Editorial

Modulation of the intestinal bacterial flora: a viable strategy to alleviate acute mesenteric ischemia?

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Introduction

The study of the gut microbiota and its relevance in different pathologies has experienced a boom in the last decade, and many diagnoses are thought to be influenced by the bacterial composition in the gut (1). In the paper entitled "Use of dexmedetomidine to alleviate intestinal ischemia-reperfusion injury via intestinal microbiota modulation in mice" by Dong et al in this issue, the authors examine the mechanistic effects of dexmedetomidine (DEX) in reducing ischemia-reperfusion injury (IRI) after a 60-minute occlusion of the superior mesenteric artery (SMA) (2). The authors suggest that the mechanism could be based on alterations in the balance between bacteria in the phylum Bacteroidetes and Firmicutes in the bowel.

The model mimics acute mesenteric ischemia (AMI), a serious condition usually affecting patients with preexisting cardiovascular disease and has a mortality rate from 60% to 80% (3). Complete occlusion of the SMA is usually caused by an embolus or a thrombosis but nonocclusive mesenteric ischemia can be caused by hypoperfusion secondary to systemic shock.

The intestine is sensitive to ischemic insults and irreversible damage rapidly ensues. The clinical picture is often one of abdominal pain when blood flow is obstructed, accompanied by nonspecific lab changes such as increasing blood lactate levels and leukocytosis. This is rapidly followed by signs of sepsis when the barrier breaks down and bacterial translocation ensues.

Physiologic and microscopic changes induced by bowel ischemia

On a microscopic level, during ischemia, fluid collects between the intestinal epithelial cell (IEC) layer and the lamina propria, which is hyperosmolar. Poor circulation in the subepithelial capillaries combined with reduced activity of energy-dependent ion pumps makes it difficult to clear this excess fluid away. Fluid accumulates most rapidly in the distal villus tip, due to this location having the highest osmotic gradient, and then gradually engages the rest of the villus in a proximal direction.

Due to the excess fluid being located between the base of the IEC and the lamina propria, the basal aspects of the IECs are affected first. The cells are unable to rid themselves of the excess sodium with its attendant water since the Na+/K+ ATPase pump is inhibited. This pump usually transports sodium out of the basal aspect of the cell and towards the subepithelial capillaries. This causes swelling and ultimately rupture of the basal part of the

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enterocyte (4). This progressive damage is easily observed histologically and scored with increasing severity using the Chiu-Park score (5).

The mechanisms of ischemia-reperfusion injury

The imbalance between metabolic supply and demand in the ischemic bowel results in tissue hypoxia and dysfunction of the microvasculature. Reperfusion subsequently increases the activation of the innate and adaptive immune responses. Following reperfusion, the initial response is the generation of reactive oxygen species (ROS). Paradoxically, reintroduction of oxygen enhances cellular injury due to changes that take place during the hypoxic period. Increased ROS production has been detected as early as 20 seconds after reperfusion and results in damage to cellular components such as proteins, nucleic acids, mitochondria, and cellular membranes. ROS can cause oxidative stress, directly damaging cellular components or they can act as mediators amplifying the inflammatory response. In turn ROS may lead to necrosis, apoptosis, or cause activation, a change in cellular phenotypes in response to injury. Several transcription factors are activated by hypoxia and oxidative stress. Of special importance for the innate immune response is nuclear factor-κB (NF-κB). NF-κB has an important role in inflammation by regulating the expression of genes which cause production of important inflammatory mediators such as interleukins, adhesion molecules, tumor necrosis factor, metalloproteinases and colony-stimulating factors (6).

Barrier injury allows the luminal contents to interact with the immune system

The epithelial barrier may be disrupted by ischemic damage alone or in combination with IRI. Disruption of the barrier allows contact between luminal contents and submucosal structures, triggering a more severe inflammatory response. Microorganisms found in the lumen express microbe-associated molecular patterns (MAMPs) which are essential in triggering the immune response. Furthermore, when the integrity of an enterocyte is disrupted, damage-associated molecular patterns (DAMPs) like heat-shock proteins and nucleic acids are released. Both DAMPs and MAMPs are recognized by receptors on epithelial and immune cells called pattern-recognition receptors (PRRs). Triggering these receptors leads to a cascade of events eliciting an inflammatory response (6).

Bacterial composition may affect the production of MAMPs

The inclusion of fecal transplantation in Dong *et al.*'s study demonstrates that bacterial composition, specifically the change in the Firmicutes/Bacteroidetes ratio, is the likely mechanism by which DEX mediates its protective effect. The phylum Firmicutes includes Gram-positive bacteria with a rigid or semi-rigid cell wall whilst the phylum Bacteroidetes includes Gram-negative bacteria. Bacterial lipopolysaccharides, endotoxins found on the cell membranes of Gram-negative bacteria are considered the prototypical MAMPs. It is therefore conceivable that a reduction in Gram-negatives relative to other bacteria helps explain the improvement to IRI described.

Interventions to save the affected bowel must be done quickly

The repair phase following AMI has been extensively studied by Grootjans' group in both animal models and humans (7). They have shown a rapid breakdown of the villi tips during ischemia with an additional 1–2 grade initial worsening after reperfusion, but also an astonishingly rapid recovery of the tissue in cases where circulation is restored rapidly (8). The short time available is clinically challenging and any strategies which may be employed to extend this time could potentially improve the outcomes for this patient group. The time of intervention is highly relevant. In clinical practice the time from symptom debut to AMI diagnosis must be short. Similarly, the time from initial suspicion to potentially curative treatment must be brief if it is to succeed. It is during this short time, when ischemia is already established, that effective pharmaceuticals could theoretically make a difference to outcomes.

Curative treatment for AMI in the form of surgery (embolectomy, mesenteric bypass) or interventional radiology (embolectomy, catheter-directed thrombolysis, balloon angioplasty) which leads to a reestablishment of patent circulation and a recovery of bowel function must be exceedingly rapid if the affected bowel is to be saved. In many cases, at the time of surgery the damaged bowel must be resected. Since reperfusion injury exacerbates the initial damage a second-look surgery is mandatory to remove part of the bowel that may have appeared viable on the initial operation but become damaged beyond repair after reperfusion. If the blockage is in the proximal part of the SMA, it may eventually become necessary to resect

the entirety of the small intestine and the proximal half of the large intestine. Such an extensive resection will lead to short bowel syndrome (SBS) with subsequent nutritional impairment. SBS may be rectified with total parenteral nutrition (TPN). However, in many cases long-term TPN leads to liver damage and the need for a small bowel transplant or, in cases where liver damage has become irreversible, a multivisceral transplant.

With these potentially disastrous clinical consequences in mind, strategies to minimize ischemic damage are welcome.

Is the use of DEX to modulate the intestinal bacterial flora a viable strategy to alleviate acute mesenteric ischemia?

The study by Dong et al convincingly demonstrates why DEX has a protective effect on the intestinal epithelium. While this is intellectually gratifying it has limited potential practical applications in its current format where the intervention must be carried out 24 hours before the ischemic insult is initiated. If an intervention must be carried out the day before an ischemic event it is not a practical solution to the problem. If one were to assess DEX as a therapeutic for the proposed indication, a study to look at the intervention at a relevant time-point needs to be done, i.e., administered after the establishment of symptomatic ischemia.

A drug which must be given 24 hours before an acute ischemic insult has very limited scenarios where it might be useful. One such case could be planned reimplantation of the SMA. In such a case it would be reasonable to assume that transient ischemia would occur and could potentially be ameliorated by such a strategy. Another potential avenue is in the case of donor pretreatment for small bowel transplantation. These grafts are always affected by ischemia during cold storage and the procurements are often planned 24 hours in advance. It may be permissible in certain jurisdictions to pretreat multiorgan donors with DEX to optimize the bacterial flora and thus reduce the severity of ischemia reperfusion injury in the transplant recipient. In its current iteration this may be a more suitable application of DEX pretreatment.

Lessons from small intestine transplant studies

Multiple groups have looked at ways to better improve the barrier function of transplanted grafts with various protective strategies (9-14). In these studies, it has been made clear that the unmitigated contact of feces with the enterocyte layer is damaging which could in part be due to goblet cell depletion and loss of the mucin layer. However, many strategies that seem very convincing in small animal models lose their allure when confirmatory experiments are done by other groups, on large animal models or in humans (15). Great care is needed when extrapolating findings from studies in small animal models to other more clinically relevant settings such as larger species or to humans (16,17). In fact, the successful conversion of a concept found to be promising in a small animal model to a confirmation study in a much more costly large animal or human study is quite uncommon. One would ideally want an independent research group to confirm the validity of a concept in another small animal model before committing to the cost and effort of conducting confirmatory studies in larger models (15).

Modified Chiu-Park scores: perfect is the enemy of good

When comparing study results between groups on small bowel ischemia, the relatively simple method of assessing and scoring histological damage with the original Chiu-Park score is easily conducted by even relatively inexperienced labs. Such scoring should be reported in a standardized way which is easy to interpret. A great many articles have been written using various modifications of the Chiu-Park score. However, most modifications are never described accurately nor validated systematically. The system most commonly cited in the literature, which is easy to score, shows consistent scoring between observers and provides information about damage ranking from mild to critical is the original Chiu-Park score (5). In a head-to-head comparison between different systems, this one has been shown to be preferable and should be favored in the standardized reporting of results until someone convincingly shows otherwise (18).

Unfortunately, one unnecessary shortcoming in the study is the use of a non-standardized modified scoring system. This makes it more difficult to interpret than it needs to be. The scoring system is described briefly in the provided reference as a modified Chiu-Park score with 10 grades (0–9) rather than the original 9 (0–8). The use of non-validated scoring systems with minor modifications makes interpretation more difficult for other researchers and should be avoided until a new formal validation study is published demonstrating superiority (18).

If authors wish to give additional valuable information in studies assessing IRI, data on the height and width of villi should be provided from serial sampling prior to and during reperfusion. In successful repair after mild to moderate ischemic damage this should show, after an initial worsening of injury grade, a shortening and a widening of the villi when repair is completed (8).

The way forward

The current work introduces a promising new avenue that may be valuable in developing strategies to ameliorate AMI and its associated reperfusion injury. Conceptually, the modulation of the bacterial flora to reduce the stimulation of the innate and adaptive immune system if very appealing. However, all small animal studies should be confirmed by other groups and in other animal models before attaching too much weight to the concepts. Differences between species can be consequential in the study of bowel ischemia (16,19). Considering that AMI primarily affects older patients, the human equivalent age of animals could make difference when conducting translational IRI studies (20). But these caveats aside, hopefully this paper heralds a new era of gut flora modulation for the purpose of reducing ischemia-reperfusion injury.

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