Pathophysiology of mesenteric ischemia/reperfusion: a review

Fisiopatologia da isquemia e reperfusão mesentérica: revisão

Nereide Freire Cerqueira², Carlos Alberto Hussni³, Winston Bonetti Yoshida⁴

1. Article from Faculty of Veterinary Medicine and Animal Science. UNESP – São Paulo State University. Botucatu.
2. Doctor of Veterinary Medicine, School of Veterinary Medicine and Animal Science. UNESP - São Paulo State University. Botucatu.
3. Associate Professor, Department of Veterinary Surgery and Anesthesiology. UNESP - São Paulo State University, Faculty of Veterinary Medicine and animal Science.
4. Associate Professor, Department of Surgery and Orthopedics. School of Medicine. UNESP - São Paulo State University. Botucatu.

ABSTRACT
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During ischemia, the cell structures are progressively damaged, but restoration of the blood flow, paradoxically, intensifies the lesions caused by the ischemia. The mechanisms of ischemia injury and reperfusion (I/R) have not been completely defined and many studies have been realized in an attempt to find an ideal therapy for mesenteric I/R. The occlusion and reperfusion of the splanchnic arteries provokes local and systemic alterations principally derived from the release of cytotoxic substances and the interaction between neutrophils and endothelial cells. Substances involved in the process are discussed in the present review, like oxygen-derived free radicals, nitric oxide, transcription factors, complement system, serotonin and pancreatic proteases. The mechanisms of apoptosis, alterations in other organs, therapeutic and evaluation methods are also discussed.

Key words: Ischemia. Reperfusion. Mesenteric vascular occlusion. Reperfusion injury.

RESUMO
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Durante a isquemia, as estruturas celulares são progressivamente lesadas, mas a restauração do fluxo sanguíneo, paradoxalmente, pode agravar o dano celular isquêmico. O mecanismo das lesões de isquemia e reperfusão (I/R) ainda não foram completamente definidos e muitos estudos têm sido realizados na tentativa de encontrar uma terapia ideal para I/R mesentérica. A oclusão e a reperfusão das artérias esplâncnicas provocam alterações locais e sistêmicas derivadas principalmente da liberação de substâncias citotóxicas e da interação entre neutrófilos e células endoteliais. As substâncias envolvidas no processo, como radicais livres derivados do oxigênio, óxido nítrico, fatores de transcrição, complemento, serotonin e proteases pancreáticas, são discutidas na presente revisão. Os mecanismos de apoptose, repercussão sistêmica, terapêutica e métodos de avaliação também são discutidos.


The ischemia and reperfusion (I/R) syndrome performs a fundamental role in the pathophysiology of several clinical-surgical conditions and may be caused by intestinal intussusception, acute mesenteric arterial occlusion, and hemodynamic shock. Lesions caused by mesenteric I/R can also occur in transplants of the small intestine.

Ischemia and reperfusion of the mesenteric blood vessels generally follows acute vascular insufficiency and the initial stages of multiple organ failure, conditions which are associated with high rates of morbidity and mortality.

Intestinal ischemia

Intestinal ischemia is generally the result of arterial occlusion by thrombi or embolisms and, more frequently, by non occlusive processes, like in situations of low mesenteric flow which occurs in cardiac insufficiency, sepsis, the administration of alpha-adrenergic agents or digitalics. The arteries most compromised by obstruction are the celiac trunk (or celiac artery), superior mesenteric (or cranial) artery (SMA) and inferior mesenteric (or caudal) artery. However, the sources of the collateral flow between the mesenteric blood vessels themselves or with adjacent circulation are numerous and may compensate the blood flow to the tissues.

Although less frequently found, the repercussions of mesenteric vein occlusion can be just as severe as arterial occlusion. Experiments on rats demonstrated that the occlusion of the cranial mesenteric vein provoked a smaller reduction in energetic metabolism than the occlusion of the cranial mesenteric artery, conversely, hemorrhage and tissue injury caused by venous occlusion elicited an irreversible tissue loss in the animals’ intestines. In humans, mesenteric venous thrombosis can result in hemorrhagic infarction with acute mesenteric ischemia and irreversible severe tissue lesions.
Arterial ischemia initiates alterations in tissues by blocking the oxygen supply, thus impeding aerobic energetic metabolism. This fact determines the depletion of intracellular levels of adenosine triphosphate (ATP) and disturbs cellular homeostasis. During the ischemia process, aside from the inadequate supply of oxygen, with the consequent compromise of oxidative phosphorylation in the mitochondria, there is an accumulation of metabolites, which, directly or through mediators, can cause cellular injury. With the evolution of ischemia, the continuous action of these elements results in progressive cellular alterations, culminating in necrosis. However, when the process which initiates ischemia is corrected before irreversible alterations can occur, oxygen return allows for the reestablishment of energetic metabolism, the removal of toxic products and the progressive return of normal cell functions.

Reperfusion

Depending on the time and intensity of the ischemia, when oxygen is reintroduced to the tissues, tissue injury can be further exacerbated (oxygen paradox). Parks & Granger reported that tissue lesions produced during reperfusion were greater than those produced during ischemia, in mesenteric I/R in felines. I/R can provoke complex interactions between the endothelium and different cell types, leading to microvascular injury, cellular necrosis and/or apoptosis. Occlusion and reperfusion of the splanchnic arteries precipitate circulatory shock, which, principally through an increase in vascular permeability, causes the activation and adhesion of polymorphonuclear neutrophils (PMNs), the release of proinflammatory substances and the formation of both nitrogen-derived and oxygen-derived free radicals (OFR). Injury provoked by splanchnic I/R is characterized by intense infiltrated inflammations, found predominantly in the mucosae and submucosae, causing endothelial destruction.

Ischemia and reperfusion of the small intestine provoke the rupture of the mucosa barrier, bacterial translocation and the activation of inflammatory responses, as well as hydroelectrolytic and acid-alkaline equilibrium disturbances, which are manifested in distant organs. Bacterial translocation is the passage of viable bacteria through the intestinal mucosa to mesenteric lymphatic nodes and other organs and tissues. The process of translocation involves the initial contact of the bacteria with the intestine wall, which alone can precipitate the production of cytokines and a subsequent inflammatory response. Once the bacteria penetrate the mucosa, they can be transported to distant organs by the circulation system. Hypoxia, followed by changes in the functioning of the intestine wall, promote a cycle of increased permeability leading to the release of toxic mediators, which results in accentuated permeability and the facilitation of bacterial translocation. A study using marked bacteria showed that translocation in rats was more evident 24 hours after mesenteric reperfusion than after 30 minutes, indicating that time is a significant factor for translocation. With the return of blood perfusion, the influx of calcium into the intracellular medium increases, which leads to an expressive increase in phospholipase A2 activity. Arachidonic acid released by phospholipase A2 is metabolized during reperfusion by the enzyme cyclooxygenase (COX), generating prostaglandins (PG), thromboxane (TX) e prostacyclins (PGI), and by the enzyme lipoxygenase (LOX), which generates leukotrienes (LT). These substances can cause vasoconstriction (TXA2, LTC4, LTD4, LTE4), vasodilatation (PGI2, PGE1, PGE2, PGD2), increased vascular permeability (LTC4, LTD4, LTE4), can stimulate platelet aggregation and chemotaxia in the PMNs (LTB4, HETE). Aydemir-Koksoy et al. observed a reduction in the velocity and intensity in platelet aggregation after reperfusion in intestinal ischemia of rabbits.

Reactive oxygen species (ROS)

Another factor that induces intestinal injury after reperfusion is the generation of free radicals from oxygen molecules, derived from the electron transport chains of the mitochondria, xanthine-oxidase (XO) metabolism, endothelial cells, prostaglandins and activated neutrophils.

Under normal conditions, the oxygen molecule (O2) is submitted to a tetravalent reduction by the cytochrome system in the mitochondria, creating water (H2O). However, one to two percent of the O2 escapes this path and suffers a univalent reduction, generating oxygen-derived free radicals. In normal conditions, these radicals are neutralised by endogenous antioxidant enzymes, but during reperfusion an excessive concentration of free radicals occurs, eliciting a process called oxidative stress, with subsequent deleterious effects. The ROS originate when O2 is reintroduced to the ischemic tissue during reperfusion. Free radicals are a class of chemicals, which have a single unpaired electron in their outer orbit. In this state, the free radicals are extremely reactive and unstable, participating in reactions with organic and inorganic chemical substances, proteins, lipids, carbohydrates and, particularly, cell membranes and nucleic acids. During the ischemic period, ATP is catabolized to produce hypoxanthine. Under physiological conditions hypoxanthine is oxidated to xanthine by way of xanthine dehydrogenase (XDH). During ischemia, an accumulation of intracellular calcium (Ca2+) occurs and the conversion of XDH into xanthine oxidase (XO), where the degree of conversion of proportional to the time of ischemia. This reaction is quicker in intestinal tissue than in other tissues, which makes the measurement of XO a safe indicator for intestinal ischemia. During reperfusion, the O2 is reintroduced into the tissue and reacts with hypoxanthine and XO to produce the superoxide anion (O2·-), which is transformed into hydrogen peroxide (H2O2) by the action of the enzyme superoxide dismutase (SOD) and through a reaction with catalase or glutatiana peroxidase, H2O2 is further transformed into H2O. In the presence of iron, the superoxide and H2O undergo the Haber-Weiss and Fenton reaction to form hydroxyl radicals (OH·), which are highly reactive and cytotoxic. Hydroxyl initiates lipidic peroxidation of the cell membrane components and the subsequent release of substances, which attract and promote the adherence of granulocytes to the microvascular endothelium. The attached granulocytes cause further injury to the endothelial cell.
through the release of superoxide and several proteases. The superoxide ions react with nitric oxide (NO) to produce peroxynitrite, which then causes accentuated lipid peroxidation, proteic and DNA modifications resulting in cellular damage. All cellular components are susceptible to the action of ROS, though the membrane is one of the most affected as a result of lipid peroxidation, which causes alterations in the structure and permeability of cellular membranes. The lipid-radical reactions release lipidic peroxides, which are in themselves free radicals, initiating the subsequent reduction of other fatty acids. Consequently there is a loss of selectivity in the ionic exchange and the release of the contents of the organelles, like the hydrolytic enzymes of the lysosomes and the formation of cytotoxic products culminating in cell death. Besides precipitating lipid peroxidation, the ROS have the capacity to attract and activate neutrophils. The utilization of inflammatory cells is regulated by chemoattractors (C5a, IL-8, LTB4, PAF) generated during I/R, and also by the induction of various adhesion molecules on the surface of both inflammatory cells and venous endothelial cells. The adhesion molecules which regulate the leukocyte-endothelium interaction are the selectins, integrins b2 (CD11/CD18) and the immunoglobulins (ICAM-1, VCAM-1, PECAM-1). The migration sequence of the leukocytes includes bordering the vessel wall, rolling along the endothelium and, finally, firm adhesion before transmigrating through the endothelium. The active neutrophils, those which show adhesion molecules, after adhesion and migration through the endothelium cause local destruction through the release of more free radicals, proteolytic enzymes and peroxidase. The capture of activated neutrophils in the lungs and other organs is an important step towards the development of multiple organ failure. In normal rat intestine, ICAM-1 and VCAM-1 are constitutively expressed in the vascular endothelium. In response to I/R, they are superexpressed in the vascular endothelial cells, connective tissue, lamina propria and inflammatory cells. The superexpression of ICAM-1 and VCAM-1 accentuates the leukocyte adhesion and promotes their migration to the injured intestine during I/R. The microrcirculation occlusion principally attributed to the mass adhesion of neutrophils in the capillaries is called the no-reflow phenomenon.

Among the free radicals produced during I/R nitric oxide (NO) is also important. It is normally synthesized by L-arginine through NO synthase, which can have constitutive (cNOS) and induced (iNOS) isoforms. The excess production of NO through induced NO synthase (iNOS) contributes to the pathophysiology of I/R in the intestine. However, NO produced through constitutive NO synthase (cNOS) can be an important protective molecule for the small intestine at the onset of I/R. Nitric oxide presents beneficial vasodilatory effects in the microvascular system, through the relaxation of the smooth vascular muscle cells, but, paradoxically, could be involved in the production of cytotoxic radicals. According to Sekhon et al., NO is beneficial as a modulator or messenger, but during oxidative stress is potentially toxic. Naito et al. observed that inflammation induced by mesenteric reperfusion in rats resulted in oxidative and nitrosative stress, characterized by the high production of NO, by the increased expression of iNOS and by an excess expression of lipidic peroxides. Tissue ischemia and reperfusion activate families of protein kinases which converge to specific transcription factors [protein activator-1 (PA-1) and nuclear factor-kB (NF-kB)], which regulate the expression of proinflammatory genes. The resulting products include enzymes (iNOS, phospholipase A2, cyclooxygenase), cytokines (TNF-a, IL-1, IL-6) and adhesion molecules (ICAM-1). NF-kB is maintained in a latent form in the cytoplasm and, under certain conditions translocates to the nucleus, where it regulates the transcription. According to Zou et al., NF-kB appears to perform an important function in the generation and resolution of intestinal I/R lesions. NF-kB is activated by the intestine due to proinflammatory stimuli, including sepsis, cytokines and oxidative stress. The activation of NF-kB leads to the coordinated expression of many genes which codify proteins involved in mediator synthesis and in the amplification and maintenance of inflammatory response. Thus, NF-kB functions as a marker for antiinflammatory treatment. The activation of the complement system can lead to the translocation of NF-kB and an increase in the transcription of iNOS. The complement system exerts a significant function in the lesion pathogenesis resulting from I/R and its activation can also contribute to the increased production of superoxide anion not only through the utilization and activation of neutrophils, but also through a reduction in the levels of SOD after reperfusion.

The depletion or inhibition of the complement could diminish many of the mediators of I/R injury. Complement component 5 (C5) measures the intestinal lesions through regulation of ICAM-1, TNF-a and IL-1a. Although it is clear that blocking the complement can protect tissues from reperfusion injury, the specific molecular mechanisms of injury induction at a cellular level are still under investigation. In gastrointestinal I/R, the complement induced iNOS expression of the intestinal epithelium of rats. The activation of neutrophils was also complement-dependent during intestinal I/R. On the other hand, Fleming et al. showed that C5a provoked limited injury, after mesenteric I/R in mice, independent of the PMNs.

Additionally, mastocytes may be involved in exacerbating injury after the activation of the complement. Mastocytes, and the reactions induced by them, appear to contribute to the increase in mucosa permeability during reperfusion, but their function in pathological injury remains unknown. Evidence suggests that besides PMNs and endothelial cells, other cells exert significant functions in the pathogenesis of posts ischemic reperfusion injury. The platelets are utilized by the posts ischemic microvasculature soon after the onset of reperfusion and, even though they are anuclear, still generate free radicals, release proinflammatory mediators like TXA2, leukotrienes, serotonin, platelet factor-4 and PDGF and modulate leukocyte function. The accumulation of platelets in the posts ischemic microvasculature could contribute significantly to the manifestation of I/R lesions.

Platelets, similar to leukocytes, roll and firmly adhere to the vascular endothelium during posts ischemic reperfusion. The cellular interactions between endothelial
cells and platelets induced by I/R are mediated through endothelial P-selectin, while platelet P-selectin promotes platelet-leukocyte interaction. The interactions between these two cell types can provoke reciprocal activation, resulting in increased expression of the adhesion molecules, in the generation of superoxide and in the phagocytic activity of the leukocytes.

Another factor involved in the lesions of intestinal I/R is serotonin (5-HT). Serotonin is a bioactive amine, which acts, in several physiological phenomena like neurotransmission, intestinal movement, platelet activation and vasoconstriction. A study realized by Teramoto et al. showed an increase in the plasmatic levels of 5-HT after mesenteric I/R in rats, probably due to an increase in the release of 5-HT by the injured intestine. According to the authors, 5-HT together with other bioactive substances could perform an important function in the pathogenesis of intestinal I/R. It has been demonstrated recently that pancreatic proteases are involved in the production of inflammatory mediators after intestinal I/R, such as trypsin and metalloproteinase-9 (MMP-9). MMP-9 is an endoprotease which has zinc in its active nucleus, and is involved in immune and inflammatory responses, including I/R of the myocardia, liver and pancreas. Rosario et al. observed that intraluminal trypsin increased the inflammatory response, with an increase in neutrophil infiltration in the intestinal wall. Pancreatic trypsin promoted a rapid proteolytic activation of MMP-9, acting as a potential mechanism for injury. Growing evidence suggests that platelet aggregator factor (PAF) exerts an important function in the initiation and amplification of I/R lesions. PAF is a lipid mediator for inflammatory response and promotes mucosal apoptosis, induced by I/R in the small intestine. I/R inhibits the activity of PAF-acetylhydrolase (PAF-AH), resulting in an increase of PAF activity. Activated PAF accentuates the expression of PECAM-1 and the secretion of IL-6 and inhibits the synthesis of IL-10, which exerts antiinflammatory functions during intestinal reperfusion.

The release of mitochondria cytochrome for cytosol occurs to activate caspase-9, which, in turn, increases apoptosis in the mucosa. In the past, necrosis was considered to be the principal effect of ischemia on epithelial cells. More recent studies indicate that apoptosis is clearly a significant and, perhaps, the principal contributor to cellular death after I/R injury. Contrary to cellular necrosis, apoptosis is an active process of genetically driven cellular self-destruction and is characterized by morphological changes, like cellular shrinkage while maintaining the organelles intact, chromatin condensation and the eventual fragmentation of the nucleus and the forming of “apoptotic bodies” by the cytoplasm.

The main executors of “programmed cell death” are the endoprotease cysteines called caspases. The administration of allopurinol, a xanthine-oxidase inhibitor and the use of epidermal growth factor (EGF) offered protection after I/R in rat small-intestine. Ilhan et al. showed that the administration of allopurinol and monoclonal antibodies to counter adhesion molecule ICAM-1 before mesenteric reperfusion diminished the lesions resulting from I/R in rats. Many other agents have been tested experimentally and have prevented or diminished mesenteric I/R injury during research. Hydrocortisone was capable of diminishing reperfusion lesions after venous ischemia in horse jejunum. 21-aminosteroid, an agent with similar properties to corticosteroids, though without their undesired effects, was not capable of protecting horse jejunum after partial vascular occlusion followed by reperfusion. Other strategies have been proposed to protect tissues from I/R injury, like hypothermia and ischemic or pharmacological preconditioning. Ischemic preconditioning is defined as a brief period of ischemia.

Systemic repercussions

Multiple organ failure is a frequent complication after intestinal I/R and involves organs like the liver, heart, kidneys and lungs. Reactive chemical species initiate a sequence of events including the activation of neutrophils and the release of harmful substances like PAF and histamine. Activated neutrophils infiltrate through the intestinal epithelium and endothelial cells, causing mucosal and submucosal injury, with the consequent increase in vascular permeability. This increase in permeability results in translocation of enteric bacterial products. Besides this, the release of neutrophils, bacterial products and PAF lead to distant pathophysiological effects, including systemic hypertension. According to Iglesias et al., acute pulmonary edema resulting from mesenteric I/R is due to the increase of pulmonary microvascular permeability to fluids and proteins, as well as the increase in hydrostatic microvascular pressure. Horton and White demonstrated that one of the mechanisms that promotes cardiac dysfunction after intestinal I/R in rats is the production of ROS and lipidic peroixdation in cardiac cell membranes. Hepatic injury induced by mesenteric I/R was diminished with the administration of ethanol, which may have inhibited the apoptosis of hepatocytes.

Therapeutics

Several endogenous mechanisms exist to inhibit I/R lesions and many drugs have shown protective effects. These agents act through various pathways: elimination of free radicals; inhibition of free radicals production; neutrophilic inhibition; blocking phospholipase A$_2$, cyclooxygenase and lipoxygenase; platelet aggregation factor inhibition; production of blockers for chemotaxic agents and monoclonal antibodies to counter adhesion molecules. However, none of the treatments in isolation were effective in limiting oxidative damage.

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followed by reperfusion (isolated or in cycles) which
precedes a prolonged period of ischemia. Ischemic
preconditioning diminished morphological lesions in rat
intestine after I/R. Abrâhão et al. found no histological
differences when comparing fragments of rat intestine
submitted to I/R with animals submitted to ischemic
preconditioning, however, the biochemical dosage showed
that preconditioning protected the intestine from I/R injury.

The protection mechanisms of hypothermia are
multifactorial and have to be defined. A number of
mechanisms have been proposed, including the reduction of
O₂ consumption, ATP depletion, neutrophil activation,
lipidic peroxidation and the production of proinflammatory
cytocines. Hassoun et al. observed that intestinal regional
hypothermia applied during mesenteric ischemia reduced
the proinflammatory responses induced by I/R, but showed
no significant effect in microvascular barrier alterations
during reperfusion in dogs. Attuwayb et al. demonstrated
that hypothermia protected rat intestines from I/R injury and
that, according to the authors, this protection was
associated with diminished NF-kB activity and the induction
of iNOS, as well as the expression of heme oxygenase-1
(HO-1). According to Stefanutti et al., moderate hypothermia
has beneficial effects on the heart, liver, intestine and lungs
after mesenteric I/R. Moderate hypothermia, characterized
by a rectal temperature between 30 and 33°C, diminished
the infiltration of neutrophils in rat lung after mesenteric I/
R. Aldemir et al., in an experimental study on rats,
observed that the in vivo freezing of SMA and pretreatment
with mannitol before reperfusion were beneficial in the
prevention of intestinal I/R lesions.

Evaluation

Mesenteric I/R can be evaluated by the detection of
various products resulting from injury, using laboratorial,
histomorphological and anatomopathological methods.
Mean arterial pressure in rats, after mesenteric I/R, was
evaluated by means of catheters introduced in to the femoral
or carotid artery. Intestinal microvascular and cranial
mesenteric arterial blood flow, as well as cardiac
frequency and amplitude of intestinal electrical activity
(basic electrical rhythms) were sensitive to ischemia and that they registered change before
histological alterations occurred. Variation in the electrical
activity of the small intestine during I/R is a dynamic process,
which reflects the metabolic state of the smooth muscle.

Sun et al. observed that measurement of D-lactate in
plasma could be an intestinal injury marker, monitoring the
increase in permeability and endotoxemia after mesenteric
I/R in rats. Günel et al. believe that an elevated level of D-
lactate may be used as a parameter to identify patients with
ischemia before laparotomy. The intestinal fatty acid
bonding proteins (I-FABP) are small intracellular proteins
which exist in the mucosa of the intestinal epithelial of many
mammals and can be detected in the plasma and urine after
cellular injury.

Conclusions

Intestinal ischemia performs a fundamental role in the
pathophysiology of several clinical-surgical conditions and
reperfusion results in more severe tissue injury than with
ischemia alone. The events which occur during I/R are
complex and well studied, although, there still remain many
doubts as to their pathophysiology and therapeutics,
revealing the need for new research to obtain a more
complete understanding and in the search for improved
treatments.

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