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Effect of intraperitoneal administration of sterile human cerebrospinal fluid in rats

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Abstract

Introduction: Distal ventriculoperitoneal shunt failure can be attributed to unabsorbed cerebrospinal fluid (CSF) from peritoneum. The objective of the experiment was to determine peritoneal reaction in rats after intraperitoneal administration of human CSF and evolution of local inflammatory response. *Materials and Methods*: Wistar rats were used divided into four groups: three groups in which intraperitoneal injection of 3 mL, 2 mL and 0.5 mL of sterile human CSF was done and a control group. After sacrificing the animals, at 24, 48 or 72 hours, macro- and microscopic examination of the peritoneal cavity and peritoneal fluid analysis were performed. The experiment was done in compliance with legislation regarding animal research. *Results*: Administration of high dose CSF (3 mL) led to death of all specimens. The dose of 0.5 mL of sterile CSF intraperitoneally administered was compatible with survival. Peritoneal response varied from necrotic-purulent reaction, with maximum intensity in group 1, and milder in group 2, to minimum inflammation in small foci and polymorphic cells in group 3. Inflammation only partially resolved in some specimens from group 3 after 72 hours, which incriminates the role of unabsorbed peritoneal CSF in pathogenesis of abdominal complications of ventriculoperitoneal shunts. *Conclusions*: Intraperitoneal administration of sterile human CSF caused inflammatory response of varying degrees and with multiple locations. High doses of CSF led to death of specimens. At 24 hours, the peritoneal response ranging from congestion to purulent reaction was acute, intense and diffuse but after 72 hours, the inflammatory response was mild, focal and limited.

Keywords: cerebrospinal fluid, hydrocephalus, peritoneum.

Introduction

Hydrocephalus is due to the impairment in production, flow, or absorption of cerebrospinal fluid (CSF). An abnormal increase in CSF volume and, usually, pressure within cerebral ventricles occur. Hydrocephalus is a health problem worldwide. It has an estimated prevalence of 1–1.5% [1].

Hydrocephalus requires prompt treatment, because otherwise it leads to increased intracranial pressure and death. Treatment is challenging, because until now no infallible therapy was found. Over the years, many surgical techniques were proposed for treatment of hydrocephalus, such as medical therapy, repeated lumbar taps, external ventricular drainage, ventriculoperitoneal shunt, lumboperitoneal shunt, ventriculoatrial shunt, ventriculosinusal shunt, ventriculopleural shunt, ventriculo–gall bladder shunt, ventriculouretheral shunt, lombouretheral shunt, ventriculomastoidian shunt, ventriculo–subarachnoid–subgaleal shunt and endoscopic third ventriculostomy (ventriculocisternostomy) [2–5]. Despite advances in neuroendoscopic surgery, the treatment of choice in hydrocephalus remains ventriculoperitoneal shunt. Kausch W performed the first ventriculoperitoneal shunt in 1908 [6], but the procedure become widely used only 50 years later, and since 1960 the surgical technique has not changed much.

Abdominal complications cause distal shunt failure and acute hydrocephalus. Ventriculoperitoneal shunt-related consequences are encountered in 24–47% of cases [7]. Distal shunt complications are shunt infection, shunt disconnection with distal catheter migration, CSF pseudocysts, CSF ascites, visceral perforations, bowel obstructions, inguinal hernia with or without hydrocele, mesenteric pseudotumor, incisional hernia, subfrenic abscess and peritoneal metastases from primary central nervous system tumors [4, 8, 9].

Opening of the peritoneal cavity in patients with ventriculoperitoneal shunt for distal shunt revision or for other abdominal pathology reveals, in some cases, extensive adhesions between distal tube and viscera. Distal shunt failure can be attributed to the presence of unabsorbed CSF in peritoneum, causing inflammatory reaction.

Aim and objective

The aim of the experiment was a better understanding of phenomena occurring within peritoneal cavity in patients with hydrocephalus and ventriculoperitoneal shunt. The objective was to determine the response and most of all the peritoneal reaction in rats after intra-peritoneal administration of human CSF and the evolution of the local inflammatory response.

Materials and Methods

The experimental study was carried out in the Center for Experimental Medicine of "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, using Wistar female healthy rats, each weighing 180–190 g. The experiment has been done in compliance with the legislation regarding animal research. The rats had standard living conditions. Euthanasia of the animals has been performed dynamically, by intra-cardiac inoculation of Vetased in lethal dose.

The cerebrospinal fluid inoculated intraperitoneally came from a human patient with hydrocephalus, was clear, with negative Pandy reaction and was harvested and stored under sterile conditions for 72 hours at 4^0C.

The experiment was designed in two steps:

▪ First step aimed to set the suitable dose of CSF to be intraperitoneally administered without causing animals' death within 24 hours;

▪ The second step aimed to assess the morphological changes of abdominal cavity and abdominal organs after prolonged exposure to CSF introduced in peritoneal cavity.

Four groups of 10 animals, each were used in each step. The first group in each step was the control group where each animal received a single dose of 0.5 mL of normal saline. The other three groups of each step received a single dose of CSF.

All subjects were kept under observation after the intraperitoneal administration in standard life conditions, according to the animal facility microclimate.

The protocol for the first step was as follows:

▪ The animals in the control group, which received normal saline, showed no behavioral changes neither immediately nor 24 hours after inoculation.

▪ The animals in the group A received 3 mL of CSF each. A sudden deterioration of general state occurred 15 minutes after inoculation: subjects developed a muscular contracture, adopting a hedgehog position, suggestive for severe abdominal cramps and had cold extremities. All subjects were dead at 24 hours, most of them within the first hours.

▪ The animals in the group B received 2 mL of CSF each. The symptoms occurred and the results were the same, all subjects being dead at 24 hours.

▪ The animals in the group C received 0.5 mL of CSF each. No behavioral changes occurred during the first six hours after administration, and all subjects were alive at 24 hours.

Thus, the dose of 0.5 mL CSF/subject was considered suitable for the second step of the experiment.

The protocol for the second step was as follows:

▪ The animals in the control group were inoculated with a single dose of 0.5 mL CSF and were sacrificed at 24 hours.

▪ The animals in the group 1 were inoculated with a single dose of 0.5 mL CSF and were sacrificed at 24 hours.

▪ The animals in the group 2 were inoculated with a single dose of 0.5 mL CSF and were sacrificed at 48 hours.

▪ The animals in the group 3 were inoculated with a single dose of 0.5 mL CSF and were sacrificed at 72 hours.

After sacrificing the animals, peritoneal fluid analysis, macroscopic and microscopic examination of the peritoneal cavity and abdominal organs were carried out.

Cytological analysis of peritoneal fluid was carried out on smears stained with May–Grünwald–Giemsa (MGG). Histopathological examination of visceral and parietal peritoneum, abdominal wall, small bowel, epiploon and uterus was carried out using formalin-fixed paraffin-embedded samples, stained with Hematoxylin–Eosin (HE).

Results

The main observations identified in each group are described below by categories of morphological parameters and summarized in Table 1.

Table 1 – ***Synopsis of the morphological study***

Group	Fluid	Outcome	Necropsy	Cytology	Histopathology
Control	Normal saline, 0.5 mL	Euthanasia at 24 hours	No changes	Few mature cells	No changes
Group 1	Sterile CSF, 0.5 mL		Parietal and visceral peritoneal congestion	Obvious inflammatory reaction Bacteria free and within the phagocytic cells ↓ Elements suggestive for *acute peritonitis*	Necrotico-purulent inflammation of parietal and visceral peritoneum Areas of desquamation and necrosis Epiploic necrosis Inflammatory process extended to the abdominal wall
Group 2		Euthanasia at 48 hours	Discrete peritoneal and abdominal wall congestion Moderate wall edema	Reduction in number of free or phagocytized bacteria Increasing in number of macrophages with significantly phagocytosis ↓ *Acute peritonitis in remission*	Histopathologic examination similar to group 1, but with less intensity
Group 3		Euthanasia at 72 hours	Almost total remission of congestion and edema	Small amount of inflammatory cells	Reduced inflammatory process, in small foci, with macrophages, neutrophils, lymphocytes, mast cells

Necropsy

The necropsy revealed no changes in the peritoneum and abdominal organs of control group animals (Figure 1).

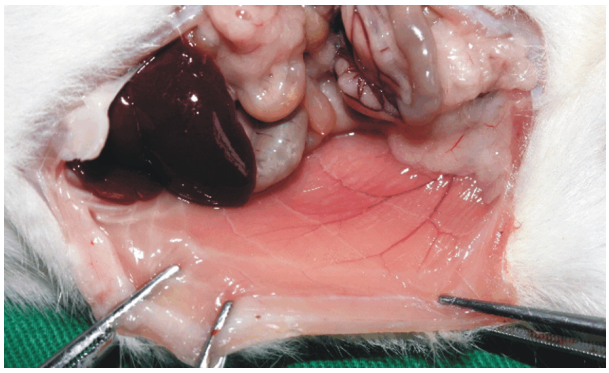

Figure 1 – ***Control group. Normal aspect of the peritoneum and abdominal organs.***

In group 1 of animals, a strong congestion of the abdominal wall, peritoneum and abdominal organs was observed (Figure 2, a and b). In some animals, increased amount of turbid, yellow-white abdominal fluid was found.

In group 2, there was a slight congestion of the peritoneum in some animals, but most of them showed no obvious macroscopic changes of the peritoneum and abdominal organs (Figure 2c).

In group 3, complete or almost complete remission of peritoneal and abdominal organs macroscopic changes was observed (Figure 2d).

Cytological examination of peritoneal fluid

The peritoneal fluid in the control group contained predominantly old neutrophils, with multilobulated nuclei, eosinophils and lymphocytes (Figure 3). Other rare cells were mononuclear cells, mast cells and mesothelial cells.

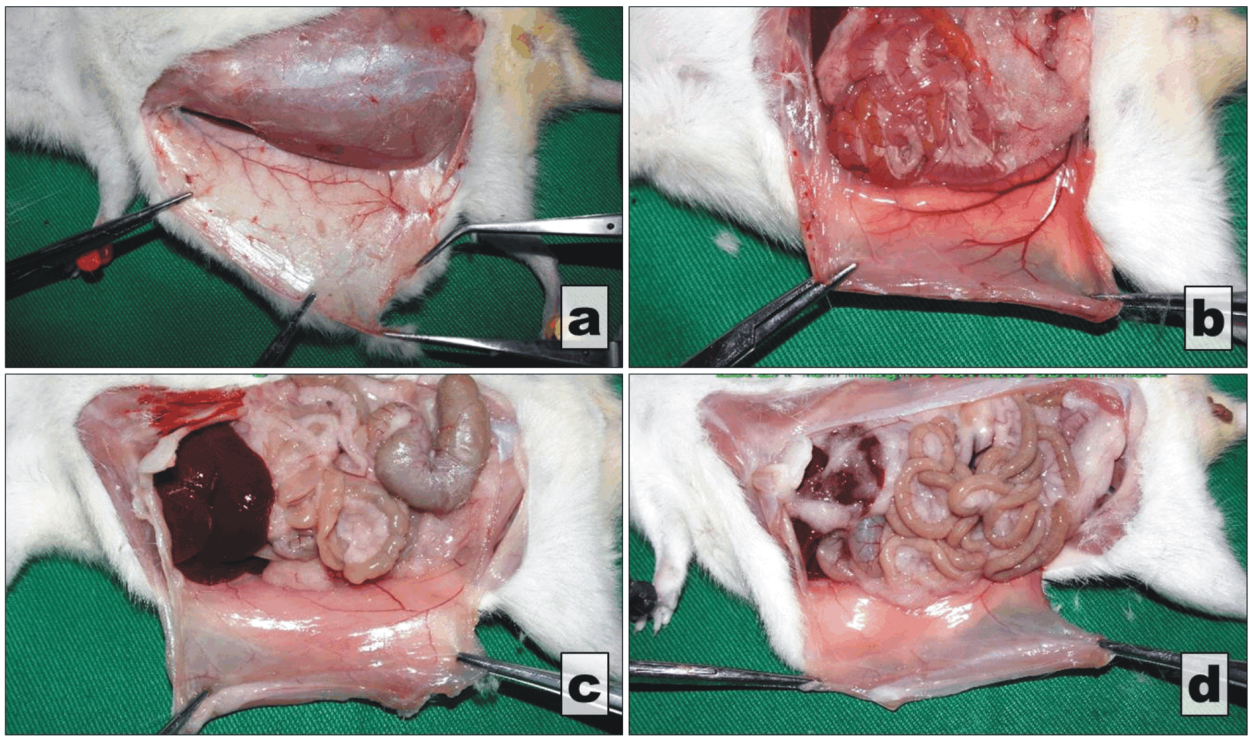

Figure 2 – ***Different aspects of macroscopic changes of the peritoneum and the abdominal wall in studied groups: Group 1 – intense congestion of the abdominal wall (a) and abdominal viscera (b); Group 2 – abdominal viscera showing only slight congestion (c); Group 3 – peritoneum and abdominal viscera with normal aspect.***

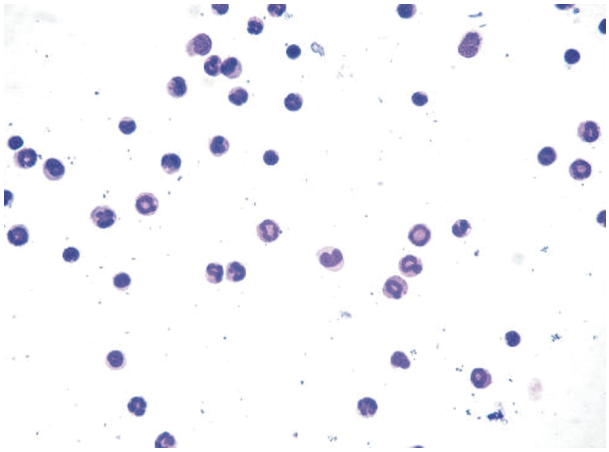

Figure 3 – ***Control group. Peritoneal fluid cytology. MGG stain, ×400.***

In group 1, the peritoneal cytology revealed a large number of young and old neutrophils and macrophages containing phagocytized bacteria with diplo and strepto arrangement (Figure 4, a and b), aspect which pleaded for the diagnosis of purulent bacterial peritonitis.

The cytological examination performed in group 2 showed a few free or phagocytized bacteria, an obviously increased number of macrophages (some of them multi-nucleated) who had phagocytized bacteria and dead cells, of neutrophils and old and young eosinophils (Figure 4c). The cytological diagnosis was purulent bacterial peritonitis, with lower intensity than in group 1.

In group 3, the peritoneal fluid cellularity was slightly higher than in the control group, with an increased number of macrophages, neutrophils and young mast cells (Figure 4d). This aspect pleaded for a milder bacterial inflammation than in groups 1 and 2.

Histopathological changes

As expected, the control group showed no obvious morphological changes of all studied structures (Figure 5, a and b).

In group 1, an intense purulent necrotic inflammation of visceral and parietal peritoneum was found. Neutrophils and macrophages containing cocci were present on the mesothelial surface. Mesothelial cells containing bacteria presented, in desquamated areas, necrosis and transformation into macrophages. The epiploon showed areas of necrosis, infiltrates of macrophages and neutrophils containing cocci, as well as free bacteria, on a background of intense congestion. All abdominal organs studied showed abundant infiltration with macrophages and granulocytes beneath the serous layer. The abdominal wall was congested, with granulocytes and macrophages infiltration beneath the peritoneum. Adherent peritoneal mesothelial cells became cuboidal or columnar in shape, with abundant cytoplasm, morphological changes defining the so-called mesothelial activation (Figure 6, a–e).

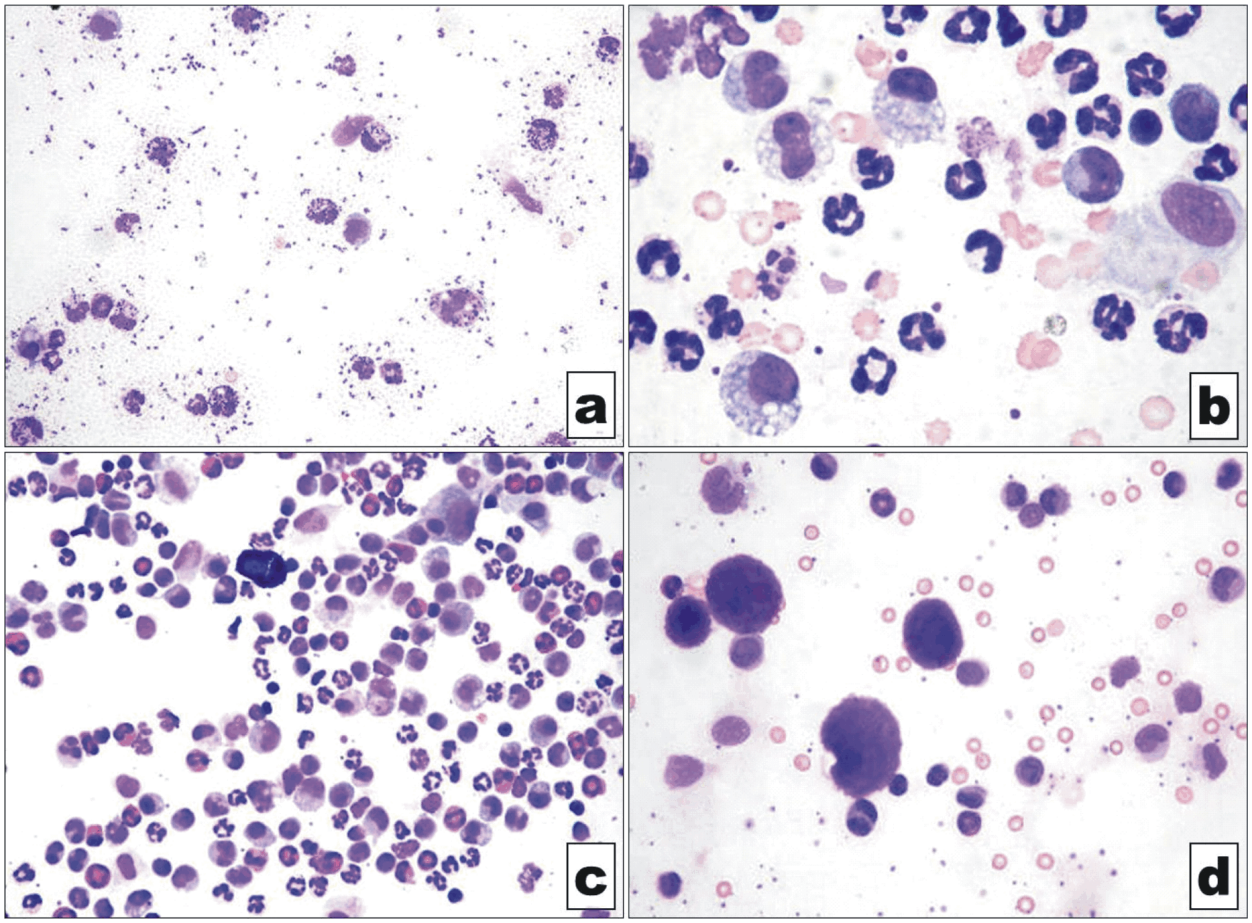

Figure 4 – ***Different aspects of peritoneal fluid cytology, MGG staining, ×400: (a and b) Group 1 – neutrophils and macrophages containing cocci, pleading for purulent inflammation; (c) Group 2 – numerous neutrophils and macrophages characteristic for purulent inflammation; (d) Group 3 – numerous monocytes and four mast cells.***

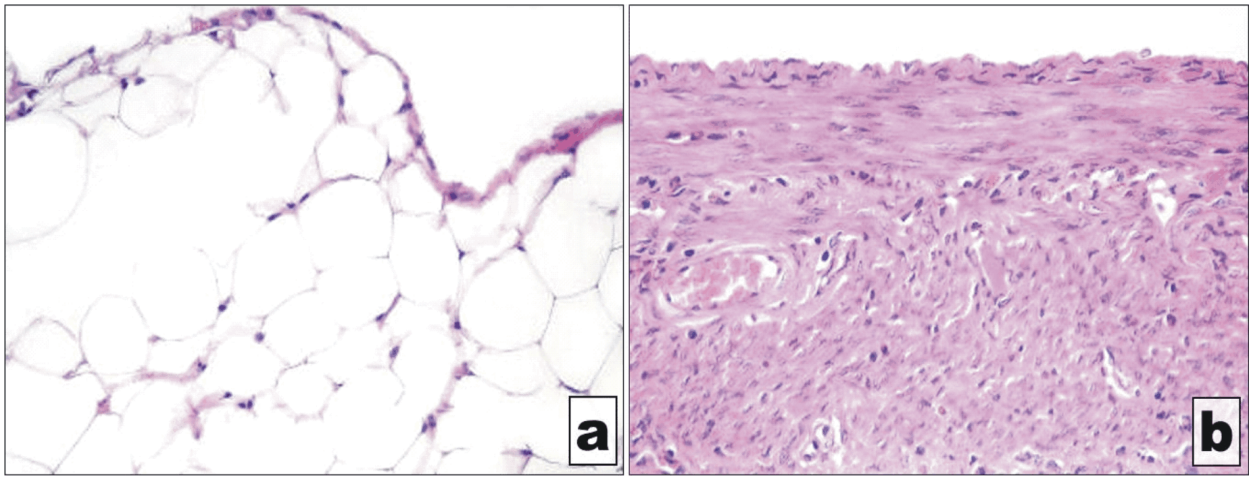

Figure 5 – ***Control group: (a) Epiploon, normal aspect, HE staining, ×200; (b) Uterine wall, serous layer with normal aspect, HE staining, ×400.***

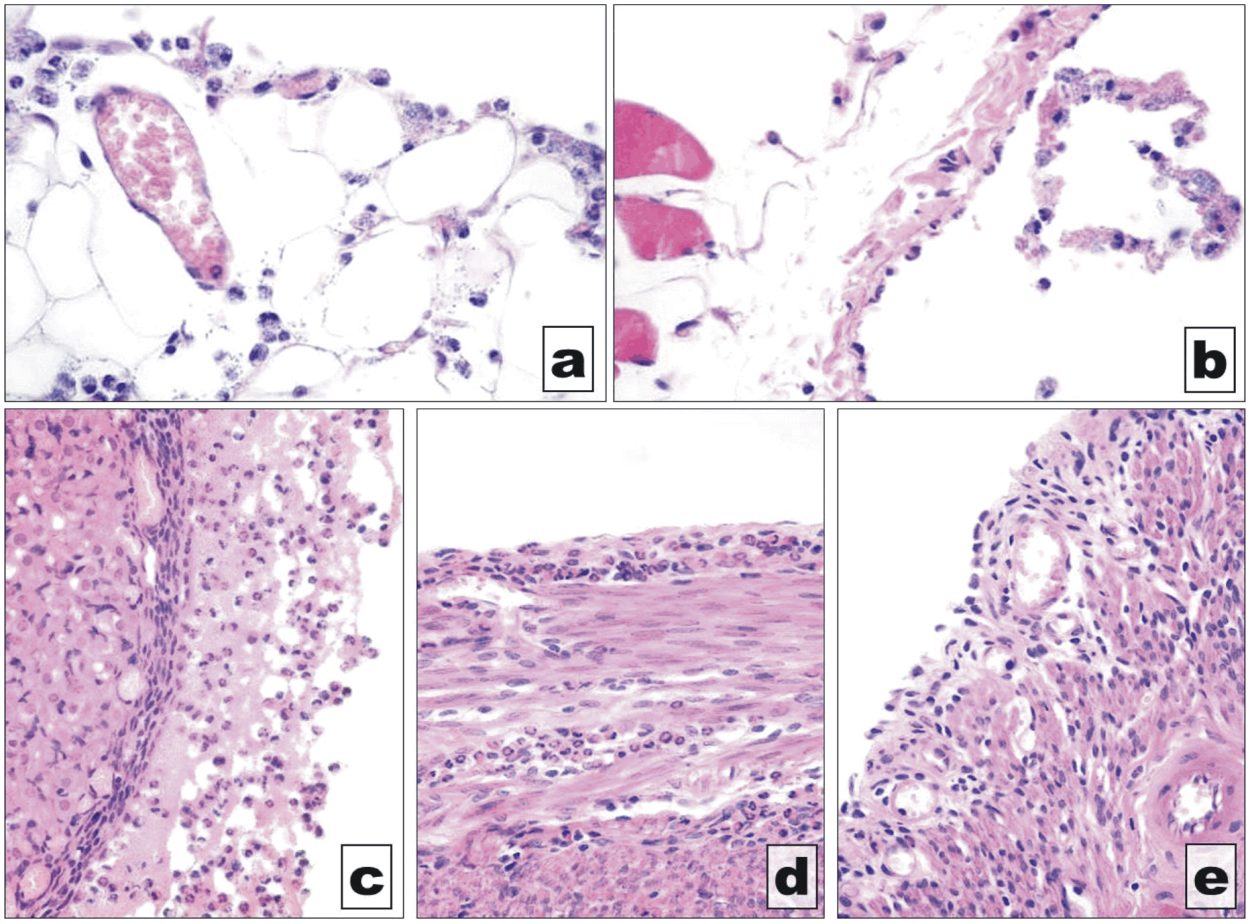

Figure 6 – ***Different histopathological aspects of peritoneal cavity structures in group 1, HE staining, ×400: (a) Epiploon: Mesothelial cells, neutrophils and macrophages containing bacteria, purulent inflammation; (b) Abdominal wall: Mesothelial cells, neutrophils and macrophages containing bacteria, purulent inflammation; (c) Ovary with purulent exudate on the surface; (d) Abdominal wall: Neutrophils and eosinophils infiltrated in the serous and muscular layers; (e) Uterine wall: Activated, hypertrophic mesothelial cells.***

In group 2, lesions were similar to those in group 1, but with a lower intensity. Congestion was much weaker, the number of bacteria free or contained within the cytoplasm of phagocytes was significantly reduced, sometimes very difficult to be identified. The inflammation was dominated by neutrophils and macrophages (Figure 7a).

In group 3, the inflammatory process was present only as small foci of multiform cellular infiltration (macrophages, neutrophils, lymphocytes, mast cells, eosinophils) beneath the peritoneal serous layer. Mesothelial cells still presented a diffuse activation feature (Figure 7, b and c).

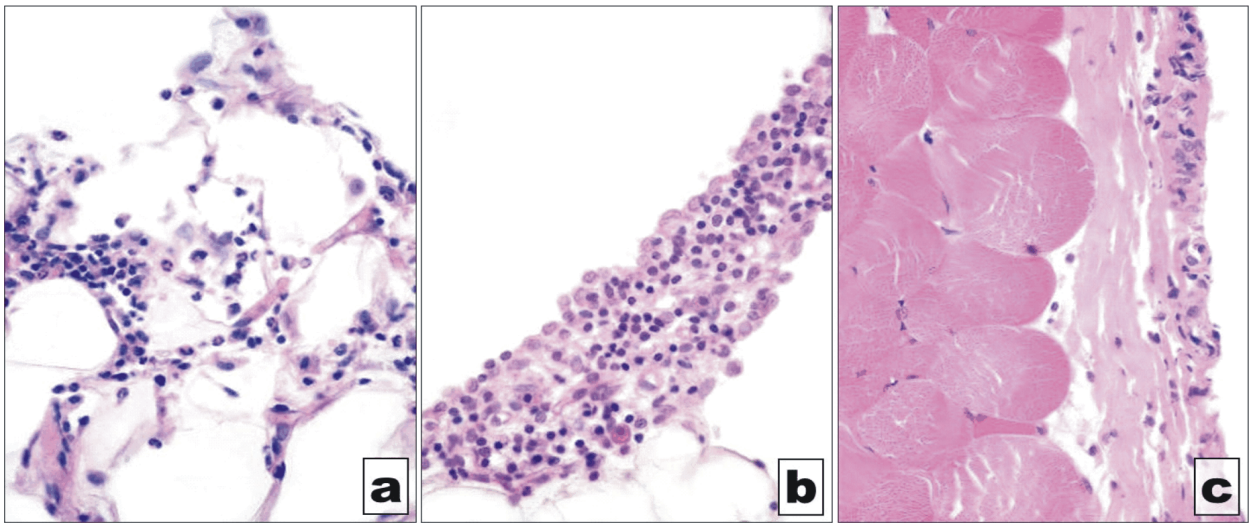

Figure 7 – ***Different histopathological aspects of peritoneal cavity structures in groups 2 and 3, HE staining, ×400: (a) Group 2: Epiploon – infiltrates with neutrophils and macrophages suggestive for purulent inflammation; (b) Group 3: Epiploon – rare infiltrates with mast cells, lymphocytes and eosinophils, activated mesothelium; (c) Abdominal wall: Rare foci with neutrophils under the serous layer.***

Discussion

There is no clear evidence until now regarding the pathophysiology of specific complications such as: extensive peritoneal adhesion syndrome, CSF pseudocysts, CSF ascites, viscus adhesion with secondary perforation or bowel obstruction through volvulus, occurring in the peritoneum of patients with hydrocephalus and ventriculo-peritoneal shunt, when the abdominal cavity is the site of shunt dysfunction [4, 8, 10]. Therefore, this study was designed to assess the local and general reactions after intraperitoneal administration of sterile CSF and the local inflammatory process using macroscopic cytological and histopathological criteria in order to better understand the appearance of these phenomena.

The control group was necessary in order to follow the outcome of subjects that received intraperitoneal normal saline. The subjects in this group had a normal evolution after intraperitoneal administration of the fluid, with no mortality or local cytological or histopathological changes, confirming once again the impressive resorption power of the peritoneum.

CSF administration in large quantities led to the death of all subjects, probably because of the toxic effects of high doses. Gradually decreasing doses made subjects' survival possible and the dose of 0.5 mL sterile CSF intraperitoneally administered was compatible with survival, confirming the presence of the peritoneum tolerance for CSF.

Subjects were divided into three groups in order to identify dynamic changes in peritoneum. Preliminary conditions, including dose of CSF were the same, the only distinguishing element being the time elapsed until subjects were euthanized: 24 hours, 48 hours, and 72 hours respectively.

In group 1, macroscopic examination revealed a pronounced congestion of the parietal and visceral peritoneum, confirming that the CSF is a chemical which can cause local reaction with possible general consequences.

Cytological examination of the intraperitoneal fluid showed polymorphonuclear cells, free bacteria and mononuclear phagocytic cells, suggesting a process of early peritonitis. Bacterial seeding occurred by chance, given the sterile administration of CSF, through translocation of intestinal bacteria amid the initial chemical peritonitis.

Aforementioned elements were confirmed by histopathological examination, which showed the presence of an inflammatory process involving both parietal and visceral peritoneum, with areas of desquamation and necrosis, dispersed throughout the peritoneal cavity.

The conclusion at this point of the experiment was that intraperitoneal administration of foreign bodies, such as the CSF, could rapidly induce non-specific reactions due to changes of local biochemical microenvironment, with fatal secondary infections.

In group 2, necropsy confirmed, from the macroscopic point of view, the peritoneal, abdominal wall and viscera inflammation, which had similar features to those found in group 1.

Peritoneal fluid cytology, although partially overlapping the picture observed in the previous group found some new changes, consisting in the reducing of the free or phagocytized bacteria number, and the tendency to replace the polymorphonuclear type elements with macrophages. Given that the histopathological elements also were of lower intensity as compared to those found in the previous group, it could be concluded that the morphological picture is that of acute peritonitis in remission.

In the third group, morphologic investigation highlighted from microscopic point of view reduced cellularity and histopathological changes.

Data analysis in the short term, showed a very prompt inflammation, developed within 24 hours of the experiment and associated with elements as the infection determined by bacterial translocation from the bowel, meeting thus all the criteria of an acute purulent peritonitis.

This inflammatory response was secondary to the presence of CSF in the peritoneum.

The reduction almost complete after 72 hours of the above-mentioned phenomena, is the result of a very active immune response, characteristic of experimental animals.

In humans, although peritoneal opening is minimal and no abdominal viscera are injured during distal catheter placement, patients with ventriculoperitoneal shunt have dynamic ileus, with absent or reduced gastric and bowel movements, nausea and vomiting just after surgery. They do not tolerate full meals in the first days after operation. These symptoms usually disappear spontaneously over time, in the vast majority of cases. However, there were cases that developed acute persistent ileus after ventroculo-peritoneal shunt insertion, which imposed the shunt removal [11].

Corroborating data from the experiment with the reported outcome of the patients with hydrocephalus and ventriculoperitoneal shunt, we can suppose that peritoneal inflammation found in many patients shortly after surgery could be largely due to the mechanisms observed in our experiment. The consequences of the presence of CSF in the peritoneal cavity probably have a more complex and mixed pathogenesis, in which chronic inflammatory, immunologic, allergic and vascular elements are overlapping.

The presence of adherence peritoneal syndrome, found in patients, with no abdominal co-morbidities, may be the result of a low, prolonged, local inflammatory response of the peritoneum to CSF aggression, which proved to be a highly irritating factor for the peritoneum.

The peritoneal inflammation causes more or less extensive peritoneal adhesions, which can lead to various abdominal complications, such as CSF pseudocyst, viscus perforation, bowel obstruction, volvulus, and CSF ascites [8, 10, 12–14].

Infection, symptomatic or clinically silent peritonitis or low-grade sepsis are also incriminated in the pathophysiology of specific abdominal complications following ventriculoperitoneal shunt [4, 8, 15, 16]. The presence of an inflammatory process leads to an impaired peritoneal capacity of absorption [15–17].

The limit of this experiment is the impossibility of assessing the long-term outcome of specimens.

Conclusions

Intraperitoneal administration of sterile human CSF caused an inflammatory response in varying degrees and

with multivisceral locations. Doses greater than 2 mL of CSF intraperitoneally injected resulted in the death of the animals whereas the dose of 0.5 mL of CSF was consistent with animals' survival. The peritoneal response at 24 hours, ranging from congestion to purulent reaction, was intense and diffuse, but after 72 hours the inflammatory response became mild, focal and limited only to small areas. The remission of acute peritonitis started at 48 hours after intraperitoneal CSF injection. Acute, congestive and, sometimes, purulent chemical peritonitis frequently causes animal's death, through cumulative toxic effect of CSF. However, the subjects who have survived the acute moment showed remission of peritoneal and general phenomena. Data from this experiment suggest that the peritoneal inflammatory response to the inoculation of CSF is dose dependent, usually transitory but, if prolonged, it could explain specific abdominal complications occurred in the peritoneal cavity following ventriculoperitoneal shunt insertion.

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