndecan-1 expression in epithelial cells is induced by transforming growth factor beta through a PKA-dependent pathway. *J Biol Chem.* 281:24365–24374. [PubMed: 16807246]
21. Yeaman C, Rapraeger AC. Post-transcriptional regulation of syndecan-1 expression by cAMP in peritoneal macrophages. *J Cell Biol.* 1993; 122:941–950. [PubMed: 8394371]

22. Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. *J Cell Sci.* 2002; 115:3719–3727. [PubMed: 12235282]
23. Edwards DR, Handsley MM, Pennington CJ. The ADAM metalloproteinases. *Mol Aspects Med.* 2008; 29:258–289. [PubMed: 18762209]
24. Pruessmeyer J, Martin C, Hess FM, Schwarz N, Schmidt S, Kogel T, Hoettecke N, Schmidt B, Sechi A, Uhlig S, Ludwig A. A disintegrin and metalloproteinase 17 (ADAM17) mediates inflammation-induced shedding of syndecan-1 and -4 by lung epithelial cells. *J Biol Chem.* 2010; 285:555–564. [PubMed: 19875451]
25. Hayashida K, Parks WC, Park PW. Syndecan-1 shedding facilitates the resolution of neutrophilic inflammation by removing sequestered CXC chemokines. *Blood.* 2009; 114:3033–3043. [PubMed: 19638625]
26. Kliment CR, Englert JM, Gochuico BR, Yu G, Kaminski N, Rosas I, Oury TD. Oxidative stress alters syndecan-1 distribution in lungs with pulmonary fibrosis. *J Biol Chem.* 2009; 284:3537–3545. [PubMed: 19073610]
27. Robinson EK, Kelly DP, Mercer DW, Kozar RA. Differential effects of luminal arginine and glutamine on metalloproteinase production in the postischemic gut. *JPEN.* 2008; 32:433–438.
28. Endo K, Takino T, Miyamori H, Kinsen H, Yoshizaki T, Furukawa M, Sato H. Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration. *J Biol Chem.* 2003; 278:40764–40770. [PubMed: 12904296]
29. Celie JW, Katta KK, Adepu S, Melenhorst WB, Reijmers RM, Slot EM, Beelen RH, Spaargaren M, Ploeg RJ, Navis G, van der Heide JJ, van Dijk MC, van Goor H, van den Born J. Tubular epithelial syndecan-1 maintains renal function in murine ischemia/reperfusion and human transplantation. *Kidney Int.* 2012 Jan 11. [Epub ahead of print]. 10.1038/ki.2011.425
30. Lundin M, Nordling S, Lundin J, Isola J, Wiksten JP, Haglund C. Epithelial syndecan-1 expression is associated with stage and grade in colorectal cancer. *Oncology.* 2005; 68(4–6):306–313. [PubMed: 16020957]

Figure 1. Effect of glutamine on syndecan-1 mRNA and ectodomain protein expression
IEC-6 cells supplemented with 2.5 to 20 mM glutamine were subjected to hypoxia with 1% O₂-5% CO₂-94% N₂ for 24 hours followed by 4 hours of reoxygenation. Cells were harvested for A. Quantitation of mRNA by quantitative real-time reverse-transcription polymerase chain reaction or B. protein expression by Western Blot analysis. B-actin was used as a loading control. The data shown indicate mean $\pm$ SEM of 3 independent experiments/group.

Figure 2. Syndecan-1 shedding is mitigated by glutamine

IEC-6 cells supplemented with 2.0 or 10 mM glutamine were subjected to hypoxia with 1% O₂-5% CO₂-94% N₂ for 24 hours followed by 4 hours of reoxygenation. A. Cells were immunostained with antisyndecn-1 monoclonal antibodies (magnification = original × 200) and the nucleolus with DAPI. Images were obtained using an Olympus 1X71 microscope. Representative images are shown in the upper panel. The relative fluorescence intensity was quantified and reported as relative fluorescence units (RFU). B. Media was collected and syndecan-1 ectodomain expression assessed by ELISA. The data shown indicate mean ± SEM of 3 independent experiments/group.

Figure 3. Glutamine's reduction in intestinal permeability is lost in syndecan-1 KO mice
 Small bowel enteral sacs $\pm$ 60 mM glutamine were fashioned in wild type and *Sdc1*^{-/-} mice. The superior mesenteric artery was clamped for one hour followed by six hours of reperfusion and compared to shams. Gut permeability is expressed as the mucosal-to-serosal clearance of FD4 which was logarithmically transformed prior to statistical analysis. The data shown indicate mean $\pm$ SEM with n= 8/group.

Figure 4. Glutamine's reduction in ex vivo intestinal fluorescence is mitigated in syndecan-1 KO mice

Small bowel enteric sacs $\pm$ 60 mM glutamine were fashioned in wild type and *Sdc1*^{-/-} mice. The superior mesenteric artery was clamped for one hour followed by six hours of reperfusion and compared to shams. Gut sacs were injected with a 3000 MW dextran

conjugated to Alexa-fluor 680 thirty minutes prior to harvest and imaged using the Caliper Instruments IVIS Lumina XR system using near-infrared detection settings that were standardized across all samples. A. Black and white and fluorescent images of excised gut sacs are shown from a representative animal in each group. The more yellow in the image, the less clearance and therefore greater degree of injury. B. Quantification of fluorescent signal from resected intestinal sacs. The data shown indicate mean $\pm$ SEM with n= 8/group.

Table 1

In vitro protection by glutamine

	Permeability	Caspase 3/7	Proliferation
Normoxia	0.86 ± 0.64	2574 ± 190	2.08 ± 0.01
HR 2mM glut	11.25 ± 2.21 *	20,938 ± 951 *	0.75 ± 0.01 *
HR10mM glut	2.54 ± 0.17 *#	2753 ± 392	1.95 ± 0.01

*
p<0.01 vs normoxia and HR 10mM glut

p<0.01 vs normoxia

Table 2

In vivo parameters of gut dysfunction

Groups	MPO (mU/ml)	Gut injury (injured villi %)
WT sham	0.24 ± 0.01	10.2 ± 1.5
KO sham	0.27 ± 0.01	7.9 ± 0.9
WT IR	1.25 ± 0.12 ^{*Φ}	47.6 ± 3.5 [*]
KO IR	1.83 ± 0.13 [#]	50.3 ± 3.6 [#]
WT IR glut	0.77 ± 0.06 [¶]	31.8 ± 2.7 [¶]
KO IR glut	1.54 ± 0.13	46.3 ± 3.7

^{*} p<0.01 vs WT sham and WT IR glut

^Φ p<0.01 vs KO IR

[#] p<0.01 vs KO sham

[¶] p<0.01 vs KO IR glut