EXPERIMENTAL MODEL OF MIXED INTESTINAL INFARCTION IN RABBIT

I. I. Jeican1*, D. Gheban2, M. Socaciu3, S. Toader4, C. Ciuce1
“Iuliu Hațieganu” University of Medicine and Pharmacy, Cluj-Napoca
Faculty of Medicine
1. 1st Department of Surgery
2. Department of Pathology
3. Medical Imaging Department
4. Experimental Medicine Center
*Corresponding author. E-mail: ionutjeican@yahoo.com

EXPERIMENTAL MODEL OF MIXED INTESTINAL INFARCTION IN RABBIT (Abstract): The article presents an experimental model of the mixed intestinal infarction obtained by ligaturing of the cranial mesenteric artery (CMA) and of the cranial mesenteric vein (CMV) in rabbit. Keywords: RABBIT, INTESTINAL INFARCTION, EXPERIMENTAL MODEL

Acute mesenteric ischemia (AMI) consists of interruption of the blood flow in the irrigation area of the superior or inferior mesenteric artery, that results in intestinal infarction (1).

The first description of mesenteric vascular occlusion was attributed to Antonio Beniviene from Florence in the latter part of the fifteenth century (2). Little progress was made until 1895, when Elliot reported the first patient recover following resection of infarcted intestine (3).

In 1926, Cokkinissaid: “Occlusion of the mesenteric vessels is apt to be regarded as one of those condition of which […] the diagnosis is impossible, the prognosis hopeless and the treatment almost useless” (4). However, the progress in understanding of the pathophysiology and diagnosis, has made it possible to save sometimes the lives of patients with mesenteric ischemia.

For the physio pathological study of intestinal ischemia/ perfusion injury, the following animals are mainly used in experimental models: mice (5), rat (6-7), rabbit (8-9), pig (10).

In this study, the mixed intestinal infarction was reproduced in rabbits by ligaturing simultaneously the cranial mesenteric artery (CMA) near the emergence and by ligaturing the cranial mesenteric vein (CMV) near its pouring point in the portal vein. The abdominal topography of the rabbit presents some particularities: the cecum occupies a considerable part of the dextroventral half of the abdominal cavity, the spleen is positioned on the caudomedial surface of the stomach, and the kidneys are asymmetrically positioned in the retroperitoneal space – the right kidney lays between the planes through the 10-11 intercostal space and through the first lumbar

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direct constant affluent of the portal vein, that helps its marking (12).

In humans, the vascular mixed mesenteric obstruction is realized rather by an extrinsic compressive mechanism: tumors from near surrounding structures, of the mesenteric roots, of the duodenojejunal flexure (13), incarcerated trans mesenteric intestinal hernia (14), aortic aneurysm situated in the pouring point of the mesenteric artery. The double occurrence in artery and vein through intrinsic mechanism is rarer found (in mesenteric vasculitis (15-16)). Some authors consider the mixed form an evolutive stage of the arterial or venous infarction (17).

MATERIAL AND METHOD

All experimental procedures were performed in the Experimental Medicine Center, “Iuliu Hațieganu” University of Medicine and Pharmacy, Cluj-Napoca, with approval of the Ethical Commission of the university. Seven adult Californian rabbits (Oryctolagus cuniculus sp.) in two lots: A–experiment (five rabbits) and B–control (two rabbits, which were undertaking the same preoperative procedures and anesthetics like the rabbits from group A).

In order to eliminate from the study, the animals with abdominal pathology or vascular malformation of the cranial mesenteric package, the preoperative exam included veterinarian clinical examination, abdominal x-ray (fig. 1) and abdominal ultrasound. For the ultrasonography, we used a GE Logiq S7 machine, with a 6-15 MHz matrix transducer. We used the B mode to identify and assess the morphology of the vessels (fig. 2). We used color Doppler flow mode to visualize the direction and the laminar nature of the blood flow and we measured the velocities and flow indexes using spectral Doppler (fig. 3-4).

![Fig. 1. Abdominal X-ray A - rabbit in orthostatic position, AP incidence: normal aspect; B - rabbit in quadrupedal position, LL incidence: normal aspect.](image-url)
Fig. 2. Ultrasound identification of CMA emerge and measuring of its diameter.

Fig. 3. Doppler ultrasound identification of the cranial mesenteric vascular package – CMA (red), CMV(blue).

Fig. 4. Doppler ultrasound measuring of hemodynamic parameters in CMA.
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Preoperative diet consisted in food deprivation for 24 hours, with hydration.

Preoperative, abdominal fur was shaved off.

Induction of anesthesia was performed with Ketamine 100 mg/ml, 0,35 ml/kg body weight and Xylazine 20 mg/ml, 0,15 ml/kg body weight, after which the animal was bound on the operating table in a dorsal decubitus position (fig. 5).

![Fig. 5. The animal on the operating table in a dorsal decubitus position.](image)

Preoperative, the instruments were sterilized through boiling for 60 minutes at 110°C in an electric boiler.

After preparing and isolating the surgical field, laparotomy was performed through midline incision.

While exploring the abdominal cavity, the aspect and the viability of the intestine, the presence of the peristalsis was followed. After the exteriorization of the intestine through the surgical incision and it’s flapping towards the right side, the left kidney was looked for as a reference point and the retroperitoneal adipose tissue was penetrated. Following the left renal artery, path the aorta was localized and through its discovery, overlying the emerge of the left renal artery, the CMA was identified, easily mobilized by the movement of the mesentery and intestine. After the discovery of the CMA for a length of ca. 5mm from the emergence (fig. 6), the CMA was ligatured with a non-absorbable 4.0 (fig. 7) and the absence of the pulse distal to the ligature was verified.

![Fig. 6. Discovery of CMA, mobilization through light pulling of mesentery.](image)
Fig. 7. Ligaturing of CMA.

After flapping the intestine on the left side, near to the CMS, the CMV was identified. After its isolation, CMV was ligatured with non-resorbable 4.0 (fig. 8).

Fig. 8. The CMV ligature close to its entry into the portal vein.

During the intervention the intestine was covered by sterile compresses soaked in physiological salt solution.

After ligaturing, the abdominal cavity was washed with physiological salt solution which was warmed to 37°C. The laparotomy was performed in anatomical layers with non-resorbable 2.0.

Post operatory, the rabbits were put into recently cleaned and disinfected cages.

RESULTS

Intraoperative and post operatory observations. The clinical state of the animals was permanently supervised intraoperatory. Short periods of tachypnea-tachycardia (<1 min) were observed, not observed in group B – control during the anesthesia.

Approximately 5 minutes after ligaturing the vessels, the intestinal loops presented hyperperistalsis, afterwards they turned pale.

After approximately 6-7 hours the animal presents respiratory perturbations and death occurs.

Necropsy and histopathological evaluation. The necropsies were effectuated in the scheduled interval of 1-3 hours after death occurred.

While the external examination, the corpses showed the signs of real death. In three of the five cases, the outflow of a serohemorrhagic liquid through the suture's fibers was observed.

While opening the abdominal cavity, in three of the five cases from group A, approximate 3 ml serohemorrhagic ascites were stated. The cytological examination revealed rarely flaked mesothelial cells, rarely erythrocytes and leukocytes.

Signs of peritonitis were not observed.

The intestine is relaxed, with parietal edema, and thrombosis in the mesenteric vascular arcades (fig. 9).

On the surface, the duodenum presents the color pink-cyanotic and an accentuation of the parietal vascular drawing (fig. 9), the microscope shows the necrosis of the duodenal villosity and stasis is stated (fig. 10).
Concluding that the duodenum presents incipient ischemic modifications.

On the surface, the proximal jejunum presents the color Bordeaux and thrombosis in the adjacent mesenteric vessels. The distal jejunum and the ileum presents strongly cyanosed loops with the color purple-black (fig. 11); the mucosa is intensely red colored. Thrombosis in the adjacent mesenteric vessels is remarked. Microscopically, massive necrosis of the intestinal mucosa and submucosa is stated, necrotic-hemorrhagic content in the lumen. Thrombosis in the mesentery (fig. 12).

On the level of the appendix, no ischemic modifications are stated.

The proximal cecum presents a purple-black color whereas the distal cecum and the proximal colon presents alternations of purple and light grey areas (fig. 9). Microscopically, this segment of the digestive tube presents partial necrosis of the mucosa, areas where the epithelium of the surface is ulcerated, stasis and thrombosis in mucosa, alternating with areas of normal aspects (fig. 13, fig. 14). The distal colon presents incipient ischemic modifications or has a normal aspect.

The mesentery presents a hemorrhagic infiltration.

The liver presents stasis and multiple Zahn infarcts (fig. 15). The spleen presents stasis and thrombosis in the splenic vein (fig. 16). The stomach presents stasis and, in two of the five cases, hemorrhagic shock ulcerations (fig. 17).

The pancreas presents stasis and necrotic areas (fig. 18). The paravertebral ganglia present stasis (fig. 19).

The microscopically control of the ligation reveals the complete halting of the bloody flux in the cranial mesenteric package (fig. 20).
Fig. 12. A - Jejunum, HE, 10x: necrosis of mucosa, thrombosis in mesenter; B - Jejunum, HE, 40x: necrosis of mucosa.

Fig. 13. Cecum, HE, 100X: severe stasis in mucosa and detail with epithelium of surface absence

Fig. 14. Colon, HE, 100x: partial necrosis of mucosa.

Fig. 15. Liver, HE, 40x: Zahn infarct.

Fig. 16. Spleen, HE, 40x: stasis; thrombosis in splenic vein.
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**Fig. 17.** Stomach: hemorrhagic shock ulcerations.

**Fig. 18.** HE, 40x: thrombosis of CMV, necrosis of pancreas and adipose tissue.

**Fig. 19.** Sympathetic ganglions, HE, 200x: stasis.

**Fig. 20.** A - CMA, HE, ob. 40X: detail of vascular ligature; B - CMV, HE, 40x: detail of vascular ligature.

**DISCUSSION**

The splanchnic circulation receives approximately 25% of the resting and 35% of the postprandial cardiac output (18-19). 70% of the mesenteric blood flow is directed to the mucosa and submucosa of the
bowel, with the remainder supplying the muscularis and serosal layers. Multiple major elements interact to provide the intestinal tract with an appropriate share of the blood supply, including the intrinsic (metabolic and myogenic) and the extrinsic (neural and humoral) regulatory systems (19-20).

Pressure-flow autoregulation, reactive hyperemia, and hypoxic vasodilation are considered intrinsic controls. In the metabolic theory, oxygen delivery rather than blood flow causes adaptive changes in splanchnic circulation. An imbalance between tissue oxygen supply and demand will raise the concentration of local metabolites (hydrogen, potassium, carbon dioxide, and adenosine), resulting in vasodilation and hyperemia. The myogenic theory suggests that arteriolar tension receptors act to regulate vascular resistance in proportion to transmural pressure. An acute decrease in perfusion pressure is compensated for by a reduction in arteriolar wall tension, thereby maintaining splanchnic blood flow (21).

The vascular network of the submucosa prevents the installation of ischemia when the irrigation's debit of the extramural vessels sinks significantly (22).

In the first place, in physiological conditions, only 20% of the mesenteries capillaries are opened; when one of the mesenteric arteries is obstructed, a reduction of pressure takes place in the distal arterial bed, which triggers the opening of the capillaries of submucosa. After a couple of hours, this compensatory mechanism is overstrained and the distal arteries enter vasoconstriction. Initially, the vasoconstriction is reversible, but after a period of time it can become irreversible, even if the trigger of the ischemia was identified and corrected (23).

In the second place, when the O₂ contribution sinks, the extraction capacity grows (once the bloody flux sinks under 30ml/min/100g tissue or the systemic blood pressure drops under 50mmHg, the O₂ extraction becomes dependent on the flux (24)).

In this way, the intestine could endure a sinking of 75% of the irrigation debit up to 12 hours without causing cellular injury (25).

Through the sudden and complete installation of the vascular obstruction during the CMA ligature, the compensatory mechanisms are rapidly overstrained and it cannot be talked about the capacity of collateral circulation. The obtained results of our team are similar to those obtained by experimentally reproducing the mixed infarction in rabbits (the bowel became pale 5 to 10 minutes after vessel ligation, 3 hours after the ligation, the necrosis of the small bowel was evident, 6 hours after the ligation, the animals had extensive patchy necrosis and dilation throughout the small bowel with free hemorrhagic intraperitoneal fluid, 9 hours after the ligation, the small bowel was dilated and black, most of the animals died 9 hours after vessels ligation (26) or in dogs (30 minutes after ligaturing, the loops are lightly cyanotic, without peristalsis; after 1h the loops are relaxed, Bordeaux colored, with parietal edema, the mesenteric vascular arcades are turgescent, ascites is present; after 1,5 hours the loops are strongly cyanotic, ascites in great amount, after 2,5 hours the loops are black, in some places perforated, the mesentery strongly infiltrated, all the vascular arcades are black, rigid, after 3,5 hours the animal dies (17)).

The fact that the proximal duodenum
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and jejunum presents incipient, respectively middle ischemic modifications are due to the anastomotic paths given by the celiac trunk through the right pancreaticoduodenal arteries, which form an anastomosis with the left pancreatic-duodenal, the first collateral of the CMA. The distal colon presents minor or absent ischemic modifications due to the vascularization which gets through the anastomosis of the caudal mesenteric artery with the Riolan arcade and through the collaterals from the caudal mesenteric artery.

The lack of an adequate oxygen supply leads to tissue ischemia, which, in turn, causes cellular dysfunction and cell death (27). Intestinal injury causes significant damage to the intestine, particularly to the mucosa and neurons. The epithelial surface of the mucosa can be lost, which allows bacteria and toxins to enter the gut wall, compromises the ordered absorption of nutrients, and disturbs regulated water and electrolyte transport (28-30). The basic electrical rhythm of the intestine is known to decrease during mesenteric ischemia (31-32).

The most abundant constituents of epithelial cellular membranes are phospholipids. After ischemia, the oxygen radicals, which are the products of this oxidative stress, destroys lipid via lipid peroxidation (33). Consequently, intestinal permeability increases, lysosomal hydrolases are released and protein is enhanced, and intestinal barrier fails through destruction of endothelial and epithelial cells. Finally, indigenous enteric bacteria translocates to extra intestinal sites, induces sepsis and multiple organ system failure (34-37).

On the other hand, 20-25% of the cardiac debit stays sequestrated after ligaturing the mesenteric vessels, thus the animal suffers a volume depletion.

The fact that multiple abdominal organs present stasis, attests the cardio-circulatory insufficiency installed post operatory.

CONCLUSIONS
By ligaturing of the CMA and of the CMV, we obtained an experimental model of the mixed intestinal infarction in rabbits.

Necropsy and histopathological evaluation showed: the duodenum presents incipient ischemic modifications, the distal jejunum and the ileum presents massive necrosis of the mucosa and submucosa, the cecum and the proximal colon presents partial necrosis of the mucosa, areas where the epithelium of the surface is ulcerated, stasis and thrombosis in mucosa, alternating with areas of normal aspects; the distal colon presents incipient ischemic modifications or has a normal aspect.

On the level of the appendix, no ischemic modifications are stated.

The mesentery presents hemorrhagic infiltration.

Multiple abdominal organs present stasis (liver, spleen, sympathetic ganglions) or shock signs (stomach, pancreas).

The rabbits died by the cardio-circulatory insufficiency and multiple organ system failure.

REFERENCES


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A NOVEL ONCE-WEEKLY DPP-4 INHIBITOR IN THE CARE OF TYPE 2 DIABETES

Omarigliptin is a novel onceweekly (q.w.) dipeptidyl peptidase IV (DPP-4) inhibitor for the treatment of patients with type 2 diabetes. This study aimed to evaluate the long-term safety of optimal dose. In a multicenter, double-blind, 12-week, dose-range finding study, 685 oral antihyperglycemic agent-native or washed-out subjects with type 2 diabetes were randomized to one of five once-weekly doses of omarigliptin (0.25 mg, 1 mg, 3 mg, 10 mg, or 25mg) or placebo. Analysis included all patients who received at least one dose of the study medication. At week 12, the omarigliptin 25-mg dose provided the greatest glycemic efficacy. Subjects who completed the base study were eligible to enter a 66-week extension study. Omarigliptin 25 mg q.w., compared with placebo, provided significant glucose lowering and was generally well tolerated throughout the base and for up to 78 weeks in patients with type 2 diabetes (Sheu WHH, Gantz I, Chen M et al. Safety and efficacy of Omarigliptin (MK-3102), a novel once-weekly DPP-4 inhibitor for the treatment of patients with type 2 diabetes. Diabetes Care. 2016;38:2106–2114 | DOI: 10.2337/dc15-0109).

Elena-Daniela Dediu Grigorescu