Elevated concentrations of angiogenin in serum and ascitic fluid from patients with severe ovarian hyperstimulation syndrome

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This study was conducted to investigate the possible role of angiogenin in the pathogenesis of ovarian hyperstimulation syndrome (OHSS). The study group consisted of 10 healthy women who developed severe OHSS (group A) following ovarian stimulation by a long protocol of gonadotrophin-releasing hormone analogues/human menopausal gonadotrophin for in-vitro fertilization. A control group B (n = 10) underwent stimulation by the same protocol and did not develop OHSS. Blood samples were taken from group A on day of admission to hospital for treatment of OHSS and, in group B, 1 week after oocyte retrieval. In group A, ascitic fluid was routinely aspirated as a treatment for severe OHSS, and a peritoneal fluid sample was aspirated transvaginally before oocyte retrieval in group B. In group A, the mean serum angiogenin, the mean ascitic fluid angiogenin, the mean serum oestradiol, and mean haematocrit were 8390 ± 6836 ng/ml, 2794 ± 1024 ng/ml, 6300 ± 2450 pg/ml and 46.6 ± 4.4 respectively, as compared with 234 ± 91 ng/ml, 254 ± 105 ng/ml, 1850 ± 1100 pg/ml and 36.8 ± 4.6 in group B respectively. The differences between groups were highly significant for all parameters. Angiogenin seems to be strongly associated with the formation of neovascularization responsible for the development of OHSS.

Key words: angiogenin/angiogenesis/ascites/cytokines/OHSS

Introduction

Ovarian hyperstimulation syndrome (OHSS) is the most serious complication of ovulation induction by gonadotrophins (Aboulghar et al., 1993). In its severe form, the syndrome is characterized by huge enlargement of the ovaries, massive ascites, hydrothorax, hypovolaemia, and haemoconcentration. Although the pathophysiology of OHSS has not been completely elucidated, the underlying mechanism responsible for the clinical manifestations of OHSS appears to be an increase in capillary permeability of the mesothelial surfaces (Polishuk and Schenker, 1969). The exact factors responsible for enhanced capillary permeability are the subject of debate (Abramov et al., 1996). Recently, several reports showed evidence that cytokines (Friedlander et al., 1993; Abramov et al., 1996) and vascular endothelial growth factor (McClure et al., 1994; Abramov et al., 1997) could possibly play an important role in angiogenesis and the increase in vascular permeability, thus promoting the circulatory sequelae that occur in OHSS.

Angiogenin is a polypeptide which is known to be a potent inducer of neovascularization (Weremowicz et al., 1990) and plays a role in the vascular development of the fetus and in the neovascularization that accompanies wound healing (Furcht, 1986). The aim of the present study was to investigate the possible role of angiogenin in the pathogenesis of OHSS. To the best of our knowledge, this is the first study of this issue in the world literature.

Materials and methods

A total of 20 patients was included in this study. The study was approved by our internal ethical committee and all patients signed a consent form before involvement in the study. All were healthy women presenting with the complaint of infertility, and were treated with human menopausal gonadotrophins using our routine long protocol of gonadotrophin-releasing hormone analogue (Aboulghar et al., 1994). They were divided into two groups of 10 patients. Group A patients had a diagnosis of severe OHSS. The diagnosis was based on ovarian enlargement (>10 cm in diameter) and massive ascites, and hydrothorax was present in two patients. All patients complained of abdominal pain, and dyspnoea. Group B was the control group of age-matched patients who underwent ovarian stimulation for in-vitro fertilization using the same protocol without the development of OHSS. All patients selected had a variable amount of peritoneal fluid visualized by vaginal ultrasound on the day of oocyte retrieval.

In group A, 10 ml of venous blood were withdrawn from each patient upon admission to the hospital for the treatment of severe OHSS. Routine transvaginal aspiration of ascitic fluid was performed (Aboulghar et al., 1990). Blood samples and ascitic fluid were assayed for angiogenin. The interval between oocyte retrieval and blood and ascitic fluid sampling ranged from 7–17 days.

In group B, 36 h after human chorionic gonadotrophin (HCG) administration, the pouch of Douglas was scanned by vaginal ultrasound before oocyte retrieval and the patients with fluid present were included in this study. The peritoneal fluid was aspirated prior to oocyte retrieval. A blood sample was withdrawn 1 week later. Ascitic fluid and serum were assayed for angiogenin. Blood and ascitic fluid samples were collected into tubes containing ethylenediaminetetra-acetic acid and 500 mIU of aprotonin. Samples were centrifuged for 10 min at 2000 g. The supernatant fluid was stored at –30°C.

Angiogenin concentrations were measured by enzyme-linked immunosorbent assay (ELISA). Reagent kits were purchased from R&D Systems, Minneapolis, MN, USA; the Quantikine® angiogenin
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Discussion

OHSS is a dramatic complication of ovulation induction. Ascites develop because of increased capillary permeability associated with relative vascular instability (Rizk and Aboulghar, 1991; Friedlander et al., 1993). The present data show that angiogenin is markedly elevated in both serum and ascitic fluid in the OHSS group compared with that in the control group.

The high concentrations of angiogenin in patients with severe OHSS suggest that it is directly or indirectly involved in the pathogenesis of OHSS and that it may be responsible for the increased angiogenesis resulting in the massive fluid shift from the intravascular to the extravascular compartment. Angiogenesis is known to be connected with increased vascular permeability (Jacob et al., 1977). However, a direct influence of angiogenin on vascular permeability was not proven by the present study. The other possibility is that angiogenin production is increased due to development of OHSS. The higher concentration of angiogenin in the serum as compared with that produced in the ascitic fluid suggests that angiogenin production may escape from the ovary to the circulation via the extensive vascular network in the hyperstimulated ovaries.

Angiogenin is a 14-kDa non-glycosylated polypeptide, so named for its ability to induce new blood vessel growth in the chick chorioallantoic membrane (Fett et al., 1985). Although originally isolated as a general angiogenic factor from the HT-29 human colon adenocarcinoma cell line, it is now known to be produced by a variety of cell types (Moennner et al., 1994) and exhibits both angiogenic and non-angiogenic activities (St Clair, 1987; Bicknell and Vallee, 1988; Bicknell and Vallee, 1989; Hu et al., 1994; Tschesche et al., 1994).

Messenger RNA encoding angiogenin is expressed in almost every tissue or culture cell line examined (Weremowicz et al., 1990) including normal epithelial cells, fibroblasts and peripheral blood cells (Rybak et al., 1987). Angiogenesis is mediated by growth factors and precise regulation of the synthesis and degradation of the extracellular matrix. Growth factors that stimulate angiogenesis include angiogenin, tumour necrosis factor-α and E-series prostaglandin (Ribatti et al., 1991; Bauer et al., 1992; Diaz-Flores et al., 1994; Cockerrill et al., 1995). Macrophages, tumour cells and platelets produce and release these angiogenic modulators (Gibbs et al., 1992). The widespread expression of angiogenin in different human cells suggests a biological function not only related to angiogenesis (Moenner et al., 1994).

The clinical characteristics of OHSS suggest that certain mediators are responsible for the vascular changes and the leakage of ascitic fluid (Rizk et al., 1997). The ovarian renin–angiotensin system was investigated as an aetiological factor in the pathogenesis of OHSS (Elchalal and Schenker, 1997). Recent data imply a possible association between inflammatory cytokines and the evolution of OHSS (Abramov et al., 1996). Cytokines have been implicated as mediators of the acute phase response in sepsis, injury, and immunological challenge (Wesselius et al., 1995). It was also reported that high amounts of cytokines occur in the follicular fluid of patients undergoing ovulation induction and this was related to the oestradiol

Results

There was no significant difference in the ages in groups A and B (± SD), which were 30 ± 5.2 years and 29 ± 4.6 years respectively. In the OHSS group (A), five patients became pregnant and, in the control group (B), three patients became pregnant.

The mean ± SD serum concentrations of angiogenin in groups A and B were 8390 ± 6837 ng/ml and 234 ± 91 ng/ml respectively (P < 0.001). The mean ± SD ascitic fluid angiogenin concentrations in groups A and B were 2794 ± 1024 ng/ml and 254 ± 105 ng/ml respectively (P < 0.001). The serum concentration ranged from 2400 to 20000 ng/ml in group A and from 80 to 400 ng/ml in group B. The ascitic fluid angiogenin concentration ranged from 1240 to 5000 ng/ml in group A and from 80 to 480 ng/ml in group B. The mean oestradiol concentrations on day of HCG injection in groups A and B were 6300 ± 2450 pg/ml and 1850 ± 1100 pg/ml, respectively, and the haematocrit was 46.4 ± 4.4 and 36.8 ± 4.6 respectively. The difference was statistically significant (P < 0.05).

The average white cell count was 21 000/ml in group A compared with 7800/ml in group B. Figure 1 shows the concentration of angiogenin in the serum and ascitic fluid in both groups.

Figure 1. Serum and ascitic fluid angiogenin concentrations in 10 patients with ovarian hyperstimulation syndrome (OHSS) and 10 control patients without OHSS. Normal serum concentration in the laboratory for angiogenin was mean 360 ng/ml, range 196–437 ng/ml, measured with Quantikine human angiogenin immunoassay, catalogue number DAN00, R&D Systems, Minneapolis, MN, USA.

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concentration, also known as the vascular permeability factor, is a heparin-binding glycoprotein with potent angiogenic endothelial cell-specific mitogenic and vascular permeability-enhancing activities, which has been strongly implicated in the pathogenesis of OHSS (McClure et al., 1994).

Currently, the factors mediating the vascular hyper-permeability in OHSS are not clearly recognized. The data presented in this study suggest that angiogenin is probably an important factor related to the pathogenesis of OHSS. Ovulation induction produces multiple follicles with impressive angiogenic activity. The agents responsible for the altered capillary permeability in ovarian follicles may not be contained within the individual follicle but may diffuse into the peritoneal cavity exerting its effect distally (Revel et al., 1996).

The exact role of different cytokines in the vascular permeability is not clearly defined. It has been suggested that the combined effects of different cytokines may be additive (Revel et al. 1996). The strong statistical correlation found between these cytokines and certain biological characteristics of OHSS, such as leukocytosis, elevated haematocrit and increased plasma oestradiol, further supports this assumption (Abramov et al., 1996). However, it is difficult to ascertain whether the elevation of plasma cytokine concentration is the result of leukocytosis or its cause. It is clear that white blood cell count does not affect the assay for the cytokine measurement itself, since the latter is performed on peritoneal or pleural fluids or serum after the cellular component has been separated (Abramov et al., 1996). Ovulation induction produces multiple follicles which improve angiogenin activity, and corpus luteum secretions are directed intravascularly and intraperitoneally (Revel et al., 1996). The direct escape of angiogenin from highly vascular ovaries to the circulation could possibly explain the higher concentration of serum angiogenin concentration compared with the ascitic angiogenin concentration. It is also possible that a higher level of serum angiogenin activity in the case of OHSS simply reflects increased ovarian activity (i.e. ovarian size, number of follicles, etc.). It is believed that angiogenin as well as other cytokines are possibly involved in the pathogenesis of OHSS, however the exact role of each cytokine is not known exactly. It is also possible that all these cytokines are elevated in response to an unidentified factor.

Recently, research has been directed towards kinetic analysis and molecular modelling to map the ribonucleolytic centre of angiogenin. It was found that 5'-phosphoadenosine 2'-phosphate might be a more potent inhibitor than any of the nucleotides tested thus far. Indeed, its K₅₅ value of 150 µM is 50-fold lower than that for the best nucleotide previously reported and 400-fold lower than the K₅₅ for the best dinucleotide substrate. This compound may serve as a suitable starting point for the eventual design of tight-binding inhibitors of angiogenin as anti-angiogenic agents for human therapy (Russo et al., 1996). The development, followed by the successful clinical trials of angiogenin inhibitor or other anti-cytokines for the treatment of OHSS, may illustrate the exact role of these factors in the pathogenesis of OHSS.

In conclusion, our data have shown a strong association between high angiogenin concentrations and increased vascular permeability in patients with OHSS. The lack of exact information on the aetiology and pathogenesis of OHSS is reflected in the absence of a real treatment for the syndrome, which is still directed towards improvement of symptoms, prevention of complications and shortening of hospital stay (Aboulghar et al., 1996).

References
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