Taiwanese Journal of Obstetrics & Gynecology 56 (2017) 657–663

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

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

Oya Sayin [a, *], Serap Cilaker Micili [b], Asli Goker [c], Gonca Kamaci [d], Bekir Ugur Ergur [b], Osman Yilmaz [d], Gul Guner Akdogan [e]

[a] Research Laboratory, Dokuz Eylul University, Faculty of Medicine, Izmir, Turkey
[b] Department of Histology and Embryology, Dokuz Eylul University, Faculty of Medicine, Izmir, Turkey
[c] Department of Obstetrics and Gynecology, Celal Bayar University, Faculty of Medicine, Manisa, Turkey
[d] Department of Laboratory Animal Science, Dokuz Eylul University, Faculty of Medicine, Izmir, Turkey
[e] Health Science Institute, Izmir University of Economics, Izmir, Turkey

ARTICLE INFO

Article history:
Accepted 28 July 2017

Keywords:
Glutathione peroxidase
Malondialdehyde
Resveratrol
Uterine injury
Wound healing

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

* Corresponding author. Dokuz Eylul University, Research Laboratory, School of Vocational Health Service, Inciraltı, 35340, Izmir, Turkey. Fax: +90 232 412 8682.
E-mail address: oya.sayin@deu.edu.tr (O. Sayin).

http://dx.doi.org/10.1016/j.tjog.2017.08.015

(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

inhibit endogenous peroxidase activity, sections were incubated in a solution of 3% H_2O_2 for 15 min and then with normal serum blocking solution. VEGF (bs-1313R, Bioss) and PDGF (bs-0196R, Bioss), antibodies were used. Sections were incubated with the primary antibodies VEGF (bs-1313R, Bioss) and PDGF (bs-0196R, Bioss) in a humid chamber for 18 h at +4 °C, after washing with PBS three times. Sections were incubated in biotinylated IgG, and then with streptavidin conjugated to horseradish peroxidase for 15 min each prepared according to kit instructions (85-9043, Invitrogen Corporation, Camarillo, UK). Sections were finally stained with DAB (diaminobenzidine) (1718096, Roche, Mannheim, Germany) and counterstained with Mayer hematoxylin and were evaluated with a light microscope [5].

Homogenization of rat tissues for biochemical examinations

All tissues were homogenized using a Tissue Lyser (Qiagen, UK) in 10 volumes of tissue extraction buffer composed of 100 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% Sodium deoxycholate, Protease inhibitor cocktail. The homogenate samples were centrifuged at 2500×*g* for 15 min. The supernatants were removed to another tube for malondialdehyde (MDA) analysis. Then, homogenates were centrifuged further at 12000×*g* for 5 min at 4 °C. Supernatants were used for GPx and SOD activity analysis. Supernatants' protein levels were detected using bicinchoninic acid protein (BCA) assay kit (Thermo, Cat No: BCA, Protein assay kits, 23225, Rockford, USA). All homogenates were stored at −80 °C until the day of analysis [19].

Analysis GPx and SOD in rat tissues

The glutathione peroxidase (GPx) activity was assayed using a colorimetric assay kit (BioAssay Systems) according to the manufacturer's instructions. GPx activity is measured indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione (GSSG), produced upon reduction of an organic hydroperoxide by GPx, is recycled to its reduced state by GR and NADPH. This assay measures NADPH consumption in the enzymes coupled reactions. The reaction was read at 340 nm using a Synergy HT, Multi-Detection Microplate Reader, BIO-TEK plate reader. GPx activity was expressed as U/mg protein.

The SOD activity was evaluated using a colorimetric assay kit (BioAssay Systems) according to the manufacturer's instructions. The superoxide dismutase assay kit measures the dismutation of superoxide radicals generated by xanthine oxidase and xanthine. Superoxide Dismutases are enzymes that catalyze the dismutation of superoxide into O_2 and H_2O_2.Superoxide radicals reacts with a dye to form a colored product. The color intensity was measured to determine the SOD activity. The reaction was read at 440 nm using a Synergy HT, Multi-Detection Microplate Reader, BIO-TEK plate reader. SOD activity was expressed as U/mg protein.

Analysis of MDA in rat tissues

The malondialdehyde levels (MDA) is a breakdown product of lipid peroxidation. MDA was commonly measured by derivatization with thiobarbituric acid (TBA) to yield a red compound. At low pH and elevated temperature, MDA readily participates in nucleophilic addition reaction with thiobarbituric acid (TBA), generating a red fluorescent MDA:TBA complex. The TBA-MDA complex was analyzed using high performance liquid chromatography (HPLC) (Shimadzu VP Series, Tokyo, Japan) as described previously [20]. The MDA levels were expressed as nmol/mg protein.

Analysis of VEGF and PDGF in rat tissues

Enzyme-linked immunosorbent assay (ELISA) kits were used to evaluate vascular endothelial growth factor (CUSABIO, CSB-E04757r, limit determination 3.9 pg/ml–250 pg/ml) and platelet-derived growth factor (CUSABIO, CSB-E14313r, limit determination 0.312 pg/ml–20 pg/ml) levels in homogenized tissue. All assays were performed in accordance with the manufacturers' protocol. Rat VEGF and PDGF specific monoclonal antibodies were precoated wells. The sample of homogenized tissue was added to well which was precoated with VEGF and PDGF monoclonal antibody and was incubated and washed with PBS buffer. Then was added biotin-conjugated antibody and Avidin conjugated to Horseradish Peroxidase (HRP). Then a TMB substrate (3,3′,5,5′ tetramethyl-benzidine) substrate solution was added to each well. HRP catalyzed TMB substrate to exhibit change in color and enzyme–substrate reaction was terminated by the addition of a acid stop solution. The color change was measured at a wavelength of 450 nm using a Synergy HT, Multi-Detection Microplate Reader, BIO-TEK plate reader. All results are expressed as pg/mg protein.

Statistical analysis was performed using SPSS 15.0 for Windows. Data expressed as mean ± SD. Differences between the two groups were analyzed by using the Mann–Whitney U-test. A value of $p < 0.05$ was considered statistically significant.

Results

Histological analysis

We examined whether treatment with resveratrol protects uterine wound healing after injury. We evaluated the histological structures of the uterine horns by hematoxylin-eosin staining. The endometrium, myometrium and perimetrium layers were defined histologically in control group.

In both groups, the simple columnar epithelium of the uterus lumen was similar to that in the control group. In the myometrium of the uterine injury group, there was no perimetrium layer and there was defective scar area. In the resveratrol treatment group, the histological layers of uterus were similar to the control group (Fig. 1).

Uterine wall thickness measurements

We observed that the uterine wall thickness in uterine injury group (641.1 ± 205.1 μm) significantly decreased when compared to the control group (917.3 ± 280.9 μm) ($p = 0.001$). Resveratrol treatment (867 ± 239.0 μm) significantly increased the uterine wall thickness compared to the uterine injury group (641.1 ± 205.1 μm) ($p = 0.008$) (Fig. 1).

Immunohistochemical analysis of VEGF and PDGF

The effects of resveratrol on uterine injury were evaluated by VEGF and PDGF immunohistochemical staining. VEGF and PDGF immunoreactivities were evaluated in randomized five field in each group (×400 Magnification). In uterine injury group (1.57 ± 0.53) the VEGF immunoreactivity significantly increased when compared to control group (0.28 ± 0.48) ($p = 0.000$). Treatment with resveratrol (2.24 ± 0.48) significantly increased VEGF immunoreactivity when compared to uterine injury group (1.57 ± 0.53) ($p = 0.045$) (Fig. 1). Compared to the control group (0.28 ± 0.48) increased PDGF immunoreactivity was observed in the uterine injury group (1.42 ± 0.78) ($p = 0.009$). Resveratrol group (0.42 ± 0.53) significantly decreased PDGF immunoreactivity when compared to the injury group (1.42 ± 0.78) ($p = 0.035$).

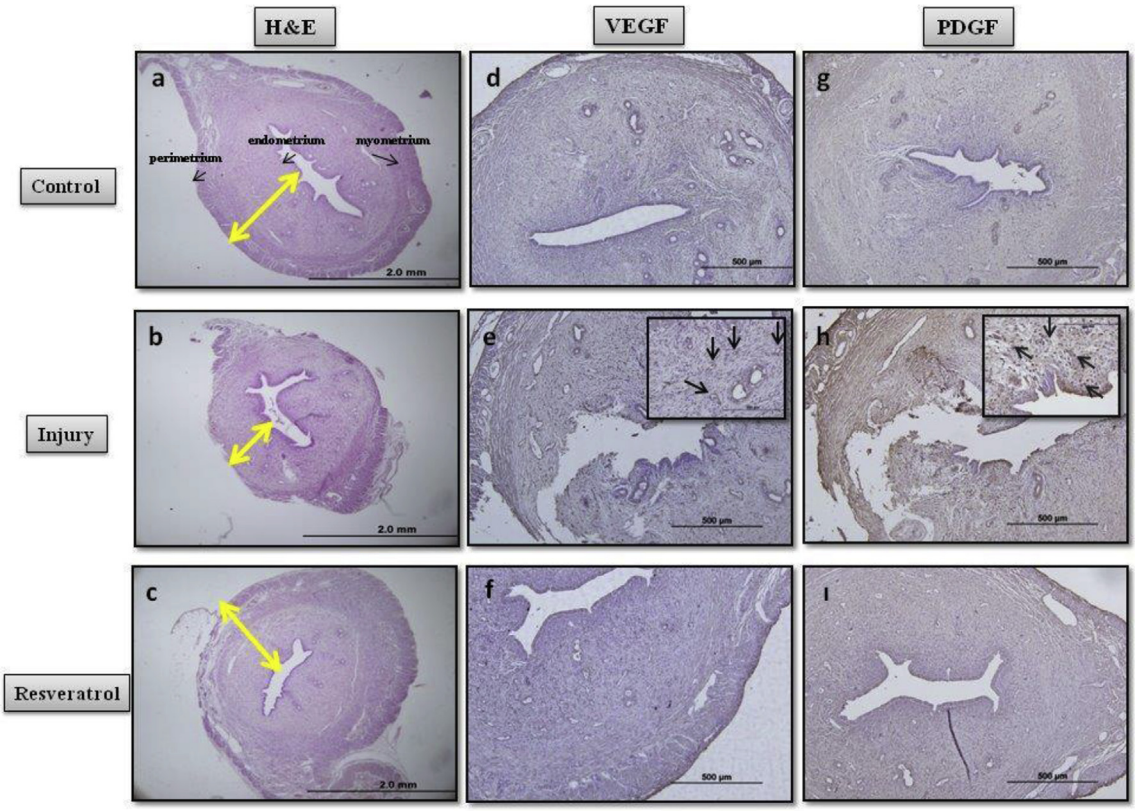

Fig. 1. (a, b, c): H&E staining of groups. Yellow arrows show thickness of uterus. (d, e, f): VEGF and (g, h, i):PDGF immunostaining. Black arrows show immunopositive cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Biochemical analysis

To investigate the effects of resveratrol after uterine injury, we evaluated GPx and SOD enzyme activities and MDA, VEGF and PDGF levels in tissue homogenate. GPx enzymes activity in uterine injury group (51.1 ± 0.99 U/mg protein) was significantly lower than in control group (71.3 ± 1.81 U/mg protein) ($p = 0.001$). Resveratrol treatment (65.4 ± 1.37 U/mg protein) increased significantly GPx enzymes activity when compared to the uterine injury group (51.1 ± 0.99 U/mg protein) ($p = 0.001$) (Fig. 2).

Similarly, when compared to the control group (61.6 ± 2.34 U/mg protein), decreased SOD enzymes activity was shown in uterine injury group (46.4 ± 1.30 U/mg protein) ($p = 0.001$). Resveratrol (58.3 ± 1.68 U/mg protein) increased SOD enzyme activity when compared to the uterine injury group (46.4 ± 1.3 U/mg protein) ($p = 0.001$) (Fig. 2).

We observed that MDA level (11.77 ± 0.81 nmol/mg protein) in uterine injury group was significantly increased compared to the control group (6.12 ± 0.51; nmol/mg protein) ($p = 0.001$). Treatment with resveratrol (7.48 ± 0.78 nmol/mg protein) significantly decreased MDA level compared to the uterine injury (11.77 ± 0.81 nmol/mg protein) ($p = 0.004$) (Fig. 2).

In uterine injury group (6.79 ± 0.21 pg/mg protein), VEGF level significantly increased when compared to the control group (3.29 ± 0.32 pg/mg protein) ($p = 0.000$). Resveratrol-treated group (8.15 ± 0.39 pg/mg protein) was observed a significant increase in VEGF level when compared to the uterine injury (6.79 ± 0.21 pg/mg protein) ($p = 0.008$) (Fig. 3).

PDGF level showed significant increase in uterine injury group (6.69 ± 0.8 pg/mg protein) compared to the control group (3.64 ± 0.7 pg/mg protein) ($p = 0.017$). Resveratrol treatment (5.63 ± 0.3 pg/mg protein) decreased significantly PDGF level compared to the injury group (6.69 ± 0.8 pg/mg protein) ($p < 0.05$) (Fig. 3).

Discussion

Wound healing is a process which consists of cellular, physiological and biochemical regeneration that develops in response to tissue injury. The wound healing consists of four phases involving blood clotting, inflammation, new tissue formation and tissue remodeling [13,21].

A variety of abdominal operations, particularly hysterectomies and cesarean sections, may cause uterine injuries and scar and also lead to infertility, amenorrhea, pregnancy loss and uterine rupture. Uterine rupture may result in maternal and neonatal morbidity and mortality [22–24]. Wound healing of the uterus is vitally important in order to maintain patient's reproductive life later. Our previous [5] and current study showed that antioxidants may have beneficial effect on uterine wound healing. The findings of our study are: (1) Full thickness uterine injury increases VEGF immunoreactivity and decreases GPX, SOD enzymes activities and increased MDA level (2) Resveratrol administration improves histological, immunohistochemical and biochemical results of injury.

In recent years, there have been various experimental studies showing resveratrol has beneficial effects. There have been studies related to effect on wound healing of internal organs of resveratrol [13,14]. In addition, two studies demonstrated an inhibiting effect of resveratrol on postoperative peritoneal adhesion formation in a rat uterine adhesion model [16,17]. These are via the anti-inflammatory, anti-adhesive and antioxidant effect of resveratrol. The aim of this study is to investigate the effects of resveratrol on the wound healing process of the uterus in rats treated with resveratrol after the incision of the uterus.

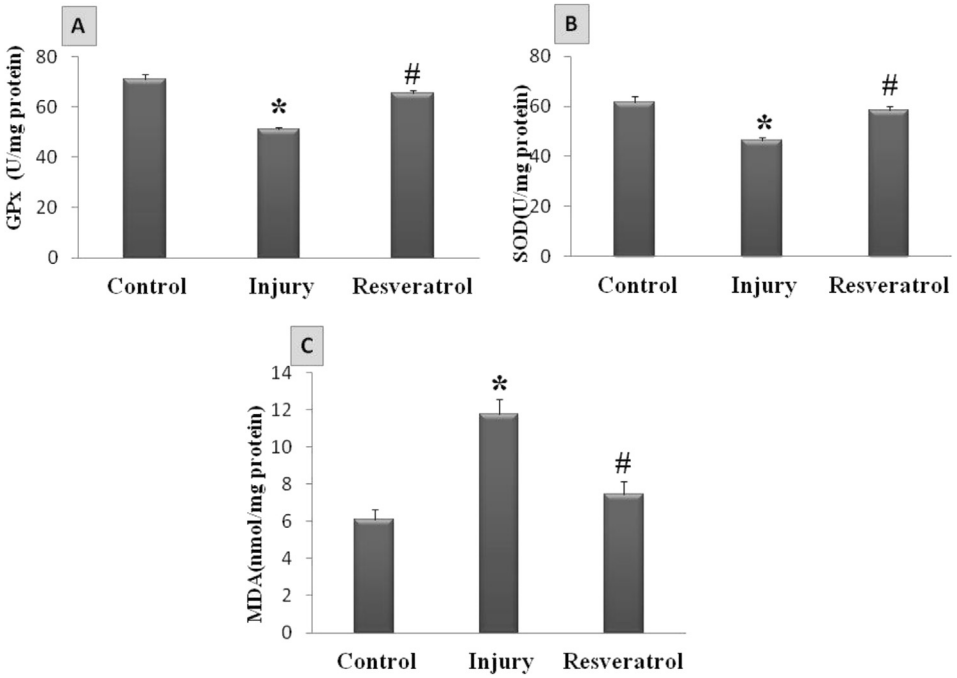

Fig. 2. (A) GPx level, (B) SOD level and (C) MDA level of groups. Data are means ± SD; $^{*}p < 0.05$ compared to Control group, $^{\#}p < 0.05$ compared to Injury group.

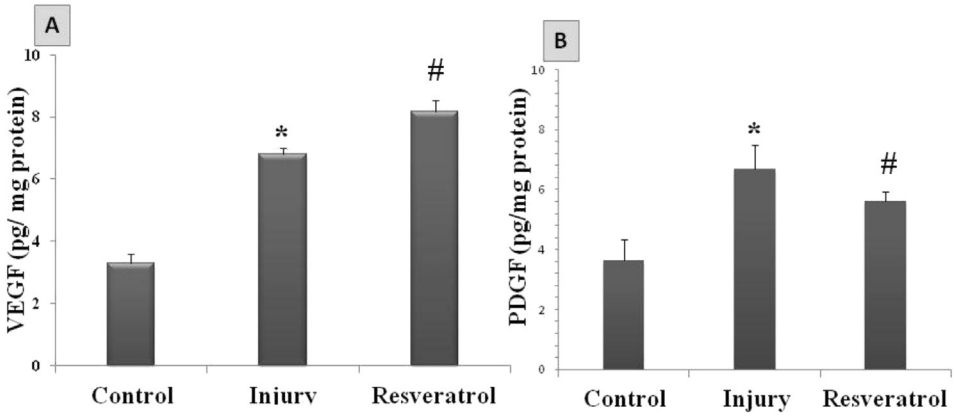

Fig. 3. (A) VEGF level and (B) PDGF level of groups. Data are means ± SD; VEGF level $^{*}p < 0.05$ compared to Control group, $^{\#}p < 0.05$ compared to Injury group. PDGF level $^{*}p < 0.05$ compared to Control group, $^{\#}p < 0.05$ compared to Injury group.

Previous studies showed that administration of basic fibroblast growth factor [25] 2011) and lipoic acid [5] improved the structure and function of uterine endometrium, muscular cells and vascularization in the rat uterine full-thickness injury model. Our findings showed that administration of resveratrol increased uterine thickness compared to the control group. This result is consistent with previous reports about the full-thickness injury model in rat uterus.

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 (PDGF) are particularly important. In the process of wound healing, growth factors regulate such processes as cell migration, synthesis of extracellular matrix proteins, proliferation and differentiation of endothelial cells in addition to angiogenesis [21]. VEGF plays a central role in endometrial neoangiogenesis during postmenstrual/postpartum repair [26]. Lin et al. [18] reported that administration of collagen-binding VEGF to a rat scarred uterus induced remodeling of the scarred uterus including the regeneration of endometrium, muscular cells, and vascularization and improved pregnancy outcomes. Micili et al. [5] showed that in groups treated with lipoic acid the percentage of VEGF immunoreactivity in a full thickness uterine injury model in rats was increased. We have also found significant increase in VEGF immunoreactivity and level by using immunohistochemical and biochemical methods in uterine injury group. In addition, we have found significant increase in VEGF immunoreactivity and level in groups treated with resveratrol when compared to uterine injury group. We have determined that groups treated with resveratrol decreased PDGF immunoreactivity and level. To the best of our knowledge, this is the first study in the literature investigating the effect of resveratrol with VEGF and PDGF immunoreactivities on wound healing in a full thickness uterine injury model in rats. These growth factors regulate cellular

proliferation, differentiation and migration, and the synthesis of extracellular matrix proteins as well as angiogenesis during wound healing. Resveratrol affects angiogenesis through different signaling pathways. It is speculatived that the effects of resveratrol on different cell types are not only dependent on its concentration but also on the physical and chemical conditions surrounding cells [27]. It was found that different concentrations of resveratrol showed different effects on VEGF expression even in the same cell. For example, Wang H et al. [28] shown that low concentration of resveratrol increased VEGF expression and angiogenesis promotion while high concentration of resveratrol decreased VEGF expression and angiogenesis inhibition in HUVEC. In our study shown that this dose of resveratrol could be a reducing effect on PDGF. We think that resveratrol increases wound healing through VEGF as the most potent stimulator.

Inflammatory cells, for example neutrophils, macrophages, endothelial cells and fibroblasts produce reactive oxigen species in wound healing process. Low levels of ROS are important for cellular signaling, especially for angiogenesis. Vascular repair and angiogenesis are the crucial factors for tissue repair and regeneration. On the other hand, excessive amounts or impaired detoxification of ROS causes oxidation products of lipids, proteins or DNA and result in cell damage [6]. MDA is a product of lipid peroxidation and is produced as a result of lipid peroxidation or the toxic effects of reaction oxygen radicals with unsaturated fatty acids in membranes [29]. Therefore, balance of ROS production and detoxification is very important for normal wound healing process. However, endogenous molecules, such as glutathione, ubiquinones, uric acid, and lipoic acid, and ROS-detoxifying enzymes such as superoxide dismutase, catalase and glutathione peroxidases are required for the regulation of cellular redox balance [6]. We therefore evaluated the effect of resveratrol on the antioxidant enzymes which has previously not been investigated in the full thickness wound healing. The results of our study showed that there is an increase in activities of glutathione peroxidase and superoxide dismutase enzymes in treated resveratrol group. In addition, MDA level in treated resveratrol group were significantly decreased compared to the uterine injury group. Resveratrol has anti-inflammatory, antioxidant, cytoprotective, anti-cancer and cardioprotective effects. Antioxidant effect of resveratrol has been shown via two different cytoprotective mechanism; it directly scavenges free radicals (i.e. hydroxyl radical and superoxide anion) and increases the activity of antioxidant enzymes [8,30]. Our results showed that treatment with resveratrol has positive effects on wound healing in a full thickness uterine injury model in rats. This may be due to the fact that resveratrol has protective effects by increasing activities of antioxidant enzymes such as GPx and SOD and decreasing MDA levels of lipid peroxidation product.

Achieving a more rapid wound healing is important if the next pregnancies are to be healthier and the time between the two pregnancies is to be shorter. Buhimschi et al. [4] reported that uterine scar could be a site of active remodeling even 15 days post-cesarean delivery (7.5 months in human equivalent) and also these changes were phenotypically dependent in mouse model. In their model, they represented approximately life span of mice 60 days is equivalent to 2.5 years in humans. Andreollo et al. [31] converted 16.7 rat days to 1 human year. In the literatüre studies showed that women who attempt vaginal birth after cesarean delivery at less than 2 years post-cesarean delivery have a higher risk of uterine rupture [4,32]. Our previous study [5], we demonstrated that lipoic acid was found to be effective in enhancing wound healing in uterine full thickness injury at 30 days when compared to 15 days, and therefore, we chose the day that corresponds to 30 days of 2 years to assess the effect of resveratrol in this study.

In the literature, doses of resveratrol administered ranged from 0.5 to 20 mg/kg orally per day in animal models. It has been shown that a dose of 0.5 mg/kg resveratrol can ensure systemic protective effects in rats. Yaman et al. [13] have given a single daily dose of 0.5 mg/kg of resveratrol on incisional wound healing in rats and had not monitored any side effects in their study. Therefore, we used 0.5 mg/kg of resveratrol daily in rat similarly to Yaman et al.'s administration.

In conclusion, the uterus has a fundamental function in sperm migration, embryo implantation and fetal nutrition. Diseases and traumas cause serious damages, which lead to scars in uterus, resulting in amenorrhea, infertility, pregnancy loss and rupture of the uterus. Healing of the wound site in the uterus is clinically important. Achieving a more rapid wound healing is important if the next pregnancies are to be healthier and the time between the two pregnancies is to be shorter. We have investigated the effects of resveratrol on wound healing in a full thickness injury model. The present study has shown that resveratrol may have positive effects on uterine wound healing. The results of this study indicate that resveratrol may confer positive effect on wound healing by increasing the activities of SOD and GPx antioxidant enzymes and the decreasing MDA level. These beneficial effects of resveratrol may be used to accelerate the regeneration in clinic.

Funding source

This study was supported by a grant (number 113S829) from The Scientific and Technological Research Council Of Turkey (TUBİTAK).

Conflicts of interest

The authors have no conflict of interest to declare relevant to this article.

References

[1] El-Khayat W, Elsharkawi M, Hassan A. A randomized controlled trial of uterine exteriorization versus in situ repair of the uterine incision during cesarean delivery. Int J Gynaecol Obstet 2014;127(2):163–6.
[2] Cakir Gungor AN, Oguz S, Hacivelioglu S, Isik S, Uysal A, Gencer M, et al. Predictive value of striae gravidarum severity for intraperitoneal adhesions or uterine scar healing in patients with previous caesarean delivery. J Matern Fetal Neonatal Med 2014;27(13):1312–5.
[3] Micili SC, Göker A, Sayın O, Akokay P, Ergür BU. Lipoic acid decreases peritoneal adhesion formation in a rat uterine scar model. J Turk Ger Gynecol Assoc 2013 Jun 1;14(2):76–80.
[4] Buhimschi CS, Zhao G, Sora N, Madri JA, Buhimschi IA. Myometrial wound healing post-Cesarean delivery in the MRL/MpJ mouse model of uterine scarring. Am J Pathol 2010;177(1):197–207.
[5] Micili SC, Göker A, Sayın O, Akokay P, Ergür BU. The effect of lipoic acid on wound healing in a full thickness uterine injury model in rats. J Mol Hist 2013;44:339–45.
[6] Schäfer M, Werner S. Oxidative stress in normal and impaired wound repair. Pharmacol Res 2008;58(2):165–71.
[7] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44–84.
[8] Sayin O, Arslan N, Güner Akdoğan G. The protective effects of resveratrol on human coronary artery endothelial cell damage induced by in vitro hydrogen peroxide. Acta Clin Croat 2012;51(2):227–35.
[9] Peng C, Wang X, Chen J, Jiao R, Wang L, Li YM, et al. Biology of ageing and role of dietary antioxidants. Biomed Res Int 2014;2014, 831841.
[10] Sayin O, Arslan N, Altun Z, Guner G. In vitro effect of resveratrol against oxidative injury of human coronary artery endothelial cells. Turk J Med Sci 2011;41:1–8.
[11] Shakibaei M, Harikumar KB, Aggarwal BB. Resveratrol addiction: to die or not to die. Mol Nutr Food Res 2009;53(1):115–28.
[12] Sayin O, Arslan N, Güner G. Resveratrol ve Kardiyovasküler sistem. Turkish J Biochem 2008;33(3):117–21.
[13] Yaman I, Derici H, Kara C, Kamer E, Diniz G, Ortac R, et al. Effects of resveratrol on incisional wound healing in rats. Surg Today 2013;43(12):1433.
[14] Bedirli A, Salman B, Pasaoglu H, Ofluoglu E, Sakrak O. Effects of nuclear factor-kB inhibitors on colon anastomotic healing in rats. J Surg Res 2011;171(1):355–60.

[15] Bashmakov YK, Assaad-Khalil S, Petyaev IM. Resveratrol may be beneficial in treatment of diabetic foot syndrome. Med Hypotheses 2011;77(3):364–7.
[16] Orçan S, Seven A, Isık H, Timur H, Caydere M, Ustün H, et al. Resveratrol inhibits postoperative adhesion formation in a rat uterine horn adhesion model. Hum Fertil (Camb) 2012;15(4):217–20.
[17] Ustün Y, Engin-Ustün Y, Ovayolu A, Meydanli MM, Temel I, Kafkasli A. The effect of resveratrol on prevention of the development of postoperative adhesions in a rat model. Gynecol Endocrinol 2007;23(9):518–22.
[18] Lin N, Li X, Song T, Wang J, Meng K, Yang J, et al. The effect of collagen-binding vascular endothelial growth factor on the remodeling of scarred rat uterus following full-thickness injury. Biomaterials 2012;33(6):1801–7.
[19] Wiechelman KJ, Braun RD, Fitzpatrek JD. Investigation of bicinchoninic acid protein assay: identification of the groups responsible for color formation. Anal Biochem 1988;175:231–7.
[20] Chang CY, Hu ML. Total plasma malondialdehyde levels in 16 Taiwanese college students determined by various thiobarbituric acid tests and an improved high-performance liquid chromatography-based method. Clin Biochem 2000;33(8):619–25.
[21] Koca Kutlu A, Ceçen D, Gürgen SG, Sayın O, Cetin F. A comparison study of growth factor expression following treatment with transcutaneous electrical nerve stimulation, saline solution, povidone-iodine, and lavender oil in wounds healing. Evid Based Complement Altern Med 2013;2013, 361832.
[22] Brahmalakshmy BL, Kushtagi P. Variables influencing the integrity of lower uterine segment in post-cesarean pregnancy. Arch Gynecol Obstet 2015;Apr;291(4):755–62.
[23] Song T, Zhao X, Sun H, Li X, Lin N, Ding L, et al. Regeneration of uterine horns in rats using collagen scaffolds loaded with human embryonic stem cell-derived endometrium-like cells. Tissue Eng Part A 2015; Jan;21(1–2):353–61.
[24] Li YR, Xiao SS, Wan YJ, Xue M. Analysis of the efficacy of three treatment options for cesarean scar pregnancy management. J Obstet Gynaecol Res 2014; Nov;40(11):2146–51.
[25] Li X, Sun H, Lin N, Hou X, Wang J, Zhou B, et al. Regeneration of uterine horns in rats by collagen scaffolds loaded with collagen-binding human basic fibroblast growth factor. Biomaterials 2011; Nov;32(32):8172–81.
[26] Fan X, Krieg S, Kuo CJ, Wiegand SJ, Rabinovitch M, Druzin ML, et al. EGF blockade inhibits angiogenesis and reepithelialization of endometrium. FASEB J 2008;22:3571e–80e.
[27] Wang H, Zhou H, Zou Y. Resveratrol modulates angiogenesis through the GSK3beta/betacatenin/TCF-dependent pathway in human endothelial cells. Biochem Pharmacol 2010;80:1386–95.
[28] Kamaleddin MA. The paradoxical pro- and antiangiogenic actions of resveratrol: therapeutic applications in cancer and diabetes. Ann N Y Acad Sci 2016;1386(1):3–15.
[29] Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Med Cell Longevit 2014;2014, 360438.
[30] De la Lastra CA, Villegas I. Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications. Biochem Soc Trans 2007;35:1156–60.
[31] Andreollo NA, Santos EFD, Araújo MR, Lopes LR. Rat's age versus human's age: what is the relationship? Arq Bras Cir Dig Rev Artic 2012;25(1):49–51.
[32] Stamilio DM, Defranco E, Paré E, Odibo AO, Peipert JF, Allsworth JE, et al. Short interpregnancy interval: risk of uterine rupture and complications of vaginal birth after cesarean delivery. Obstet Gynecol 2007;110:1075–82.