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Protective effects of dexmedetomidine in pneumoperitoneum-related ischaemia–reperfusion injury in rat ovarian tissue

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ABSTRACT

Objectives: To determine the effects of dexmedetomidine on pneuomoperitoneum-related ischaemia–reperfusion (I/R) injury in rat ovarian tissue.
Study design: Animals were randomized into three groups: Group S ($n = 8$), no pneumoperitoneum; Group C ($n = 8$), pneumoperitoneum; and Group D ($n = 8$), 100 μg intraperitoneal dexmedetomidine 30 min before pneumoperitoneum. Ovarian tissue was collected from all rats 30 min after desufflation, and fresh frozen for histological and biochemical evaluation.
Results: Body weight was similar in all three groups (202.62 ± 28.86, 211.00 ± 14.45 and 212.87 ± 15.71 g in Groups S, D and C, respectively). The mean malondialdehyde level was higher in Group C than the other groups ($p < 0.03$). When the histological samples of ovarian tissue were compared, vascular congestion, haemorrhage, follicular cell degeneration and infiltrative cell infiltration scores were higher in Group C compared with the other groups ($p < 0.05$). Significantly lower scores for the histological parameters were found in Group D compared with Group C ($p < 0.05$). Similar scores for follicular cell degeneration and inflammatory cell infiltration were found in Group D and Group S ($p > 0.05$). Although vascular congestion and haemorrhage scores were significantly lower compared with Group C, higher scores were found for Group D compared with Group S ($p < 0.05$).
Conclusion: Pneumoperitoneum caused oxidative injury in rat ovarian tissue. Dexmedetomidine reduced oxidative stress and histological injury related to I/R.

1. Introduction

Laparoscopic methods represent the gold standard for many gynaecological procedures. Laparoscopy was initially used for diagnostic purposes, but now almost all gynaecological procedures can be performed via laparoscopy. An increasing number of studies, however, have focused on concerns related to pneumoperitoneum and its pathophysiology [1]. Experimental and clinical studies have shown that increasing the intra-abdominal pressure (IAP) during pneumoperitoneum decreased macro- and micro-circulation in organs and tissues, and the ischaemic organs were reperfused following abdominal desufflation. Free oxygen radicals released during reperfusion were determined to be an important mediator following tissue injury [2–5].

Experimental studies have shown that laparoscopic surgery creates oxidative stress [6–8] even at low IAP (<12 mmHg), and prophylactic administration of anti-oxidants decreases laparoscopy-related oxidative stress [9–11]. Very few studies have focused on laparoscopy-related ovarian reperfusion injury [4,12]. Pneumoperitoneum leads to formation of free radicals and subsequent reperfusion injury by decreasing adnexial circulation [4,5]. This is very important for women of reproductive age who have fertility problems.

Dexmedetomidine is a selective and potent α2 adrenoceptor agonist that is often used for sedation, anxiety and analgesia. It is known to decrease tissue ischaemia by decreasing catecholamine release and oxygen consumption [13–15]. This study aimed to evaluate the effects of dexmedetomidine on pneumoperitoneum-related ischaemia reperfusion (I/R) injury in rat ovarian tissue.

2. Materials and methods

2.1. Animal preparation and study design

This study was approved by the Animal Care and Ethics Committee, and was compliant with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

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Twenty-four adult female Sprague-Dawley rats weighing 200–250 g were used in this study. The animals were kept in a windowless, light-controlled environment at 20 ± 2 °C, and were allowed free access to food and water. They were subjected to an overnight fast for the night preceding the experiment.

2.2. Anaesthesia and surgical protocol

The rats were anaesthetized with 50 mg/kg ketamine (Ketalar; Parke Davis, Berlin, Germany) and 20 mg/kg xylazine (Rompun; Bayer, Leverkusen, Germany), and placed in a supine position on an operating table. The tail vein was cannulated with a 24G intravenous catheter. After the tracheal region was cleaned, the trachea was isolated with a midline incision and cannulated with a 16G intravenous catheter. Mechanical ventilation was initiated in volume-controlled mode with a respiratory frequency of 40 breaths/min, tidal volume of 10 ml/kg, inspiratory/expiratory ratio of 1:1 and fractional inspiratory oxygen concentration of 1.0. Spontaneous respiration was suppressed with intravenous pancuronium (1 mg/kg).

Pneumoperitoneum was established by inserting an 18G intravenous catheter into the abdominal right lower quadrant of the peritoneal cavity, and insufflating the abdomen with CO_2 to a pneumoperitoneal pressure of 12 mmHg. IAP was maintained for 60 min with an electronic laparoflator (Karl-Storz, Tutlingen, Germany).

2.3. Experimental protocol

Following initial stabilization, the rats were randomized into three groups ($n = 8$ in each group). In Group S (sham group), no pneumoperitoneum was established. In Group C (control group), pneumoperitoneum was established for 60 min under 12 mmHg pressure. In Group D (ischaemia–reperfusion/dexmedetomidine treatment group), intraperitoneal dexmedetomidine (100 μg) was administered 30 min before abdominal insufflation, and pneumoperitoneum was established for 60 min under 12 mmHg pressure.

The researchers conducting the biochemical and histological assessments were unaware of the randomized groups until the end of the study.

2.4. Malondialdehyde measurement

The level of malondialdehyde (MDA) was used as proof of lipid peroxidation in order to determine oxidative stress. The level of ovarian injury due to free radicals formed by ischaemia–reperfusion was determined indirectly. Ovarian tissue was used to measure the level of MDA. Ovarian tissues were weighed and homogenized in ice-cold 1.15% KCl (2 and 10%, w/v, respectively). The homogenate was centrifuged at 2000 × g for 10 min. MDA levels in tissue samples were determined using the method of Uchiyama and Mihara [16]. Tetramethoxypropane was used as the standard, and tissue MDA levels were calculated as nmol/g wet tissue.

2.5. Histological staining and analysis

For histological evaluation, ovarian tissues were collected from all rats. The ovarian tissues were fixed immediately in 10% neutral buffered formalin solution for 24 h, dehydrated in increasing concentrations of ethanol, and embedded in paraffin blocks. Serial sections of 5-μm thickness were sliced using a microtome (Leica RM 2255; Tokyo, Japan) and stained with haematoxylin and eosin. All ovarian histology was examined under a light microscope (Olympus BX-51; Olympus Co., Tokyo, Japan) and assessed blindly by a histologist. Photographs were taken using a light microscope with a camera attachment (Olympus DP 71; Olympus Co.). Five samples of ovarian tissue were selected at random for each group, and scored semi-quantitatively from 0 to 3 in terms of vascular congestion, haemorrhage and inflammatory cell infiltration (0 = none; 1 = mild; 2 = moderate; 3 = severe) [4].

2.6. Statistical analysis

Statistical analysis was performed using two- and three-way analysis of variance. All values are expressed as mean ± standard deviation. Significance was set at $p < 0.05$.

3. Results

All rats survived until the end of the experiment. Body weight was similar in all three groups (202.62 ± 28.86, 211.00 ± 14.45 and 212.87 ± 15.71 g in Groups S, D and C, respectively).

3.1. Malondialdehyde levels

The mean tissue MDA level was higher in Group C compared with Group S ($p = 0.03$). There were no differences in mean MDA levels in Group D compared with Groups C and S. Fig. 1 shows the MDA levels in the three groups.

3.2. Histological findings

When histological samples were compared, vascular congestion, haemorrhage, follicular cell degeneration and infiltrative cell infiltration scores were found to be higher in Group C compared

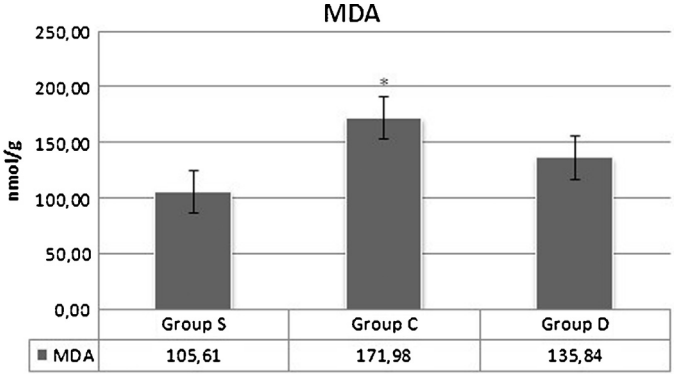

Fig. 1. Malondialdehyde (MDA) levels of all groups.

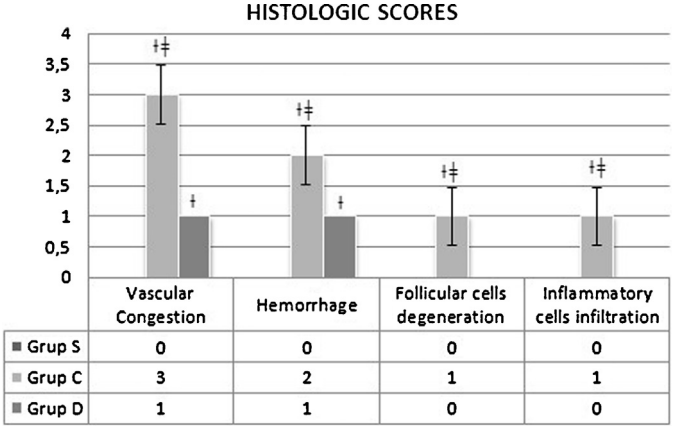

Fig. 2. Histopathological investigation scores.