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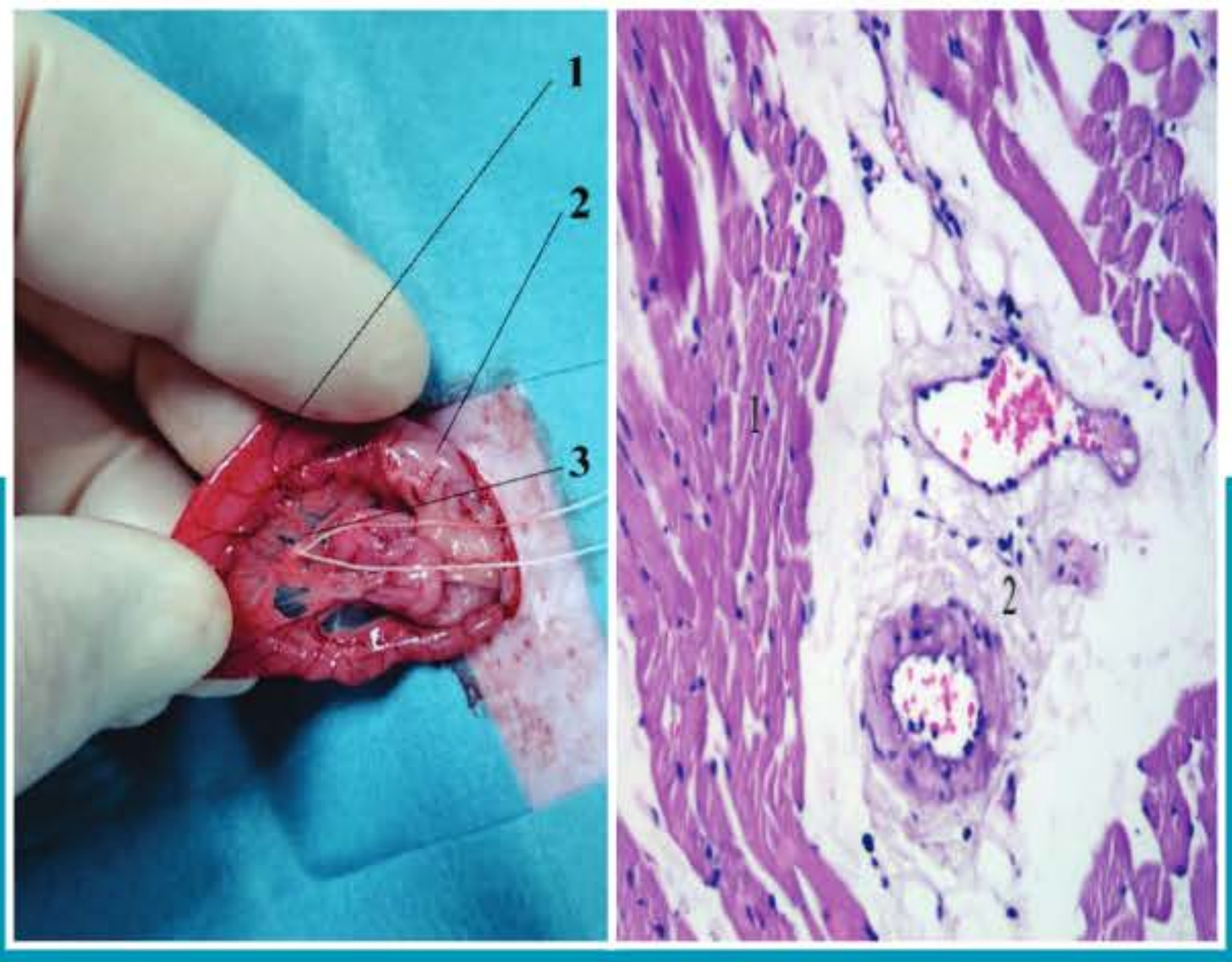

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ORIGINAL PAPER

CHARACTERISTICS OF MORPHOLOGICAL CHANGES IN THE LUMBAR PART OF THE DIAPHRAGM IN CASES OF OBSTRUCTIVE JAUNDICE AND PNEUMOPERITONEUM

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Abstract

Introduction. Patients suffering from obstructive jaundice (OJ) represent a particularly challenging cohort.
The objective of the study was to examine the morphological features of the right and left cruses of diaphragm under conditions of obstructive jaundice and exposure to pneumoperitoneum (PP).
Materials and methods. The present study was conducted on a total of 75 rats, with an average age of six months, who were divided into five distinct groups. The animals were employed as a model for obstructive jaundice and pneumoperitoneum.
Results. Structural changes because of bilirubin intoxication were detected, such as oedema, dystrophic and degenerative lesions of the muscle fibers (MFs), and the presence of cellular infiltrates. The additional "high pressure" PP resulted in greater destruction, and after one hour, foci of cytolysis were observed. In the adjacent MFs, myofibril disintegration was present, and in other areas, variants of dystrophic changes were observed. The heterogeneity of fibres

Résumé

Caractéristiques des modifications morphologiques de la partie lombaire du diaphragme en cas d'ictère obstructif et de pneumopéritoine

Introduction. Les patients souffrant d'ictère obstructif représentent une cohorte particulièrement difficile.
L'objectif de l'étude a était d'examiner les caractéristiques morphologiques des croisillons droit et gauche du diaphragme dans des conditions d'ictère obstructif et d'exposition au pneumopéritoine (PP).
Matériel et méthodes. La présente étude a été menée sur un total de 75 rats, d'un âge moyen de six mois, qui ont été divisés en cinq groupes distincts. Les animaux ont été utilisés comme modèle pour l'ictère obstructif et le pneumopéritoine.
Résultats. Des modifications de la structure dues à l'intoxication à la bilirubine ont été détectées, qui se sont manifestées par un œdème, des lésions dystrophiques et dégénératives du CF et la présence d'infiltrations cellulaires. L'utilisation supplémentaire de PP

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was clearly visualised in transverse sections, revealing the presence of hypertrophied MFs alongside rounded atrophic MFs. Within a 2-hour time frame, the nuclei underwent hyperplasia, a rare occurrence. Significant oedema manifested proximally to the vessels, arterioles and small veins, which exhibited weak blood filling. The endothelium underwent exfoliation. In a later stage, along with previous changes, there were cellular debris, necrotic changes, loss of internal optical contrast, characteristic pattern and indistinct borders.

Conclusions. The results obtained demonstrate that OJ alone and in combination with PP causes morphological and structural changes in the diaphragmal cruses.

Keywords: diaphragm, jaundice, morphology, laparoscopy, intra-abdominal pressure.

List of abbreviations:
PP – pneumoperitoneum
OJ – obstructive jaundice
CO_2 – carbon dioxide
LS – laparoscopic surgery
ER – experimental research
MFs – muscle fibres

"haute pression" a entraîné une plus grande destruction et, après 1 heure, des foyers de cytolyse ont été observés. Dans les fibres musculaires adjacentes, une désintégration des myofibrilles était présente et dans d'autres zones, diverses variantes de modifications dystrophiques ont été observées. L'hétérogénéité des fibres a été clairement visualisée dans les sections transversales, révélant la présence de fibres musculaires hypertrophiés à côté de MF atrophiques arrondis. Dans un laps de temps de 2 heures, les noyaux ont subi une hyperplasie, un phénomène rare. Un œdème important s'est manifesté à proximité des vaisseaux, des artérioles et des petites veines, qui présentaient un faible remplissage sanguin. L'endothélium a subi une exfoliation. À un stade ultérieur, parallèlement aux changements précédents, on a observé des débris cellulaires, des changements nécrotiques, une perte de contraste optique interne, un motif caractéristique et des bordures indistinctes.

Conclusions. Les résultats obtenus démontrent que l'ictère obstructif seul et en combinaison avec le PP provoque des changements morphologiques et structurels dans les vaisseaux diaphragmatiques.

Mots-clés: diaphragme, ictère, morphologie, laparoscopie, pression intra-abdominale

Introduction

Jaundice, characterised by elevated total and direct bilirubin levels in the blood serum, in conjunction with impaired biliary outflow, may serve as an indicator of choledocholithiasis or potentially more serious diagnoses in patients experiencing pain and exhibiting characteristic symptoms[1-3]. The safety and outcomes of urgent laparoscopic cholecystectomy in patients with jaundice and suspected choledocholithiasis have been discussed in scientific papers[4,5]. However, the effects of this condition and the presence of pneumoperitoneum (PP) caused by carbon dioxide on the organs and systems of the body have not yet been studied.

Patients suffering from obstructive jaundice (OJ) are one of the most severe groups of patients with abdominal pathology, with the frequency of emergency surgery ranging from 2.6% to 23.7%[2, 5]. Currently, minimally invasive methods of treating patients with OJ have become a leading area in surgery[4,5]. Biliary surgery continues to exhibit a tendency towards multistage minimally invasive interventions, considering the severity of the condition of patients with OJ[5].

PP is essential for laparoscopic surgery, as it provides visibility and mobility in the workplace. However, PP alters abdominal homeostasis and may contribute to metabolic changes through mechanical and biochemical effects[6,7]. In the course of the development of laparoscopic surgery (LS), the effects of several gases have been investigated and used in this type of surgery, to reduce the incidence of local and systemic adverse effects. Helium and argon are classified as inert gases, characterised by their minimal solubility in biological matrices. Available evidence points to a heightened prevalence of subcutaneous emphysema and venous gas embolism following helium administration[8]. In contrast, carbon dioxide (CO_2) has 50 times the solubility of helium. Argon, like helium, is almost insoluble in blood, which suggests an association with a higher risk of embolism. In addition, it has been demonstrated to cause cardiac depression[6,9,10]. Carbon dioxide (CO_2) has become a staple in laparoscopy due to its cost-effectiveness, non-flammability and solubility, and rapid excretion via the lungs. However, recent studies have indicated potential adverse effects associated with the use of CO_2 during LS. The utilisation of this gas has been observed to induce metabolic, immune and structural modifications, which may have clinical implications[5,10].

It has been hypothesised that patients with and without OJ have differing tolerances to laparoscopic cholecystectomy. It is logical to hypothesise that patients without jaundice will have an easier postoperative period and shorter hospital stay. Jaundice

can cause renal and hepatic failure, which can have a negative effect on the intraoperative and postoperative periods. It has been reported that constant high CO_2 pressure during laparoscopic surgery can lead to ischaemic damage to many organs, and the longer the operation lasts, the more serious the injury[5,7,10].

The objective of the study was to examine the morphological features of the right and left cruses of diaphragm under conditions of obstructive jaundice and exposure to PP formed by CO_2 of different duration.

Materials and methods

This experimental study was carried out in the vivarium of the I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine. The study was performed in 75 healthy mature rats, aged 6 months and weighing 220±25 g. Throughout the entire period of the animal experiment, the provisions of the Law of Ukraine 'On the Protection of Animals from Cruelty', the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 18th of March 1986, and Council Directive 2010/63/EU were complied with.

In the course of the experimental research (ER), the animals were divided into five groups of 15 animals each. Group I, the intact group, was used to determine the morphological norm of the lumbar part of the diaphragm. Group II was modelled with OJ to determine changes in the structure in this pathology. Group III was modelled with OJ and on day 7, the PP was modelled for 1 hour. Group IV included animals who were modelled with OJ and on day 7, PP lasted 2 hours. Group V included OJ and PP for 3 hours.

All animal manipulations were conducted during the morning hours in an indoor environment, with temperature maintained at 20-22°C and relative humidity set at 60-80%. Surgical procedures were performed by the same experimenter, employing the same technique. The rats were not provided with food for a 12-hour period prior to anaesthesia, however, they had unrestricted access to water. Notably, two hours prior to the induction of anaesthesia, the water supply was withdrawn from the cage. Animals were anaesthetised by injection of 10 mg/kg Xylazine and 90 mg/kg Ketamine solutions, administered intramuscularly in separate syringes. They were then fixed in a supine position on a table. Prior to the surgical procedure, the anterior abdominal wall was shaved, and the surgical field was treated three times with 10% povidone-iodine solution and covered with sterile napkins. Subsequently, a midline laparotomy

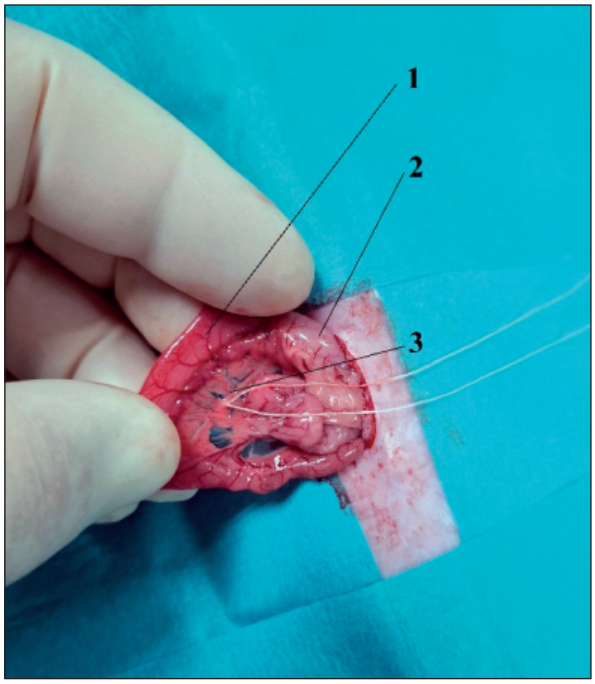

Figure 1. Modelling of obstructive jaundice:
1 – duodenum; 2 – stomach; 3 – ligated common bile duct.

approach was performed, and the stomach and duodenum were carefully removed through the gentle approach. The common bile duct was then visualised and tied with silk suture material (Fig. 1).

On the 7th day, repeated anaesthesia was performed with the above drugs and the formation of PP was performed according to the copyright certificate for the work no. 126409[12] (Fig.2). Following a seven-day observation period, the animals in the OJ group exhibited symptoms including yellowing of the ears, urine and sclera. Upon opening the abdominal cavity, the presence of jaundice in the adipose tissue, and slight characteristics of muscle tissue, along with dilatation of the common bile duct, were noted (Fig. 3). It is noteworthy that this period was not chosen by chance; according to the literature, a period of 5 to 7 days is sufficient for the severity of jaundice in rats and the occurrence of changes in organs and body systems, and is optimal for studying this pathology[13].

In accordance with the protocol, the animals were euthanised by intraperitoneal injection of 75 milligrams of Thiopental sodium per kilogram of body weight[14]. According to the group number, the rat diaphragm was taken in accordance with the copyright certificate for the work no. 126059 of 29th of April 2024. Only the lumbar part of the diaphragm, i.e., the right and left cruses of the diaphragm, was selected from the macro preparations and placed in a 10% solution of neutral formalin. Following fixation, the histological material was dehydrated using ethyl alcohols of

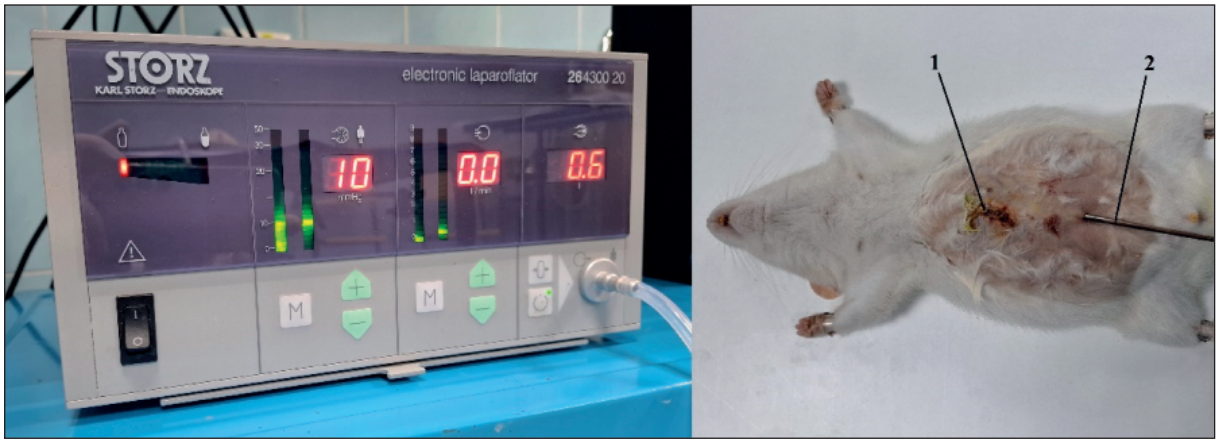

Figure 2. Simulated pneumoperitoneum of 10 mmHg: 1 – laparotomy wound; 2 – Veress needle.

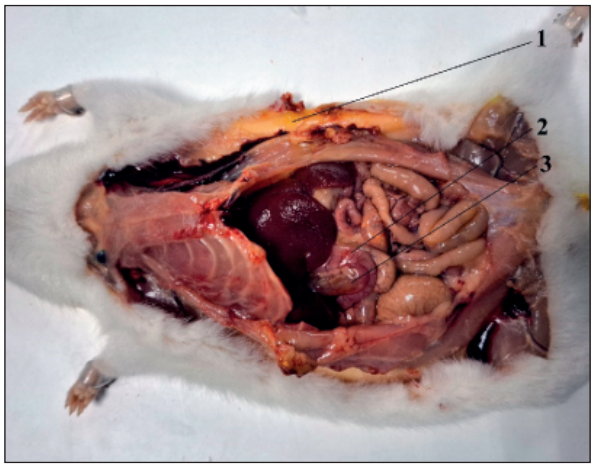

Figure 3. Seventh day after modelling obstructive jaundice: 1 – jaundice of subcutaneous fat; 2 – duodenum; 3 – dilated choledochus.

increasing concentration and embedded in paraffin. From each paraffin block, histological sections 5–7 μm thick were made using a microtome. Following deparaffinisation, staining was performed using hematoxylin and eosin, as well as Masson's stain.

Two rats from group II died on the 5th and 6th day after modelling OJ, from group III – 2 animals on the 2nd and 5th day, from group IV – 2 on the 5th and 6th day and from group V – 3 on the 6th day. OJ is a severe pathology that can trigger multiorgan failure, which can result in mortality. This was observed in the present experiment. Rats who died during the experiment were excluded from the study, and no new rats were added to replace them. All other rats were euthanised at the end of the experiment.

Results

In the present study, preparations obtained from intact animals were examined to determine the morphology of the diaphragm. The right and left cruses of the diaphragm were found to contain transversely striated muscle fibres (MFs), which formed a dense layer when viewed transversely. When viewed longitudinally, the striated fibres exhibited histological features that were typical of this tissue type. The striated fibres varied slightly in thickness, and the sarcoplasm was light in colour. The MFs varied slightly in thickness, the sarcoplasm had a light eosinophilic colour, and the presence of adipose tissue in the perimysium was noted. The elongated-oval nuclei are located beneath the sarcolemma, and the transverse striation is arranged in straight or arcuate stripes. However, visualisation is not always clear. The stroma is very thin and is represented by blood-filled capillaries and a small number of fibrocytes (Fig. 4).

The results obtained from the selected tissues in the group of animals with OJ demonstrated the presence of oedema. In addition, foci of dystrophic and degenerative changes in muscle fibres were detected. The thickness of OJ compared to the intact group was greater due to intracellular oedema. Cellular infiltrates were always present and their structure was dominated by lymphocytes (Fig. 5). Such structural changes are explained by the increasing bilirubin intoxication in the conditions of OJ.

In the subsequent cohort of animals, where, in addition to the modelling of OJ, a CO_2 PP was created for a duration of one hour, prominent foci of cytolysis were observed in the cruses of diaphragm. In the adjacent MFs, myofibril disintegration was evident. In other areas, various variants of dystrophic changes, intracellular oedema, and increased interfibrillar spaces were observed. Dense cellular infiltrates were observed within the endomysium and perimysium, featuring the presence of erythrocytes and plasmacytes. The heterogeneity of fibres was clearly visualised in the transverse sections, with the presence of hypertrophied MFs alongside rounded atrophic MFs. Arterioles

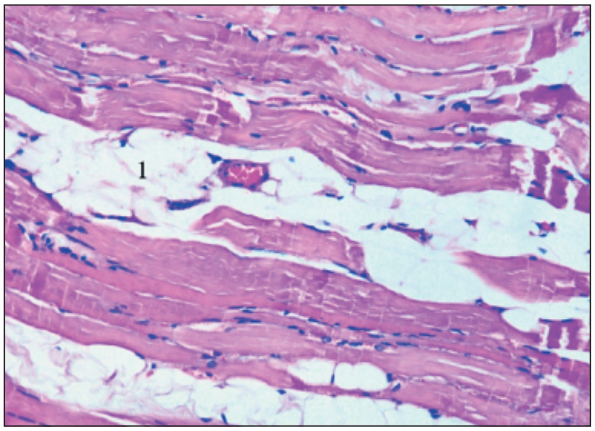

Figure 4. Transverse section of the cruses of diaphragm of an intact rat: 1 – adipose tissue in the perimysium. Hematoxylin and eosin staining. × 200.

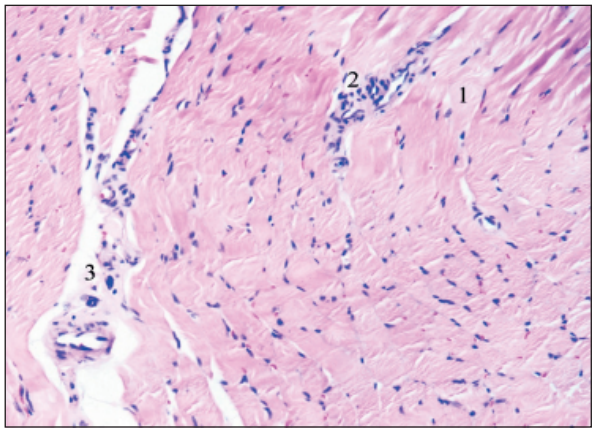

Figure 5. Histological structure of the muscle of cruse of diaphragm of rats with modelled jaundice: 1 – disintegration of muscle fibre sarcoplasm; 2 – cellular infiltrates; 3 – distinct stromal edema. Hematoxylin and eosin staining. × 100.

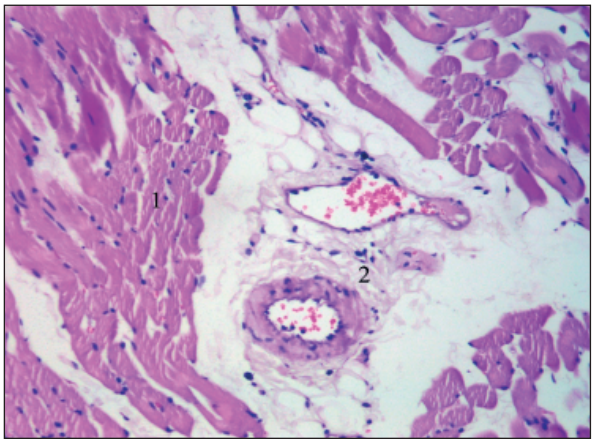

Figure 6. Histological structure of the muscle of cruse of diaphragm of a rat with modelled jaundice after one hour of PP: 1 – disintegration of sarcoplasm and sarcolemma; 2 – oedema of the perimysium, fatty infiltration and increased collagen formation around the vessels. Hematoxylin and eosin staining. ×100.

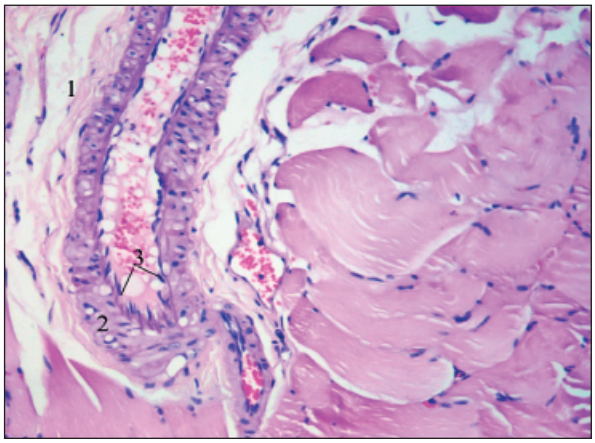

Figure 7. Histological structure of the muscle of cruse of diaphragm of a rat with modelled jaundice after two hours of PP: 1 – perivascular oedema; 2 – vascular wall oedema; 3 – endothelial exfoliation. Hematoxylin and eosin staining. ×400.

and venules contained a minimal amount of blood, and the endothelium was partially exfoliated. The arteriolar walls exhibited signs of oedema, resulting in the disruption of their normal structure. Perivascular oedema is expressed to a significant degree (Fig. 6).

In the group of animals in whom PP was created for a period of two hours, small cell infiltrates in the muscles of the cruses of the diaphragm were observed, and these were characteristic of the lesions in the MF. Hypertrophied MFs with nucleus hyperplasia were rare, and significant oedema associated with fatty infiltration was observed in the area near the vessels. The arterioles and small veins were poorly filled with blood, and the endothelium was exfoliated over a large area, resulting in plasmorrhages both in the arteriolar wall and perivascularly (Fig. 7).

The examination of the next term of PP revealed the presence of cellular debris and necrotic changes in degenerating MFs, as indicated by the loss of internal optical contrast, characteristic pattern and indistinct borders. The spatial location of the lesions was found to be in larger volumes compared to previous observations, and at the same time, they were combined with proliferation of the endomysial connective tissue and mild regenerative processes in the form of fibre hypertrophy and hyperplasia of their nuclei (Fig. 8, 9).

Discussion

It is known that jaundice affects the biliary tree, hepatocytes and induces a systemic inflammatory response, potentially leading to systemic complications

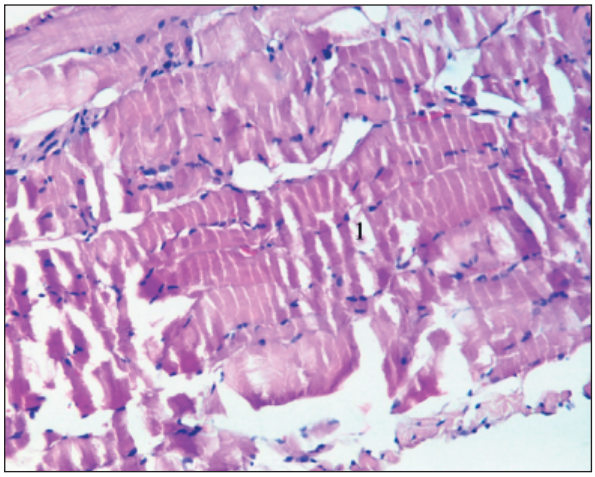

Figure 8. Fragment of the muscle of cruse of diaphragm of a rat with modelled jaundice after 3 hours of PP: 1 – breakdown of fibres into sarcomeres and their cytolysis. Hematoxylin and eosin staining. ×200.

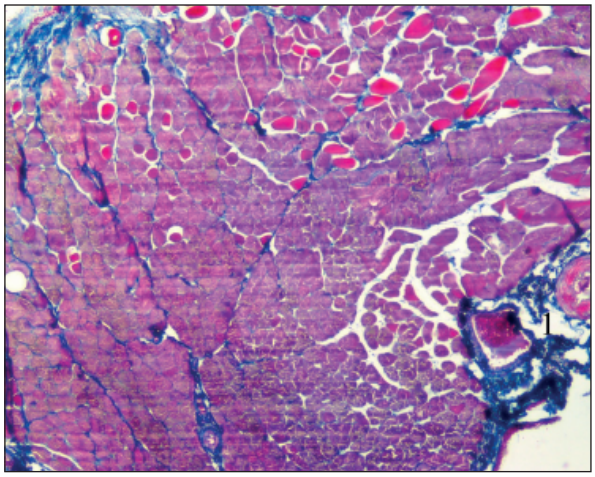

Figure 9. Histological structure of the crus of diaphragm V of the experimental group: 1 – fibrosis of the endomysium and perimysium (blue). Masson's stain. ×200.

and increased postoperative morbidity[15]. The impact of PP on early laparoscopic cholecystectomy and biliary interventions in patients with acute jaundice is hardly described in the literature. In this study, the results are presented with the aim of understanding the pathological effects of OJ and PP of the so-called "high pressure", which is most often used in LS and is commonly referred to as having a pressure of 12-15 mmHg. It is documented in the literature that a pressure of 15 mmHg in the human body causes similar systemic changes in the rat body at a pressure of 10 mmHg[16,17]. From an experimental research perspective, rats are ideal animals and economically viable.

The results of the study are unexpected and are of great interest. We might have expected the group of jaundiced animals to have dystrophic changes in the diaphragm, as it causes similar changes in other abdominal organs[2,18]. However, it was not possible to predict that the presence of high-pressure PP would deepen the observed histological changes, and it was logical to hypothesise that these changes would be more pronounced the longer the PP remained. This was indeed observed in the present study. It would be interesting to know how the presence of OJ and "low pressure" PP affects the structure of the diaphragm. Recent studies in this area demonstrate that PP at the level of 7 mmHg in the LS exerts a significantly lesser effect on the organs and systems of the human body and the postoperative period in general[16,17], raising many questions that need to be studied.

A review of the literature on the effects of PP and OJ on the respiratory muscle reveals that the present study is the first to address this topic. The majority of studies are based on the effects of intra-abdominal pressure on the body as a whole during LS. However, there have been several reports related to the adverse effects of CO_2 PP on the diaphragm after laparoscopic surgery, especially the occurrence of the so-called phrenicus syndrome[19,20]. It was also observed that during PP, the diaphragm shifts upwards, reducing lung compliance, thereby reducing the respiratory and pleural cavity volumes and increasing the maximum airway pressure. In an experiment conducted on rabbits, which simulated chronic elevated intra-abdominal pressure that caused changes in the diaphragm, an adaptation of this respiratory muscle equivalent to intense contraction was observed, which led to changes in the diaphragm to make it functionally more efficient in relation to the workload, but made it vulnerable to oxidative stress[20].

Conclusions

The results obtained demonstrate that OJ, both in isolation and in combination with PP, induces morphological and structural alterations in the cruses of diaphragm, the manifestation of which is contingent on the duration of these pathological factors. OJ has been shown to induce oedema, dystrophic and degenerative changes in the MF, and the presence of cellular infiltrates, primarily in the form of lymphocytes. The addition of PP resulted in the appearance of cytolysis foci, presence of erythrocytes and plasma cells within the cellular infiltrates, and development of perivascular oedema and endothelial exfoliation. After 3 hours of PP, cellular debris, necrotic changes in degenerating MFs, loss of characteristic pattern and indistinct borders were observed.

Authors' Contributions:

Conceptualization, methodology, design of the work M.K., wrote the manuscript, analysis M.K., T.G., project administration, draft preparation O.Ya., M.I., interpretation of data for the work, editing, validation O.S., O.K.

All authors have read and agreed to the published version of the manuscript.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law."

"All institutional and national guidelines for the care and use of laboratory animals were followed"

"No funding for this study"

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None

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