IS THERE AN ACUTE GALLBLADDER’S INFLAMMATION AFTER THE EXPERIMENTAL LAPAROSCOPIC LIGATURE AND SECTION OF CYSTIC DUCT AND ARTERY?

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Summary

The experimental study aimed to induce the acute alithiasiatic cholecystitis by application of clips and then sectioning the cystic duct and cystic artery in 10 pigs using laparoscopy, to verify if the ischaemia of the gallbladder and the oclusion of the cystic duct produce inflammation and so the acute cholecystitis. The laparoscopic cholecystectomy was performed after 3 and 7 days. Hematoxylin and eosin stain was done from the sections of the gallbladder. At the reintervention moment, after 3 days, the sections presented the consequences of an important ischaemia of the tissues and we noticed that there aren’t any signs of an acute inflammation after the ligature and section of cystic duct and artery. After 7 days the restoration of the vascularization with the regeneration of the structures are signs of the overcome of the ischaemia period and the reperfusion of the tissues.

Key words: cystic duct, cystic artery, acute cholecystitis, laparoscopic cholecystectomy, inflammation, ischaemia

Introduction

Acute cholecystitis (AC) was induced over the time in experimental animals by many procedures. After a review of the literature, if the gallbladder’s wall inflammation is secondary to the lithiasiatic phenomenon, on the acute acalculus cholecystitis (AAC) the physiopathological mechanisms are not well clarified yet.

In imagining an experimental laparoscopic model for AC we have considered one of the three factors that determine the inflammatory response in the gallbladder (VB), namely mechanical inflammation produced by intraluminal pressure, increasing with the extension and having as result the ischemia of mucosa and of VB’s wall. The other two factors relate to chemical and bacterial inflammation.

So successful were used in combination or not with cystic duct ligation, the pancreatic or gastric juice injection into the VB. Other authors have reproduced the AC after the ligature of the cystic duct and artery in dogs, injecting colonic flora in VB and making conventional cholecystectomy (CC) after 72 hours. (Rulli et al., 1996)

Although these factors cause the inflammatory response, they may not be involved in the natural etiology of the disease in humans because they are not normally found in the VB.

Experimental data show however that cystic duct ligation alone does not produce AC in animal models only if the bile is supersaturated with cholesterol (CST) or if is inserted concentrated bile into the VB after the cystic duct ligation

Some authors describe the AC induction in rabbits after the ligation of the main biliary pathway (CBP) for 1 to 4 days. It was suggested that the initial factor that triggers AC is the transient obstruction of the cystic duct. (Trondsen et al., 1997)
Material and methods
The experimental research project included four groups of 10 female pigs (Sus scrofa), with 30-35 kg weight, the pig being the most appropriate laboratory animal from the human anatomical point of view for the laparoscopic cholecystectomy (CL). During the experiment the pigs were maintained on standard laboratory conditions.

For laparoscopic approach we used a videoscopic trocar of 10 mm who was placed above the umbilicus. The second trocar (10 mm) was placed at a few centimeters under the xiphoid appendix and a little bit on the left side of the mediane line. Through this trocar we operated with forceps, electrocoagulation hook, scissors, clip applier, suction-irrigation canula and other instruments for the removal of the VB. The third trocar (5 mm) was placed on the medioclavicular line, at 4 cm under the right costal margin. Through this canula we operated with the forceps which pull the VB’s infundibulum to expose the cystic pedicle. The fourth trocar (5 mm) was introduced on the anterior axillary line, at 4 cm under the right costal margin. Through this canula we actioned with the forceps which elevated the fundus of the VB together with the liver.

In the first operative time was accomplished the application of clips on the cystic duct. On 5 pigs the CL was performed after 3 days (Sublot 1a) and in the other 5 pigs after 7 days (Sublot 1b). (Fig. No. 1)

During the second intervention, CL, the VB was removed and sections were sent for histopathological examination. Hematoxylin eosin staining were done from sections of the VBs and then their aspects were studied with the microscope. (Stancu, 2011 a).

Results
In animals that were applied to the cystic duct and artery clips, were found at 3 days changes over the entire the wall thickness. In the upper half the surface epithelium is affected on a big area (at least 50%). (Fig.No.2). Lesions are characterized by cellular necrosis relatively large in size, nuclei appear pyknotic, hyperchromatic, intercellular spaces are visible, the cells become individually, become more narrow than normal and there is a tendency of epithelial disruption in some areas, but not very large. (Fig. No. 3)

There are also areas where alterations are more advanced, with extensive epithelial necrosis and denudation appearance on some areas, in some places large enough. There is stasis and interfibrillar edema, vessels appear slightly congested and only in the slightly higher venules are observed processes of stasis with a discrete trend of fibrin network organization and early alterations of vascular wall.

Fig. 1 Application of the first clip at the cystic duct

Fig. 2 Aspect of VB’s wall with surface epithelium damage - Sublot 1a
Fig. 3 Changes in zonal epithelial disorganization - hyperchromatic nuclei, intercellular spaces visible, narrowed cells - *Sublot 1a*

Fig. 4 Ensemble aspect of VB’s wall after 7 days - *Sublot 1b*

The edema is more pronounced in the deep half of the mucosa. Here vessels, especially veins, have very dilated lumen and occupied mostly by fibrin networks, where are attached in some of them, some red blood cells (RBCs). Some vessels have alterations of components, with pronounced change of permeability so that in addition to liquid component, RBCs also cross the vessel wall. They can be found in a large number in the region close to the vessel’s wall, and fewer at long-distance from vessels, on large areas, especially where the swelling fluid is high or very high volume.

We noted that in the deep mid part there are colagenolysis processes, and their intensity is in some way different from one area to another. The most advanced processes of colagenolysis are present in areas where the edema is most pronounced.

After 7 days from the cystic duct and artery application of clips and sectioning, lesions can be detected but they are subtle compared with the situation in animals operated at 3 days. In the epithelium, the folds are present and are numerous. They were comparable to normal height and are covered by simple prismatic epithelium apparently normal, except for very small areas located in the highest portion of some of the wrinkles. Others appear thicker than normal, have discrete edema and their chorion appears heavily infiltrated with mononuclear. (Fig. No. 4)

Blood vessels appear congested. Pronounced vascular disorders that were present after 3 days aren’t noticed in the profound area. Vessels appear congested but interfascicular swelling can be found only on certain portions and not with very high intensity. Note that most vessels are small.

There is the question of neovascularisation appearance at this level. There are also identified veins with alterations in wall and organizing tendency of fibrin networks but are much less, compared to the situation for 3 days. (Stancu, 2011 b)

**Disccusions**

Usually, when a gallstone is inclavated into the Hartmann's pouch or obstructs the cystic duct, a cascade of events are going to be held involving cellular injury and release of lysosomal enzymes, phospholipase, lecitin, and prostaglandins, all leading to an inflammatory process. This cascade of chemical and mechanical reactions initiates the progressive process which ends with AC.

Prostaglandins are important mediators of the inflammatory process. When prostaglandins are secreted, swelling occurs, causing the increase of obstruction and increasing smooth muscle contractions. Furthermore, bradykinin, histamine and
lizolecithin are secreted in response and accelerate the progression of chain reaction. The severity of biliary colic is increased, leading to obstruction irreversible of the cystic duct.

Many times AAC occurs in critical patients, burned or traumatized, or after surgery, diabetes, malignant tumors, with vasculitis, congestive heart failure, so that all can lead to shock or cardiac arrest. Mortality in these cases remains about 30% due to difficult diagnosis, patients with critical illness and due to rapid progression of AC with an increased incidence of gangrene (> 50%) and perforation (> 10%). (Laurila et al., 2005)

In these cases a central pathogenesis role belongs to ischemia/reperfusion lesions, but biliary stasis, opioid therapy, positive pressure ventilation and parenteral nutrition have also been implicated as cofactors. It seems however that the injuries of ischemia/reperfusion can induce AC in some cases, but requires an altered terrain of the patients and a number of adjuvant factors such as bacterial invasion of VB’s ischemic tissues. (Vakkala et al., 2007)

Histopathological aspects of Sublot 1a reveal that on the animals where we applied clips on the cystic duct and artery, the main disorders are vascular. These are very pronounced at 3 days after surgery when there are stasis processes almost generalized with dilated lumen, especially of veins, with marked changes in vascular permeability and extravasations of fluids in large quantity which causes a generalized edema.

The intensity of these processes is greater in the deep half part of the wall where vessels are of large caliber, here the edema fluid being in a greater amount than the in the upper half part. Moreover, here some vessels present some more advanced changes, with altered zonal components, which results in cancellation of the permeability function, practically all components can get out of these vessels.

In the connective tissue in the immediate vicinity of these vessels (perivascular area) RBCs are found in large numbers in the interfibrillar edema fluid and away from them, RBCs are present in the edema fluid but in a smaller number.

The appearance suggests that RBC extravasation is still an active process in those vessels. Moreover, the appearance of vascular components and processes running in the vicinity of the vessels and away from them, suggests that the pathologic process is still underway and has no tendency to stop yet.

There aren’t noticed even discrete reparative processes, being still an evolving process. All alterative processes seem to be the consequence of ischemic vascular disorders.

In the upper half part, vascular disorders are present but less pronounced compared to those from depth and therefore stasis and edema, although present, are of lesser intensity. They appear somewhat more pronounced only at the level of some venules (the larger ones) where can be observed zonal alterations of the wall.

Lesions are present in epithelium but they do not cover the entire surface but only about 50%. The most affected is the epithelium covering the upper half part of the folds, and in those areas, the vascular changes from chorion seems to be more pronounced than in neighboring areas.

We believe that also epithelial necrosis are the consequence of vascular disorders which are comprising chorionic vessels, with the disruption of the diffusion processes by the basal membrane of nutrients to cells, knowing that epithelial cells are dependent on this input, because the epithelium isn’t vascularized.

Due to changes in chorionic vessels’ permeability with extravasation of higher than normal amounts of fluids and resulting edema above mentioned, and in terms of quality the edema fluid is not suitable for exchanges by basal membrane to run properly. We can appreciate that the issue is
of the consequences of a major ischemia of tissues and that are not present signs of an acute inflammation after the ligation and section of cystic duct and artery.

Histopathological aspects of the Sublot 1b shows that after 7 days the situation is much changed from the previous appearance, vascular disorders can be highlighted, but are more discreet than after 3 days. It seems that the organ is able to largely restore vascularization using probably collateral vessels and apparently appear neoformation vessels.

This statement is supported by the presence in some areas of small caliber vessels but relatively numerous. Is clearly in evidence that circulatory disorders, even advanced ones, although causing brutal changes, they are not necessarily irreversible, collateral circulation succeeding to reestablished the vascularization, which has as result the structural restoration in a very high proportion, a sign of overcoming the period of tissues’ ischaemia and reperfusion. On the other side vascular disorders alone determine changes which may be even of a greater intensity, but they are mostly reversible.

Mucosal folds are present and many of epithelium present in most of the surface area. Longer maintain a discrete edema and presence of mononuclear. In the lamina propria is loose connective tissue and congested small vessels. There are noticed intussusceptions crossing the muscular layer, so-called Rokitansky-Aschoff crypts. In the muscular layer fibrin deposits are outlined.

Restoring vascularization of VB’s wall or by neovascularisation or by vessels that connect strongly with hepatic vascular bed crossing the adventitia determines repair processes after the ischaemic episode, close to normal. (Stancu, 2011b).

Conclusions
Our experimental study invalidated the ischemic theory of AC production by the initial application of clips and section of the cystic duct and cystic artery, this being demonstrated both macroscopically and histological by performing CL after 3 and 7 days.

In terms of histopathology in experimental animals where we applied clips the cystic duct and artery, alternative processes identified after 3 days are due to ischemic vascular disorders with lumen expansion especially in veins, with the increase of vascular permeability and extravasation of a large amount of fluids producing a generalized edema without inflammatory signs.

After 7 days there is vascularization recovery with the restore of structures and we found signs of the exceeding this period of ischaemia and tissues reperfusion.

References