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Original Article

The effects of resveratrol on ovarian hyperstimulation syndrome in a rat model

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ABSTRACT

Objective: The aim of the present study was to investigate effects of resveratrol (RSV) over ovarian hyperstimulation syndrome (OHSS) in rat model.
Materials and Methods: 24 female Wistar rats (22 days old) were divided into four groups. Group 1 (control group; n = 6) received 0.1 ml intraperitoneal (IP) saline from days 22–26; group 2 (mild-stimulated group; n = 6) received 10 IU pregnant mare serum gonadotropin (PMSG) on day 24 and 10 IU of hCG 48 h later (day 26); group 3 (OHSS group; n = 6) was given 10 IU of PMSG for 4 consecutive days from day 22 and 30 IU hCG on the fifth day to induce OHSS; group 4 (OHSS + RSV group; n = 6) was treated the same as group 3, but received 60 mg/kg RSV 2 h before PMSG injection for 4 consecutive days and 2 h before the hCG injection on the fifth day.
Results: Weight gain was highest in the OHSS group. Ovarian weights were lower in the treatment group than OHSS group. Peritoneal fluid VEGF levels were lower for RSV group compared to group 2 and 3. Total VEGF immunoreactivity was higher in OHSS group than group 1, 2 and 4.
Conclusion: These results indicate that RSV is beneficial for prevention of OHSS by reducing the increases in body and ovarian weight and VEGF activity. These effects may be mediated by anti-inflammatory, anti-oxidant and anti-angiogenic capacity of RSV.

Introduction

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication in connection with ovarian stimulation for the treatment of infertility. OHSS occurs in 5–10% of patients undergoing ovulation induction, and severe form of the disease take place in 0.5–5% [1,2]. Serious complications such as renal failure, hypovolemic shock, thromboembolic events and adult respiratory distress syndrome may happen. Clinical symptoms of OHSS include severe extravascular fluid accumulation and hemoconcentration similar to syndromes owing to capillary leakage. In spite of the fact that the pathophysiology of OHSS has not been completely clarified, it is generally accepted that increased capillary permeability which is triggered by the release of vasoactive substances from hyperstimulated ovaries under the stimulation of human chorionic gonadotropin (hCG). The vascular endothelial growth factor (VEGF) system and its receptors have been identified as the main angiogenic factors responsible for increased capillary permeability and are therefore discussed as crucial for the occurrence of OHSS [3]. In addition the role of proinflammatory cytokines was investigated in the pathogenesis of OHSS and capillar permeability. Previous work has indicated that both serum and ascitic or follicular fluid from women with OHSS contained greater amounts of interleukin-6 (IL-6), a proinflammatory cytokine [4–6].

Resveratrol (RSV) (3,5,4′-trans-trihydroxystilbene) is a natural phytoalexin that is mostly found in peanuts, grapes, mulberries and red wine. This compound has miscellaneous pharmacological effects including anti-inflammatory, anti-proliferative, anti-oxidant,

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anti-platelet, anti-atherogenic, and anti-carcinogenic activities [7]. It has beneficial effects on aging, inflammation and metabolism, which are thought to result from the activation of the lysine deacetylase, sirtuin 1 (SIRT1), the cyclic adenosine monophosphate (c-AMP) pathway, or AMP-activated protein kinase [8]. RSV possesses multiple protective properties in the vasculature, including anti-oxidative and anti-inflammatory effects and improvement of endothelial function [9].

Since RSV has anti-inflammatory and anti-oxidant activities and protective properties on endothelial function and vasculature, the aim of the present study was to identify the effects of RSV in an animal model of OHSS. Therefore, it would be clarified whether this agent with anti-inflammatory, anti-proliferative and anti-oxidant properties can be used to prevent or overcome the negative effects of OHSS.

Materials and methods

The design of the present study was approved by the Experimental Research Board and Animal Care and Use Committee of Gazi University Faculty of Medicine with the reference number GUTF-11072. A total of 24 female Wistar rats were obtained from Gazi University Research Center animal laboratory. All research animals were treated in compliance with the guidelines for the care and use of animals approved by the institution in accordance to principles of laboratory animal care (NIH *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Researches, National Research Council, Washington, D.C.). The rats were caged in a controlled environment of 22 °C with 12 h light/dark cycles. Standard rat feed and reverse-osmosis-purified water were provided ad libitum. All of the rats were weighed at the beginning of the study and 48 h after the HCG injection.

Twenty two-day-old female rats (weight 30–50 g) were randomly divided into four groups: Group 1 (control group; n = 6) received 0.1 ml intraperitoneal (IP) saline from days 22–26; group 2 (mild-stimulated group; n = 6) received subcutaneous (SC) 10 IU pregnant mare serum gonadotropin (PMSG; Folligon®, Intervet) on day 24 and and 10 IU of hCG (Chorulon®, Intervet) 48 h later (day 26) to mimic a routine ovarian stimulation; group 3 (OHSS group; n = 6) was given 10 IU of PMSG for 4 consecutive days from day 22 and 30 IU hCG on the fifth day to induce OHSS; group 4 (OHSS + RSV group; n = 6) was treated the same as group 3, but received 60 mg/kg/d RSV (polygonum cuspidatum®, Solgar) via oral gavages 2 h before PMSG injection for 4 consecutive days and 2 h before the hCG injection on the fifth and sixth day. The OHSS animal model of this study was based on the model of a previous study by Ujioka et al. [10]. Resveratrol dosage administered in this trial was based on the results of a previous study [11].

48 h after the final hCG injection all of the rats were weighed and anesthetized with an intramuscular administration of 50 mg/kg ketamine hydrochloric acid (Ketalar; Eczacibasi Warner-Lambert Ilac Sanayi, Levent, Istanbul, Turkey) and 7 mg/kg xylazine hydrochloric acid (Rompun; Bayer, Sisli, Istanbul, Turkey). They were immobilized on a standard rat surgery board. Using aseptic technique, a ventral midline incision was made. Peritoneal lavage with 5 ml saline was performed to assess the VEGF and IL-6 levels in the peritoneal fluid such as described by Cenksoy et al. [12]. Saline administered to the intraperitoneal cavity was collected with a cannula after 30 s shaking without any tissue and vessel injury. Bilateral oophorectomy was done to measure the ovarian weights and for histopathologic examination. Afterwards all animals underwent euthanasia by taking intracardiac blood. Eventually, all animals were sacrificed 48 h after hCG injection on seventh day (day 28) (Table 1). All procedures were performed by a physician blinded to the groups.

VEGF and IL-6 levels in the peritoneal fluid were quantitatively assessed using commercially available enzyme-linked immunosorbent assay kits (RayBiotech®, Inc., Norcross, Georgia, USA) according to the manufacturer's instructions. The enzyme immunoassay has an intra-assay variability of <10% and inter-assay variability of <12% both for VEGF and IL-6. The minimum detectable doses were <10 pg/ml for VEGF and <3 pg/ml for IL-6.

Histopathologic examination was performed by a pathologist blinded to the groups. Formalin-fixed ovarian tissues were embedded in paraffin blocks, sectioned at 5-mm thickness (four sections per sample), stained with haematoxylin and eosin, and examined under a light microscope. Atretic follicles, antral follicles, and corpora lutea were evaluated. Tissue blocks were then sectioned, deparaffinized and rinsed under running tap water. Sections were incubated in 3% hydrogen peroxide for 10 min, rinsed under distilled water and pretreated at a high temperature in a microwave oven in Tris-EDTA buffer (pH 9) for 20 min. After waiting at room temperature for 20 min, sections were washed in PBS (phosphate buffered saline, pH 7.6) for 5 min. After application of the protein block for 10 min, sections were incubated with the primary antibody at room temperature for 1 h (VEGFAb-1 Rabbit Polyclonal Antibody, Thermo Scientific RB222) and then washed with PBS for 5 min. The sections were incubated for 20 min with a biotinylated secondary antibody, washed with PBS for 5 min, incubated with streptavidin/peroxidase complex for 20 min, washed with PBS for 5 min and incubated with AEC chromogen for 5 min. Tissue sections were analyzed for protein localization and staining intensity which was scored from negative (−) to faint (+), medium (++) and strong (+++).

Statistical analysis was performed using Statistical Package for the Social Sciences version 18.0 (SPSS, Chicago, IL, USA). Variables were expressed as mean ± standard deviation (SD). Normal distributions of continuous variables were assessed by Shapiro Wilk test. Non-normally distributed metric variables were analyzed by the Kruskal Wallis test, Mann–Whitney U-test with the post hoc Bonferroni correction. When the p value from the Kruskal Wallis test statistics are statistically significant Conover's non-parametric multiple comparison test were used to know which group differ from which others. P values of <0.05 were considered statistically significant.

Results

Table 2 shows the anatomical, biochemical and histomorphometrics of the study groups. Weight gain was the highest in the OHSS group; it was almost twice as much in the OHSS group as it was in the control and the OHSS + RSV groups (Table 2, Fig. 1). The hyperstimulation treatment caused an increase in ovarian weight in all treatment groups. Group 2 (0.13 g vs. 0.07 g, $p < 0.001$), group 3 (0.31 g vs. 0.07 g, $p < 0.001$) and group 4 (0.26 g vs. 0.07 g, $p < 0.001$) showed higher ovarian weights compared to control group. Group 3 (0.31 g vs. 0.13 g, $p < 0.001$) and group 4 (0.26 g vs. 0.13 g, $p < 0.001$) also exhibited higher ovarian weights than group 2. Ovarian weights of OHSS + RSV group were lower than that of OHSS group (0.26 g vs. 0.31 g, $p = 0.019$) indicating the protective role of RSV (Fig. 2).

Peritoneal fluid VEGF levels were also lower for OHSS + RSV group as compared to OHSS (33.1 pg/ml vs. 58.4 pg/ml, $p < 0.001$) and mildly stimulated groups (33.1 pg/ml vs. 47.2 pg/ml, $p < 0.001$) indicating diminished VEGF activity associated with RSV treatment. All treatment groups showed higher VEGF levels than control group ($p < 0.001$) (Fig. 3).

Peritoneal IL-6 levels were lower in control group than that of mildly stimulated (222.4 pg/ml vs. 5762.7 pg/ml) and OHSS (222.4 pg/ml vs. 5719.1 pg/ml) groups, as expected. OHSS + RSV