Animal models of ischemia-reperfusion-induced intestinal injury: progress and promise for translational research

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Gonzalez LM, Moeser AJ, Blikslager AT. Animal models of ischemia-reperfusion-induced intestinal injury: progress and promise for translational research. Am J Physiol Gastrointest Liver Physiol 308: G63–G75, 2015. First published November 20, 2014; doi:10.1152/ajpgi.00112.2013.—Research in the field of ischemia-reperfusion injury continues to be plagued by the inability to translate research findings to clinically useful therapies. This may in part relate to the complexity of disease processes that result in intestinal ischemia but may also result from inappropriate research model selection. Research animal models have been integral to the study of ischemia-reperfusion-induced intestinal injury. However, the clinical conditions that compromise intestinal blood flow in clinical patients ranges widely from primary intestinal disease to processes secondary to distant organ failure and generalized systemic disease. Thus models that closely resemble human pathology in clinical conditions as disparate as volvulus, shock, and necrotizing enterocolitis are likely to give the greatest opportunity to understand mechanisms of ischemia that may ultimately translate to patient care. Furthermore, conditions that result in varying levels of ischemia may be further complicated by the reperfusion of blood to tissues that, in some cases, further exacerbates injury. This review assesses animal models of ischemia-reperfusion injury as well as the knowledge that has been derived from each to aid selection of appropriate research models. In addition, a discussion of the future of intestinal ischemia-reperfusion research is provided to place some context on the areas likely to provide the greatest benefit from continued research of ischemia-reperfusion injury.

animal model; intestine; ischemia; mucosal injury; reperfusion

The survival rate for human and veterinary patients with ischemic intestinal injury is generally less than 50% (17, 69, 172, 183) because tissue hypoxia, inflammation, and cell infiltration result in loss of the mucosal barrier. This barrier is primarily composed of a single layer of columnar epithelial cells bound together by interepithelial junctions, which collectively prevents the translocation of bacteria and associated toxins into the systemic circulation (140). Intestinal ischemia is associated with a broad range of clinical conditions including neonatal necrotizing enterocolitis (125), acute mesenteric ischemia (AMI) (183), volvulus (155), trauma (41), cardiopulmonary disease (151), hemorrhagic shock (41, 53), and intestinal transplant rejection (112) (Fig. 1). Ischemic injury may be coupled with subsequent reperfusion of tissue that is known to exacerbate injury in some of these disease processes (112). Since intestinal ischemia is rarely preventable, most research in the field has focused on advancing techniques for early detection of ischemia and the development of novel therapeutic approaches that target the postischemic insult (the reperfusion period). Injury attributed to reperfusion is thought to be primarily attributable to reactive oxygen metabolites, associated with activation of oxidant-producing mucosal enzymes, release of lipid chemoattractants from injured cellular membranes, and subsequent infiltration of neutrophils (Fig. 2). Therefore, proposed therapeutic interventions to protect against reperfusion injury have targeted inhibition of oxidant injury and of neutrophil activation (144, 157). However, owing to the relative failure of clinical therapy directed at reperfusion injury following early studies, the importance of targeting reperfusion injury in cases of intestinal ischemic injury has been brought into question (105). The most recent clinical perspectives on the efficacy of these treatments are derived from studies of tissues more commonly affected by reperfusion, such as the myocardium following infarction (7). These too have failed to demonstrate the clinical utility of these therapies. Encouragingly, recent studies have recognized the modulation of cell death...
pathways as a novel target for therapeutic intervention (87, 181). Mechanisms of cell death mediated by necrosis and apoptosis have been shown to further exacerbate injury induced by ischemia and reperfusion. In fact, insufficient clearance of these dying cells has been shown to lead to increased inflammation and impaired tissue repair (181). As a result, there has been a shift of emphasis toward modulation of pathways of cell death (87, 181) and regenerative processes (113, 161). However, initial consideration of the animal model to select for study is of paramount importance, given the highly variable clinical presentation of diseases that involve ischemia. Animal models have been indispensable to the study of mechanisms of ischemia and reperfusion injury (22, 39, 134–137), but each model has distinct advantages and disadvantages so that none of these models can perfectly recapitulate the natural onset and progression of human disease (Tables 1 and 2). The aim of this review is to provide an in-depth assessment of key animal models used for the study of ischemia-reperfusion injury, with the goal of allowing investigators to understand and select from available models for translational research. In addition, the future of intestinal regenerative medicine in light of recent advances will be discussed.

Mechanisms of Ischemia

Ischemia is the reduction or complete occlusion of blood flow to a target organ that results in a state of tissue oxygen deprivation. In the intestine, overt obstruction of the blood supply can result from mesenteric vascular occlusion derived from thrombus formation or emboli secondary to cardiopulmonary disease (143, 166). Compromise of normal blood flow to the intestine can also be associated with severe intestinal distension and mechanical obstruction as occurs in cases of intestinal strangulation associated with hernia, volvulus and intussusception (4, 183). Additionally, ischemic injury is thought to contribute to the pathogenesis of neonatal necrotizing enterocolitis, the most common life-threatening gastrointestinal emergency in neonatal patients (69, 125, 127). Other major sources of intestinal ischemic injury result from diseases that reduce systemic blood flow such as cardiopulmonary diseases and shock states (53, 151). Finally, the interruption of blood flow is an important component of the process of intestinal transplantation that inevitably results in a period of ischemia (106, 112) (Fig. 1).

Decreases in blood flow compromise the oxygen supply required for normal cellular function and result in both cellular damage and death. The intestinal epithelium has a particularly high energy demand, largely as a result of basolateral Na\(^+-\)K\(^+-\)ATPase used to drive secretion and absorption, and is therefore particularly sensitive to reductions in blood flow. The process of epithelial cell loss that results from ischemic injury is well documented in many research animal species (39, 135, 137, 147). Cells closest to the intestinal lumen on the villus tips in the small intestine and intercrypt surface epithelium in the colon initially lose their attachment to the basement membrane with progressive loss of cells extending toward the crypt base as the duration of ischemia increases (39, 135) (Fig. 3). Interestingly, the cells closest to the luminal surface normally function in a state of “physiological hypoxia” due to an oxygen gradient that is derived from the mucosal vascular architecture (146, 171). Although there are species differences in the anatomy of the mucosal vasculature (Fig. 4), a general construct of countercurrent exchange exists whereby oxygen diffuses from the arterial blood supply that ascends the mucosa toward the lumen and venous drainage flowing in the opposite direction, progressively decreasing the arterial concentration of oxygen as the vessels near the luminal surface (2, 9). This creates a steep oxygen gradient that makes the villus tips hypoxic (33, 159). A similarly decreasing gradient of oxygen from the crypt base to the luminal surface exists within the colon (171). Therefore, further decreases in oxygen concentrations, as occurs with intestinal ischemia, impact the cells at the villus tips and colonic surface epithelium first. This initially creates a distinct lesion identified histopathologically, called Gruenhagen’s space (16, 39) (Fig. 3). Originally, this space was solely attributed to the accumulation of cytoplasmic fluid...
from ischemically damaged cells (39). More recent studies using in vitro techniques and a novel in vivo human study have shown that contraction of the myofibroblasts within the lamina propria also contribute to the creation of this initial defect (46, 141). In fact, the creation of the space was shown to occur within 30 min of complete ischemia and reestablish by 60 min if the blood supply was subsequently returned (46). This demonstrates the remarkable capacity of the intestine to repair rapidly in cases where vascular compromise can be reversed. However, in most cases the duration of ischemia is likely to be lengthier and the tight junctions that serve to anchor the cells together break down, resulting in cell loss into the intestinal lumen. Epithelial cell separation from the basement membrane causes marked compromise of barrier function, allowing luminal bacteria and toxins to gain access to the underlying vasculature within the lamina propria (75, 111, 140). The sequelae are septicemia and multiple organ dysfunction, which are responsible for the high morbidity and mortality rates associated with severe intestinal ischemic injury (86, 143, 166, 183).

**Models of Ischemia**

**Complete vascular occlusion.** Complete ischemia by temporary vascular occlusion (with atraumatic vascular clamps) or permanent vascular occlusion (by ligation) of the superior mesenteric artery (SMA, technically the cranial mesenteric artery in animals) in rodent models is currently the most commonly used method of inducing intestinal ischemic injury. The SMA is approached through a midline incision and occluded for varying lengths of time (87). In most of these studies, heparin is injected intravenously to prevent thrombus formation within the SMA, which enables the circulation to be reestablished once the atraumatic vascular clamps are removed for measures of reperfusion injury. However, an early study by Megison et al. (116) noted a highly variable degree of injury and often high mortality of rats in studies in which occlusion of the SMA was performed. That study noted that SMA occlusion

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**Table 1. Comparison of models of ischemia**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA Ligation</td>
<td>Models occlusive causes of ischemia</td>
</tr>
<tr>
<td></td>
<td>Ease of surgical model creation</td>
</tr>
<tr>
<td>SMA Embolization</td>
<td>Models occlusive causes of ischemia</td>
</tr>
<tr>
<td></td>
<td>Intestinal tract not exposed or manipulated (1, 25, 96, 148)</td>
</tr>
<tr>
<td>Low-Flow</td>
<td>Ideal for diagnostic imaging studies</td>
</tr>
<tr>
<td>Segmental Vascular Occlusion</td>
<td>Poor model of chronic ischemia</td>
</tr>
<tr>
<td></td>
<td>Only arterial supply occluded</td>
</tr>
<tr>
<td></td>
<td>Unreliable to create consistent injury (116)</td>
</tr>
<tr>
<td></td>
<td>High mortality rate (116)</td>
</tr>
<tr>
<td></td>
<td>No reduction of blood flow to distal colon (142)</td>
</tr>
<tr>
<td></td>
<td>Different segments of intestine vary in resistance to ischemic injury (37, 107, 147)</td>
</tr>
</tbody>
</table>

SMA, superior mesenteric artery.

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**Table 2. Comparison of large and small animal models of digestive disease**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large animal (pig, dog, cat)</td>
<td>High cost</td>
</tr>
<tr>
<td></td>
<td>More difficult to manage</td>
</tr>
<tr>
<td></td>
<td>Special housing facilities</td>
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<tr>
<td></td>
<td>Few transgenic models available</td>
</tr>
<tr>
<td></td>
<td>Time consuming</td>
</tr>
<tr>
<td></td>
<td>Pathophysiological differences, specifically with regard to reperfusion injury (cat)</td>
</tr>
<tr>
<td></td>
<td>Morphological differences in mucosal epithelial cell populations compared with humans</td>
</tr>
<tr>
<td></td>
<td>Pathophysiological differences, specifically with regard to reperfusion injury</td>
</tr>
<tr>
<td></td>
<td>Absence of clinical signs of human disease in some models</td>
</tr>
<tr>
<td></td>
<td>Technically difficult to create surgery models due to size</td>
</tr>
<tr>
<td></td>
<td>Mortality rate</td>
</tr>
<tr>
<td></td>
<td>Genome is approximately 14% smaller than humans</td>
</tr>
</tbody>
</table>

Small animal (rodent)  

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low cost</td>
</tr>
<tr>
<td></td>
<td>Ease of maintenance</td>
</tr>
<tr>
<td></td>
<td>Ease of genetic manipulation</td>
</tr>
<tr>
<td></td>
<td>Rapid reproduction rate</td>
</tr>
<tr>
<td></td>
<td>Number of available and well established models</td>
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alone was unreliable in creating consistent and reproducible injury, whereas SMA occlusion combined with ligation of collateral arcades created a greater degree of injury and a higher but consistent level of mortality in the animals. These findings lent support to a similar study performed in cats in which SMA occlusion reduced blood flow to the mucosa an average of 35% in the proximal duodenum, 61% in the distal duodenum, 71% in the jejunum and ileum, and 63% in the proximal colon. No reduction of blood flow was identified in the mid and distal colon (142). Thus the effect of SMA occlusion is highly dependent on the intestinal segment being evaluated. These findings may be complicated further by the fact that different segments of the intestine may be more or less sensitive to ischemic injury. For example, differences in resistance to ischemic injury have been identified between the jejunum, ileum, and large colon, with studies indicating a progressive increase in resistance to ischemic injury in aboral segments (37, 107, 147). Furthermore, a porcine study of small bowel transplantation demonstrated microscopic injury in the ileum only after 5 h of ischemic injury compared with 1.5 h in the jejunum (35). Little reperfusion injury was noted in either segment. It should be noted, however, that this study only evaluated injury histologically.

Unfortunately, many studies of ischemic injury either assess injury in a single segment of intestine, many times the ileum, or do not specify which segment is being evaluated (Table 3). Additionally, few studies have focused on the colon despite the fact that colonic ischemia is a more common clinical entity in people than small intestinal ischemia (4, 51, 73, 122). The colon does, in fact, respond differently to ischemia. For example, a study compared regional differences in susceptibility to injury between the small intestine and colon and demonstrated that the colon was more resistant to ischemia in rats (107).

A more recent technique used to achieve complete irreversible ischemia was established in a porcine model by embolization of the SMA. The SMA was accessed either percutaneously (96) or via endovascular catheterization (1, 25, 148) and a solution of either butyl-2-cyanoacrylate (96) or polyvinyl alcohol particles and gel foam (1, 25, 148) was injected intravascularly. These models are particularly useful for imaging studies aimed at improving diagnosis in patients suspected of having AMI. 
not exposed or manipulated in creating the vascular obstruction. Since bowel manipulation alone creates inflammation that otherwise would not be present in clinical cases of AMI, minimally invasive approaches to the SMA may be optimal for studies modeling AMI.

The critical assessment of the type of ischemia model with regard to studies of AMI cannot be overstated. Acute mesenteric ischemia is a complex of diseases that can be classified on the basis of whether the mechanism of obstruction is occlusive or nonocclusive. Occlusive intestinal ischemia is further subdivided into acute or chronic and whether arteries or veins are affected. Nonocclusive mesenteric ischemia is a disease process that is more poorly understood but occurs with patent mesenteric arteries and is associated with disease processes such as congestive heart failure, aortic insufficiency, renal or hepatic disease, and patients following cardiac surgery (172). Therefore, other approaches to create ischemic injury such as the low-flow model likely better recapitulate this form of injury.

**Low-flow ischemia.** Most of the original low-flow ischemia studies were performed in cats (Table 3). This model likely best recreates the type of intestinal injury that would be expected following hemorrhagic shock or other events that dramatically decreases the volume of blood delivered to an intestinal segment. In the low-flow ischemia model, blood flow is reduced to 20% of baseline levels (~25–35 mmHg). With this method, the abdomen is approached through a midline incision and a segment of ileum is typically isolated. An arterial circuit is then established between the superior mesenteric and femoral arteries and pressure cannulas are used within the vessels to measure vascular pressure. Reduced blood pressure within the SMA (25–30 mmHg) is achieved by use of an adjustable vascular clamp. The majority of injury associated with this model has been attributed to reperfusion as a result of increases in xanthine oxidase and neutrophil activation, making the ischemic event more of a priming mechanism for subsequent injury. This may or may not adequately represent clinical scenarios, in which injury caused by ischemia may be so severe that reperfusion injury is of limited significance. Again, careful selection of models depending on the human condition being studied is critical.

Segmental mesenteric vascular occlusion. An additional model of ischemia is segmental mesenteric vascular occlusion, in which the blood flow within a discrete loop of intestine is interrupted by clamping the local mesenteric vascular supply and cross-clamping the bowel. Within a single animal, multiple loops can be subjected to varying lengths of ischemia with or without reperfusion, thereby titrating the degree of injury within each loop. This model can be readily performed in large animals such as the pig and in rodents (73, 74) and has the advantage of providing multiple treatment groups and controls within a single animal (Tables 4, 6, and 7). However, this model of strangulating obstruction relies on complete occlusion of the arterial and venous blood supply, whereas clinically the venous circulation is commonly compromised prior to the arterial blood supply owing to differences in vessel wall thickness and compliance (135). This results in continued arterial supply of oxygenated blood for a variable period of time, until the tissue becomes so congested in the absence of venous drainage that the arterial vascular supply ultimately collapses. This complex form of ischemic injury includes elements of ischemia as well as excessive interstitial pressure, which together likely affect the level of epithelial sloughing.

Perhaps the most interesting and significant application of the mesenteric vascular occlusion model of intestinal ischemia has been its application in human subjects (46, 47). In these studies, a normal segment of jejunum from patients undergoing Roux-en-Y or similar intestinal reconstruction were isolated and subjected to 30–45 min of mesenteric vascular occlusion, with varying lengths of reperfusion, prior to removal from the patient (46, 47, 75, 115). The limitation of these studies is that severe ischemic damage as applies to most patients suffering from ischemia-reperfusion injury cannot be induced in these clinical studies.

With regard to each of these models of ischemia, the research animal species used may contribute significantly to the outcome. As previously noted, variations of the mucosal vascular supply, particularly in the small intestine, exist between species (Fig. 4). The pig and human villus microvascular architecture are very similar, arborizing at the tip of the villus into a fountain-like pattern and converging into one or two venules located beside the centrally located arteriole (9, 33). In contrast, in the rat, the flattened leaf-shaped villi are supplied by a single arteriole that passes unbranched from the base to the tip of the villus where it bifurcates into a capillary network, a so-called “netted bag” pattern (38). The arterioles begin to converge and form two venules as the vasculature approaches the base of the villus (33, 38). In the mouse, two arterioles supply oxygenated blood to the villus and then divide into a capillary network that rejoins to form a single venule at the tip of the villus. The venule then travels down the center toward the base of the villus (34). These differences may impact the physiological function of the intestinal mucosa between species and potentially their susceptibility or response to injury.

<table>
<thead>
<tr>
<th>Type of Experimental IR Injury</th>
<th>Segment of Intestine</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA occlusion</td>
<td>Ileum</td>
<td>5, 94, 163</td>
</tr>
<tr>
<td>Mesenteric vascular occlusion</td>
<td>Jejunum</td>
<td>27, 71</td>
</tr>
</tbody>
</table>

Table 3. Experimental details of in vivo ischemia-reperfusion models in cats

<table>
<thead>
<tr>
<th>Type of Experimental IR Injury</th>
<th>Segment of Intestine</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA complete occlusion</td>
<td>Ileum</td>
<td>95</td>
</tr>
<tr>
<td>SMA complete occlusion</td>
<td>Jejunum + ileum</td>
<td>52</td>
</tr>
<tr>
<td>Mesenteric vascular occlusion</td>
<td>Small intestine</td>
<td>80</td>
</tr>
<tr>
<td>Low-flow SMA occlusion</td>
<td>Jejunum + ileum</td>
<td>98</td>
</tr>
<tr>
<td>Low-flow SMA occlusion</td>
<td>Jejunum</td>
<td>130</td>
</tr>
<tr>
<td>Low-flow SMA occlusion</td>
<td>Small intestine</td>
<td>91, 99, 184</td>
</tr>
</tbody>
</table>

IR, ischemia-reperfusion.

Table 4. Experimental details of in vivo ischemia-reperfusion models in dogs

<table>
<thead>
<tr>
<th>Type of Experimental IR Injury</th>
<th>Segment of Intestine</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA occlusion</td>
<td>Ileum</td>
<td>95</td>
</tr>
<tr>
<td>Mesenteric vascular occlusion</td>
<td>Jejunum</td>
<td>27, 71</td>
</tr>
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</table>
Multiple in vivo and in vitro intestinal permeability studies have demonstrated significant differences between humans and rodents and greater correlation between humans and pigs (15, 45, 126). In particular, Bijlsma et al. (15) hypothesize that the differences in permeability between rodents and humans are due to the interspecies variation in villus blood vessel architecture resulting in varying countercurrent exchange efficiency that directly impacts villus epithelial cell function.

Mechanisms of Reperfusion Injury

The concept of progressive injury that occurs following the restoration of normal vascular circulation to an ischemically compromised region seems counterintuitive. After all, reestablishing blood flow is ultimately critical to rescue and maintain cell function. However, ischemia creates an environment primed for the production of injurious metabolites, the influx of inflammatory cells and epithelial cell apoptosis and necrosis. Early studies of reperfusion in cats and rodents attributed the exacerbation of injury during this period to the production of reactive oxygen metabolites that directly contribute to injury as well as generation of neutrophils chemoattractants (26, 48, 65, 66, 68, 70, 77, 78, 82, 98, 99, 101, 128 –130, 136 –138, 153, 164). During ischemic conditions, as tissues continue to utilize adenosine triphosphate as a source of energy, the metabolic by-product hypoxanthine accumulates. In addition, in this oxygen-deprived environment xanthine dehydrogenase, which would normally function to metabolize hypoxanthine, is converted to xanthine oxidase by locally produced tissue proteases (130). When tissue perfusion is reestablished, xanthine oxidase utilizes the now freely available and abundant oxygen as an electron acceptor as it metabolizes hypoxanthine, producing superoxide (66 – 68, 70) (Fig. 2). The direct injury induced by oxygen free radicals combined with their role in the activation and chemoattraction of neutrophils creates the severe tissue injury attributed to reperfusion.

Neutrophils inflict tissue injury by a multitude of proposed mechanisms that include occlusion and increased permeability of the microvasculature (44, 83), release of reactive oxygen metabolites (48, 130, 165), cytotoxic enzyme release (186), and mechanical injury induced by migration (58). The vast majority of the studies used to determine these findings used a feline low-flow ischemia model. Upon activation, neutrophils release the contents of their granules that include potent proteases such as elastase, myeloperoxidase, protease-3, and metalloproteases as well as reactive oxygen species including H₂O₂ and hypochlorous acid (108, 153, 186). These compounds are bactericidal and aid in neutrophil migration as connective tissues are lysed but concomitantly cause extensive collateral damage to surrounding matrix proteins and nearby cells. Additional injury is created by the mechanical damage induced by their migration across the epithelial monolayer, which has been shown to increase paracellular permeability (58). The detrimental role of neutrophil accumulation on epithelial barrier function postischemia was supported by findings in a porcine model of complete mesenteric vascular occlusion (58) in which pretreatment of animals with anti CD11/CD18 monoclonal antibody, targeting neutrophil adhesion, significantly reduced neutrophil infiltration and improved measures of epithelial barrier function (58).

Model-Dependent Reperfusion Injury

The significance of reperfusion to intestinal injury following an ischemic event has been and continues to be controversial. This is partly due to the fact that the degree of reperfusion injury appears to depend on the type of ischemia (complete vs. incomplete vascular occlusion) and duration of ischemic injury as well as the research animal species and segment of affected intestine.

Complete vascular occlusion. Currently, the complete vascular occlusion method for induction of ischemic injury in rodents is the most commonly used approach for the study of ischemia-reperfusion injury (Tables 5 and 6). Rodents are advantageous to use because of their relatively low cost, ease of maintenance, and rapid reproduction rate. In recent ischemia-reperfusion research, mouse models have been used to determine genes associated with oxidative stress (12) as well as to provide evidence of increased epithelial proliferation and barrier function in response to endogenous factors such as keratinocyte growth factor administration (30). Furthermore, mice are highly amenable to genetic manipulation. Attenuation of reperfusion injury in mice genetically modified to overexpress superoxide dismutase has provided further proof of the deleterious role of reactive oxygen metabolites in reperfusion (48). Additionally, other transgenic mouse models have been used to demonstrate the protective role of mu opioid receptor signaling (61) and the detrimental effects of cytokines such as IL-17A (104) in ischemia-reperfusion injury. A large number of studies have also utilized rat models. Aside from transgenic
Another important species difference is the role of neutrophils in the induction and perpetuation of reperfusion injury. The resident mucosal population of neutrophils, as opposed to neutrophils recruited from circulation during onset of injury, appears to play a significant role in reperfusion injury in rodents and cats (48, 101, 130, 153). In a low-flow cat model, acute vs. chronic pretreatment with a CD18-specific monoclonal antibody enabled the investigators to assess the role of resident mucosal neutrophils, with chronic administration of the antibody depleting mucosal neutrophil reserves. These studies indicated that it was the resident neutrophils, rather than those infiltrating from the microvasculature, that were responsible for the majority of the reperfusion injury documented (101, 130, 153). However, humans have a relatively small population of resident neutrophils (22), a finding that was also found to be the case in horses. Interestingly, equine ischemia studies have shown very limited evidence of reperfusion injury (21, 120). Together, differences in oxidant enzyme expression and neutrophil populations likely affect the reliability of basic studies to translate findings to the treatment of humans with ischemia-reperfusion injury.

**Segmental mesenteric vascular occlusion.** Most of the segmental mesenteric vascular occlusion studies have been performed in pigs (Table 7), although other species have been used (Tables 3, 4, and 6). This is likely because the larger size of the pig facilitates temporary occlusion of smaller vessels, such as those found within the mesentery, without compromising the integrity of the vessels, thereby allowing reperfusion. There are other advantages to using the porcine model for the study of reperfusion injury. Pigs share a similar pattern of xanthine oxidase expression as humans (22), whereas in rodents enzyme expression is four- to fivefold higher (14, 22). Additionally, pigs, like humans, have a relatively small population of resident neutrophils, unlike rodents and cats (22).

The larger size of pigs is also advantageous for models requiring surgical manipulation, such as Thiry-Vella loops in which an isolated cannulated segment of intestine can be subject to ischemic injury (19), or where research involves tissue transplantation (182) or testing advanced diagnostic imaging (28). Similar surgical manipulations in rodents have been fraught with complications (6).

Although evidence supports the use of pigs to more closely recapitulate human intestine, obvious morphological differences exist that may influence the use of pigs for the study of reperfusion injury (10, 45, 92, 149). In many mammalian species, including humans, the small intestinal epithelium is composed of stem cells that reside within the crypt base and four postmitotic cell types: absorptive enterocytes, goblet cells, enteroendocrine cells, and Paneth cells. However, the existence of Paneth cells in pigs remains controversial and there is currently no definitive evidence to prove or disprove the existence of a similarly functioning cell type (63, 123, 124). Paneth cells are unique in that they reside adjacent to the

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**Table 7. Experimental details of in vivo ischemia-reperfusion models in pigs**

<table>
<thead>
<tr>
<th>Type of Experimental IR Injury</th>
<th>Segment of Intestine</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMV occlusion</td>
<td>Jejunum, ileum, colon</td>
<td>170</td>
</tr>
<tr>
<td>Mesenteric vascular occlusion</td>
<td>Jejunum + ileum</td>
<td>35</td>
</tr>
<tr>
<td>Mesenteric vascular occlusion</td>
<td>Ileum</td>
<td>3, 18–20, 22–24, 28, 58, 89, 96, 102, 110, 117–119, 133, 154, 160, 170</td>
</tr>
<tr>
<td>SMA embolism</td>
<td>Jejunum + ileum</td>
<td>148</td>
</tr>
<tr>
<td>SMA embolism</td>
<td>Small intestine + colon</td>
<td>96</td>
</tr>
<tr>
<td>SMA embolism</td>
<td>Small intestine segment not specified</td>
<td>1, 25</td>
</tr>
</tbody>
</table>

SMV, superior mesenteric vein.

murine models, the role of a host of factors has been successfully investigated in rodents, highlighting the beneficial effects of epidermal growth factor (11, 59), heparin-binding epidermal growth factor (36, 50, 114), anti-apoptotic signaling (86), and xanthine oxidase blockade (157) to prevent injury or improve healing. Other studies using rats have shown that atenolol (31), a beta-blocker, and L-arginine (62), a substrate of nitric oxide biosynthesis, both attenuate enteric nervous system dysfunction that occurs as a result of ischemia-reperfusion injury. However, multiple studies have demonstrated that the degree of injury attributed to reperfusion following mesenteric vascular occlusion is variable. The degree of reperfusion injury appears to depend on the duration of preceding ischemia and the segment of intestine (107, 135). Evidence of reperfusion injury has been shown to occur following intervals between 30 and 60 min of ischemia but not when the duration of ischemia is shorter or longer (107, 135). Additionally, the colon appears to be more resistant to reperfusion injury compared with the small intestine and susceptibility to injury along the length of the small intestine is variable, with the ileum appearing to be more resistant to reperfusion injury than the jejunum. The reason for these differences remains poorly understood (35, 37, 107), although the lack of oxidant-producing enzymes in the colon may be one reason that injury differs from the small intestine (76, 120, 121).

**Low-flow ischemia.** As noted above, in models utilizing the low-flow method of ischemia, the majority of tissue damage occurs during reperfusion. In vivo feline studies have shown a direct correlation between elevated xanthine oxidase levels and tissue damage as well as microvascular injury (65, 68, 164, 184). However, important differences exist between species that influence their susceptibility to reperfusion injury. The robust reperfusion injury identified in cats and rodents as a result of mucosal xanthine oxidase-induced oxidant release is not found in other species (22). In fact, humans and pigs lack mucosal xanthine oxidase at birth, and levels remain low even into adulthood, making the clinical relevance of the feline and rodent studies questionable (14). Furthermore, no definitive clinical utility has been identified for xanthine oxidase inhibitors in patients with intestinal ischemia-reperfusion injury (105, 132). Perhaps with the development of the novel human model of ischemia-reperfusion injury (47) the controversy regarding the contribution of xanthine oxidase to reperfusion injury will be resolved.

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**Table 8. Experimental details of in vivo ischemia-reperfusion models in humans**

<table>
<thead>
<tr>
<th>Type of Experimental IR Injury</th>
<th>Segment of Intestine</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Mesenteric vascular occlusion</td>
<td>Jejunum</td>
<td>46, 47, 75, 115</td>
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<tr>
<td>Mesenteric vascular occlusion</td>
<td>Proximal colon</td>
<td>73, 74</td>
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</table>
intestinal stem cells within the small intestinal crypt base and are thought to migrate toward the crypt base following complete maturation, in contrast to other maturing cell lineages that migrate toward the lumen. Paneth cells are thought to play an integral role in modulating stem cell self-renewal or differentiation especially during times of insult (40, 57, 152), making the study of regenerative events following ischemic injury potentially more complex in porcine models. However, there is recent evidence that Paneth cells may actually contribute to the injury induced by ischemia-reperfusion injury (72, 93, 104). Work in rodent models attribute injury within the gut, systemic inflammation, and remote organ injury following intestinal ischemia-reperfusion injury to Paneth cell degranulation. In fact, it appears that Paneth cell depletion may attenuate ischemia-reperfusion injury (104).

Knowledge regarding the advantages and disadvantages to each model is critical to appropriate experimental design and data interpretation.

Future Directions

New insight into mechanisms of cell death. Recent insight into the process of cell death by apoptosis and necrosis in both rodent and human models of intestinal ischemia-reperfusion injury have begun to shed light into the role of these pathways in the process of injury and subsequent repair (32, 46, 115, 156, 181). An important component to the combined injury attributed to ischemia and reperfusion is the direct impact on the intestinal epithelial cells. Controlled cell death is critical to the maintenance of normal barrier function and serves to maintain epithelial continuity along the luminal surface while expelling dying cells. Under homeostatic conditions, cells mature as they gradually migrate along the crypt-villus axis. As they near the luminal surface a normal process of desquamation occurs that results in epithelial cell shedding. Briefly, this process is thought to be driven by an interaction between the extracellular matrix and integrins and cadherins, the transmembrane proteins that anchor cells to the basement membrane and that signal cell survival through pathways including focal adhesion kinase (p125fak), phosphatidylinositol 3'-kinase/Akt, and mitogen-activated protein kinases (ERK, JNK, p38, MAPK) (49, 54, 55, 84, 174). Changes in these surface proteins and signaling pathways occur as cells approach the villus tip that signal a normal process of cell shedding into the intestinal lumen (29). During ischemia and reperfusion, cell injury results in death by both apoptotic and necrotic processes (75, 87, 109). Sustained tissue injury appears to be associated with inflammation that is perpetuated by the presence of excessive apoptotic and necrotic cells mediated by the complement system (79, 162, 178, 185). However, the exact pathophysiological mechanisms that occur are complex and incompletely understood (181). Nonetheless, inhibition of apoptosis as well as the facilitated clearance of apoptotic cells appears to reduce bacterial translocation and promote repair by minimizing prolonged inflammation (46, 86, 115, 181). On the basis of research dedicated to understanding ischemia-reperfusion injury, it seems that the attenuation or modulation of the inflammatory response would be critical to future therapeutic approaches aimed at reducing tissue destruction. This is discussed in a few recent reviews: one that addresses the contribution of oxidative stress to the pathogenesis of gastro-intestinal disease (13) and another that summarizes the therapeutic potential for neutrophil elastase inhibitors to decrease inflammatory tissue injury (81). These reviews note that despite the large amount of preclinical research dedicated to these particular approaches to modulation of inflammation, the challenges to translate findings to clinical application remain. However, compared with the field of gastrointestinal research, more current and novel therapeutic strategies appear to be available for ischemic cardiovascular disease (81, 139). Nevertheless, little progress has been made in improving clinical outcome in either field. Therefore a better understanding of reparative processes may provide an alternative means to improve care.

Advances in understanding epithelial renewal. Recent advances in the field of intestinal stem cell biology and regenerative medicine have improved our understanding of epithelial renewal (145). The recent discovery of biomarkers to clearly distinguish intestinal stem cell populations has contributed to the rapid advancement of the field (8, 64, 167, 175, 176). Interestingly, rodent models of intestinal ischemia have shown architectural preservation of the intestinal epithelial stem cells (56, 88, 134, 173). Additionally, the ability of the intestinal epithelial stem cell compartment to expand after intestinal resection, radiation injury, and doxorubicin treatment has been demonstrated in rodent models (42, 43, 85, 177). Together this makes the intestinal epithelial stem cells a promising therapeutic target to hasten mucosal epithelial regeneration following ischemia-reperfusion injury. It is our hope that this new knowledge and advanced technology will facilitate targeted therapies to improve treatment and outcome of this devastating disease.

Summary. Ischemia and reperfusion contribute to intestinal injury in a multitude of clinical gastroenterological conditions. Despite this, advances in the field have remained modest. This review aimed to shed light on the most commonly used animal models for the study of ischemia and reperfusion to aid in interpretation of study results and perhaps influence model selection in the future. For example, if investigation of reperfusion injury were of particular interest, a feline model of ischemia would be helpful, whereas strangulating intestinal ischemia would be more appropriately studied via a porcine mesenteric vascular occlusion model. Appropriate modeling of disease states or model selection are arguably components of the failure to translate benchtop discoveries to clinical application.

REFERENCES

ANIMAL MODELS OF ISCHEMIC INJURY

Physiology in Medicine


