Dopamine receptor 2 activation inhibits ovarian vascular endothelial growth factor secretion in an ovarian hyperstimulation syndrome (OHSS) animal model: implications for treatment of OHSS with dopamine receptor 2 agonists

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Objective: To explore whether a dopamine receptor 2 agonist (D2-ag) can prevent ovarian hyperstimulation syndrome (OHSS) in a rat model by decreasing ovarian vascular endothelial growth factor (VEGF) production.
Design: Experimental study in an OHSS animal model.
Setting: University-affiliated infertility center.
Patient(s): Immature Wistar rats.
Intervention(s): Immature rats were stimulated with gonadotropins to mimic OHSS and treated with a D2-ag and/or D2-antagonists (D2-ant). Vascular permeability (VP) was measured at the endpoint, and ovaries were collected to assess the effects of these drugs on VEGF production.
Main Outcome Measure(s): VP was estimated by measuring the peritoneal extravasation of a previously injected dye. Ovarian VEGF mRNA expression and VEGF protein levels were assessed by quantitative real-time PCR and Western blots, respectively.
Result(s): The D2-ag exerted a reduction in VP that was associated with a drastic decrease in VEGF protein production in OHSS rat ovaries. The effects of this D2-ag on VP and VEGF protein levels were partially reversed by concomitant administration of a D2-ant. Ovarian VEGF mRNA expression levels were unaffected by these drugs in OHSS rats.
Conclusion(s): D2-ags prevent increased VP in OHSS rats by decreasing ovarian VEGF production, very likely through a D2-mediated post-transcriptional mechanism. Given the dose-dependent inhibitory effect of D2-ags on ovarian VEGF production reported herein, we infer that current OHSS therapies used in humans may be improved by increasing the intraovarian concentration of D2-ags in these patients. (Fertil Steril® 2014;102:1468–76. ©2014 by American Society for Reproductive Medicine.)
Key Words: OHSS, VEGF, animal model, dopamine receptor 2 agonists

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Received May 14, 2013; revised July 15, 2014; accepted July 25, 2014; published online September 11, 2014.
H.F. has nothing to disclose. C.M.G.-P. has nothing to disclose. M.G. has nothing to disclose. C.M. has nothing to disclose. C.S. has nothing to disclose. F.G. has nothing to disclose. A.P. has nothing to disclose. R.G. has nothing to disclose.
H.F. and C.M.G.-P. should be considered similar in author order.
This work was supported by the Spanish Ministry of Economy and Competitiveness, through the Miguel Servet Programme (CP13/00077) cofounded by FEDER and the grants BFU2009-0831 (to R.G.) and SAF2008-03546 (to A.P.) from the Spanish National Government.
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Fertility and Sterility® Vol. 102, No. 5, November 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.07.1240

In spite of a better knowledge of its etiopathology and improvements in prevention protocols, ovarian hyperstimulation syndrome (OHSS) has not yet been completely erased from IVF clinics. The syndrome almost always onsets in its iatrogenic form in women subjected to controlled ovarian stimulation protocols. It is assumed

that the severity of OHSS symptoms (1, 2) reflects the duration and extent of the increased vascular permeability (VP) that each patient suffers.

Vascular endothelial growth factor (VEGF) is the most important mediator of hCG-dependent increases in VP. Ovarian VEGF is oversecreted in response to hCG (3–6) after binding to VEGF receptor 2 (VEGFR2) (7) on endothelial cells (ECs) (8) and makes local capillaries leaky (9–11). The extravasation of fluid leads to its accumulation in the abdominal and pleural cavities (12), while fluid depletion in the intravascular system leads to serious coagulation anomalies, electrolytic imbalances, and pre–renal failure, which is potentially lethal (12).

Activation of dopamine receptor 2 (D2) by the neurotransmitter dopamine (Dp) has been hypothesized to be a physiological regulation mechanism of ovarian VEGF production and secretion, and further supporting this, Cristina et al. (13) observed that both VEGF/VEGFR2-mediated VP and angiogenesis were augmented in mice lacking D2. Given their reported capacity to interfere with VEGF/VEGFR2-mediated VP in an OHSS rat model (14), we proposed that D2 agonists (D2-ags) might also prevent the syndrome and, given their relatively benign profile and lack of teratogenic effects (15–19), that these drugs might be ideal candidates for the clinical treatment of OHSS. In spite of the demonstrated efficacy of D2-ags in decreasing ovarian VP and related OHSS, the mechanism through which they exert this effect remains unknown; there is therefore no consensus on how to improve the efficacy of D2-ag OHSS therapies.

Previous in vitro and in vivo studies suggest that D2-ags decrease VEGF/VEGFR2-mediated VP by promoting VEGFR2 internalization through autocrine activation of D2 on ECs (20, 21). On the basis of this assumption, we searched for differential D2 expression on the surface of ovarian ECs from different patients in the hope that this would provide clues as to why D2-ags prevent OHSS in some patients but not in others (22). Surprisingly, we found that ovarian ECs do not express D2, thus challenging our assumptions. To find an alternative explanation for the differential efficacy of D2-ags, we focused our attention on the effects of dopaminergic drugs on VEGF secretion. Our in vitro studies with human lutein granulosa cells (LGCs) showed that D2-ags decrease VEGF secretion in a dose-dependent fashion by interacting with the D2 expressed on these cells (23). Assuming that LGCs become the major source of ovarian VEGF during OHSS (24), we hypothesized that D2-ags might prevent the onset of the syndrome by interfering with VEGF production by LGCs. Moreover, as the decrease in VEGF secretion by LGCs is exerted by D2-ags in a dose-dependent manner, we also speculated that OHSS prevention might be improved by increasing the intraovarian concentrations of D2-ags. To test this idea in vitro, we analyzed whether D2-ags decrease ovarian VEGF production (which induces the inhibition of increased VP) in a well-established and validated OHSS rat animal model (10, 25) supplemented with PRL (14).

MATERIALS AND METHODS

Drugs and Reagents

HCG (Profasi) was obtained from Serono Laboratories, and the D2 agonist Cb2 (Dostinex) was obtained from Pharmacia & Upjohn. PRL pellets were obtained from Innovative Research; and pregnant mare's serum gonadotropin (PMSG), the Evans blue (EB) dye, and the D2 antagonist (D2-ant) L-741,626 were obtained from Sigma-Aldrich. Primary mouse anti-rat platelet EC adhesion molecule-1 (PECAM1) and immunoabsorbed biotinylated conjugated secondary antibodies were obtained from Ptarmigan. Primary rabbit anti-dopamine D2 receptor antibody was obtained from Chemicon (Millipore), and the secondary biotinylated antibody was obtained from Ptarmigan. The 3,3′-diaminobenzidine (DAB) substrate kit for peroxidase staining to detect PECAM and D2 by immunohistochemistry was obtained from Vector Laboratories. The hormones progesterone (P_4) and PRL were measured using an RIA kit from Diagnostic Products Corporation. Trizol reagent was obtained from Life Technologies, Inc., the RNA was reverse-transcribed using an Advantage RT-for-PCR kit from Clontech, and TaqMan assays for rat VEGF (Rn01511601_m1) and rat β-actin (Rn00667869_m1) were obtained from Applied Biosystems. The Bradford protein assay was obtained from Bio-Rad Laboratories, and Western blots were performed using a VEGF protein primary antibody from R&D Systems and secondary rabbit anti-goat IgG-horseradish peroxidase (HRP) from DakoCytomation. The primary antibody used to detect GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was obtained from Abcam, and secondary goat anti-mouse IgG-HRP was obtained from Santa Cruz Biotechnology. The blot was incubated with SuperSignal West Femto Chemiluminescent Substrate (Thermo Scientific).

Animals

Immature 15-day-old female Wistar rats were obtained from Harlan Interfauna Iberica and kept for 1 week in our laboratory. Animals were fed ad libitum with a 12-hour light and 12-hour dark schedule. In vivo studies were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. Protocols for animal handling were approved by the animal ethics committee at the Valencia University School of Medicine.

The PRL-supplemented OHSS Rat Model

To induce OHSS, immature rats received 10 IU PMSG by SC injection for 4 consecutive days, starting on day 22 of life, to promote follicular development. Animals were given 30 IU hCG on the fifth day to induce ovulation. Exogenous D2-ag administration causes PRL deprivation, which leads to luteolysis in rats. To prevent PRL-dependent D2-ag-induced luteolysis, PRL pellets (5 mg) were implanted SC in Cb2-treated animals on the day of hCG administration.