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The effects of iloprost, a prostacyclin analogue, in experimental ischaemia/reperfusion injury in rat ovaries

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Abstract

Ovarian torsion is a surgical emergency affecting not only the ipsilateral ovary but also contralateral ovary. Although the conventional treatment is salpingo-oophorectomy, recent studies advocate detorsion. We hypothesized that iloprost, an analogue of prostacyclin with cytoprotective properties, may prevent the harmful effects of ischaemia–reperfusion injury in bilateral ovaries after unilateral ovarian torsion–detorsion in rat. In this study, 24 female Wistar-albino female rats were divided into four groups. Ovarian torsion was produced by applying vascular clamps to right ovaries. In Group I, bilateral oophorectomy was performed. In group II, bilateral oophorectomy was performed after a unilateral torsion period of 4 h. In group III, bilateral ovaries were removed, following unilateral torsion–detorsion periods each lasted for 4 h. Saline was injected i.p. 30 min before detorsion. In group IV, same experimental protocol, which was conducted in group III, was repeated. Iloprost was injected i.p. 30 min before detorsion instead of saline in group IV. Tissue levels of malondialdehyde (MDA) and nitric oxide (NO), which are the indicators for oxidative stress were determined and histopathological evaluation was performed in bilateral ovaries in all groups. The MDA and NO levels for ipsilateral ovaries of four groups were compared and no significant difference was found ($p > 0.05$). The same comparison were done for the contralateral sides and no difference was seen either ($p > 0.05$). In histological examination, iloprost produced improvement in I/R-induced alterations in ipsilateral and contralateral ovaries. In conclusion, these results showed that iloprost has beneficial effect on the histological appearences in both the ipsilateral and contralateral rat ovaries after unilateral torsion–detorsion.

Keywords: Iloprost; PGI_2; İschaemia/reperfusion; Rat; Ovary

Introduction

Ovarian torsion is an infrequent but serious cause of gynecological surgical emergency, which is mostly seen in the first 3 decades (Houry and Abbott, 2001). It may

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occur in normal ovaries, but is more frequent in association with pre-existing tubal/ovarian pathologies (McWilliams et al., 2008). Traditional approach to treat adnexal torsion is salpingo-oophorectomy without untwisting adnexa, in order to avoid potential thrombotic emboli from the ovarian vein (Bayer and Wiskind, 1994; Hibbard, 1985). On the other hand, recent studies advocate a conservative approach including detorsion and releasing the pedicle to preserve fertility (Bayer and Wiskind, 1994; Oelsner et al., 1993). However, maintaining the circulation of the ovary after detorsion worsens the tissue injury and leads to a pathologic process called ischaemia/reperfusion (I/R) injury, which is characterized by oxidative stress (Li and Jackson, 2002).

Histological and ultrastructural changes have been reported in contralateral ovaries after ipsilateral ovarian ischaemia (Cakmak et al., 1993). Unilateral torsion and ovariectomy also affects the ovulation in contralateral ovary (Chaves et al., 1987; Coleman et al., 1984). It has been suggested that unilateral ovarian ischaemia may stimulate the sympathetic system of the contralateral ovary resulting in decrease in regional blood flow smilar to testicular torsion (Akgur et al., 1994).

Low levels of reactive oxygen species (ROS) are formed and play important roles in cellular homeostasis including mitosis, differentiation and signaling in healthy conditions (Irani et al., 1997). Following I/R, large amounts of ROS are produced and the antioxidant defense mechanisms are overwhelmed (McCord, 1985). Increased generation of ROS causes injury in cells by peroxidation of membrane lipid, denaturaton of proteins including enzymes and ion channels and breaks in DNA strands (Gutteridge, 1993; Carden and Granger, 2000). Lipids are the most involved class of biomolecules, which were targeted by oxidative stress. Malondialdehyde (MDA) is the principal product of polyunsaturated fatty acid peroxidation and is a highly toxic molecule. Therefore, this molecule is assessed in order to quantify the level of oxidative stress in vivo and in vitro (Del Rio et al., 2005). Nitric oxide (NO), which is produced by the normal endothelium, is a principal determinant of normal endothelial and vascular function. In states of inflammation, overproduction of NO occurs by the vasculature and, in conjunction with other ROS, contributes to oxidative stress (Lubos et al., 2008). Accumulation of activated neutrophils also contributes to pathophysiology (Chan et al., 2003). Although, the results are not conclusive, antioxidant therapies including *N*-acetylcysteine, vitamin E, superoxide dismutase, catalase, allopurinol and vitamin C have been reported to attenuate I/R injury (Rangan and Bulkley, 1993; Sagsoz et al., 2002).

Iloprost (ZK 36 374) is a long-acting analogue of epoprostenol, which is also named prostacyclin or PGI_2 mainly synthesized in the vascular endothelium (Grant and Goa, 1992). It has been reported to have a similar profile of action as PGI_2 (Kisch-Wedel et al., 2005). Pharmacodynamic properties include vasodilatation, inhibition of platelet aggregation and cytoprotection as well as antioxidant effect (Moncada, 1982; Pajdo et al., 2001). Iloprost has also been shown to improve I/R injury in distinct tissues (Bozkurt, 2002; Emrecan et al., 2006).

The present study was conducted in order to clarify the effects of iloprost on I/R injury in ipsilateral (torsed) and contralateral (untorsed) ovaries in rats. Therefore, histopathological examination were performed and NO and MDA levels as oxidative stress markers were assessed in ovarian tissues.

Materials and methods

In the present study, the recommendations of the Declaration of Helsinki (1964) for animal care were taken into consideration. Experiments were performed in agreement with the "Principles of laboratory animal care (NIH publication No. 86-23, revised 1985)" and this study was approved by Gazi University Ethics Committee for Animals.

A total of 24 Wistar-albino female rats in cycling reproductive age weighing 250–300 g were used in the experiments. Animals were randomly divided into four groups and study groups were defined as follows:

1. C (control group; $n = 6$)
2. I (ischaemia group; $n = 6$)
3. I/R (ischaemia/reperfusion group; $n = 6$)
4. I/R+iloprost (ischaemia/reperfusion+iloprost group; $n = 6$)

Each rat was anesthetized with ketamin (50 mg/kg i.m.) and xylazine (9 mg/kg i.m), which were repeated as necessary to maintain anesthesia troughout the experiments. Rats were placed in dorsal recumbent position and covered with a steril drapes. After the skin preparation and cleaning, a midline incision of 2.5 cm was performed for laparatomy. Ovarian torsions were produced by applying vascular clamps, which were placed just below the right (ipsilateral) ovary above the uterine horne (Uguralp et al., 2005). Incisions were closed with 4/0 silk sutures and after 4 h of torsion the vascular clamps were removed. Following the torsion period, reperfusion was allowed for 4 h. Bilateral ovaries were excised for biochemical and histological examinations at the end of reperfusion. The incisions were kept closed troughout the experiments. The rats were killed by exsanguination through the carotid arteries at the end of experiments. In control group (group I), sham operation and bilateral oophorectomy were performed. In ischaemia group (group II), bilateral