Ovarian hyperstimulation syndrome is correlated with a reduction of soluble VEGF receptor protein level and a higher amount of VEGF-A

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Submitted on November 10, 2010; resubmitted on September 7, 2011; accepted on September 22, 2011

BACKGROUND: Ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening condition associated with increased vascular permeability. 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. Recently, a number of soluble receptors for the VEGFs have been detected (sVEGF-Rs) and it has been shown that these sVEGF-Rs compete with the membrane-standing VEGF-R to bind VEGFs.

METHODS: We analyzed the serum levels of soluble VEGF-R1, -R2 and -R3 in 34 patients suffering from OHSS and in 34 controls without this disease. In a subgroup analysis, we correlated the severity of the OHSS with the detected amounts of VEGF-R1, -R2 and -R3. In addition, we determined the amount of total VEGF-A in the samples.

RESULTS: All the three soluble VEGF receptors tended to be higher in the control group compared with that in the OHSS group but this difference only reached significance for sVEGF-R2 (mean ± SEM: 15.5 ± 0.6 versus 13.8 ± 0.5 ng/ml, respectively, P < 0.05). In the subgroup analysis, sVEGF-R2 levels decreased as the severity of OHSS increased (OHSS-I: 16.8 ± 1.9 ng/ml and OHSS-III: 12.7 ± 1.0 ng/ml, P < 0.05) Moreover, the serum levels of total VEGF-A were higher in the OHSS group than those in the controls (537.7 ± 38.9 versus 351 ± 53.4 pg/ml, respectively P < 0.05).

CONCLUSIONS: We propose that VEGF-A plays a role in the occurrence of OHSS, that the amount of biologically available VEGF-A is modulated by sVEGF-Rs and that different combinations of VEGF-A and sVEGF-R levels might contribute to the severity of OHSS.

Key words: assisted reproduction / ovarian hyperstimulation syndrome / angiogenesis / follicle development / vascular endothelial growth factor

Introduction

Ovarian hyperstimulation syndrome (OHSS) is a rare but severe complication resulting from the use of gonadotrophins during IVF therapy. However, a small number of spontaneous cases of OHSS were also reported (De leener et al., 2006). This pathology occurs in ~0.5–5% of all IVF treatment cycles (Delvigne et al., 2002). OHSS is accompanied by a shift in serum from the intravascular space to the third space—mainly to the abdominal cavity—which may lead to hemoconcentration and the risk of thromboembolism. Although the pathophysiology of this syndrome is far from being completely elucidated, it is assumed that vasoactive substances secreted by the ovaries under hCG stimulation may play a key role in the increased capillary permeability observed in OHSS (Soares et al., 2008).

To date there are ~25 factors known which are reported to be involved in the regulation of cellular permeability (Nagy et al., 2008). One of them, the vascular endothelial growth factor (VEGF)-A is up-regulated after hCG administration during IVF therapy and exhibits a strong permeability effect on endothelial cells. VEGF-A is therefore considered as a possible candidate stimulating the increased permeability observed in OHSS. However, studies measuring the serum levels of VEGF-A in patients suffering from OHSS are largely conflicting. Whereas studies by Agrawal et al. (1999) reported a link between VEGF-A levels and the development of OHSS, other researchers did...
not confirm these findings (Kobayashi et al., 1998; Chen et al., 2000; Enskog et al., 2001). Therefore, the debate about the role of VEGF-A in the occurrence of this pathology is still ongoing.

The potential role of the VEGF/VEGF-receptor (VEGF-R) system in the occurrence of OHSS is based on three lines of evidence. First, it was demonstrated by various authors that VEGF increases the vascular permeability of endothelial cells which could lead to a shift in fluids from the inner space of the endothelial vasculature to the third space (reviewed in Bates and Harper, 2002). Second, the occurrence of OHSS is strongly correlated with the application of hCG during IVF therapy, leading to a strong increase in VEGF expression by granulosa cells of the corpus luteum (Koos RD, 1995; Neulen et al., 1998; Walz et al., 2005). Third, in OHSS patients the amount of VEGF in the follicular fluid is frequently higher than in persons not affected by this complication (Wang et al., 2002).

To fulfill its specific function on endothelial cells, VEGF-A binds to VEGF-Rs 1 and 2 (VEGF-R1 and VEGF-R2). After binding, the receptors become activated through phosphorylation and transduce its signaling to various cell signaling pathways (Eriksson and Altaloo, 1999; Ferrara, 1999). However, heterodimerization between VEGF-R1 and VEGF-R2, as well as between VEGF-R2 and VEGF-R3, is reported and seems to modulate the activation process. Recent data reported that in the serum of humans, soluble variants of these receptors exist, which are able to bind various VEGFs (Jaroszewicz et al., 2008; Kuemmel et al., 2009; Mouawad et al., 2009; Ni et al., 2010). These receptors therefore could contribute to the amount of free, biologically active VEGFs in the serum by binding VEGFs and thereby depleting the amount of free circulating biological active VEGFs. In this context VEGF-Rs may play a role as modulators of available VEGF in the serum of OHSS patients. We therefore determined the amount of total VEGF-A and soluble receptors for the VEGFs (sVEGF)-Rs in serum from patients with, or without, OHSS. In a subgroup analysis, we correlated the amounts of sVEGF-Rs and VEGF-A with the severity of OHSS.

Materials and Methods

Patients

The study group comprised 34 patients who developed OHSS during their IVF therapy. The control group comprised 34 patients who were undergoing IVF but did not suffer from OHSS during their stay in hospital. All patients had both ovaries and had regular menstrual cycles every 27–32 days. All had blood pressure within the normal range and were not involved in intensive exercise. Patients underwent IVF therapy because of male factor or unexplained infertility. All patients were treated with the same GnRH antagonist protocol. Ovarian stimulation was performed with Pregnyl (Organon). All subjects received a single dose of hCG (Ovitrelle 250 μg SC, Merck-Serono, Geneva, Switzerland) to trigger final oocyte maturation when two or more than two follicles of ≥18 mm were observed. No significant differences were detected with regard to BMI, smoking status and age. Patients with endometriosis or polycystic ovary syndrome were excluded from this study because an increase in circulating VEGF levels in these patients has been debated in the literature (Gagné et al., 2003; Matalliotakis et al., 2003). Also, women with hypertension, hypotension, orthostatic hypotension, recurrent syncope or any contra-indication to the use of gonadotrophins or dopamine agonists were prohibited from inclusion. The classification of OHSS was performed according to the system proposed by Golan et al. (1989). In this system, OHSS is classified into three groups depending on the severity of OHSS. In our institute, OHSS-I (n = 5) is characterized by serum estrogen levels >4000 IU/ml at the day of hCG application; ovarian enlargement/small cysts are observed. OHSS-II (n = 20) is characterized by an additional abdominal distension. OHSS-III (n = 9) is characterized by large ovarian cyst and ascites or hydrothorax and hemoconcentration, with or without coagulation defects.

The collection of serum samples was approved by the Ethics Committee of the Medical University of Vienna and patients had to give written informed consent.

Blood samples were allowed to clot and the supernatant was separated by centrifugation and stored at –80°C until assays were performed. Serum samples were analysed in duplicate for sVEGFR-1, sVEGFR2 and sVEGFR3 using an enzyme-linked immunosorbent assay (ELISA, Bender Medical Systems, Austria Vienna) according to manufacturer’s instructions. These assays were not proven to distinguish membrane-bound VEGF-Rs from sVEGF-Rs. Although we cannot completely rule it out, based on the preparation method of the samples the probability of measuring both membrane-bound and sVEGF-Rs seems to be low in this study.

Total VEGF-A was determined using the Platinum ELISA (eBioscience Vienna, Austria). The serum probes were diluted 1:2 with provided dilution buffer and subsequently the amount of VEGFRs and VEGF-A was determined using a photometer (BenderMedsystems). Inter- and intra-assay variations for VEGF-R were 6.5% and 11.2%, sVEGFR-2, 8.7% and 9.7% and sVEGFR-3, 6.6 and 10.2%, respectively. Inter- and intra-assay variations for VEGF-A were 4.3% and 6.2%, respectively.

Results

Our analyses revealed that the mean level of sVEGFR-R1 in serum ranges from 1.99 ± 0.9 ng/ml in the OHSS group to 2.22 ± 1.5 ng/ml in the control group, the amount of sVEGFR-R2 ranges from 13.8 ± 0.5 to 15.5 ng/ml and for sVEGFR-R3 from 33.7 ± 2.4 to 39.3 ± 2.1 ng/ml, respectively. Patients in the OHSS group tended to have lower amounts of sVEGF-Rs, however, these differences only reached statistical significance for sVEGFR-R2, which was significantly lower in the OHSS group versus controls (Fig. 1, P < 0.05).

Mean VEGF-A levels were significantly higher in the OHSS group compared with the control group (537.7 ± 38.9 versus 351.4 ± 53.4 pg/ml, P < 0.05). In the OHSS subgroup analysis, we showed that the amount of sVEGFR-R1 is generally low but was highest in the group with severe, i.e. Grade III OHSS (1.5 ± 1.9 ng/ml) and lowest in the group with Grade I (0.6 ± 0.4 ng/ml) but these differences were not significant. sVEGFR-R2 levels decrease with the severity of the OHSS, from 16.8 ± 1.9 to 12.7 ± 1.0 ng/ml: the difference between OHSS-I and OHSS-II as well as the difference between OHSS-I and OHSS-III was statistically significant. The amounts of sVEGFR-R3 are highest in the OHSS-I subgroup (42.9 ± 7.5 ng/ml) and lowest in the OHSS-II subgroup (30.2 ± 2.2 ng/ml) with a value of 36.9 ± 6.4 ng/ml in the OHSS-III subgroup (Fig. 2, all NS).

With regard to VEGF-A, we found no significant differences in the subgroups of various OHSS grades ranging from 490.3 ± 137.6 pg/ml in OHSS-I to 548.7 ± 55.4 in OHSS-II and 471.8 ± 78.8 in OHSS-III.
Discussion

The VEGF system compromises the VEGFs and their specific receptors with soluble variants, and plays an important role in angiogenesis, lymphangiogenesis and vascular permeability. The results of our study indicate that sVEGF-Rs as well as VEGF-A appear to play a role in the occurrence of OHSS in women undergoing IVF therapy. Our novel findings that lower serum levels of sVEGF-R2 are associated with the occurrence of OHSS and that the severity of the disease increases with the decrease in sVEGF-R2 serum levels, may add new impact on the explanation of OHSS pathology. Based on our findings, we hypothesize that the amount of free, biologically active VEGF-A is, at least in part, modulated by binding to sVEGF-R2. Interestingly, in our samples the amount of total VEGF-A is higher in the OHSS group compared with that in the non-OHSS group confirming results by others (Agrawal et al., 1999). With regard to the subgroup analysis of OHSS severity, we found no increase in the amount of VEGF-A with an increase in the severity of OHSS, however, this might be related to the low number of participants in the individual subgroups. Based on these findings, our hypothesis is that the amount of VEGF-A is at least partially responsible for the occurrence of OHSS but its severity is increased when the amount of sVEGF-R2 is low. As a result a higher amount of biologically active VEGF-A might be available for binding to its membrane-bound receptor on endothelial cells in OHSS patients. In this hypothesis, the amount of free VEGF-A, which is assumed to be responsible for the increased vascular permeability in OHSS, is dependent on the amount of sVEGF-R2 in the serum. Therefore, women with a high amount of sVEGF-R2 have a lower amount of free VEGF-A and therefore a lower risk of developing severe OHSS. In contrast, women with a lower amount of sVEGF-R2 exhibit higher free VEGF-A levels and therefore are at an increased risk of developing OHSS. This hypothesis might help to explain the conflicting findings by other authors regarding the serum levels of VEGF-A and its relationship with OHSS.

A number of studies have reported the amount of sVEGF-Rs in various diseases, mainly in different types of cancer. For example, the serum level of sVEGF-R3 correlates with the clinical outcome of patients suffering from metastatic melanomas (Mouwad et al., 2009) and the progression of neuroblastomas is correlated with a down-regulation of sVEGF-R2 (Becker et al., 2010).

Although there are remarkable numbers of articles dealing with the occurrence of OHSS and its relationship with the amount of VEGF-A, there are only few articles dealing with the membrane-bound VEGF receptors and the occurrence of OHSS. For example, Gomez et al. (2002) found that in gonadotrophin hyperstimulated rats the inhibition of VEGF-R2 by a specific VEGFR-2 inhibitor (SU5416) prevented an increase in vascular permeability and Rodewald et al. (2009) demonstrated that the addition of antibody-conjugated VEGFR-1 in a granulosa lutein cell/endothelial cell co-culture system decreased vascular permeability. In both studies, it was shown that the blocking or depleting of membrane-bound VEGF-Rs in a biological system leads to a decrease in vascular permeability, highlighting the crucial function of the VEGF/VEGF-R system in the occurrence of OHSS.

Pau et al. (2006) analyzed the plasma levels of sVEGF-R1 in women who developed early or late OHSS and in women who did not show any signs of OHSS. Their findings demonstrated that the amount of sVEGF-R1 is lower and the amount of VEGF-A (free and total) is higher in OHSS groups. These results conflict somewhat with our own results regarding sVEGF-R1; in our study sVEGF-R1 levels tended to be higher in the OHSS group, but this difference was not statistically significant. This discrepancy might be explained by the different characteristics of the patients allocated to individual groups in their study compared with ours. Whereas Pau et al. (2006) distinguish between early- and late onset of OHSS, we did not. Additionally, in Pau et al. (2006) sVEGF-R1 was lower only in the severe form of early-onset OHSS but in the mild form of early-onset OHSS it is higher. This also might contribute to the observed differences.

In summary, our data indicate that sVEGF-R2 levels are lower in women suffering from OHSS compared with that in the non-OHSS group, and confirm the results by others that the serum level of
VEGF-A is higher in patients with OHSS. Taken together, these findings may provide new insights into the pathomechanisms of OHSS and might help to find new preventive approaches to this disease.

Parts of this study were presented at ESHRE 2011 / Stockholm.

Authors’ roles

D.P. involved in the study proposal, manuscript writing and editing, data mining, extraction and transfer; L.S. contributed in data collection and data mining; M.S. involved in the supervision, manuscript review and editing of manuscript; A.J. involved in the data mining and editing manuscript; C.E. contributed in supervision, study proposal and editing.

References


