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Does Omegaven have beneficial effects on a rat model of ovarian ischemia/reperfusion?

Ayse N. Cakir Gungor [a,*], Hakan Turkon [b], Aynur Albayrak [c], Mehmet Ovali [d], Mine Islimye [e], Meryem Gencer [a], Servet Hacivelioglu [a], Sibel Cevizci [f], Ismet Cesur [g], Emine Cosar [a]

[a] Canakkale Onsekiz Mart University, Faculty of Medicine, Department of Obstetrics and Gynecology, Canakkale, Turkey
[b] Canakkale Onsekiz Mart University, Faculty of Medicine, Department of Biochemistry, Canakkale, Turkey
[c] Diskapi Education and Research Hospital, Department of Pathology, Ankara, Turkey
[d] Canakkale Onsekiz Mart University, Faculty of Medicine, Department of Physiology, Canakkale, Turkey
[e] Balikesir University, Faculty of Medicine, Department of Obstetrics and Gynecology, Balikesir, Turkey
[f] Canakkale Onsekiz Mart University, Faculty of Medicine, Department of Public Health, Canakkale, Turkey
[g] Canakkale Onsekiz Mart University, Faculty of Medicine, Department of Pathology, Canakkale, Turkey

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ABSTRACT

Objective: The beneficial effects of omega-3 fatty acids on an intestinal ischemia/reperfusion (I/R) model was shown previously. Therefore, we aimed to examine the potential beneficial effects of parenteral omega-3 fatty acids, a safe and inexpensive product, on a rat model of ovarian I/R.

Study design: A group of 39 rats was divided into six groups. Group 1 (Sham Group; $n = 6$) underwent two laparotomies with a 3-h interval and their ovaries were removed 3 h later. Group 2 (torsion–detorsion Group; $n = 7$) had their ovaries torsioned clockwise and fixed at 720°; 3 h later a detorsion operation was done and after another 3 h, their ovaries were removed. Group 3 ($n = 7$) and Group 4 ($n = 7$) received the same treatment as Group 2; however, half an hour prior to detorsion, these rats received Omegaven at 1 mL/kg and 5 mL/kg, respectively. Group 5 ($n = 6$) and Group 6 ($n = 6$) received the same treatment as Group 1; however, half an hour prior to the second laparotomy, these rats received Omegaven at 1 mL/kg and 5 mL/kg, respectively. One ovary from each rat was evaluated histologically by hematoxylin and eosin (H&E) staining and the other ovary was homogenized and evaluated for total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI).

Results: While we failed to show any significant relationship among groups in oxidative parameters, there was a significant worsening in the torsion–detorsion group in histological evaluation. High Omegaven doses, but not low doses, improved tissue injury scores of torsioned and detorsioned ovaries to the levels observed in the control group.

Conclusion: Omegaven improves the detrimental effects of ovarian I/R when used in sufficient doses. Its effects and dose adjustment on women with ovarian torsion must be investigated by further studies.

Introduction

Ovarian torsion is a rare problem that can have serious complications since many patients have not completed making their family. Its current management methods involve surgical detorsion of the affected ovary independent of the necrotic appearance of the ovary. In this way, clinicians have been attempting to spare the patient's fertility. However, a recent study with rats showed that ovary detorsion alone cannot solve the fertility problem [1]. In addition to the ischemic damage caused by torsion, detorsion can also have devastating effects on the reperfused tissue. An overproduction of reactive oxygen radicals has been shown to have additive toxic effects at the cellular level [2].

Before detorsion operations, several medications have been administered with the goal of preserving ovarian reserve; these medications may have either anti-inflammatory or anti-oxidant properties. Previously, several medications such as selenium [3], ozone [4], growth hormone [5], alpha-lipoic acid [6] and aprotinine [7] have been used experimentally to decrease the devastating

* Corresponding author. Tel.: +90 2862635950; fax: +902862180516.
E-mail address: dr_aysecakir@hotmail.com (A.N.C. Gungor).

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effects of torsion and detorsion. Unfortunately, most of the experimentally used medications cannot be used in clinical practice for the management of ovarian torsion. An ideal medication must be safely usable parenterally in humans so that it can effectively and rapidly protect the ovary when applied during the short interval between torsion and detorsion surgery.

Omega-3 fatty acids have many beneficial effects in humans. In a study conducted in 2011, intraperitoneal usage of omega-3 fatty acids was found to be beneficial in a rat ischemia/reperfusion (I/R) model of intestines [8]. Parenteral omega-3 fatty acids can be used safely in humans [9] and is an inexpensive and easily accessible product. Additionally, omega-3 fatty acids can be used during pregnancy without adverse effects; this makes it eligible for pregnant ovarian torsion patients. Moreover, because of its beneficial effects on maternal and fetal health status, some authors suggest the use of omega-3 fatty acids routinely during pregnancies [10].

We aimed to examine the potential beneficial effects of Omegaven, a parenteral form of omega-3 fatty acids, on an ovarian I/R model in rats.

Materials and methodS

Animals

Animals were maintained in accordance with international guidelines and the studies were approved by the Canakkale Onsekiz Mart University Animal Care and Use Ethical Committee. In this study we used 39 Wistar albino female adult rats that were 4–6 months old and weighed 200–250 g. Animals were housed in, and the experiment was conducted in, the Canakkale Onsekiz Mart University Experimental Research Center. Animals were fed standardized pellets and all food and water was given ad libitum. Animals were housed according to a 12-h light–dark cycle.

Groups

Animals were randomly divided into six groups as follows:

1. Group 1 (Sham Group, $n = 6$): after sham operation at 0- and 3 h-point both ovaries of the rats were removed for evaluation at 6 h-point.
2. Group 2 (torsion–detorsion Group, $n = 7$): after torsion and detorsion operation, both ovaries were removed at the 6 h-point [7,11].
3. Group 3 (torsion–detorsion + low-dose Omegaven Group, $n = 7$): rats underwent torsion and detorsion operation. Half an hour prior to detorsion, 1 mL/kg Omegaven (Fresenius Kabi, Austria) [100 mL Omegaven contains 1.25–2.82 g of eicozapentaenoic acid (EPA) and 1.44–3.09 g of docosahexaenoic acid (DHA)] was applied intraperitoneally [8,12]. At the 6-h point, both ovaries of the rats were removed.
4. Group 4 (torsion–detorsion + high-dose Omegaven Group, $n = 7$): rats underwent torsion and detorsion operation. Half an hour prior to detorsion, 5 mL/kg Omegaven was applied intraperitoneally. At the 6-h point, both ovaries of the rats were removed.
5. Group 5 (Sham + low-dose Omegaven Group, $n = 6$): rats underwent torsion and detorsion operation. Half an hour prior to the second laparotomy, 1 mL/kg Omegaven was applied intraperitoneally. At the 6-h point, both ovaries of the rats were removed.
6. Group 6 (Sham + high-dose Omegaven Group, $n = 6$): rats underwent torsion and detorsion operation. Half an hour prior to second laparotomy, 5 mL/kg Omegaven was applied intraperitoneally. At the 6-h point, both ovaries of the rats were removed.

Anesthesia

Anesthesia was administered with intraperitoneal applications of a ketamine hydrochloride (85 mg/kg) and xylazine (15 mg/kg) compound at a 0.5–1.0 mL/kg dose.

Surgical procedures

Sham operation

Following anesthesia, rats were placed on operation desk in supine position and a 2-cm mid-line vertical incision was made, both ovaries were found and picked-up gently with forceps for 30 s. The abdomen was then closed with interrupted, size 0 vicryl sutures.

Torsion–detorsion model

Rats underwent laparotomy procedure as formerly defined. After finding the adnexes, both ovaries were torsioned 720° clockwise and fixed to the abdominal wall with a size 00 silk suture [4,5] and the abdomen was closed with interrupted, size 0 vicryl sutures. After three hours, the fixed ovaries were freed by cutting the suture and both ovaries were detorsioned with a re-laparotomy procedure and the abdomen was closed with size 0 vicryl sutures.

In summary, rats in Group 1, 5 and 6 underwent sham operation two times before ovarian removal and Omegaven in low and high doses were applied intraperitoneally in the last two groups subsequently. Rats in Group 2, 3 and 4 underwent torsion and detorsion operation before ovarian removal and Omegaven in low and high doses were applied intraperitoneally before detorsion in the last two groups subsequently

For better standardization, every stage of the operations was performed by the same surgeon.

Histopathologic evaluation

Ovarian tissues were numbered and the right ovaries were placed in 10% formaldehyde and sent to the pathology laboratory. They were processed in the pathology laboratory, stained with hematoxylin and eosin (H&E) and evaluated by a pathologist who was blind to the study groups.

Ovarian tissues were fixed in a 10% formalin solution and embedded in paraffin. The paraffin blocks were cut using a microtome thickness of 4–5 μm and the sections were stained with H&E. The histopathologic sections were examined under a microscope (Olympus BX51) for the presence of interstitial edema, congestion (vascular dilation), and hemorrhage. Sections were also photographed. Five microscopy fields were used to determine the presence of, or severity of, tissue damage. The scoring system used for histopathologic evaluation of the ovarian tissues was previously described by Bozkurt et al. [3]. Congestion, hemorrhage and interstitial edema scores ranged from 0 to 3 according to the severity (0 = no pathologic findings; 1 = less than 25%; 2 = between 25% and 75%; and 3 = more than 75% of the ovarian section). The total tissue damage score was calculated by summing these scores.

Laboratory

Left ovarian tissues were prepared at 4 °C to measure their oxidant and antioxidant levels. After washing with 0.9% NaCl, left ovaries were stored at −80 °C in Eppendorf tubes until the biochemical analysis. After thawing the ovarian tissues, they were weighed to a precision of 0.001 g, cut into small pieces and a