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PRACE ORYGINALNE
ginekologia

Montelukast is effective in preventing of ovarian hyperstimulation syndrome; an experimental study

Montelukast jest skutecznym lekiem w zapobieganiu zespołowi hiperstymulacji jajników; badania eksperymentalne

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Abstract

Objectives: *To determine the efficacy of montelukast in comparison with cabergoline in the prevention of ovarian hyperstimulation syndrome (OHSS) in rats.*

Material and methods: *An experimental OHSS model was formed in 35 female Wistar rats. Rats (22 days old) were randomized into 5 groups, each containing 7 animals. The control group received no therapy; the mild OHSS group was administered pregnant mare serum gonadotropin (PMSG) 10 IU for 4 days, hCG 10 IU on the 5th day; the severe OHSS group received PMSG 10 IU for 4 days, hCG 30 IU on the 5th day. The montelukast group: received montelukast 10 mg/kg/day and the cabergoline group was administered cabergoline 100μg/kg/day via oral gavage for 6 days (days 22–27), in addition to those of severe OHSS. All groups were sacrificed on 28th day. Body weight, ovarian diameter and weight, vascular permeability, vascular endothelial growth factor (VEGF), semiquantitative VEGF receptor-1, and VEGF receptor-2 (VEGFR-2) immunohistochemistry were evaluated.*

Results: *Ovarian diameter and VEGF expression were significantly lower in the montelukast and cabergoline groups than in the severe OHSS group. While montelukast was more effective in limiting vascular permeability in the severe OHSS, cabergoline was superior to montelukast with respect to the limiting effect on increased body weight and VEGFR-2 expression.*

Conclusions: *The VEGF/VEGFR-2 interaction plays an important role in OHSS pathogenesis. Montelukast limits VEGF expression, and cabergoline reduces both VEGF and VEGFR-2 expressions; they are both effective therapies for the prevention of severe OHSS.*

Key words: **ovarian hyperstimulation syndrome** / **VEGF, VEGFR-2** / **cabergoline** / / **montelukast** /

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Streszczenie

Cel: *Ocena skuteczności montelukastu w porównaniu z kabergoliną w zapobieganiu zespołowi hiperstymulacji jajników (OHSS) u szczurów.*

Materiał i metoda: *Model doświadczalny OHSS stanowiło 35szczurów rasy Wistar, płci żeńskiej. Szczury (22 dniowe) podzielono na 5 grup, każda zawierająca 7 zwierząt. Grupa kontrolna nie otrzymała żadnej terapii. Grupa z łagodnym OHSS otrzymała gonadotropinę z surowicy ciężarnych klaczy (PMSG) w ilości 10IU przez 4 dni, hCG 10IU w 5 dniu, grupa z ciężkim OHSS otrzymała PMSG 10IU przez 4 dni, hCG 30IU w 5 dniu. Grupa z montelukastem otrzymała montelukast w dawce 10mg/kg/dzień a grupa z kabergoliną otrzymała kabergolinę 100μg/kg/dzień przez doustny zgłębnik przez 6 dni (dni 22-27). Wszystkie zwierzęta zabito w 28 dniu. Oceniono masę ciała, wymiar i wagę jajników, przepuszczalność naczyń, czynnik wzrostu śródbłonka naczyń (VEGF) oraz w immunohistochemii półilościowo receptor – 1 VEGF i receptor-2 VEGF.*

Wyniki: *Wymiar jajnika oraz ekspresja VEGF były istotnie niższe w grupach z monelukastem i kabergoliną niż w grupie z ciężkim OHSS. Podczas gdy montelukast był bardziej skuteczny w ograniczaniu przepuszczalności śródbłonków w ciężkim OHSS, to kabergolina okazała się lepsza od montelukastu po uwzględnieniu ograniczającego efektu zwiększonej masy ciała i ekspresji VEGFR-2.*

Wnioski: *Wzajemne oddziaływanie VEGF/VEGFR-2 odgrywa istotną role w patogenezie OHSS. Montelukast ogranicza ekspresję VEGF, a kabergolina zmniejsza zarówno ekspresję VEGF jak i VEGFR-2; obie terapie są skuteczne w zapobieganiu ciężkiemu OHSS.*

Słowa kluczowe: **zespół hiperstymulacji jajników** / **VEGF** / **VEGFR-2** / **kabergolina** / / **montelukast** /

Introduction

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication of assisted fertilization techniques. It is characterized by cystic enlargement of the ovaries, increased vascular permeability, a fluid shift from intravascular to extravascular space, and ovarian neoangiogenesis. Increased vascular permeability causes ascites and pleural and pericardial effusion and results in hemoconcentration, reduced organ perfusion, and thromboembolic events. Severe OHSS, which may at times be life-threatening, is seen in 0.3–5% of stimulated cycles. OHSS occurs in conjunction with human chorionic gonadotropin (hCG) applications. It is rare without antecedent hCG administration [1, 2].

OHSS has no specific therapy. Its physiopathology is not entirely clear, but it has been reported that increased vascular permeability and vascularity in the region surrounding ovaries play a role [3]. The critical step is the stimulation of vascular endothelial growth factor (VEGF) m-RNA expression in luteinized granulosa cells by hCG administration [4]. VEGF exerts its angiogenic action via two tyrosine kinase receptors, namely VEGF receptor-1 (VEGFR-1 or Fms-like tyrosine kinase-1:Flt-1) and VEGF receptor-2 (VEGFR-2 or fetal liver kinase/kinase-insert domain receptor:Flk-1/KDR) [5, 6]. VEGF stimulates neoangiogenesis in the ovaries as well as vascular permeability via VEGFR-2 stimulus [4].

Cabergoline is a dopamine receptor 2 (Dp-r2) agonist. In low doses, it partially inhibits ovarian VEGFR-2 phosphorylation and reduces VEGFR-2 dependent vascular permeability increases without affecting luteal angiogenesis. It appears possible to reduce the risk of OHSS with prophylactic cabergoline use [4]. Currently, it is accepted that it does not affect oocyte maturation, rates of fertilization, and clinical outcomes [7].

Montelukast is a receptor antagonist of cysteinyl leukotrienes (cysLTs). CysLTs induce vascular permeability increases, causing plasma exudation. It is known that their receptor antagonists reduce VEGF expression. Hence, it has been proven that montelukast may prevent inflammation characterized by increased vascular permeability and plasma exudation in asthmatic airways [8, 9].

Therefore, we wondered whether montelukast, an agent that reduces VEGF expression, may be an alternative therapy in preventing severe OHSS. As far as we knew, no studies have been done examining the efficacy of montelukast in OHSS prophylaxis. The aim of our study was to examine the efficacy of montelukast in preventing OHSS, as evidenced by the changes occurring in vascular permeability, physical properties of the ovaries, and VGEF and its receptors, which have a key role in the physiopathology of OHSS. To validate the efficiency of montelukast in a more robust way, we compared it with cabergoline, an agent that is widely recognized as an established therapy in OHSS.

Materials and methods

This study was performed in Dokuz Eylul University Medical Faculty, Experimental Animals Research Center, in March 2014. It was approved by the Local Ethics Committee for Animal Research, and it complied with the standards for the care and use of laboratory animals published by the United States National Health Institutes.

Study design

The study was conducted on a total of 35 female Wistar rats of 21 days of age under laboratory conditions. The animals were exposed to 12-hour dark, 12-hour light cycles (light cycle between 07:00-19:00), fed with standard feed and water ad libitum. Animals reaching 22 days of age were weighed. The animals were then randomized into 5 groups, each containing 7 animals. The control group received no therapy; the mild OHSS

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group was administered pregnant mare serum gonadotropin (PMSG) 10 IU for 4 days and hCG 10 IU on the 5th day. The severe OHSS group received PMSG 10 IU for 4 days and hCG 30 IU on the 5th day. The montelukast group: received PMSG 10 IU for 4 days, hCG 10 IU on the 5th day, and montelukast 10 mg/kg/day for 6 days. The cabergoline group received PMSG 10 IU for 4 days, hCG 30 IU on the 5th day, and cabergoline 100µg/kg/day for 6 days.

To form OHSS, 22-day-old rats, except those in the control group, were administered PMSG (Folligon®-Intervet; Schering-Plough Animal Health, Pune, India) 10 IU/day subcutaneously for 4 days. On the 5th day (when the animals were 26 days old), hCG (Chorulon®-Intervet; Schering-Plough Animal Health, Boxmeer, The Netherlands) was administered at a dose of 10 IU for Group 2; and 30 IU for each of Groups 3, 4, and 5 to stimulate ovulation. Group 4 was administered montelukast sodium (Singulair®, 4 mg oral granule, Merk Sharp &Dohme, İstanbul, Turkey) 10 mg/kg/day via oral gavage for 6 doses 24 hours apart between days 22 and 27. Montelukast was diluted with distilled water to reach a concentration of 20 µg/ml and homogenized with Auto Vortex Mixer SA (Stuart Scientific, UK) to prevent sticking onto injector's inner wall. Montelukast was applied via oral gavage within 15 minutes of dilution. Group 5 received cabergoline (Dostinex®, Pharmacia&UpjohnSpA, Italy) 100 µg/kg/day via oral gavage for 6 doses 24 hours apart between days 22 and 27. Cabergoline, which was supplied as tablets, was crushed to become pulverized and diluted in distilled water to reach a final concentration of 0.2 µg/ml.

The animals were reweighed 48 hours after hCG injection. All rats were anesthesized with 35 mg/kg of ketamin (Ketalar®; Eczacibasi-Werner Lambert, İstanbul, Turkey) and 5 mg/kg of xylazine (Ksilazol®, 20 mg/ml, Provet, İstanbul, Turkey) given via intraperitoneal route. Then, to assess vascular permeability, 5mM of Evans Blue (EB) diluted in 0.2 ml of distilled water was administered with an insulin injector intravenously via the jugular vein exposed by neck dissection. After a 30-minute waiting period, the peritoneal cavity was filled with 5 ml of 0.9% NaCl solution (21°C), a mild abdominal massage was applied for 30 seconds, and peritoneal lavage fluid was redrawn gently with a vascular catheter in a way to avoid damaging tissues and blood vessels. Peritoneal fluid was put into tubes containing 0.05 ml of 0.1 N NaOH. Respiratory status and maximal cardiac impulse were monitored throughout the procedure. The animals were protected against hypothermia by conducting the study procedure at room temperature (22–26°C). The last stage was the sacrifice of the animals and the removal of bilateral ovaries. The ovaries were weighed on a precision scale (±0.001). The organs were then fixed with suitable fixatives for histochemical and immunohistochemical procedures.

Histopathological evaluation

Removed ovaries were fixed by overnight immersion in 10% formaldehyde and rinsed in running tap water (overnight). Dehydration was done in graded 60%, 80%, and 95% alcohol series for 30 minutes each. Afterwards, they were embedded in paraffin and sectioned via microtome. Sections were stained with standard protocols of Hematoxylene and Eosin (HE) and immunohistochemistry.

For histochemical analyses, 5µm-thick sections were used for the HE; the slides were deparaffinized with xylene for 1 hour. They were then rehydrated with descending alcohol series for 2 minutes each and stained with hematoxylin for 30 minutes (01562E, Surgipath, Bretton, Peter Borough, Cambridgeshire). After washing with running tap water, they were immersed in acid alcohol for a few seconds. The slides were stained with eosin for 2 minutes (01602E, Surgipath, Bretton, Peter Borough, Cambridgeshire), and after washing them with tap water, they were mounted with entellan (UN 1866, Merck, Darmstadt, Germany).

For immunohistochemical analyses, 5µm-thick sections were deparaffinizated with xylene for 1 hour. Rehydration was done in sequential descending alcohol series for 2 minutes each. After washing with distilled water for 5 minutes, the sections were immersed in phosphate buffered saline (PBS) for 10 minutes and incubated with 0.5% trypsin (EK001-10K, Biogenex, San Ramon, USA) for 15 minutes. The primary antibodies VEGF (Dako), VEGFR-1 (RB-1527-P, Neomarkers, Fremont, CA9), and VEGFR-2 (RB-1526-P, Neomarkers, Fremont, CA) in 1/100 dilution were then applied at +4°C overnight. They were washed with PBS and thenthe biotinylated secondary antibody (DAB500, Millipore) and streptavidine-peroxidasewere applied for 30 min each and washed with PBS before incubating with the enzyme conjugate and 3,3-diaminobenzidine tetrahydrochloride (DAB, K007, DBS, California, USA). Then, sections were counterstained with Mayer's hematoxylin (W01030707, DDK) and mounted with mounting medium.

Immunostaining for VEGF, VEGFR-1, and VEGFR-2 in the granulosa and vessel endothelial cells was evaluated semiquantitatively by HSCORE analysis. All slides were examined under light microscopy (Olympus BX40, Tokyo, Japan). Immunostaining intensity was categorized into the following scores: 0 (no staining), 1 (weak but detectable staining), 2 (moderate staining), and 3 (intense staining). An HSCORE value was derived for each specimen by calculating the sum of the percentage of cells for granulosa cells and vascular endothelial cells in the ovary that was stained at each intensity category multiplied by its respective score, by means of the formula H-score=$\sum$Pi (i+1), where i=intensity of staining with a value of 1, 2, or 3 (weak moderate or strong respectively) and Pi is the percentage of stained epithelial cells for each intensity, varying from 0 to 100%. For each slide, ten different fields were evaluated microscopically at 200x magnification. HSCORE evaluations were performed independently by at least two investigators blinded to the source of the samples as well as to each other's results. The average score of both was utilized.

To determine ovarian diameters, each sample was microscopically measured in millimeters with the Olympus image-analysis software (DP21).

Biochemical Evaluation

The peritoneal fluids were centrifuged at 900 X g for 12 minutes. The EB concentration was evaluated with a Shimadzu UV 1800 Visible spectrophotometer (Tokyo, Japan) at 600 nm to obtain absorbance levels. Vascular permeability was standardized by calculating the level of extravasated stain as EB millimole (mM) per 100 gr body weight.

Statistical Analyses

Statistical analyses were performed using the Rstudio software version 0.98.501 via R language. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk test) to evaluate the distribution pattern. Data were expressed as the mean and standard deviation. The Mann-Whitney U test was used for non-normal distributed data sets. In case of nonparametric condition, the Kruskal-Wallis H test was used for comparison more than two groups. P values of less than 0.05 were accepted as significant.

Results

Body weight

There was no significant difference between the groups with respect to body weight on the 22nd day (body weight-1) (p=0.49). Body weight increase (the difference between the 28th day:body weight-2 and the 22nd day:body weight-1) was significantly lower in the cabergoline group compared to both severe OHSS and montelukast groups (p=0.025 and p=0.013, respectively) (Table 1).

Ovarian weights and diameters

Ovarian weights and diameters were significantly greater in the severe OHSS group thanthe control group (p=0.002 and p=0.002). Although montelukast and cabergoline groups were associated with lower ovarian weight than severe OHSS, the difference was statistically non-significant (p=0.085 and p=0.180, respectively). As for the ovarian diameters, montelukast and cabergoline groups had significantly lower ovarian diameters compared to the severe OHSS group (p=0.005 and p=0.006, respectively) (Table 1).

Vascular permeability

While there was no significant difference between the control and mild OHSS groups, the difference between the mild and severe OHSS groups was significant (p=0.085 and p=0.004, respectively). The vascular permeability level was lower in the cabergoline group than the severe OHSS group, albeit statistically non-significant (p=0.180). It was significantly lower in the montelukast group than the severe OHSS group (p=0.018). The montelukast group had a lower, albeit statistically non-significant, vascular permeability level compared to the cabergoline group (p=0.225) (Table I, Figure 1).

Immunohistochemical evaluation of VEGF and its receptors

VEGF showed a significantly higher expression in the granulosa cells of ovarian tissues obtained from severe OHSS than those obtained from the controls and mild OHSS subjects (p=0.002 and p=0.003, respectively). Montelukast and cabergoline groups showed a significantly lower VEGF expression than the severe OHSS group (p=0.002 and p=0.002) (Table 2, Figure 2). VEGFR-2 was significantly lower in the control group than the mild OHSS and severe OHSS groups, while VEGFR-1 was significantly higher in the same comparison (p<0.001 and p<0.001). VEGFR-1 and VEGFR-2 expressions were greater in the montelukast group than the severe OHSS group (p<0.001 and p<0.001). VEGFR-1 expression was lower in the severe OHSS group than the cabergoline group, whereas VEGFR-2 expression was significantly lower in the cabergoline group than the severe

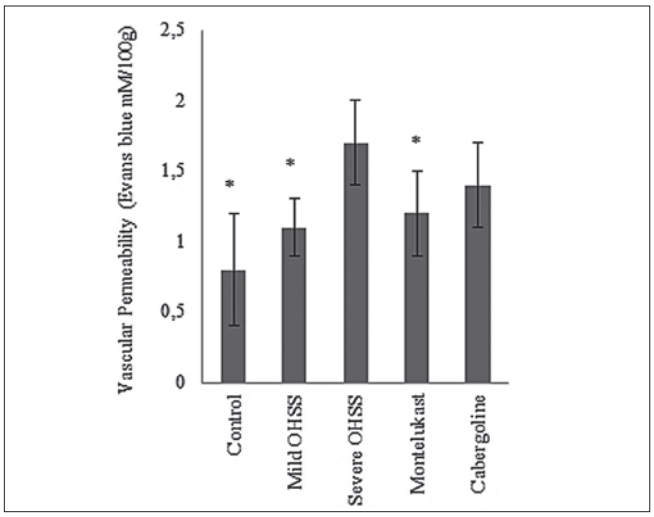

Figure 1. Comparison of vascular permeability values between groups. Bars present mean±SD (standard deviation). *p <0.05 versus severe OHSS group.

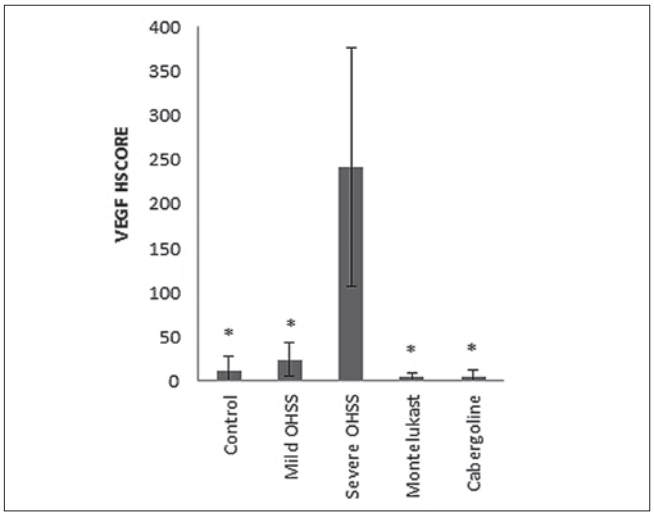

Figure 2. Comparison of HSCORE values of vascular endothelial growth factor (VEGF) in the granulosa cells between groups. Bars present mean±SD (standard deviation). *p<0.05 versus severe OHSS group.

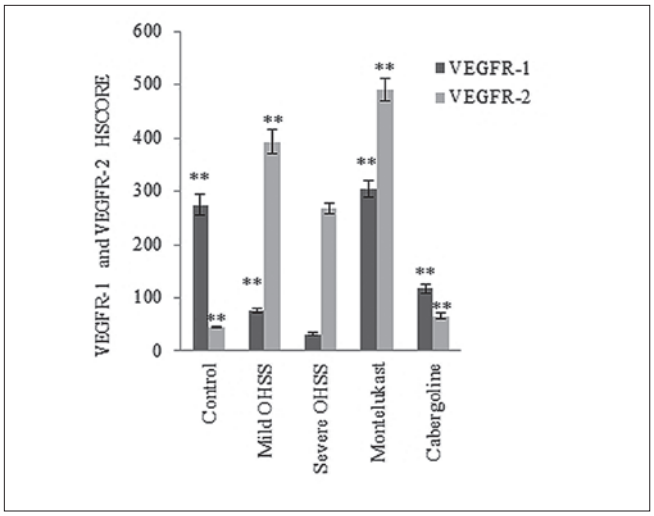

Figure 3. Comparison of HSCORE values of vascular endothelial growth factor receptors (VEGFR-1 and VEGFR-2) in the granulosa cells between groups. Bars present mean±SD (standard deviation). **p<0.001 versus severe OHSS group.

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Table I. Evaluated parameters in the groups (excluding histopathological evaluation).

	Control (n=7)	Mild OHSS (n=7)	Severe OHSS (n=7)	Montelukat (n=7)	Cabergoline (n=7)
Body Weight -1 (g)	30.2 ± 3.8	29.5 ± 3.4	31.1 ± 3.0	32.4 ± 1.6	32.2 ± 1.5
Body Weight -2 (g)	42.9 ± 6.0	43.2 ± 5.8	45.9 ± 5.2	47.4 ± 3.6	43.7 ± 2.9
Body Weight Gain (g)	12.6±2.2	13.7±2.4	14.7±2.3	14.9±2.1**	11.4±2.09*
Ovary Weight (mg)	16.5 ± 8.8*	122.4 ± 28.3	141.3 ± 34.2	110.4 ± 30.3	118.7 ±28.2
Ovary diameter (mm)	1.7±0.4*	6.8±2.5	6.9±1.4	3.8±1.3*	4.4±0.9*
Vascular Permeability (Evans Blue mM/100 g)	0.8 ± 0.4*	1.1 ± 0.2*	1.7 ± 0.3	1.2 ± 0.3*	1.4 ± 0.3

Data are presented as mean±SD (standard deviation) . Ovarian hyperstimulation syndrome (OHSS).
* p<0.05 versus severe OHSS group ** p<0.05 versus cabergoline group

Table II. HSCORE values of vascular endothelial growth factor (VEGF) and receptors (VEGFR-1 ve VEGFR-2) in the granulosa cells.

	Control (n=7)	Mild OHSS (n=7)	Severe OHSS (n=7)	Montelukast (n=7)	Cabergoline (n=7)
VEGF	12.0±16.4*	24.3±19.0*	241.4±135.0	4.6±4.6*	5.1±7.4*
VEGFR-1	274±19.4**	76.6±4.2**	32±3.4	304±16**	118±8.4**
VEGFR-2	44.8±1.3**	392±22.8**	268±10.9	490±20**	65.6±6.2**

Data are presented as mean±SD (standard deviation). Ovarian hyperstimulation syndrome (OHSS).
* p<0.05 versus severe OHSS group ** p<0.001 versus severe OHSS group

OHSS group (p<0.001 and p<0.001). Expression of both receptors was significantly lower in the cabergoline group than the montelukast group (p<0.001 and p<0.001) (Table II, Figure 3). The staining samples for VEGF and its receptors in granulosa cells are shown in Figures 4–8.

VEGF immunoreactivity in vascular endothelium was the same in all groups and was weakly positive (+). VEGFR-1 immunoreactivity showed similar staining (3.0±0.0) for the control, severe OHSS, and montelukast groups. The cabergoline group showed higher staining (4.0±0.0) than these three groups, while the mild OHSS group showed lower staining (0.5±0.54) than these groups (p<0.001 and p<0.001). VEGFR-2 showed similar staining in the control and cabergoline groups (4.0±0.0), while it demonstrated a (3.0±0.0) staining in the severe OHSS group, a (2.0±0.0) staining in the montelukast group, and a (1.0±0.0) in the mild OHSS group. The differences between all groups were significant (p<0.001), with the exception of the difference between the control and cabergoline groups.

Discussion

In this animal model on OHSS, which has neither a clear pathophysiological basis nor a specific therapy, we found that montelukast had a significant limiting effect on VEGF expression, vascular permeability, and increased ovarian diameter in severe OHSS. However, it lacked similar efficacy for VEGF receptors. In order to determine the efficacy of montelukast, we compared it with cabergoline, which is currently a recognized agent in OHSS prophylaxis and treatment (7). While montelukast was more effective in limiting vascular permeability in severe OHSS, cabergoline was superior to montelukast in limiting an increase in body weight and VEGFR-2 expression.

OHSS is an iatrogenic complication that typically develops after administering hCG to trigger the final stage of oocyte maturation following the stimulation of ovulation with gonadotropins. It usually emerges as a result of an exaggerated ovarian response and is characterized by high estrogen levels and dilated ovaries [1, 2]. Risk factors for OHSS include polycystic ovaries, young age, low body mass index, and history of allergies [10]. Increased vascular permeability in OHSS leads to 2 clinical problems; the first is abdominal pain and heaviness and respiratory difficulty secondary to restricted diaphragmatic mobility caused by fluid accumulation in the abdomen as well as other body spaces as a result of extravasation [11]; the second is hemoconcentration and resultant reduced organ perfusion. As a consequence, oliguria, renal failure, and abnormal liver functions may ensue. Hemoconcentration caused by excessive fluid extravasation increases the risk of thromboembolic events. In extreme cases, this disorder may cause renal failure, reduced perfusion of vital organs such as brain and heart, coma, and even death [12].

The pathophysiology of OHSS has not been fully elucidated. Recently, increased vascular permeability secondary to the hypersecretion of VEGF, an angiogenic molecule, from ovaries has been implicated as the main cause [13]. Gomez et al. [14] showed in an OHSS animal model that ovulation induction with gonadotropins increased VEGF, VEGFR-2 mRNA expression in ovaries, and vascular permeability prior to hCG administration. HCG injection further increases the levels of these parameters. The linear correlation between increased VEGF/VEGFR-2 mRNA expression and vascular permeability begins 2 hours after hCG injection and peaks 48 hours later. VEGF mRNA expression also increases in luteinized granulosa cells of oocytes picked up from patients who were stimulated with hCG and developed OHSS [15]. In addition to VEGF expression, VEGF receptors (VEGFR-2) are also produced by human luteinized granulosa cells upon hCG injection [16]. VEGF receptors are mainly found in endothelium and ovarian follicles in humans [14, 17].

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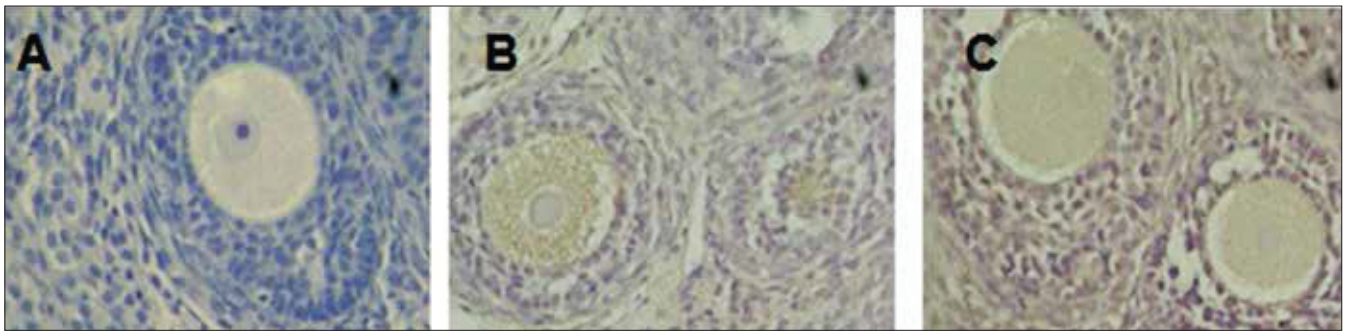

Figure 4. Control group: representative ovarian sections showing the immunolocalizations of VEGF (A), VEGFR-2 (B) and VEGFR-1 (C) (Original Magnification: 400x).

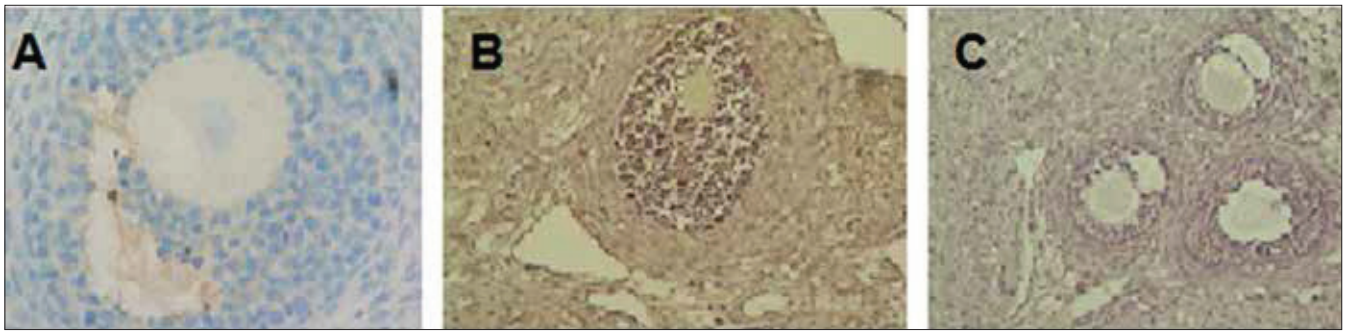

Figure 5. CMild OHSS (ovarian hyperstimulation syndrome) group: representative ovarian sections showing the immunolocalizations of VEGF (A), VEGFR-2 (B) and VEGFR-1 (C) (Original Magnification: 400x).

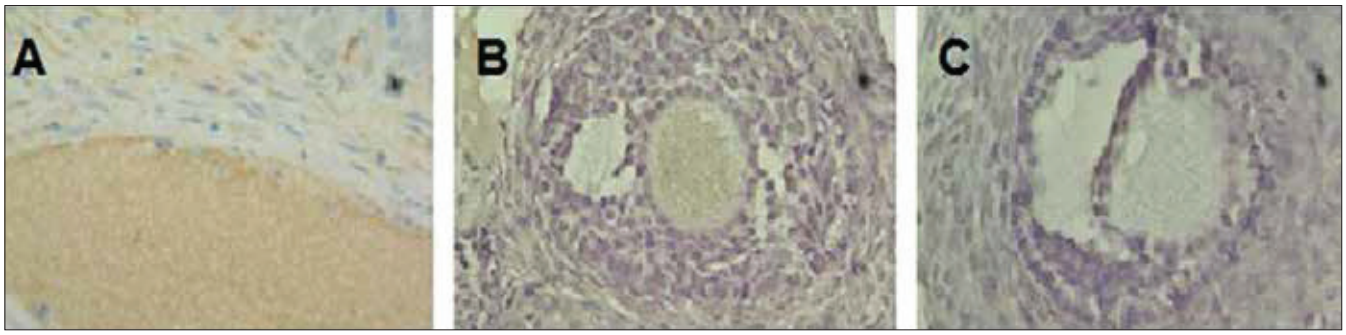

Figure 6. CoSevere OHSS (ovarian hyperstimulation syndrome) group: representative ovarian sections showing the immunolocalizations of VEGF (A), VEGFR-2 (B) and VEGFR-1 (C) (Original Magnification: 400x).

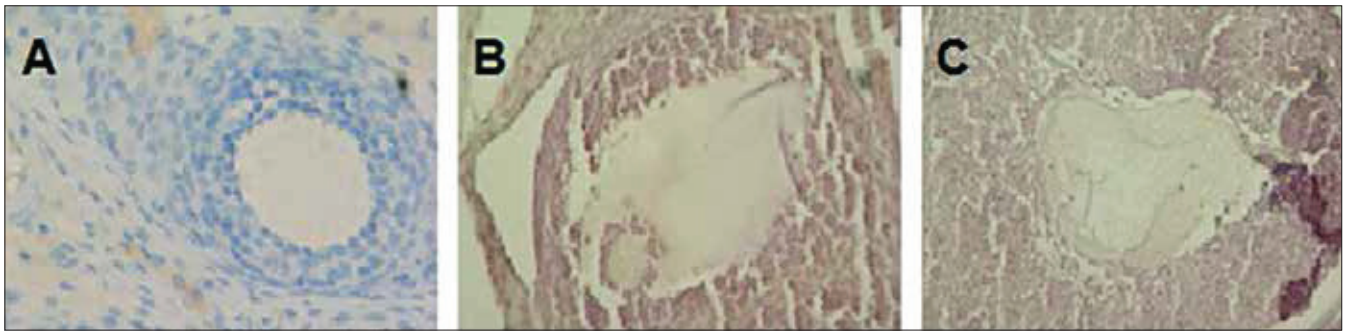

Figure 7. Montelukast group: representative ovarian sections showing the immunolocalizations of VEGF (A), VEGFR-2 (B) and VEGFR-1 (C) (Original Magnification: 400x).

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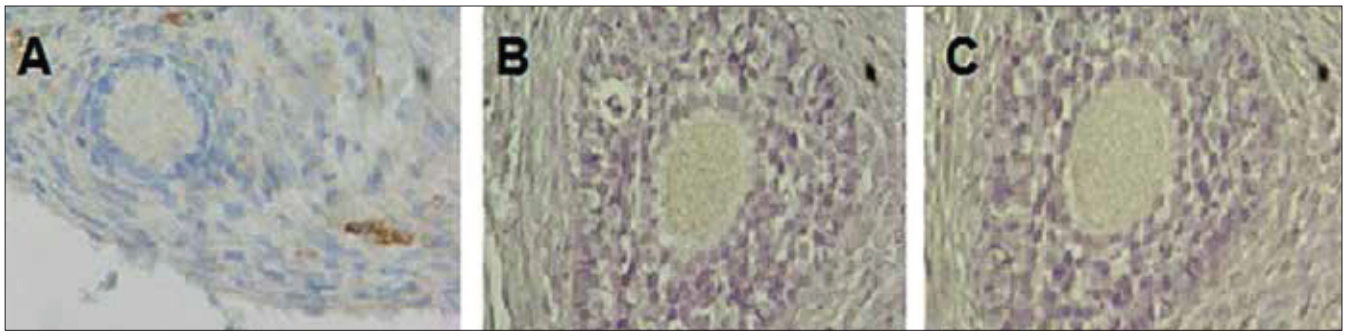

Figure 8. Cabergoline group: representative ovarian sections showing the immunolocalizations of VEGF (A), VEGFR-2 (B) and VEGFR-1 (C) (Original Magnification: 400x).

Diedrich et al. [18] suggested that the administration of gonadotropin-releasing hormone analogue in androgen LH/FSH surges and the induction of oocyte maturation may reduce the incidence of OHSS. Manau et al. [19] similarly showed that substituting shorter half-life recombinant luteinized hormone for hCG in the induction of ovulation reduced the incidence of OHSS. Gomez et al. [20], in an OHSS model in rodents, showed that injections of SU5416 (Z-3-[(2,4-dimethylpyrrol-5-yl)methylidenyl]-2-indolinone) inhibited vascular permeability increases induced by hCG. SU5416 is a small synthetic tyrosine kinase molecule that inhibits VEGFR-2 phosphorylation without affecting the receptor's surface expression and VEGF affinity. The ability of the intervening VEGF/VEGFR-2 signaling pathway to prevent gonadotropin-induced vascular permeability increases established, for the first time, the relationship between VEGF expression and capillary permeability in vivo. However, the side effect profile of SU5416 prevents its use in clinical practice [21]. It also adversely affects implantation by blocking angiogenesis, thus leading to the inactivation of corpus luteum [22].

Another approach to VEGF ligand-receptor complex blockage is the dopamine/Dp-r2 pathway. The dopamine agonist cabergoline in low doses decreases VEGFR-2-dependent vascular permeability without preventing luteal angiogenesis and exerting a luteolytic effect (without affecting serum progesteron levels and luteal apoptosis) [4]. Gomez et al. [4] showed that a low dose of (100 µg/kg) cabergoline reduces VEGFR-2 phosphorylation by 42% compared to a control group. In higher doses, it decreases the density of VEGFR-2 on the endothelial cell membrane by causing its internalization. The access to receptors is prevented, inhibiting the VEGF/VEGFR-2 pathway. However, that effect achieved at higher doses is not limited to a decrease in vascular permeability, but angiogenesis is also blocked [23].

Leukotrienes (LTs) are lipid mediators that are produced from arachidonic acid via 5-lipoxygenase activation. They are known to play an important role in the pathogenesis of allergic reactions [24]. They are proinflammatory substances referred to as LTC4, LTD4, LTE4, and cysLTs. The cysLTs cause plasma extravasation by increasing vascular permeability [25]. Montelukast, a specific antagonist of LTs, blocks the binding of cysLTs to CysLT1 receptor [26]. Montelukast is currently used for the treatment of asthma and allergies. Regular, daily, long-term oral use is easily tolerated, and no clinically important side effect has been reported [27]. In asthma, it reduces VEGF expression and regulates vascular permeability [9]. Montelukast, at a dose of 10 mg/kg/day via oral gavage, has been shown to reduce VEGF levels in rats [28, 29]. One additional reason for studying montelukast in severe OHSS was that allergic events creating susceptibility for OHSS are treated with montelukast [10, 27]. We demonstrated that montelukast significantly reduced VEGF expression in severe OHSS and, additionally, limited vascular permeability mediated by VEGF expression. The limiting effect on VEGFR-2 expression, on the other hand, was limited to vascular endothelial cells. It may be suggested that it exerted its clinical efficacy on severe OHSS by reducing VEGF/VEGFR-2 interaction via decreasing VEGF expression in granulosa cells. As is known, the angiogenic effect of VEGF is mediated by two tyrosine kinase receptors. VEGFR-2 shows a strong tyrosine kinase activity response to pro-angiogenic signals and thus positively affects angiogenesis. VEGFR-1, on the other hand, functions as an endogenous VEGF inhibitor during early embryogenesis and under certain conditions and possesses a tyrosine kinase activity that is approximately one-tenth of that of VEGFR-2 [5,6]. This, in turn, suggests that a significant increase in the VEGFR-1 levels mediated by montelukast may contribute to its limiting effect on vascular permeability.

We took into consideration the study by Saylan et al. [30] while designing the OHSS animal model. In animals, varying hCG doses produce different effects on VEGF and vascular permeability despite the same doses of gonadotropins [14]. Therefore, although we applied the same daily dose of gonadotropin, we administer hCG in two separate doses to form the mild and severe OHSS groups. We statistically revealed the differences between the control, mild OHSS, and severe OHSS groups with respect to vascular permeability and VEGF expression. Ovarian weight and diameters as well as VEGFR-2 expression were significantly greater in the severe OHSS group than the control group. VEGFR-1 expression, which may limit angiogenesis, also exhibited significant expression differences between the control, mild OHSS, and severe OHSS groups, with decreasing values from the former to the latter. One interesting finding was that in the mild OHSS group, not only the VEGFR-1, but also VEGFR-2 expression was greater than the severe OHSS group. However, VEGF, which is required for the VEGF/VEGFR-2 interaction to occur in vascular permeability increases, was significantly higher in the severe OHSS group. When we also consider the VEGF inhibitory effect of higher VEGFR-1 expression in the mild OHSS group, we can more clearly understand the difference observed in vascular permeability [5,6].

In an OHSS animal model study, Saylan et al. [30] explored the comparative efficacy of meloxicam, a cyclooxygenase-2 enzyme inhibitor, relative to cabergoline in preventing OHSS. They introduced meloxicam on PMSG and hCG days, while

cabergoline was given only on the hCG day. They reported that neither drug could limit VEGF expression [30]. Nevertheless, ovulation induction with gonadotropins stimulates VEGF and VEGFr-2 mRNA expression and vascular permeability before administering hCG. This effect peaks 48 hours after hCG injection [14]. We believe that their finding that cabergoline was not effective was related to cabergoline being administered only on the hCG day. Thus, we administered cabergoline on PMSG and hCG days and 1 day after hCG, as we did for montelukast. Saylan et al. [30] could not demonstrate any effect of cabergoline on VEGF expression (observational data) and other parameters. In our study, on the other hand, we demonstrated that cabergoline provided a significant reduction in VEGF in granulosa cells and, additionally, in VEGFR-2 expression compared to the severe OHSS group. Furthermore, this result was not observational, but rather proven by semiquantitative data.

We found montelukast to be superior to cabergoline with respect to its effect on VEGF expression and vascular permeability, although these differences were not statistically significant. Furthermore, VEGFR-1 expression, which has been shown to have a possible negative effect on angiogenesis, was significantly higher in the montelukast group. In addition, compared to the severe OHSS group, montelukast, but not cabergoline, significantly decreased vascular permeability. However, cabergoline was superior to montelukast with regard to the limiting effect on increased body weight. There may have been inequalities among the animals in terms of increased body weight and the level to which they were affected by stress. Therefore, it is difficult to draw solid conclusions about parameters like weight increases in animal experiments with a limited number of subjects. More robust conclusions can be reached with studies using a larger sample size.

Conclusion

In OHSS pathophysiology, the VEGF/VEGFR-2 interaction plays an active role. Montelukast limits VEGF expression in ovarian tissue and thus significantly reduces vascular permeability increases in severe OHSS. Unlike montelukast, cabergoline limits VEGFR-2 expression in addition to VEGF expression. Montelukast and cabergoline are effective therapies for the prevention of OHSS. The results of this study should be confirmed by other studies.

Authors' contribution:

1. Fatma Eskicioğlu – conception and design study, acquisition of data,analysis and interpretation of data, article draft, writting draft,corresponding author.
2. Gülüzar A. Turan – conception and design study, acquisition of data, analysis and interpretation of data, revised article critically.
3. Oya N. Sivrikoz – acquisition of data, analysis and interpretation of data, revised article critically.
4. Hakan Cengiz – acquisition of data, analysis and interpretation of data, revised article critically.
5. Zafer Akan – analysis and interpretation of data, revised article critically.
6. Nur Şahin – analysis and interpretation of data, revised article critically.
7. Osman Yılmaz – acquisition of data.
8. Hayrunnisa Yeşil – acquisition of data.
9. Seda Vatansever – acquisition of data.

Authors' statement

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References

1. Abramov Y, Elchalal U, Schenker JG. Severe OHSS: An 'epidemic' of severe OHSS: A price we have to pay? *Hum Reprod.* 1999, 14, 2181–2183.
2. Schenker JG, Weinstein D. Ovarian hyperstimulation syndrome: a current survey. *Fertil Steril.* 1978, 30, 255–268.
3. Morris RS, Paulson RJ. Ovarian derived prorenin-angiotensin cascade in human reproduction. *Fertil Steril.* 1994, 62, 1105–1114.
4. Gomez R, Gonzalez-Izquierdo M, Zimmermann RC, [et al.]. Low-dose dopamine agonist administration blocks vascular endothelial growth factor (VEGF)-mediated vascular hyperpermeability without altering VEGF receptor 2-dependent luteal angiogenesis in a rat ovarian hyperstimulation model. *Endocrinol.* 2006, 147, 5400-5011.
5. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med.* 2003, 9, 669-676.
6. Shibuya M. Vascular endothelial growth factor and its receptor system: physiological functions in angiogenesis and pathological roles in various diseases. *J Biochem.* 2013, 153, 13-19.
7. Seow KM, Lin YH, Bai CH, [et al.]. Clinical outcome according to timing of cabergoline initiation for prevention of OHSS: a randomized controlledtrial. *Reprod Biomed Online.* 2013, 26, 562-568.
8. Yang HS, Wang L, Yang W, [et al.]. Effects of leukotriene receptor antagonists on vascular endothelial growth factor and its receptors in a sensitized rat model. *Zhonghua Jie He He Hu Xi Za Zhi.* 2009, 32, 177-181.
9. Lee KS, Kim SR, Park HS, [et al.]. Cysteinyl leukotriene receptor antagonist regulates vascular permeability by reducing vascular endothelial growth factor expression. *J Allergy Clin Immunol.* 2004, 114, 1093-1099.
10. Golan A, Ron-El R, Herman A, [et al.]. Ovarian hyperstimulation syndrome: an update review. *Obstet Gynecol Surv.* 1989, 44, 430–440.
11. Delvigne A, Rozenberg S. Review of clinical course and treatment of ovarian hyperstimulation syndrome. *Hum Reprod Update.* 2003, 9,77–96.
12. Rizk B. Ovarian Hyperstimulation Syndrome—Epidemiology. Pathophysiology, Prevention and Management. 1st edn. New York: *Cambridge University Press,* 2006, 10–33.
13. McClure N, Healy DL, Rogers PA, [et al.]. Vascular endothelial growth factor as capillary permeability agent in ovarian hyperstimulation syndrome. *Lancet.* 1994, 344, 235–236.
14. Gomez R, Simon C, Remohi J, Pellicer A. Administration of moderate and high doses of gonadotropins to female rats increases ovarian vascular endothelial growth factor (VEGF) and VEGF receptor-2 expression that is associated to vascular hyperpermeability. *Biol Reprod.* 2003, 68, 2164–2171.
15. Yan Z, Weich HA, Bernart W, [et al.]. Vascular endothelial growth factor (VEGF) Messenger ribonucleic acid (mRNA) expression in luteinized human granulose cells in vitro. *J Clin Endocrinol Metab.* 1993, 77, 1723–1725.
16. Wang TH, Horng SG, Chang CL, [et al.]. Human chorionic gonadotropin-induced ovarian hyperstimulation syndrome is associated with upregulation of vascular endothelial growth factor. *J Clin Endocrinol Metab.* 2002, 87, 3300–3308.
17. Gomez R, Gonzalez M, Simon C, [et al.]. Tyroxine hydroxylase (TH) downregulation in hyperstimulated ovaries reveals the dopamine agonist bromocriptine (Br2) as an effective and specific method to block increased vascular permeability (VP) in OHSS. *Fertil Steril.* 2003, 80, 43–44.
18. Diedrich K, Ludwig M, Felberbaum RE. The role of gonadotrophin-releasing hormone antagonists in in vitro fertilization. *Sem Reprod Med.* 2001, 19, 213–220.
19. Manau D, Fabregues F, Arroyo V, [et al.]. Hemodynamic changes induced by urinary human chorionic gonadotrophin and recombinant luteinizing hormone used for inducing final follicular maturation and luteinization. *Fertil Steril.* 2002, 78 1261–1267.
20. Gomez R, Simon C, Remohi J, Pellicer A. Vascular endothelial growth factor receptor-2 activation induces vascular permeability in hyperstimulated rats, and this effect is prevented by receptor blockade. *Endocrinol.* 2002, 143, 4339–4348.
21. Glade-Bender J, Kandel JJ, Yamashiro DJ. VEGF blocking therapy in the treatment of cancer. *Expert Opin Biol Ther.* 2003, 3, 263–276.
22. Zimmermann RC, Hartman T, Bohlen P, [et al.]. Preovulatory treatment of mice with anti-VEGF receptor 2 antibody inhibits angiogenesis in corpora lutea. *Microvasc Res.* 2001, 62, 15–25.
23. Basu S, Nagy JA, Pal S, [et al.]. The neurotransmitter dopamine inhibits angiogenesis induced by vascular permeability factor/vascular endothelial growth factor. *Nat Med.* 2001, 7, 569–574.
24. Barnes PJ, Chung KF, Page CP. Inflammatory mediators of asthma: an update. *Pharmacol Rev.* 1998, 50, 515-596.
25. Henderson WR Jr. The role of leukotrienes in asthma. *Ann Allergy.* 1994, 72, 272-278.
26. Holgate ST, Bradding P, Sampson AP. Leukotriene antagonists and synthesis inhibitors: new directions in asthma therapy. *J Allergy Clin Immunol.* 1996, 98, 1–13.
27. Knorr B, Franchi LM, Bisgaard H, [et al.]. Montelukast, a leukotriene receptor antagonist, for the treatment of persistent asthma in children aged 2 to 5years. *Pediatrics.* 2001, 108, 48.
28. El-Swefy S, Hassanen SI. Improvement of hepatic fibrosis by leukotriene inhibition in cholestatic rats. *Ann Hepatol.* 2009, 8, 41-49.
29. Canbay E, Agachan B, Ozturk T, [et al.]. Dual inhibition of wound healing and oxidative process by montelukast in experimental colon anastomoses. *Surg Innov.* 2010, 17, 248-255.
30. Saylan A, Arioz DT, Koken T, [et al.]. Prevention of ovarian hyperstimulation syndrome in a rat model: efficacy comparison between cabergoline and meloxicam. *Acta Obstet Gynecol Scand.* 2010, 89, 692-699.

