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An Overview on Jejunal Ischemia-Reperfusion Injury and The Promising role of Mesenchymal Stem Cell-Derived Microvesicles

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Abstract:

Intestinal ischemia-reperfusion (I/R) injury is a serious pathological condition resulting from temporary interruption of blood supply followed by restoration of circulation, which leads to oxidative stress, inflammatory responses, and severe mucosal damage. The jejunum is particularly susceptible to ischemic injury due to its high metabolic demand and complex vascular supply. Recently, regenerative and immunomodulatory therapies have gained increasing attention for reducing tissue injury and enhancing intestinal recovery. Mesenchymal stem cell-derived microvesicles (MSC-MVs) represent a promising cell-free therapeutic strategy due to their ability to transfer bioactive molecules that regulate inflammation, apoptosis, and tissue repair.

Keywords: Intestinal ischemia-reperfusion injury; Mesenchymal stem cells; Microvesicles; Jejunal mucosa; Regenerative therapy.

Introduction:

Ischemic damage is defined as a pathological condition that occurs due to the deprivation of oxygen in tissues or organs. The condition is due to a decrease or complete interruption of the blood flow to tissues for multiple causes. Reperfusion is the restoration of the blood flow to the ischemic tissues. If blood flow to the ischemic tissue is not regained, a series of pathological changes as cellular dysfunction and cell necrosis will happen. In spite of that, it has been found that rapid reperfusion of ischemic tissue can cause much more massive damage compared with that caused by ischemia alone **(1).**

Mesenchymal stem cells can release mediators in extracellular vesicles such as exosomes and microvesicles. Extracellular vesicles are double layer membrane-bound structures that are derived from the plasma membrane and exocytosed into the extracellular environment transferring bioactive components like proteins, lipids and DNA. These extracellular vesicles cannot only transport proregenerative factors, but also mRNA and microRNA and even mitochondrial components over a long distance **(2).**

Extracellular vesicles can act as mediators for intercellular communication with neighboring cells by exchanging bioactive molecules or disseminating genetic contents toward distal organs. They are classified into exosomes and microvesicles according to their respective size, origin, biogenesis and composition. Exosomes vary in size ranging from "50 to 200" nm and generated through the invagination of multivesicular bodies endosomal membrane followed by fusion of them with the plasma membrane then exocytosis. On the other hand, microvesicles (MVs) are heterogeneous in size ranging from "50 to 1000" nm and released through the direct shedding or budding from the plasma membrane **(3)**.

Microvesicles had been showed to play a pivotal role as a promising therapeutic approach for various pathological conditions such as intestinal barrier dysfunction, neural degeneration, liver fibrosis, kidney injury and myocardial infarction **(4)**.

Histology of the Jejunum:

Mucosa of the jejunum:

The absorptive surface area of the small intestine is amplified by tissue and cell specializations of the submucosa and mucosa. These specializations include plicae circulares, villi and microvilli of enterocyte. Plicae circulares (circular folds) also known as the valves of Kerckring, are permanent transverse folds containing core of submucosa. Each circular fold is circularly arranged and extends about one-half to two thirds of the way around the circumference of the lumen. These folds begin to appear around 5 to 6 cm beyond the pylorus **(5).**

Villi are unique, finger-like and leaf-like projections of the mucosa that extend from the mucosal surface for about 0.5 to 1.5 mm into the lumen. They completely cover the surface of the jejunum, giving it a velvety appearance when viewed with the unaided eye. They consist of a core of loose connective tissue covered by a simple columnar epithelium. The core of each villus is an extension of the lamina propria, which contains numerous fibroblasts, smooth muscle cells, lymphocytes, plasma cells, eosinophils, macrophages, and a network of fenestrated blood capillaries located just beneath the epithelial basal lamina. In addition, the lamina propria of the villus contains a central, blind-ending lymphatic capillary, the lacteal. Smooth muscle cells derived from the muscularis mucosae extend into the villus and accompany the lacteal. These smooth muscle cells may account for reports that villi contract and shorten in an intermittent way forcing lymph from the lacteal into the lymphatic vessel network that surrounds the muscularis mucosa **(6)**.

The intestinal glands, or crypts of Lieberkühn, are simple tubular glands that extend from muscularis mucosa onto the luminal surface of the jejunum at the base of the villi. The glands are composed of a simple columnar epithelium continuous with the epithelium of the villi. The lamina propria surrounds the glands and contains numerous immune cells. The lamina propria also contains numerous lymphatic nodules that represent a major component of the GALT (Gut Associated Lymphatic Tissue). The muscularis mucosa consists of two thin layers of smooth muscle cells, an inner circular and an outer longitudinal layer **(7)**.

In brief, the mucosa of the jejunum, consisting of a lining epithelium of the villi and the crypts, an underlying connective tissue, the lamina propria, and the muscularis mucosa smooth muscles. At least five types of cells are found in intestinal mucosal epithelium. The mature cells of the intestinal epithelium are found both in the intestinal glands and on the surface of the villi. They include the following: • Enterocytes, whose primary function is absorption, Goblet cells, unicellular mucin-secreting glands, paneth cells, whose primary function is to maintain mucosal innate immunity by secreting antimicrobial substances, ~~e~~nteroendocrine cells, which produce various paracrine and endocrine hormones **(5).**

The mucosal cells in the jejunum are called enterocytes. Enterocytes are absorptive columnar cells with a basal nucleus. They have a brush border made up of numerous microvilli lining their apical surface. It is lined on its luminal side by a layer that is rich in neutral and amino sugars, the glycocalyx. The membranes of the mucosal cells contain the glycoprotein enzymes that hydrolyze carbohydrates and peptides. The mucous coat overlying the cells also continues as a significant barrier to diffusion. Most substances pass from the lumen if the intestines into the enterocytes and then out of the enterocytes to the interstitial fluids **(8).**

Microvilli increase the apical surface area as much as six hundred times; recognized in the light microscope as a striated brush border on the luminal surface. Each microvillus has a core of vertically oriented actin microfilaments linked to villin located in the tip of the microvillus and attach to the microvillus plasma membrane by myosin I molecules **(9).**

The actin microfilaments extend into the apical cytoplasm and insert into the terminal web, a network of horizontal contractile microfilaments forming a layer in the most apical cytoplasm and attach to the intracellular density associated with the zonula adherens. Contraction of the terminal web brings microvilli to spread apart, so increasing the space between them allowing more surface area exposed for absorption. In addition, contraction of the terminal web can help in “closing” the holes left in the epithelial sheet by exfoliation of aging cells **(6)**.

Enterocytes are bound to one another and to other epithelial cells by junctional complexes. Tight junctions establish a barrier between the intestinal lumen and the intercellular compartment. The tight junctions between the intestinal lumen and the connective tissue compartment of the body allow selective retention of substances absorbed by the enterocytes **(5).**

The lateral surface of the enterocytes shows, flattened cytoplasmic processes (plicae) interdigitating with those of adjacent cells increasing the lateral surface area of the cell, thus the amount of plasma membrane containing transport enzymes is raised. During active absorption, especially of solutes, water, and lipids, these lateral plications separate, enlarging the intercellular compartment. The increased hydrostatic pressure from the accumulated solutes and solvents leads to a directional flow through the basal lamina into the lamina propria **(5).**

Elongated mitochondria that provide energy for transport are concentrated in the apical cytoplasm. Tubules and cisternae of the smooth endoplasmic reticulum (sER), which are

involved in the absorption of fatty acids and glycerol and in the resynthesis of neutral fat, are found in the apical cytoplasm beneath the terminal web. Enterocytes are also secretory cells, producing enzymes needed for terminal digestion and absorption as well as secretion of water and electrolytes. The secretory function of enterocytes, primarily the synthesis of glycoprotein enzymes that will be inserted into the apical plasma membrane, is represented morphologically by aligned stacks of Golgi cisternae in the immediate supranuclear region and by the presence of free ribosomes and rER lateral to the Golgi apparatus. Small secretory vesicles containing glycoproteins destined for the cell surface are present. Histochemical methods are needed to distinguish these secretory vesicles from endocytotic vesicles or small lysosomes **(7).**

Goblet cells are unicellular glands are found dispersed among other cells of the intestinal epithelium. Goblet cells produce mucus. In the small intestine, goblet cells increase in number from the duodenum to the terminal part of the ileum. Water-soluble mucinogen is lost during preparation of routine H&E sections, the part of the cell that normally contains mucinogen granules appears empty or foamy **(6)**.

Examination with TEM demonstrates a large accumulation of electron lucent mucinogen granules in the apical cytoplasm that distends the apex of the cell deforms the shape of neighboring cells and the basal portion of the cell appears as a narrow stem giving the shape of glass hence the name "goblet". This basal portion is intensely basophilic in histological preparations as it is occupied by a heterochromatic nucleus, numerous RER, and free ribosomes. Mitochondria are also concentrated in the base of the cytoplasm. A comprehensive array of Golgi cisternae forms a wide cup around the newly formed mucinogen granules adjacent to the base of the cell. Microvilli of goblet cells are restricted to a thin rim of cytoplasm that surrounds the apical lateral portion of the granules. Microvilli are obvious on the immature cells in the deep one half of the intestinal gland **(5).**

Paneth cells are important in regulation of normal bacterial flora of the intestine. Paneth cells are found in bases of the intestinal glands. They can also occasionally be found in the normal colon in small numbers; may increase in certain pathologic conditions. They have a basophilic basal cytoplasm; a supranuclear Golgi apparatus; and large, intensely acidophilic, refractile apical secretory vesicles allowing their easy identification in routine histologic examination. The vesicles contain antibacterial enzymes as lysozyme, -defensins, other glycoproteins, an arginine-rich protein (probably responsible for the intense acidophilia), and zinc. Lysozyme digests the cell walls of certain types of bacteria. Defensins are homologs of peptides that mediate in cytotoxic CD8 T lymphocytes. Their antibacterial activity and ability to phagocytose certain bacteria and protozoa prove that **(5).**

Enteroendocrine cells belong to diffuse neuroendocrine system (DNES) or amine precursor uptake and decarboxylation (APUD). The “closed cells” are concentrated in the lower portion of the intestinal gland, whereas the “open cells” can be found at all levels of villi. By light microscope, they appear as small pyramidal cells where the nucleus is apically located. The cytoplasm is clear in routine H&E examination. They are stained black with silver hence

the name argentaffin cells (argent equals silver) and with bichromate salts so called enterochromaffin cells. Transmission electron microscope (TEM) examination of these cells appear to have all the ultrastructural features of (APUD) cells. They contain basal small electron dense secretory granules as they discharge their secretory products into adjacent capillaries of the lamina propria. Spherical or elongated mitochondria, RER and Golgi apparatus are also noticed **(6).**

The open type, that reach the lumen, has chemoreceptors similar to that of the taste buds. Stimulation of these receptors on the apical cell membrane of "open cells" activates G protein– signaling cascade, releasing peptides regulating a variety of gastrointestinal functions. These include regulating pancreatic secretion, induction of digestion, absorption, and managing energy homeostasis by acting on neural pathways of the brain-gut-adipose axis **(10).**

Cholecystokinin (CCK), secretin, gastric inhibitory polypeptide (GIP), and motilin are the most active regulators of gastrointestinal physiology that are released in this portion of the gut. CCK and secretin increase pancreatic and gallbladder activity and inhibit gastric secretory function and motility. GIP stimulates insulin release in the pancreas, and motilin initiates gastric and intestinal motility. Although other peptides produced by enteroendocrine cells have been isolated, they are not considered hormones and are therefore called candidate hormones. Enteroendocrine cells also produce at least two hormones, somatostatin and histamine, which act as paracrine hormones that have a local effect and do not circulate in the blood stream). In addition, several peptides are secreted by the nerve cells located in the submucosa and muscularis externa. These peptides, called neurocrine hormones, are represented by VIP, bombesin, and the enkephalins **(11)**

Submucosa

The submucosa consists of irregular dense connective tissue containing blood vessels, lymphatic vessels, nerve plexus and occasional glands. The larger blood vessels that send branches to the mucosa, muscularis externa, and serosa are present her. The extensive nerve network in the submucosa contains visceral sensory fibers mainly of sympathetic origin, parasympathetic (terminal) ganglia, and preganglionic and postganglionic parasympathetic nerve fibers. The nerve cell bodies of parasympathetic ganglia and their postganglionic nerve fibers represent the enteric nervous system. This system is mainly work for innervating the muscle layers and can function totally independent of the central nervous system. In the submucosa, the network of unmyelinated nerve fibers and ganglion cells constitute the submucosal plexus (called Meissner's plexus) **(12).**

Muscularis Externa

The muscularis externa consists of inner layer of circular smooth muscle cells and an outer layer of longitudinal smooth muscle cells. The main components of the myenteric plexus (Auerbach's plexus) are located between these two muscle layers. Two types of muscular contractions occur here, segmentation and peristalsis. Local contractions moving intestinal contents both proximally and distally; called segmentation. These contractions mainly belong

to the circular muscles. They also share in circulating the chyme locally, mixing it with digestive juice and moving it into contact with the mucosa for absorption. Peristalsis, which is the second type of contractions, coordinates action between both circular and longitudinal smooth muscle layers and pushes the intestinal contents distally **(12)**

Serosa

The serosa is a serous membrane possessing a layer of simple squamous epithelium, called mesothelium, occupied by a small amount of underlying connective tissue. It is equal to the visceral peritoneum. Serosa is the most superficial layer of jejunum suspended in the peritoneal cavity. Large blood vessels, lymphatics and nerve trunks travel through the serosa (from and to the mesentery) to reach the wall of the digestive tract. Amounts of adipose tissue can also be detected in the connective tissue of the serosa **(13)**.

Jejunal ischemia-reperfusion (IR) injury:

Ischemic damage is defined as a pathological condition that is brought up by deprivation of oxygen in tissues or organs. Anaerobic pathways take over cellular metabolism when oxygen and nutrients are decreased. Cell death may ensue from this change, causing a loss of cell function. Reperfusion is the regaining of blood flowing to the ischemic tissues. Restoring blood flow can be viewed as a healing procedure, but ironically, the reverse happens. Overproduction of free radicals, such as superoxide ($O2^-$) and hydrogen peroxide ($H2O2$), which have a high potential to harm cellular structures, results from the abrupt rise in oxygen supply. Compared to ischemia alone, fast reperfusion of ischemic tissue can result in significantly greater damage **(14)**

The jejunum as a part of the intestine has high sensitivity to ischemia as among the internal organs, the intestine is probably the most sensitive organ to Ischemia Reperfusion injury (IRI). About one in 1000 hospital admissions has intestinal ischemia that needs to be re-perfused. Intestinal ischemia reperfusion injury (IIRI) is a life-threatening abdominal emergency condition with high morbidity and mortality in surgical and trauma patients **(15).**

Intestinal ischemia-reperfusion injury (IIRI) is induced by interrupted blood flow as in small bowel transplantation, aortic aneurysm surgery, strangulated hernias, cardiopulmonary bypass and neonatal necrotizing enterocolitis. It can also occur as a consequence of collapse of systemic circulation, as in hypovolemic and septic shock. The pathophysiological mechanisms of IIRI are highly complex, as intestinal local tissue necrosis stimulates the release of oxygen free radicals and inflammatory mediators and enhances energy metabolism disturbance **(16)**.

It has been demonstrated that Intestinal ischemia reperfusion injury (IIRI) induces alteration in the intestinal barrier, also triggers enteral infections of opportunistic pathogen through mechanisms including intestinal inflammatory injury, releasing of bacteriocins, upregulation of antimicrobial peptide and utilization of nutrients. Thus, allowing enteric bacterial endotoxin and locally produced free radicals to penetrate the blood, translocate into systemic

circulation to the mesenteric lymph nodes and to the peripheral organs and cause extraintestinal multiple organ dysfunction or even failure (**17).**

Intestinal ischemia-reperfusion injury (IIRI) represents a serial of cascade of pathophysiologic case triggered not only by sudden disruption of blood flow but also by the subsequent regaining of blood flow, resulting in production of oxidative stress (OS). OS is caused by accumulation of intracellular reactive oxygen species (ROS) such as, hydrogen peroxide (H2O2) and hydroxyl radical. ROS can also be caused by influx of oxygen which is triggered by IIRI **(18)**.

Intestinal-Ischemia Reperfusion injury (IIRI) induces the inflammatory reaction by activating intestinal epithelium, polymorphonuclear neutrophils, macrophages secreting inflammatory cytokines, and other intermediate species all contribute further to OS. OS causes damage to cellular lipids, proteins and DNA, disrupting intestinal epithelial barrier, bacteria translocation and yet induces cells inflammation and consequently induces programmed cells response such as, apoptosis, autophagy, necroptosis (programmed form of necrosis) and eventually cell death **(19).**

Major pathologic events are contributing to ischemia/reperfusion injury. When the blood supply is markedly reduced or absent, ischemic cells switch to anaerobic metabolism to provide ATP. However, this comes to end with cellular acidosis and insufficient production of ATP to meet metabolic demand. This will be followed by ATPases inactivation, while active Ca2+ efflux and Ca2+ reuptake by the endoplasmic reticulum are clearly reduced, with resultant Ca2+ overload in the cell

Reactive oxygen species (ROS) produced by this and other mechanisms can damage literally every biomolecule found in cells, promote opening of mitochondrial permeability transition pores (PTPs), and activate inflammatory and thrombogenic cascades to exacerbate cell injury. The latter events are further amplified by release of danger signals (e.g., ATP) and other proinflammatory and thrombogenic mediators from damaged cells. The ensuing massive influx of immunocytes at ischemic sites share in cell injury via the NADPH oxidase-driven respiratory burst, release of hydrolytic enzymes, and production of MPO-derived hypochlorous acid plus N-chloramines **(20)**

Mesenchymal Stem Cell-Derived Microvesicles

Mesenchymal stem cell derived microvesicles (MMVs) are emerging therapeutic strategies for many diseases due to their bioactive substances and its low immunogenicity. They could polarize macrophages, attenuate the colonic inflammatory response in inflammatory bowel disease and guard the function of the intestinal barrier by delivering metallothionein-2. Mesenchymal stem cell derived microvesicles (MMVs) were documented as an effective treatment in severe bacterial pneumonia by delivering growth factors to inflammatory cells **(21).**

Microvesicles (MVs) as a type of EVs, carry a lot of biologically active molecules such as antigens, growth factors, mRNAs, proteins and lipids which are proved to participate in the

regulation of various pathophysiological processes and can interact with recipient cells through specific receptor–ligand interactions. Also, they contain high amounts of phosphatidylserine-containing proteins associated with lipid rafts. Also, they are rich in the surface marker CD40, as well as cholesterol, sphingomyelin, and ceramide **(21)**

Microvesicles (MVs) participate in intercellular communication, cell signaling, angiogenesis, and apoptosis. A significant therapeutic effect of MSCs-derived vesicles is also accomplished via reducing oxidative stress and enhancing antioxidant enzyme production. It was hypothesized that mesenchymal stem cells derived microvesicles (MMVs) might improve sepsis-induced intestinal barrier dysfunction, by their therapeutic proteins. Previous studies showed that they usually exerted therapeutic effects via modulating genes and proteins in target cells **(22).**

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