ABSTRACT. Introduction. The experimental study aimed to induce the acute alithiasic cholecystitis in 20 pigs, injecting L-α-Phosphatidylcholine in the gallbladder using laparoscopy. Materials and methods. After the induction the laparoscopic cholecystectomy was performed after 3 and 7 days. Results. The histology emphasized the gallbladder’s inflammation, colagenolysis and vascular alteration. The laparoscopic cholecystectomy on the first group had a medium time of 71 minutes and at the second group medium time was 94 minutes. Conclusions. The experimental induction of acute acalculous cholecystitis is possible injecting using laparoscopy in the gallbladder L-α-Phosphatidylcholine, the inflammatory lesions being emphasized.

Keywords: acute cholecystitis, L-α-Phosphatidylcholine, laparoscopic cholecystectomy, inflammation, colagenolysis

INTRODUCTION

The acute cholecystitis is one of the frequent complications of gallbladder’s lithiasis (12%). In 10% cases we also found the acute acalculous cholecystitis. Thus, the acute cholecystitis represents almost 25% of all cholecystitis, presenting higher technical difficulties than chronical cholecystitis because of the intensity of the inflamatory process and of the pericholecystic adherences which lead often to conversion.

Nowadays, after a review of the literature many authors recommend as the most favourable operative moment for laparoscopic cholecystectomy for the acute cholecystitis, the first 48-72 hours from the beginning. (Duca, 2001, Alecu, 2003).

For the experimental induction of the acute cholecystitis in this study was used L-α-Phosphatidylcholine (Laph) also known as L-α-Lecithin. We pursued the macroscopic aspect and then the histopathological one of the gallbladders after the laparoscopic cholecystectomy. After a review of the literature, if the gallbladder’s wall inflammation is secondary to the lithiasic phenomenon, on the acute acalculous cholecystitis the physiopathological mechanisms are not well clarified yet. Besides the acute distension of the gallbladder and the chronic presence of biliary calculus, for chemical stimulation of the gallbladder’s epithelium, since 1969 was used Laph by Gottfries, which is a product a hydrolysis constituent of bile and because of the remark of it’s concentration increase in the gallbladder of patients with acute cholecystitis. Laph has cytolythic features and of membrane perturbation, being capable to induce acute inflammation at the gallbladder on experience animals (rabbits, guinea pigs, cats, etc.). (Rybar et al., 2007)

MATERIALS AND METHODS

The experimental research project included two groups of 10 female pigs (Suis scrofa), with 30-35 kg weight, the pig being the appropriate laboratory animal from the human anatomical point of view for the laparoscopic cholecystectomy. During the experiment the pigs were maintained on standard laboratory conditions.

For laparoscopic approach were used 4 surgical trocars: 2 trocars of 10 mm and 2 trocars of 5 mm. (Fig. 1)

The operatory disposal was the one frequently used in Second Surgical Clinic Cluj Napoca which achieve a joining of the classical disposals described by the north-american surgery school and by the french surgery school, the surgeon being placed on the left side. Beside him, at the distal extermity of the animal is the cameraman, and on the opposite side the assistant surgeon. The pressure used on the peritoneal cavity by insufflation of CO2 was of 12 mmHg. (Radu, 2005). (Fig. 2)

The first trocar (videoiscopic, 10mm) 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 operate with forceps, electrocoagulation hook, scissors, clip applier, suction-irrigation canula and other instruments for the removal of the gallbladder. The third trocar (5 mm) was placed on the medioclavicular line, at 4 cm under the right costal margin. Through this canula we operate with the forceps which pull the gallbladder’s infundibulum to expose the cystic pedicle. This trocar was used for the...
introduction using a puncture needle, into the gallbladder of the Laph. (Fig. 3)

After the injection the puncture orifice was sealed by the application of a clip at this level. (Fig. 4)

The fourth trocar (5 mm) was introduced on the anterior axillary line, at 4 cm under the right costal margin. Through this cannula we actioned with the forceps, that elevated the fundus of the gallbladder, together with the liver.

At both pig groups, in the first operative time was accomplished the induction of the acute cholecystitis, by the laparoscopically injection into the gallbladder at the fundus level of the Laph after the initial application of clips on the cystic duct. 2 bottles of 250 mg Laph were injected on every experience animal’s gallbladder, achieving in this way a concentration of 1 mmol/l.

The laparoscopic reintervention was done precocious in the first group at 3 days and in the second group the laparoscopic cholecystectomy was done at 7 days. During the second intervention the gallbladder was removed and sections were sent for histopathological examination.

RESULTS AND DISCUSSIONS

Regarding the macroscopic aspect, at both groups during the reintervention were noticed important inflammatory alterations at gallbladder’s level. There were many adherences: gallbladder-epiploic, gallbladder-gastric, gallbladder-enteral and parietal-gallbladder. The section of the adherences and gallbladder dissection was difficult, being necessary to perform the hemostasis with the electrocoagulation hook.

Macroscopic, the intensity of the gallbladder and near gallbladder inflammatory process was strongly marked in the group which was operated later making more difficult the cholecystectomy. (Fig. 5, 6)
Histopathological aspects of the acute acalculous cholecystitis experimentally induced by laparoscopy with L-α-phosphatidylcholine

Studia Universitatis "Vasile Goldiş", Seria Științele Vieții
Vol. 19, issue 1, 2009, pp. 53-58
© 2009 Vasile Goldis University Press (www.studiauniversitatis.ro)

Hematoxylin eosin staining was done from sections of the gallbladders and then their aspects were studied with the microscope.

The medium operative time to induce the acute cholecystitis was 28 minutes. The laparoscopic cholecystectomy on the first group had a medium time of 71 minutes and at the second group medium time was 94 minutes.

From the histopathological point of view on the first group, after 3 days the inflammation lesions are very marked and comprise all the layers. (Fig. 7)

On large areas the epithelium is missing. On several areas the epithelium is present but presents changes with a higher intensity, the lesions are irreversible, until the epithelium disintegration and denudation of these areas is just a matter of time. (Fig. 8)

There weren’t noticed areas with normal epithelium. The folds are keeping the shape to certain degree but they have a vestigial aspect (Fig. 9), and the parts of its structure are all comprised from strong degenerative changes, on different stages. There are differences between an area from another. We can also notice conjunctive cells but all of them comprised by those alterative changes.

On some folds, it shows polymorph particles intense acidophile which suggest the evolvement of some generalised proteic degrading processes. There are comprised from those processes also the small vessels which pass through these areas. In some other places those processes are more discrete. (Fig. 10)

There were present also colagenolysis processes and vascular alterations more marked at small vessels from the mucous chorion. The oedema from the chorion is very pronounced in the wall’s thickness, but especially in the external half part where is accompanied by cruelly colagenolysis so that only a small number of fiber fragments can be noticed with a severe modified tinctorial affinity. (Fig. 11)

On the superior half part the colagenolysis processes are also relatively advanced but without the amplitude of those from the external part. The colagenolysis comprises not only the thin collagen fibers from the chorion but also the thick ones from the depth which are divided into fragments, swollen, with the alteration of tinctoriality. There are also present processes of miolysis which seems to have a progressive and generalisation features. Muscular cells are also comprised on alterative processes which seem
to be irreversible, although they are the most resistant from all the structures, being damaged at last.

Most blood vessels present different intensity lesions, regardless the caliber or their type (veins or arteries). At some of them, although they present advanced lesions, it can be described structural components, especially on large diameter arteries. The lumen is occupied almost totally by detritus which tend to organize in an embolus that blocks the lumen, making in that way the vessel functionless. (Fig. 12)

There are arteries in an advanced stage of alteration, where neither of the components can be appreciated anymore but only the shape, and the detritus resulted from the embolus degrade tend to mix up with those from the degrade of vessel structures, with a final aim to homogenize. All the veins have the lumen blocked, with fibrin networks which comprise also a small number of red cells as well as with material resulted from proteins degradation.

On the second group of study, at 7 days from the first intervention the inflammatory lesions are more moderate, especially in the profound half part. In the superficial half part the changes are still advanced, the epithelium is still damaged and presents the tendency to detach from the basal membrane so that large areas appear to be denudated.

In the profound part the situation is totally different, the lesions are resigning most partly and there are noticed the initiation of some repairing processes, which, even they didn’t evolve at the same intensity, comprehend large areas. There are present numerous fibroblasts which prove that the repairing processes are in fully development. Here are also present vessels, most of them of small caliber which are neoformation blood vessels looking at their aspect and distribution. The repairing processes encompass 25% to 60% from the surface dependig on the examined area. (Fig. 15)

DISCUSSIONS

The acute cholecystitis was induced along the time at the experience animals by multiple proceedings. There were used with or without the ligature of the cystic duct, the injection of pancreatic or gastric juice into the gallbladder or by intravenous injection of pathogenic agents such as Escherichia coli endotoxin, ellagic acid, or a flavonoid complex such as Rutin. Even those factors produce inflammatory response; they can’t be involved in the natural etiology of this pathology because they aren’t normally present in the gallbladder. (Myers et al., 1988)
Laph is a phospholipid which is encountered in the composition of the bile of patients with acute cholecystitis. The effect of Laph at the gallbladder level was studied for the first time by Gottfries which injected, by cystic duct cannulation, Ringer solution alone or combined with Laph in the ocluded rabbit’s gallbladder for 48 hours. On rabbits’ gallbladder treated with Laph weren’t noticed anomalies, but on the rabbits treated with Laph at gallbladder level he found the necrose of the mucous membrane, the oedema of the gallbladder’s wall and inflammation achieving in that way the acute acalculous cholecystitis. (Gottfries, 1969, Thornell et al., 1986)

Other authors like Neiderhiser in 1986 introduced Laph dissolved in bile and then injected in guinea pigs gallbladder and he noticed an effect depending on its concentration. At lower concentrations (< 0.5 mmol/l), the gallbladder wasn’t affected and the mucosa absorbed Laph but at concentrations over 1 mmol/l, Laph induced lesions of acute cholecystitis. Those histological changes were similar with those encountered in human patients. The difference was that in human gallbladder we found extensive hemorrhage and in some cases gangrene and perforation. The explanation is that in human patients occurs major complications caused by a longer sufferance. (Neiderhiser, 1986, Kaminski et al., 1985)

Texeira also induced the acute cholecystitis in pigs using Laph after the ligation of cystic duct but performing the classic open technique and conventional cholecystectomy. Some of those animals were treated with Diclofenacum and the others weren’t. (Texeira et al., 2002, Colles et al., 2000)

So that, the inflammatory response at gallbladder’s level can be expalined by 3 factors: a) Mechanical inflammation produced by the intraluminal pressure, increased by the distension and having as a result the ischaemia of mucosa and gallbladder’s wall; b) Chemical inflammation produced by the discharge of Laph and other tissue factors; c) Bacterial Inflammation, which can have a significant role in over 50% patients with acute cholecystitis. The bacterial agent most frequently found at those patients is Escherichia coli, Klebsiella species, Streptococcus group D, Staphilococcus species and Clostridium species. (Nicolau, 2004, Lai et al., 1998)

The injection of Laph produced at 3 days severe alterations of the gallbladder’s wall and comprised all of his structures from the surface to depth. Vascular changes were extremely marked. The consequence is the stifle of all structures with oedema liquid which compress on the other structures and stops the nutrition and their good functioning. Many vessels had altered walls, the circulation being washed out in a higher proportion, all the other alterations being its consequence.

At 7 days the changes were present and they had a comparable intensity with the one from 3 days, only in the superior half part of the wall, where the epithelial structure is totally altered and the collagenous structures from depth appears to be swollen by the oedema liquid which is reduced quantitatively in comparison with the first group of study. Repairing processes weren’t noticed in the superior half part but in the profound half part the situation is totally different. Here the inflammatory processes resigned mostly and we found active repairing processes.
emphasized by the presence of numerous fibroblasts and collagenous fibers, their synthesis products, which had a different thickness. The presence of those fibroblasts demonstrated that the repairing processes are very active and the presence of numerous vessels of neof ormation proves that the vascularisation was mostly rehabilitated and was capable to sustain those repairing processes. It is obvious that those repairing processes initially started from the profound areas and tend to extend to the superior part. (Rosai, 2004)

CONCLUSIONS
The experimental induction of the acute acalculous cholecystitis is possible by injection using laparoscopy into the gallbladder L-α-Phosphatidylcholine in concentration over 1 mmol/l, the marked inflammatory lesions being emphasized also macroscopic and by histopathology.

The most favourable operative moment for laparoscopic cholecystectomy for the acute cholecystitis is during the first 48–72 hours from the beginning, sustained by the shorter and easier technical procedure, because of the facility of adherences dissection, achieving in that way an analogy between the experimental pattern and the human one.

Histopathological examination proved that at 3 days L-α-Phosphatidylcholine produced a severe inflammation of the gallbladder’s wall and comprised all of his structures from the surface to depth with marked vascular changes.

The presence at 7 days of numerous fibroblasts, collagenous fibers and the presence of numerous vessels of neof ormation demonstrated that the repairing processes are very active, initially started from the profound areas and tending to extend to the superior part.

This experimental pattern is very useful for laparoscopic surgeons’ training in the treatment of the acute cholecystitis emphasizing the possibility of its introduction in the programme of postgraduate laparoscopic surgery courses.

REFERENCES