ORIGINAL PAPERS

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Histological Investigation of the Effects of Tenoxicam on Pulmonary Complications of Pneumoperitoneum – Tenoxicam Reduces Lung Injuries Caused by Pneumoperitoneum

Badanie histologiczne wpływu tenoksykamu na powikłania płucne odmy otrzewnowej – tenoksykam zmniejsza urazy płuc spowodowane przez odmę otrzewnową

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Abstract

Objectives. Pneumoperitoneum increases intra-abdominal pressure and generates oxidative stress, which mediates tissue injury. One of the causes of oxidative stress production is an inflammatory reaction. Taking this into consideration, the current animal study was designed, using tenoxicam before a laparoscopy procedure in order to ascertain whether tenoxicam can prevent lung injury caused by pneumoperitoenum.
Material and Methods. Fourteen female Wistar rats were randomly divided into two groups: the tenoxicam group (seven rats) and the control group (seven rats). The tenoxicam group was given two doses (totalling 0.5 mg/kg) of intraperitoneal tenoxicam, and the control group was given 0.5 cc of 0.9% NaCl, 12 hours and ½ hour before the operation. Under intra-peritoneal anesthesia, a Veress needle was placed in the peritoneal cavity and a 15 mm-Hg pneumoperitoneum was established and maintained for 20 minutes; the peritoneal gas was then desufflated. The lungs were resected at the 180th minute from the beginning of the operation and were evaluated histopathologically. Histopathological evaluations including intra-alveolar hemorrhage, alveolar edema, congestion and leukocyte infiltration were carried out for both groups.
Results. A statistical comparison of the evaluation scores revealed significant differences between the two groups for intra-alveolar hemorrhage (p = 0.007), alveolar edema (p = 0.023) and congestion (p = 0.005) and a non-significant difference for leukocyte infiltration (p = 0.114).
Conclusions. Pneumoperitoneum causes injuries to lung tissue; tenoxicam reduces that damage and protects the lungs by decreasing intra-alveolar hemorrhage, alveolar edema and congestion (**Adv Clin Exp Med 2011, 20, 4, 503–507**).

Key words: pneumoperitoneum, lung injury, tenoxicam, rat.

Streszczenie

Cel pracy. Odma otrzewnowa zwiększa ciśnienie w jamie brzusznej i wywołuje stres oksydacyjny, który pośredniczy w uszkodzeniu tkanek. Jedną z przyczyn stresu oksydacyjnego jest reakcja zapalna. Biorąc to pod uwagę, opisane badanie na modelu zwierzęcym z użyciem tenoksykamu przed laparoskopią zostało zaprojektowane w celu ustalenia, czy tenoksykam może zapobiec uszkodzeniom płuc spowodowanym przez odmę otrzewnową.
Materiał i metody. Czternaście samic szczurów Wistar podzielono losowo na dwie grupy: grupę z tenoksykamem (7 szczurów) i grupę kontrolną (7 szczurów). Grupa z tenoksykamem otrzymała 2 dawki tenoksykamu (w sumie 0,5 mg/kg) dootrzewnowo, a grupa kontrolna 0,5 ml 0,9% NaCl 12,5 godz. przed operacją. W znieczuleniu jamy

otrzewnej umieszczono igłę Veressa w jamie otrzewnej, wywołano 15 mm Hg odmę otrzewnową i utrzymywano przez 20 min; następnie gaz został wypompowany z otrzewnej. W 180. minucie operacji wycięto płuca i badano histopatologicznie. Przeprowadzono ocenę histopatologiczną pod względem krwawienia wewnątrzpęcherzykowego, obrzęku pęcherzyków płucnych, przekrwienia i infiltracji leukocytów.

Wyniki. Statystyczne porównanie wykazało znaczące różnice między grupami pod względem krwotoku wewnątrz pęcherzyków płucnych (p = 0,007), obrzęku pęcherzyków płucnych (p = 0,023) i przekrwienia (p = 0,005). Nie wykazano istotnej różnicy w infiltracji leukocytów (p = 0,114).

Wnioski. Odma otrzewnowa powoduje urazy tkanki płucnej; tenoksykam zmniejsza uszkodzenia i chroni płuca przez zmniejszenie krwotoku wewnątrz pęcherzyków płucnych, obrzęku i przekrwienia (**Adv Clin Exp Med 2011, 20, 4, 503–507**).

Słowa kluczowe: odma otrzewnowa, uszkodzenia płuc, tenoksykam, szczur.

Laparoscopy was introduced in late 1980s as a minimally invasive form of surgery and became the method of choice for some diagnostic and therapeutic procedures, since it is associated with smaller skin incisions, less pain, shorter hospitalization and faster recovery times as compared with open surgery [1, 2]. However, some postoperative morbidity has been noted in patients undergoing laparoscopy, and various studies published in last decade have noted complications associated with the pneumoperitoneum that is used to improve visibility during laparoscopy [3–8]. It has been reported that pneumoperitoneum has hazardous effects on pulmonary function, cardiovascular hemodynamics, hepatic perfusion and acid-base balance [3].

Pneumoperitoneum is insufflation of a gas – typically carbon dioxide (CO_2) – into the peritoneal cavity [3]. The insufflation of gas results in increased intra-abdominal pressure and produces oxidative stress, which mediates tissue injury [3, 8]. The most likely causes of oxidative stress as a consequence of CO_2 pneumoperitoneum are an inflammatory reaction due to tissue trauma, ischemia/reperfusion due to changes in abdominal pressure, and diaphragmatic dysfunction [3].

The present study was designed on the basis of the assumption that an inflammatory reaction is the cause of tissue injury during pneumoperitoneum. The authors used a non-steroidal anti-inflammatory agent preoperatively and investigated the complications of surgery on the histological level in a laparoscopic animal model. What is new and different about the current study is histological investigation; most published studies have examined only the biochemical parameters of oxidative stress caused by pneumoperitoneum.

Material and Methods

Fourteen female Wistar rats weighing 220–240 g were included in the study. They were randomly divided into two groups: the tenoxicam group (n = 7) and the control group (n = 7). The tenoxicam group was given two doses (totalling 0.5 mg/kg) of intraperitoneal tenoxicam (Oksamen, Mustafa Nevzat İlaç Sanayii AŞ, İstanbul, Turkey), 12 hours and 1/2 hour before the operation, and the control group was given 0.5 cc of 0.9% NaCl at the same time intervals.

The animals underwent anesthesia with 50 mg/kg of ketamine hydrochloride (Alfamine, Alfason Woerden, Holland) and 5 mg/kg of xylazine hydrochloride (Alfazyne, Alfason Woerden, Holland). The anesthetics were administered by the intraperitoneal route. The animals were placed in a supine position. A Veress needle was placed into the peritoneal cavity and a 15 mm-Hg pneumoperitoneum was established with a CO_2 insufflator. The pneumoperitoneum was maintained for 20 minutes, and then the peritoneal gas was desufflated. After that reperfusion period, the lungs of the animals were removed through a median sternotomy at the 180th minute from the beginning of the operation.

The excised specimens were put in a 10% neutral buffered formalin solution and embedded in paraffin wax for histological examination. One pathologist blindly evaluated the tissues after they were stained with hemotoxylin-eosin and trichrome stains.

Each specimen was evaluated for histopathological changes, including intra-alveolar hemorrhage, alveolar edema, congestion and leukocyte infiltration. Intra-alveolar hemorrhage, alveolar edema and congestion were scored on a scale from 0 to 3 where 0 = absence of pathology (< 5% of maximum pathology), 1 = mild (<10%), 2 = moderate (15–20%) and 3 = severe (20–25%) [9]. Each section was divided into 10 subsections, and leukocytic infiltration was examined in each of the subsections at a magnification of 400; the following scale was used for the evaluation: 0 = no extravascular leukocytes; 1 = fewer than 10 leukocytes; 2 = 10–45 leukocytes; 3 = more than 45 leukocytes. An average of the numbers was used for the comparison [9, 10].

The statistical analysis of the group comparison was carried out with the Mann-Whitney U-test. The value of $p < 0.05$ was considered significant.

The study was reviewed and approved by the Ethics Committee for Animal Studies at the Medical Faculty of Düzce University, Turkey. All the animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals [11].

Results

Table 1 presents the scores from the histopathological evaluation, including intra-alveolar hemorrhage, alveolar edema, congestion and leukocyte infiltration in both groups.

The statistical comparison of the scores in both groups revealed significant differences for intra-alveolar hemorrhage ($p = 0.007$, $p < 0.05$), alveolar edema ($p = 0.023$, $p < 0.05$), and congestion ($p = 0.005$, $p < 0.05$). However, the scores for leukocyte infiltration ($p = 0.114$, $p > 0.05$) did not differ significantly (Figs 1 and 2).

Discussion

Pneumoperitoneum is insufflation of a gas into the peritoneal cavity to provide good exposure of the abdominal organs during laparoscopic surgery. It is also used in thoracic surgery to elevate the diaphragm in order to prevent prolonged air leaks after lower lung resections. CO_2 is the most commonly used gas for pneumoperitoneum in laparoscopy because it is low cost, nonflammable and has a presumed lower risk of gas embolism [4, 12, 13].

During laparoscopy, intra-abdominal pressure as high as 8–20 mm-Hg is established and maintained [4]. Even at 10 mm-Hg, increased intra-abdominal pressure considerably decreases splanchnic blood flow [4, 6]. At the end of the surgery, the desufflation of the gas reduces the intra-abdominal pressure and increases splanchnic blood flow [4]. These decreases and increases in splanchnic blood flow represent an ischemia/reperfusion model, which produces oxygen-derived free radicals and oxidative stress [3, 5]. Oxidative stress induced by free radicals is believed to mediate tissue injury [14]. Free radicals can oxidize all macromolecular cell constituents, such as the lipids of membranes, proteins and DNA [3].

Increased intra-abdominal pressure elevates the diaphragm and increases intra-thoracic pressure. In addition, arterial CO_2 is increased during CO_2 pneumoperitoneum [5]. Increased intra-thoracic pressure and increased intra-arterial CO2 affect the pulmonary ventilation/perfusion ratio

Table 1. Average scores from the histopathological examination

Tabela 1. Średnie wyniki badania histopatologicznego

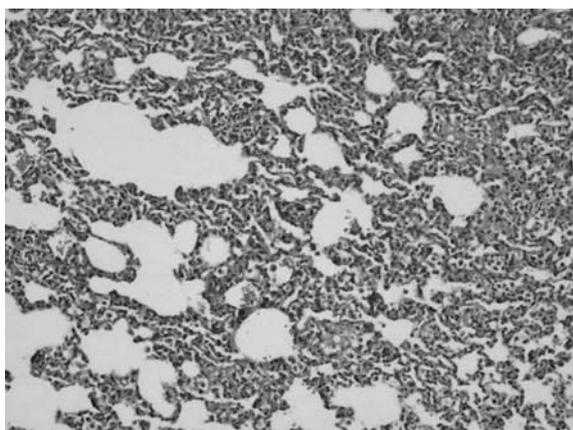| Group (Grupa)	Intra-alveolar hemorrhage (Krwotok wewnątrz-pęcherzykowy)	Alveolar edema (Obrzęk pęcherzyków płucnych)	Congestion (Przekrwienie)	Leukocyte infiltration (Infiltracja leukocytów)
Control 1	1	0	3	2
Control 2	1	1	3	3
Control 3	1	0	2	3
Control 4	1	0	2	2
Control 5	1	1	3	3
Control 6	1	1	3	2
Control 7	1	1	3	2
Tenoxicam 1	0	0	2	1
Tenoxicam 2	0	0	2	2
Tenoxicam 3	1	0	1	2
Tenoxicam 4	0	0	1	1
Tenoxicam 5	0	0	2	3
Tenoxicam 6	1	0	2	2
Tenoxicam 7	0	0	1	2

Fig. 1. Photomicrograph from the control group displaying marked intra-alveolar hemorrhage, alveolar edema, congestion and leukocyte infiltration (HE ×100)

Ryc. 1. Mikrofotografia wycinka z grupy kontrolnej ukazująca krwotok wewnątrz pęcherzyków płucnych, obrzęk pęcherzyków płucnych, przekrwienie i infiltrację leukocytów (HE 100×)

Fig. 2. Photomicrograph from the tenoxicam group displaying less intra-alveolar hemorrhage, alveolar edema and congestion compared with control group (HE ×100)

Ryc. 2. Mikrofotografia wycinka z grupy z tenoksykamem ukazująca mniejszy krwotok wewnątrz pęcherzyków płucnych, obrzęk pęcherzyków płucnych i przekrwienie niż w grupie kontrolnej (HE 100 ×)

(normally 5 liters per minute/4 liters per minute = 0.8), which impairs the efficiency of the lungs resaturating venous blood with oxygen [5]. Furthermore, an elevated diaphragm and/or the laparoscopic surgery itself may cause direct tissue trauma.

Several suggestions for preventing pneumoperitoneum-induced tissue injury can be found in the literature. Ypsilantis et al. used mesna (2-mercaptoethane-sulfonate) – "a thiol used in chemotherapy regimens for the prevention of hemorrhagic cystitis induced by the oxazaphosporines cyclophosphamide and ifosphamide" – to protect splanchnic organs from pneumoperitoneum-induced oxidative stress [8]. Pross et al suggested that patients' nutritional status should be considered, with special attention to their intake of vitamins and other antioxidants [3]. Uzunkoy et al. suggested using isothermic CO_2 for pneumoperitoneum, since their findings indicated that it has fewer negative effects than hypothermic CO_2 on respiratory function [15]. Ischemic preconditioning, establishment of low intra-abdominal pressure, insufflation with helium, pre-treatment with erythropoietin and usage of melatonin as an antioxidant are other strategies that have been suggested [8].

The current study was based on the assumption that inflammatory reactions are the most likely cause of oxidative stress as a consequence of
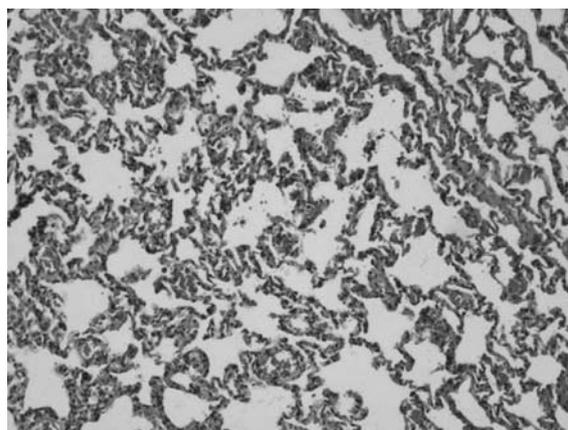
CO_2 pneumoperitoneum, and tested the results of using a non-steroidal anti-inflammatory drug in a rat model. As far as the authors are aware, such an experiment had not previously been carried out. Another distinction of the present study was the investigation of the early results on the histological level instead of only the biochemical parameters. Using a histopathological injury search model used by some previous authors [9, 10] changes in intra-alveolar hemorrhage, alveolar edema, congestion and leukocyte infiltration were examined by microscopy. The results for the rats in the tenoxicam group for intra-alveolar hemorrhage, alveolar edema and congestion were better than in the control group. However, no significant difference was found in the leukocyte infiltrations of the two groups. Leukocyte infiltrations could be observed 6 to 24 hours after the procedures in some other injury studies [9]. The present study could probably have evaluated leukocyte infiltration better if the experiment had been continued 24 hours after the pneumoperitoneum was established; this was a limitation of the study.

In conclusion – whatever the cause – pneumoperitoneum leads to lung tissue injury, and tenoxicam reduces that injury and protects the lungs against it. However, further experiments continuing for more than 24 hours are needed.

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