Original Article

Ovarian dysfunction after ischemia-reperfusion of the ovarian blood vessel in experimental rats

Koichi Nariai^{1,2}, Risa Fukumoto¹, Shin Onoto¹, Ken Watanabe¹, Hiroshi Uchiyama³,
Kiichi Kanayama⁴, Kahei Sato³.

¹ Institute of Clinical Medicine and Research (ICMR), Research Center for Medical Sciences,
The Jikei University School of Medicine,
163-1 Kashiwa-shita, Kashiwa, Chiba 277-8567, Japan

² Laboratory Animal Facilities (LAF), Research Center for Medical Sciences,
The Jikei University School of Medicine,
3-25-8 Nishi-shinbashi, Minato-ku, Tokyo 105-8461, Japan

³ Department of Applied Biology, College of Bioresource Sciences, Nihon University,
1866 Kameino, Fujisawa 252-8510, Japan

⁴ Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University,
1866 Kameino, Fujisawa 252-8510, Japan

Keywords:

reactive oxygen species (ROS),
superoxide anion radical, oxidative
stress, superoxide dismutase (SOD),
ischemia-reperfusion, ovarian function

Correspondence:

Koichi Nariai, DVM, Ph.D, Laboratory
Animal Facilities (LAF), Research Center
for Medical Sciences, The Jikei
University School of Medicine, 3-25-8
Nishi-shinbashi, Minato-ku, Tokyo 105-
8461, Japan

Mail Address:

nariai@jikei.ac.jp

Received September 29, 2009

Accepted October 24, 2009

Abstract

Excessive production of reactive oxygen species (ROS) lead to oxidative stress in tissue or organ dysfunction. To confirm ovarian dysfunction by ROS, ischemia-reperfusion (I/R) treatment of the ovarian blood vessels was performed using experimental rats in this study. Preventive effect of superoxide dismutase (SOD) as a radical scavenger against ovarian I/R injury was also examined. Hormonal sensitivities to equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) in the ovary decreased after the I/R. Ovarian tissue and ovum destruction were also observed. These changes could be prevented by SOD administration. Our data suggest that I/R injury related to ROS production causes ovarian dysfunction. The dysfunction is prevented by administration of a radical scavenger.

Introduction

Reactive oxygen species (ROS) are important molecules which play a role for homeostasis. When ROS generated excessively in a living body, however, it bring about cell destruction [1] and/or genetic mutation [2]. This is called as oxidative stress. Excessive production of ROS such as a superoxide anion radical ($O_2^{\cdot-}$) and its related radicals including a hydroxyl radical ($HO^{\cdot}$) are also caused by ischemia-reperfusion (I/R) of afferent blood vessels to the organs [3]. In organs with ischemic hypoxia, vascular reperfusion after ischemia causes reoxygenation and exacerbates tissue damages [4]. It can be thought that this situation

also occur after detorsion for treatment in ovarian pedicle torsion. If this is a fact, the ovarian I/R may cause serious female infertility. We examined influence of the I/R in ovarian blood vessels on ovarian tissue including follicular eggs in this study.

Materials and Methods

Experimental animals

Twelve to fifteen week-old female Wister strain rats (Slc:Wister, Japan SLC, Inc. Hamamatsu, Japan) were used in this study. They were kept in plastic cages and

were given commercial pellets (Certified Rodent Diet 5002, PMI Nutrition International, Brentwood, MO, USA) and ultrafiltered water *ad libitum*. The animal room was air-conditioned with $23 \pm 2^\circ\text{C}$, $55 \pm 5\%$ relative humidity and 15 air changes per hour, and was illuminated for 12 hrs (07:00-19:00) with 300 lux using a day-light fluorescent lamp. Handling and treatment of the animals were followed by "Guideline on Animal Experimentation of Jikei University" containing ethical roles on animal experimentation.

Surgery and I/R treatment

Three experimental groups by difference of the following treatment were prepared in this study. Three animals (6 ovaries) were examined in each group. In each group, xylazine hydrochloride (13 mg/kg) and ketamine hydrochloride (87 mg/kg) were intramuscularly injected to induce general anesthesia. Whenever righting reflex was observed during the following surgery, ketamine hydrochloride (40 mg/kg) was supplementally injected to maintain anesthesia. Heparin (50 units) was injected into the tail vein to prevent blood clotting during ovarian ischemia. Blood flow of the ovarian artery and vein was stemmed using a micro vascular clip (Bear Medic Corporation, Tokyo, Japan) under laparotomy (Fig. 1) in group I and II. Fifty thousand units of superoxide dismutase (SOD, from bovine erythrocyte [Cat. No. 190117], Wako pure chemical industries, Ltd., Tokyo, Japan) was intravenously injected immediately before the clipping in group II. Only saline as a solvent of the SOD was administered in group I. Four hours after the ischemia, the clip was

Fig.1 Ovarian ischemia using a microvascular clip in ovarian blood vessels

removed to restart blood flow. This ischemia time (4 hours) was designed according to the previous description [5]. Finally the muscular, subcutaneous and dermal wounds were sutured with 2-0 nylon suture. In control group, only sham-operation and the solvent administration were performed.

Evaluation of ovarian function and ovum quality after the ovarian I/R

Ovarian function was evaluated as hormonal sensitivity at the time of superovulation induction. Fifty international units (iu) of quine chorionic gonadotropin (eCG) was injected intraperitoneally 24 hours after the reperfusion. Fifty iu of human chorionic gonadotropin (hCG) was injected intraperitoneally 48 hours after the eCG injection. Twenty-four hours after the hCG injection, the animals were euthanized with excessive exposition of diethyl ether. The ovaries and the oviducts were removed. To evaluate hormonal sensitivity of the ovary, its weight was measured. The number of ovulation points was also counted. The ovarian tissues were fixed by 10 % of buffered formalin. The paraffin-embedded microscopic sections ($4\mu\text{m}$) with hematoxylin and eosin (H-E) stain were prepared for histological examination after these measurements. To evaluate damage of ova by the I/R, morphological observation of ovulated ova in the tubal ampulla was performed under microscopy.

Statistical analysis

Significant differences between each group in the

Fig.2 Effect of the ovarian I/R on ovarian weight after superovulation induction

Values are expressed as mean $\pm$ SD. Significant differences were analyzed by Tukey-Kramer's post-hoc test.

Fig. 3 Effect of the ovarian I/R on number of ovulation after superovulation induction

Values are expressed as mean ± SD. Significant differences were analyzed by Tukey-Kramer's post-hoc test.

Fig. 4 Effect of the ovarian I/R on rate of degenerating ova ovulated after superovulation induction

Values are expressed as mean ± SD. Significant differences were analyzed by Tukey-Kramer's post-hoc test.

Fig. 5 Degenerated ova appearing in the tubal ampulla after superovulation in group I (I/R treated)

Ova with cell fragmentation and/or cumulus-free ova (arrows) were observed

Fig. 6 Microscopic observation of the I/R treated ovarian tissue

Abnormal follicles with a cell fragmented oocyte (star) and/or with the granular layer detached from follicular wall (arrows) were conspicuously observed. (x 100, H-E stain).

ovarian weight, the number of ovulation and the rate of degenerating ova were analyzed by Tukey-Kramer's post-hoc test.

Results

Results of ovarian weight are shown in Fig. 2. Ovarian weight in group I (I/R treated) was significantly ($p < 0.05$) lighter than that in control group. In group II (I/R + SOD), its decline was prevented. As shown in Fig. 3, numbers of ovulation (per ovary) in both group I and II significantly ($p < 0.05$) decreased as compared to those in control group. Significant ($p < 0.05$) high numbers of degenerating ova were observed in group I as compared with those in control group (Fig. 4 and 5). In group I, abnormal follicles with a cell fragmented oocyte and/or with granular layer detached from follicular wall were conspicuously observed in histology (Fig. 6).

Discussion

Although ROS are essential molecules for homeostasis, excessively ROS existing act on cytotoxicity to the organ (or tissue). As the result, the organ falls into dysfunction. This situation is generally called as oxidative stress. Reperfusion after ischemia causes oxidative stress [4]. On the other hand, weight of the neoplasm occasionally twists the ovarian pedicle including ovarian blood vessels in ovarian tumor or ovarian cyst. This is the ovarian pedicle torsion. This ectopia causes tissue necrosis through ischemia in course of time. Generally surgical detorsion (taxis) to restart the ovarian blood flow as soon as the diagnosis

[6]. In ischemic tissue, however, there is a risk to induce hyperoxygenation with reperfusion of blood. Ischemia induces neutrophil adhesion to endothelial tissue in the ischemic organ [4, 7]. Ability for ROS production is elevated in adhesive neutrophil [8]. When the blood flow restarts, respiratory burst with production of high amount of ROS such as superoxide and hypochlorous acid occurs in neutrophil [9]. These molecules induce oxidative stress. Tissue destruction is deteriorated by these reasons.

In our study, ovarian weight and ovulation number with induction of superovulation decreased in ovarian I/R treated rats compared to in the control. These results indicate depression of hormonal sensitivity to eCG and hCG. Marked degeneration of the ovarian tissue and ovulated ova was also observed. The SOD administration prevented these deteriorations. SOD chemically inactivates superoxide by its dismutation [10]. Thus, it is strongly thought that the ovarian I/R causes superoxide production and subsequently initiate ovarian dysfunction. Administration of a radical scavenger such as SOD is able to protect ovarian functions from oxidative stress with the I/R. On the other hand, it has been reported that other radical scavengers such as vitamin C and mannitol may protect from ovarian injury in the ovarian I/R [5]. Our data supported this report.

In conclusion, our results suggest that I/R of the ovary caused ovarian oxidative stress. The damage can be reduced by administration of a radical scavenger.

References

- 1) Higuchi Y. 2003. Chromosomal DNA fragmentation in apoptosis and necrosis induced by oxidative stress. *Biochem Pharmacol.* 66: 1527-1535.
- 2) Emerit I. 1994. Reactive oxygen species, chromosome mutation, and cancer: possible role of clastogenic factors in carcinogenesis. *Free Radic Biol Med.* 16: 99-109.
- 3) Grace PA. 1994. Ischaemia-reperfusion injury. *Br J Surg.* 81: 637-647.
- 4) Granger DN, Kvietys PR. 1993. Leucocyte-endothelial cell adhesion induced by ischemia and reperfusion. *Can J Pharmacol.* 71: 67-75.
- 5) Sağsöz N, Kisa U, Apan A. 2002. Ischaemia-reperfusion injury of rat ovary and the effects of vitamin C, mannitol and verapamil. *Hum Reprod.* 17: 2972-2976.
- 6) Hasiakos D, Papakonstantinou K, Kontoravidis A, Gogas L, Aravantinos L, Vitoratos N. 2008. Adnexal torsion during pregnancy: report of four cases and review of the literature. *J Obstet Gynaecol Res.* 34: 683-7.
- 7) Yoshida N, Granger DN, Anderson DC. 1992. Anoxia/reoxygenation induced neutrophil adherence to cultured endothelial cells. *Am J Physiol.* 262: 1891-1898.
- 8) Kaminski KA, Bonda TA, Korecki J, Musial WJ. 2002. Oxidative stress and neutrophil activation -the two keystones of ischemia/reperfusion injury. *Int J Cardiol.* 86: 41-59.
- 9) Hammond B, Kontos HA, Hess ML. 1985. Oxygen radicals in the adult respiratory distress syndrome, in myocardial ischemia and reperfusion injury, and in cerebral vascular damage. *Can J Physiol Pharmacol.* 63: 173-187.
- 10) Hassan HM. 1988. Biosynthesis and regulation of superoxide dismutases. *Free Radic Biol Med.* 5: 377-385.