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Human albumin enhances expression of vascular endothelial growth factor in cultured human luteinizing granulosa cells: importance in ovarian hyperstimulation syndrome

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Ovarian hyperstimulation syndrome (OHSS) is a severe complication of ovarian stimulation for assisted reproductive techniques. Clinical manifestations are massive extravascular fluid accumulation and haemoconcentration. Vascular endothelial growth factor (VEGF) has been demonstrated to mediate the development of OHSS. Intravenous albumin at the time of oocyte aspiration has been suggested as an effective prophylactic treatment against the occurrence of severe OHSS. Here it is reported that in cultured human luteinizing granulosa cells, VEGF mRNA expression was enhanced by human albumin and maximum expression was observed in cultured granulosa cells obtained from patients with serum oestradiol concentrations >2000 pg/ml on the day of human chorionic gonadotrophin injection (P < 0.05).

Key words: albumin/granulosa cells/ovarian hyperstimulation syndrome/vascular endothelial growth factor

Introduction

The most serious complication of ovulation induction for assisted conception is ovarian hyperstimulation syndrome (OHSS) which occurs in 1–10% of in-vitro fertilization (IVF) cycles (Forman et al., 1990; Wada et al., 1990; MacDougall et al., 1992). The main risk factor is the presence of a sensitive ovarian response to ovulation induction agents, usually associated with ultrasound detected polycystic ovaries (Asch et al., 1991; MacDougall et al., 1993). Another risk factor is the administration of human chorionic gonadotrophin (HCG) to trigger the ovulatory process, or when used for luteal support, or by its endogenous release during an early pregnancy (Wada et al., 1990; McClure et al., 1992). The main pathological feature of OHSS is increased capillary permeability, leading to fluid sequestration into the peritoneal cavity. New capillary vessel formation (angiogenesis) presumably accompanies OHSS but has been shown to be a main pathological feature. These features are most pronounced in the ovarian vasculature. Evidence for a role of vascular endothelial growth factor (VEGF) is strong. Hybridization studies have demonstrated VEGF mRNA in the rat and primary ovary, predominantly in the corpus luteum (Philips et al., 1990; Ravindranath et al., 1992; Doldi et al., 1997). VEGF from the multiple corpora lutea may enter the systemic circulation and increase vascular permeability, thus prompting the circulatory sequelae that occur in OHSS. Furthermore luteal phase treatment with gonadotrophin-releasing hormone antagonist (GnRHa), to suppress luteinizing hormone (LH) secretion, decreased VEGF mRNA (Ravindranath et al., 1992). McClure et al. demonstrated that VEGF is the major capillary permeability factor in OHSS ascites (McClure et al., 1994).

Recently, the use of human i.v. albumin at the time of oocyte retrieval has been described in patients at risk of OHSS with different results (Asch et al., 1993; Shoham et al., 1994; Mukherjee et al., 1995; Lewit et al., 1996). Because the pathophysiology of OHSS is not clearly elucidated, the mechanism of albumin action remains elusive.

The primary object of this study was to analyse the in-vitro effects of albumin on VEGF expression in luteinizing granulosa cells.

Materials and methods

Patients

Human luteinizing granulosa cells were obtained by follicular aspiration from 20 regularly menstruating women participating in an in-vitro fertilization programme at the Reproductive Endocrinology Center of the Department of Obstetrics and Gynecology of the University of Milan. All patients had a tubal factor and a history of normal menstrual cycles. They were treated with GnRHa, buserelin (Suprefact®; Hoechst, L’Aquila, Italy), beginning in the mid-luteal phase of the prior menstrual cycle for 1 week and then continuing until the day of HCG administration, at the dose of 0.6 mg/day. In all cycles, three ampoules of follicle-stimulating hormone (FSH) (Urofollitrofín®; 75 IU; Metrodin®; Serono, Rome, Italy) were administered i.m. from cycle day 3 onward. The dosage of gonadotrophin was maintained or increased appropriately until an adequate oestradiol response was achieved. Ovarian response was monitored by measurements of serum oestradiol concentrations and by follicular growth, using transvaginal ultrasonography. HCG (Profasi®; 5000 IU; Serono) was administered i.m. when sonography revealed at least two follicles measuring ≥16 mm in diameter, in association with adequate serum oestradiol concentrations.

Granulosa cell cultures

Human luteinizing granulosa cells were obtained by ultrasound-guided transvaginal follicular aspiration of individual follicles that was accomplished 35 h after HCG injection from three groups of patients with increasing serum oestradiol: group 1 (n = 7) oestradiol <1000 pg/ml, group 2 (n = 7) oestradiol ≥1000 <2000 pg/ml, group 3 (n = 6) oestradiol ≥2000 pg/ml.

After removing the oocytes, the remaining cells from each patient

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Granulosa cells and red blood cells were transferred to a 12 ml tube containing 3.5 ml lymphocyte separation medium (Flow Laboratories, Milan, Italy) and separated by centrifugation at 600 g for 5 min. Granulosa cells were dispersed by gentle shaking at 37°C for 30 min in 5 ml culture medium containing 0.1% collagenase and 20 mg DNase/ml. The dispersed cells were washed in culture medium, counted and plated at a density of 4–5×10^5 cells/10 cm plastic culture dish (Falcon) in serum-free medium 199 containing 2 mM glutamine and 50 mg/ml gentamycin. Cells were cultured at 37°C in a 95% air–5% CO₂ humidified environment. After 2 days, the cells had attached to the wells. At this time the medium was removed and 24 h incubations with and without 10 mg/ml of human albumin in medium 199 were initiated. Granulosa cells were washed twice with medium 199 and then frozen immediately at –80°C until RNA extraction.

RNA extraction and analysis

Undegraded total RNA was prepared from frozen granulosa cells by guanidine thiocyanate/phenol chloroform single-step extraction (RNAfast®; Molecular Systems, San Diego, CA, USA). For dot blot hybridization, 5 µg of total RNA from each sample were denatured in 6% formaldehyde and 50% deionized formamide in 1× standard saline citrate buffer (SSC; 0.75 mol/l NaCl and 0.075 mol/l sodium citrate).

The RNA was then transferred to nitrocellulose and nylon membranes (Hybond-N®; Amersham). The cDNA probe used for hybridizations of VEGF was a 930 bp EcoRI fragment coding for human VEGF (Genentech, South San Francisco, CA, USA). Hybridizations with cDNA probe (VEGF) were performed in Northern hybridization buffer (Celbio, Milan, Italy) in 50% formamide and 100 µg/ml salmon sperm DNA. Hybridizations were performed at 42°C for 16 h with 2–4×10^6 cpm/ml labelled with [α-32P]dCTP cDNA probe. After hybridization the blots were washed twice with ×2 SSC with 0.1% sodium dodecyl sulphate (SDS), then with ×0.1 SSC with 0.1% SDS for 15 min at room temperature. After washing, the blots were exposed to X-ray film (Kodak XAR 5®; Eastman Kodak, Rochester, NY, USA) at –80°C with intensifying screens. The relative amount of mRNA examined was determined by rehybridizing all blots for mRNA encoding actin. Between consecutive hybridizations, the blots were stripped in 0.1% SDS at 100°C for 30 min before rehybridizing. The relative intensity of signals for VEGF on the dot blots was compared to that for actin using computer-assisted densitometry. The ratio of VEGF to actin was used because no up-regulation of actin was observed in the presence of albumin. The ratios between VEGF intensity and actin intensity are indicated as arbitrary units. Variations in arbitrary units between samples are indicated as the SD. Statistical analyses were performed by using Student’s paired t-test for comparison within groups.

Results

Figure 1 shows VEGF mRNA expression of human luteinizing granulosa cells cultured with and without human albumin. Dot blot analysis showed that VEGF mRNA expression was enhanced by human albumin and the enhancement was present in all the cultures obtained from patients with different serum oestradiol. Furthermore the maximum expression of VEGF mRNA was observed in cultured granulosa cells obtained from patients with serum oestradiol concentrations >2000 pg/ml on the day of HCG injection (P < 0.05).

Discussion

The present study demonstrates the in-vitro effects of human albumin on VEGF mRNA expression in cultured human luteinizing granulosa cells obtained by follicular aspiration.
VEGF mRNA in luteinizing granulosa cells


