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Original Article

The role of resveratrol on full – Thickness uterine wound healing in rats

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ABSTRACT

Objective: Healing of the uterus after cesarean section and myomectomy operation is clinically important. In this study, we aimed to investigate the effects of resveratrol (3,5,4′-*o*-trihydroxystilbene) on the wound healing process of the uterus in rats treated with resveratrol following full thickness injury of the uterus.

Materials and methods: Twenty-one female wistar albino rats were divided randomly into three groups (1) control group with no intervention (2) injury group with uterine full thickness injury (3) resveratrol group with uterine full thickness injury and treated with resveratrol. Resveratrol was injected by oral gavage at the doses of 0.5 mg/kg/day for 30 days following uterine full thickness injury. Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) distributions were assessed using the immunohistochemical methods in tissue and ELISA methods in the tissue homogenate. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were evaluated with colorimetric method and malondialdehyde (MDA) levels also were measured using high performance liquid chromatography in the tissue homogenate. The effects of resveratrol on the uterine histology also were evaluated histologically with the light microscopy.

Results: Histological evaluation and immunohistochemical evaluations showed that treatment with a resveratrol significantly increased the thickness of the uterine wall and VEGF expression and decreased expression PDGF during wound healing. Biochemically, GPx and SOD activities were increased significantly after treatment with resveratrol. Additionally, resveratrol administration decreased MDA levels.

Conclusion: These results showed that the antioxidant effects of resveratrol has been shown to have a positive influence on wound healing of the uterus.

Introduction

Cesarean deliveries are the most common surgical procedure applied among women worldwide and major according to the latest international statistics. It has been reported that cesarean delivery is rate of 30% of all births previous abdominal operations like hysterotomy, laparoscopic processes and caesarean section may lead to intraperitoneal adhesions and this situation also may cause to preterm deliveries, future infertility and miscarriages [1]. Uterine scar rupture may result in mortality for this reason, healing of the wound site in the uterus is clinically important [2–4]. Because of difficulty to obtain tissue after hysterectomy, uterine tissue remodeling process has not been fully clarified. To the our knowledge, there is only one study in the literature on the effect of antioxidant on uterine wound healing as published our previous study [5].

During the wound healing process, various growth factors, hormones and cytokines take part. Of the growth factors, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF) and platelet-derived growth factor

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(PDGF) are particularly important. In addition, recent studies showed that reactive oxygen species (ROS) are main regulators of this process. Under the physiologic conditions, the formation and elimination of ROS are in balance. ROS are played for the protect from pathogenesis of the disease. While low levels of hydrogen peroxide are important for efficient wound angiogenesis and are actived many cellular signal pathway, high level of hydrogen peroxide produces the hydroxyl radical forming oxidized proteins and lipid and causes fractures in DNA [6–8]. Increase in the oxidative enzymes activity and/or reduction in the antioxidative enzymes activity cause oxidative stress and this situation leads to cell damage, non-wound healing in the pathogenesis of chronic, premature aging and even neoplastic transformation. Therefore, ROS production and elimination is essential for the normal repair process [6]. The defense system for decreasing ROS is achieved by a variety of exogenous and endogenous low molecular weight antioxidants. Defense sources of low molecular weight antioxidants include the glutathione peroxidase, superoxide dismutase and catalase. Superoxide radical anions are produced by various oxidases, are dismutated to H_2O_2 and water by superoxide dismutase (SOD). Subsequently H_2O_2 converts to water and oxygen by catalase, glutathione peroxidase (GPx) [9,10].

Resveratrol is a polyphenolic compound belonging to the subgroup of stilbenes found in grape skins, peanuts, mulberries and red wine. Resveratrol has antioxidant, anti-inflammatory, cardioprotective, cytoprotective, anti-cancer, hepatoprotective effects and also protects vascular endothelial function [11,12].

Our previous study [13] and other studies [14,15] have shown that resveratrol may have a beneficial effect on incisional wound healing. Additionally, it has been shown that resveratrol may have the anti-adhesion effect in a rat uterine horn model [16,17]. In literature, to date there is no study investigating the effect of resveratrol on uterine wound healing.

The aim of this study was to evaluate the effects of resveratrol on the wound healing process of the uterus in rats treated with resveratrol after a full thickness injury. With this aim, we investigated: (I) the distributions of angiogenetic factors VEGF and PDGF with immunohistochemical analysis in the uterus tissue (II) levels of VEGF and PDGF with ELISA methods in the tissue homogenate (III) activities of antioxidant parameters GPx and SOD with colorimetric assay kit in the tissue homogenate (IV) level of malondialdehyde (MDA) as a lipid peroxidation product with the high performance liquid chromatography (HPLC) method in the tissue homogenate.

Materials and methods

Animals

All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the care and use of laboratory animals and were approved by Ethics Committee of the Research of Laboratory Animals, Dokuz Eylul University, Medical School (Izmir, Turkey; approval number; 47/2012). All procedures were performed in accordance with the principles of laboratory animal care.

Twenty-one female, non-pregnant Wistar albino rats were used (aged 10 weeks and weight 200–220 g). All rats were housed in separate cages in a 22–24 °C temperature-controlled room. The rats were given a standard laboratory diet and water *ad libitum* and were maintained to have free access to water and standard rat chow.

The rats were randomly separated into three groups: a control group, an injury group and a resveratrol treatment group, each having seven rats. A control group with no intervention and medication (2) an uterine injury group with uterine full thickness injury (3) a resveratrol group with uterine full thickness injury and treated with a dose of 0.5 mg/kg/day resveratrol for 30 days.

Experimental procedure: full-thickness injury model in rats

We performed the full-thickness injury model in rats as modified from Micili et al. [5] and as described previously by Lin et al. [18]. In order to standardize the hormonal changes in rats, their menstrual cycle was determined by vaginal smears and the experiment was started on day of diestrus phase of rats.

Briefly, the rats were anaesthetized by intraperitoneal injection of 35 mg/kg of ketamine hydrochloride and 5 mg/kg of xylazine hydrochloride. After sterile preparation with 70% ethanol, an incision was performed in the abdominal wall in all groups, except control group. Then, a full-thickness injury was formed by incising a segment of approximately 1.0 cm in length and 0.5 cm in width from each uterine horn, leaving the mesometrium intact. The edges of the uterine defect were marked with a 4-0 nylon line. The abdominal incision was closed with a monofilament 3/0 polyglactin suture for the peritoneum and 2/0 polyglactin suture for the skin. Then, rats were housed in cages under a heating lamp to maintain the body temperature at approximately 37 °C and allowed to recover completely from anesthesia. All rats were treated with intramuscular injection of penicillin (80,000 units/100 mg) for 3 days after the surgery [5].

Injection of resveratrol

Resveratrol (0.5 mg/kg/day; Resveratrol, 99% pure, from Sigma R5010, 3050 Spruce Street, Saint Louis, MO 63103, USA) was administered through an oral gavage for 30 days after uterine incising. Fresh resveratrol was prepared according to the manufacturer's protocol.

At the end of the 30th day, the rats were anaesthesized, relaparatomy was performed, the extent and severity of intra-abdominal adhesions were recorded and animals were sacrificed. The injury region of each left uterine horn was excised for histological evaluation, right uterine horns of each rat in all study groups were removed for biochemical examination.

Histochemical analysis

For histochemical analysis all sections were performed with Hematoxylin-eosin using routine procedures. After removing tissue samples in left uterine horn, they were kept in 10% formalin and then were blocked in paraffin. Paraffin blocks were sectioned at 5 μm serial sections using a rotary microtome [RM 2135, Leica, Nussloch, Germany] with disposable metal microtome blades (Type N35, Feather Company, Osaka, Japan). Then the serial sections were stained with hematoxylin-eosin for histomorphological assessment under the light microscope. The images were analyzed using a computer assisted image analyzer system consisting of a microscope [BX-51, Olympus, Tokyo, Japan] equipped with a high-resolution video camera [DP-71, Olympus, Tokyo, Japan].

Immunohistochemical assessment of VEGF and PDGF

For VEGF and PDGF immunohistochemistry, sections were deparaffinized at 60 °C overnight and xylene for 30 min. Sections were first rehydrated in a series of baths with decreasing amounts of ethanol. Sections were washed with distilled water for 10 min and then were treated with phosphate buffered saline (PBS) for 10 min. Sections were incubated with trypsin (Cat No: 00-3008 Digest All 2A, Zymed, San Francisco, CA, USA) at 37 °C for 15 min. To