The effects of dexmedetomidine on oxidant - antioxidant systems in the experimental model of carbon dioxide pneumoperitoneum

[DeneySEL karbondioksit pnömoperitoryum modelinde deksametomidininın oksidan-antioksidan sistem üzerine etkisinin araştırılması]

ABSTRACT

Objective: The aim of the study was to investigate the changes of oxidative and anti-oxidative systems in the splanchnic area during carbon dioxide pneumoperitoneum and to determine whether the administration of dexmedetomidine has effects on these systems.

Methods: Forty rats were randomized into four groups: Group I; Control, Group II; No pneumoperitoneum, Dexmedetomidine administration, Group III; Pneumoperitoneum, no Dexmedetomidine administration and Group IV; Pneumoperitoneum and Dexmedetomidine administration 30 minutes before insufflation. The rats were rested 30 minutes after desufflation and blood samples were obtained for; ischaemia modified albumin (IMA), myeloperoxidase (MPO), advanced oxidation protein products (AOPP), catalase (CAT), paraoxonase (PON1) and platelet-activating factor acetylhydrolase (PAF-AH) analyses.

Results: When compared with the control group; the serum IMA levels significantly decreased in group II, and also increased in group III as compared to control (p<0.05). IMA levels were also significantly decreased in both groups II and IV as compared to group III (p<0.001). Serum MPO activity increased in group III as compared to control (p<0.05). Serum AOPP levels were significantly increased in group III as compared to group II (p<0.01) and decreased in group IV as compared to group III (p<0.01). Serum CAT activity was higher in group II than controls (p<0.05). Serum PON and plasma PAF-AH activities significantly decreased in group III as compared to group II (p<0.05) and plasma PAF-AH activity were decreased in group III as compared to controls (p<0.05).

Conclusion: In conclusion, administration of dexmedetomidine; prior to ischemia reperfusion injury caused by pneumoperitoneum; reduces the oxidative injury and increases the antioxidant activity in the acute period.

Key Words: Dexmedetomidine, ischemia-reperfusion injury, oxidant-antioxidant system, pneumoperitoneum.

Conflict of Interest: The authors declare no conflict of interest.
Introduction
The insufflation of the abdomen with carbon dioxide to provide the required space for laparoscopic surgery is named as carbon dioxide pneumoperitoneum (CO₂ Pp). The periods of insufflation and desufflation constitutes a typical ischemia reperfusion injury (I/R injury) in the splanchnic area during this procedure. Today, laparoscopic surgery has become the gold standard for many procedures over the past decade but it is also accompanied by I/R injury as a result of CO₂ Pp. Hypoperfusion of the splanchnic area and the I/R injury, precipitate to release free radicals. It is known that according to injury of these radicals to the cell membrane, tissue integrity is damaged and the antioxidant capacity of the organism is reduced [1-3]. The laparoscopic procedures with possible I/R injury and major abdominal surgery especially accompanying sepsis, serious trauma are very important acute inflammation cascades and they require meticulous management of anesthesia [4].

It is known that some type of anesthetics have protective potency from I/R injury [5]. Dexmedetomidine (DEX) is an adjuvant anesthetic drug that has sedative, hypnotic and analgesic potency. DEX can be used as an adjuvant anesthetic drug and some experimental and clinical studies demonstrated that DEX has protective effects against injury in the oxidative stress since it attenuates the inflammatory response [6-11]. There are various biochemical markers that indicative of oxidative stress and antioxidant defense system and which were also used to investigate the effects of DEX against I/R injury [12-16].

Ischaemia modified albumin (IMA) is the metabolic variant of albumin. The N-terminal amine group of human albumin is the metal binding part of the protein that binds ions like cobalt, nikel, and copper in plasma. The superoxide anions and the other reactive oxygen molecules which appears during acidosis, ischemic and oxidative reactions make changes on the structure of N-terminal and finally a re-constituted metabolic variant of albumin takes place in plasma [9,17]. Myeloperoxidase (MPO) is a lysozome enzyme that appears as a response to oxidative stress [18]. Advanced oxidation protein products (AOPP) occurs as a result of aggregation and fragmentation of proteins due to reactive oxygen radicals. AOPP is a new marker that shows the effect of oxidative stress on proteins in inflammation and widely used in many studies as an indicator of I/R injury [18,19]. Toxic oxygen radicals and its derivates which appear during I/R injury are cleared by free radical cleaner such as catalase (CAT) that is an antioxidant, enzymatic defender already exists in the metabolism naturally [20,21]. Paraoxonase (PON1) is an ester hydrolase glycoprotein that has arylesterase, paraoxonase and lactonase activity [22,23]. Platelet-activating factor acetylhydrolase (PAF-AH) is a pro-inflammatory molecule which has anti-oxidant and anti-inflammatory properties that protects metabolism by hydrolysing platelet activating factor (PAF) and oxidated phospholipids. According to other routine markers PAF-AH is relatively new biochemical marker [24].

The main hypothesis of the present experimental study is based on whether DEX, an adjuvant anesthetic drug, administration has a positive impact on I/R injury in CO₂ Pp model in rats using the biochemical markers such as: IMA, MPO, AOPP and antioxidant enzymes; CAT, PON1 and PAF-AH.

Materials and Methods

Animals
This experimental study was approved by Ondokuzmayıs University Animal Care and Ethics Committee (2013/06). Female Sprague–Dawley rats (n: 40, weight: 200–250g) were randomized into four groups. There was no mortality in the study animals and all rats were stable throughout the perioperative period. Rats were anesthetized with ketamine hydrochloride (i.m. 60 mg/kg) and xylazine (i.m. 10 mg/kg) and anesthesia was maintained with xylazine (i.m.10 mg/kg per hour). Group I; Control group, Group II; No CO₂ Pp, DEX administrated in dose 100 µg/kg (Precedex 200µg/2ml, Hospira Inc, USA) i.p., Group III; CO₂ Pp with intra abdominal pressure of 12 mmHg for 60 minutes, no DEX administration and Group IV; CO₂ Pp with intra abdominal pressure of 12 mmHg for 60 minutes and DEX administrated in dose 100 µg/kg i.p. 30 minutes before insufflation of CO₂ Pp. In Group I and III, an equal volume of serum physiologic with Dexametomidine, was administered i.p. The rats were placed in supine position with their extremities to the operating table. CO₂ Pp was achieved by peritoneal cavity puncture with an 18 G cannula, placed caudally to the sternum and CO₂ was insufflated by this canulla with Karl Storz insufflator in animal research laboratory. Pp was performed by insufflation of CO₂ to maximum intra abdominal pressure of 12 mmHg and intraabdominal pressure was checked over 60 minutes with this insufflator. The rats were rested 30 minutes after desufflation and each of 6 ml blood samples were obtained for; IMA, MPO, AOPP, CAT, PON1 and PAF-AH analyses by cardiac puncture. After this procedure the animals were sacrificed by exsanguination. The blood samples were centrifuged at 2000 X g for 10 min at 4°C and serum samples were frozen at -70 °C for two months.

Biochemical analyses

Serum IMA Assay
Levels of IMA were measured using the commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (Cusabio Biotech Wuhan, China) with detectable IMA ranging from 7.8 to 500 ng/mL.

Serum MPO Assay
Serum MPO activity was determined by the method of Bradley et al. and was based on kinetic measurement of
the formation rate of the yellowish-orange product of the oxidation of o-dianisidine with MPO in the presence of 
H$_2$O$_2$ at 460 nm. One unit of MPO was defined as that 
degradating 1 µmol of H$_2$O$_2$ per minute at 25°C. A molar 
extinction coefficient of 1.13 x 10$^4$ M$^{-1}$cm$^{-1}$ of oxidized 
o-dianisidine was used for the calibration. MPO activity 
was expressed in units per litre of serum (U/L) [25].

**Serum AOPP Assay**

Determination of AOPP was based on a spectrophotometric 
assay according to Witko-Sarsat et al. One millilitre of 
serum diluted 1:5 with phosphate-buffered saline (PBS, 
pH7.4), 1 ml of chloramine-T (0-100 µmol/L) for cali-
bration and 1 ml of PBS as blank were placed on cor-
responding tubes; 50 µL of 1.16 M potassium iodide (KI) 
was then added to each tube, and 2 min later 200 µL gla-
cial acetic acid was added. The absorbance of theproduct 
mixture was read immediately at 340 nm. AOPP level was 
expressed in µmol of chloramine-T equivalents per litre 
of serum (µmol/L) [26].

**Serum CAT Assay**

The serum CAT activity was determined by Goth’s colo-
rimetric method in which serum samples were incubated 
with H$_2$O$_2$ substrate and the enzyme reaction were stopped 
by the addition of ammonium molybdate. The intensity of 
the yellow complex formed by molybdate and H$_2$O$_2$ was 
measured at 405 nm. Serum CAT activity was expressed 
as U/L [27].

**Serum PON1 Assay**

Paraoxonase activity was measured according to Gan et 
al. using phenylacetate as substrate at a final concentra-
tion of 1 mM. Formation of phenol at 25°C was moni-
tored at 270 nm, in the presence of 50 mM Tris-HCl (pH 
8) containing 1 mM CaCl$_2$ and 40 µM eserine. Enzymatic 
activity was calculated from the molar extinction coeffi-
cient of phenol ($C_{270} = 1310$ M$^{-1}$cm$^{-1}$) and corrected for 
the non-enzymatic hydrolysis. One unit of paraoxonase activ-
ity is defined as 1 µmol of substrate hydrolyzed per min, 
under the defined assay conditions [28].

**Serum PAF-AH Assay**

PAF-AH activity was determined spectrophotometrically 
using a modification of the method described by Staffo-
rini et al [29]. 2-thio-PAF (Cayman Chemical) was used 
as substrate at a final concentration of 1 mM in 100 mM 
Tris-HCl buffer (pH 7.4). Hydrolysis of thioester sub-
strate was measured at 37°C, using Ellman’s procedure 
for monitoring at 412 nm the accumulation of free sulphy-
dryl groups reacting with DTNB. Enzymatic activity was 
calculated from the molar extinction coefficient of DTNB 
($C_{412} = 13600$ M$^{-1}$cm$^{-1}$). One unit of PAF-AH activity is 
declared as 1 µmol of substrate hydrolyzed per min, under 
the defined assay conditions.

**Statistical analyses**

The data are expressed as mean (X) ± Standart deviation
Kolmogrov-Smirnov (K-S) Goodness of Fit Test was used to control whether the distribution of parameters was normal or not. Then groups of data were compared with an analysis of variance (One-way ANOVA) followed by Tukey’s multiple comparison tests. In addition, Pearson correlation analysis was carried out for determination of linear relationships among the variables. Statistical significance was considered as 5% for all statistical computations.

Results
Kolmogrov-Smirnov (K-S) Goodness of Fit Test and ANOVA results for for all variables are presented in Table 1. The comparisons of measured parameters are also presented in Table 2. As shown in Table 2; the serum IMA level significantly decreased in group II, and also increased in group III as compared to control (p<0.05). However, IMA levels were significantly decreased in both groups II and IV as compared to group III (p<0.01). Significantly increased serum MPO activity was obtained in group III as compared to controls (p<0.05). Serum AOPP levels were increased significantly in group III as compared to group II (p<0.01) and significantly decreased in group IV compared to group III (p<0.01). The serum CAT activity was higher in group II than control (p<0.05). Serum PON1 activity significantly decreased in group III as compared to group II (p<0.05). Serum PAF-AH activity were decreased in group III as compared to control and group II (p<0.05). There were no significantly differences among the other parameters in all groups (p>0.05). The correlations between all parameters in Group II, III and IV are presented in Table 3, 4 and 5. There were significantly correlation between MPO and AOPP (p<0.05, r=0.78), PAF-AH and IMA (p<0.05, r=0.71) in group II, MPO and CAT (p<0.05, r=0.66) in group III, and also MPO and CAT (p<0.01, r=0.90), PON1 and AOPP (p<0.05, r=0.61) in group IV.

Discussion
I/R injury precipitate to release free radicals. It is known that according to injury of these radicals to the cell membrane, tissue integrity is damaged and the antioxidant ca-
DEX is a potent and specific α2 adrenoceptor agonist that decreases proapoptotic proteins via α2 adrenoceptor stimulation. Sedative and analgesic features of DEX provide this drug as an adjuvant therapy for perioperative period. Moreover it is known that, DEX reduces mortality by reducing the level of TNFα and increases the functions of macrophages especially phagocytose and finally reduces proinflammatory cytokines and inflammatory response [5,10].

IMA is an early and sensitive marker of myocardial, pulmonary, mesenteric and cerebral ischemic processes that indicates the oxidative stress [9,17]. IMA was demonstrated as a useful marker that shows ischemic changes in splanchnic area during Pp in previous studies [31]. It is observed that IMA levels were increased in only Pp group and also decreased in Pp with DEX administration group in our study. Previous studies conclude that DEX is a useful drug in I/R injury by preventing lipid peroxidation [32,33]. Recently Geze et al. showed that DEX administration before Pp caused to lower IMA levels in their experimental study and they concluded that DEX prophylaxis reduced the post-pneumoperitoneum I/R injury [9]. It was observed in a histopathological study on ovarian tissue of rats with Pp that DEX administration resulted in decrease for vascular congestion, hemorrhage and follicular degeneration. Moreover, it was also reported that DEX reduces oxidative stress in the same study [34]. DEX administration resulted in significantly decreasing level of IMA and increasing CAT activity when compared to control group. It was also observed statistically significant correlation between IMA levels and PAF-AH activity in the group II. We believe that antioxidant effect of DEX derives from increasing CAT activity. Acute inflammatory response secondary to neutrophil activation in I/R injury releases reactive oxygen molecules and cytotoxic proteins such as MPO from neutrophils to the extracellular fluid [35]. An experimental study reported by Zhang et al. showed that an intestinal I/R injury by clamping superior mesenteric artery shows that MPO levels are increasing in I/R injury [30]. It is reported that MPO increases during the ischemic period due to Pp and the reperfusion period after decompression [35]. When compared with the control group, MPO activity was higher in the Pp only group and it was thought that it increases as a result of I/R injury in our study. The levels of MPO in DEX administration before Pp group were lower but it was not statistically significant. MPO is a commonly used enzymatic marker in order to show the level of inflammation. Uysal et al. reported that flap necrosis associates with high MPO levels and they stated that inflammatory response and MPO levels significantly decrease in DEX administration group [7]. Kontoulis et al. showed that Pp induced I/R injury causes higher MPO levels and also stated that long duration of Pp and reperfusion causes more increasing in MPO levels [36]. MPO activities in our study were measured in the blood samples that were taken 60 minutes after Pp. We consider the difference between the results of our study and the others originate from the timing of the blood sampling. For that reason we think that further studies are needed to investigate the protective effects of DEX during pneumoperitoneum with different dose regimens in late period of the injury.

Measurement of serum AOPP level may be a useful marker of protein oxidative damage [16]. It is demonstrated that I/R injury secondary to Pp in laparoscopic surgeries increases AOPP levels in a previous study [37]. In the only DEX administration group, there was statistically significant correlation between MPO and AOPP which are the markers of I/R injury. Likewise, AOPP levels in Pp only group were found to be increased in our study. The decrease in AOPP level in DEX administration before Pp group was thought to be a result of its anti-inflammatory effect.

Toxic oxygen radicals and its derivates which appear during I/R injury are cleared by free radical cleaner such as CAT. It degrades hydrogen peroxide into water and oxygen [20]. Bulbuloglu et al. reported in an experimental study that CAT activity decrease in I/R injury secondary to Pp and increases after administration of antioxidant [38]. An experimental model of I/R injury of muscle tissue which assess antioxidant activities with DEX administration, CAT activity in I/R injury group was found lower, on the other hand CAT activity in DEX administration group before reperfusion was higher than control group. Dong et al. concludes that DEX has protective effects in I/R injury [39]. In our present study, significantly higher CAT activity was observed in only DEX administration group as compared with control group. Although it was not statistically significant, a relative increase of CAT activity in DEX administration before Pp group would be accepted as a positive effect of DEX administration on antioxidant system. In this study, there was statistically significant correlation between MPO and CAT activity in Group III and IV. MPO and CAT are the enzymes that use hydrogen peroxide radicals as a substrate. Although CAT activity in Group III and IV was not significantly higher than control group, MPO activity in these groups was significantly higher than control group. We believe that obtained significant correlation between MPO and CAT activity is related to increasing level of hydrogen peroxide secondary to Pp.
PON1, that decrease in inflammation and oxidative stress is used as an indicator of antioxidative capacity [23]. An experimental study results showed that DEX administration before I/R injury caused to decrease total antioxidant capacity and PON1 activity in rats with hepatic I/R injury [8]. In our present study PON1 levels in Pp only group were significantly lower than DEX administration without Pp group. This result proves that Pp results in I/R injury. PON1 levels in DEX administration before Pp group were not statistically significant but it shows that DEX administration makes PON1 levels closer to control group. DEX administration caused to significant decrease on levels of AOPP and PON1 activity in Group IV. Although DEX administration didn’t cause to increase on the antioxidant enzyme activity in group IV, there was also statistically significant correlation between AOPP and PON1. The results of the present study demonstrate that DEX protects the metabolism by attenuating the oxidative stress.

In the presence of ischemia and inflammation, PAH-AH is inactivated by oxygen radicals in the circulation irreversibly [14]. The level of PAH-AH is low in patients with oxidative stress based diseases. The examples of these diseases are; asthma, systemic lupus erythematosus, acute myocard infarction, multiple organ failure and sepsis [40]. In our study, the levels of PAH-AH were found to be significantly low in the Pp only group and this result was an expected result because of its antioxidant nature of this marker which tends to decrease in ischemia. In the review of the literature, there was no study that investigates the effects of DEX on PAH-AH levels in Pp model. Our present study is the first experimental study in this manner. PAH-AH levels were low as it was expected I/R injury during Pp. Even it was not found to be statistically significant, PAH-AH levels in DEX administration before Pp group were close to control group and this result may suggest that DEX administration can tip the balance in favor of antioxidative side.

**Conclusion**

Our experimental I/R injury model demonstrated not only the increasing levels of IMA, MPO and AOPP which are oxidative markers but also the decreasing levels of PON1 and PAH-AH activity that have antioxidant potential during pneumoperitoneum. The administration of DEX may minimize the harmful effects of oxidative injury caused by Pp.

**Ethical Issues**

This experimental study was approved by Ondokuz Mayis University Animal Care and Ethics Committee (2013/06), Samsun, Turkey.

**Supporting organizations**

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**Conflict of Interest**

There are no conflicts of interest among the authors.

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