The role of enalapril in the prevention of ovarian hyperstimulation syndrome: a rabbit model

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BACKGROUND: The purpose of our study was to investigate the role of enalapril in the prevention of ovarian hyperstimulation syndrome. METHODS: Twenty New Zealand female rabbits were included in the study. A total of 75 IU FSH + 75 IU LH was given daily by i.m. route for the first 7 days and additionally 2500 IU HCG was given on the last day of ovarian stimulation. Between days 0 and 9, oral enalapril tablets (2 mg/kg) were given twice daily to 10 rabbits (group 1). The remaining 10 rabbits did not receive enalapril (group 2). Laparatomy was performed on all rabbits at day 9. The amount of peritoneal fluid and the weight of the ovaries were recorded during laparotomy. Serum renin, interleukin-6 (IL-6), oestradiol, progesterone, prolactin and aldosterone concentrations were assayed at day 0 and again at day 9 for all rabbits. RESULTS: Serum renin and IL-6 concentrations at day 9 increased significantly compared with basal values in both groups (P < 0.05). Renin was correlated with IL-6 at day 9 in both groups (P < 0.05). The amount of peritoneal fluid and the increase in body weight observed at day 9 were not significantly different between groups 1 and 2. Administration of enalapril did not prevent the formation of ascites in group 1 despite the low serum aldosterone concentrations. In group 1 the weight of ovaries was significantly higher than the control group (P < 0.05). CONCLUSIONS: Renin–angiotensin system and IL-6 may play a role in the aetiopathogenesis of ovarian hyperstimulation syndrome. Administration of enalapril did not seem to have any beneficial effect in reducing the severity of ovarian hyperstimulation syndrome.

Key words: enalapril/IL-6/OHSS/rabbit model/renin

Introduction

Ovarian hyperstimulation syndrome (OHSS) has become a common problem since the introduction of assisted reproductive technologies and ovarian stimulation methods into infertility practice. OHSS is an iatrogenic syndrome; therefore it cannot be totally prevented. However, the incidence of OHSS can be reduced by appropriate therapeutic approaches. OHSS is classified into three categories, mild, moderate and severe OHSS. The overall incidence of OHSS varies between 3 and 23%. Severe and moderate forms of OHSS have been reported at a frequency of 1–10% and 1–7% respectively in patients undergoing ovarian stimulation (Navot et al., 1988; Beerendonk et al., 1998). Golan et al. (1989) proposed classifying OHSS in five degrees and three categories (Golan et al., 1989).

Renin is an enzyme produced in the kidney, formed from pro-renin. Renin acts on angiotensinogen, an alpha-2 globulin produced by the liver, forming angiotensin I. The converting enzyme contained in the lung acts on angiotensin I in the plasma converting it to angiotensin II, the most powerful pressor substance known. It causes contraction of the arteriolar smooth muscle and has other indirect actions mediated through the adrenal cortex. Enalapril, a competitive inhibitor of angiotensin I-converting enzyme, is used essentially for the treatment of hypertension. Enalapril is most effective in conditions associated with high plasma renin activity (Katzung, 2001). Enalapril may produce fetal hypocalvaria and renal defects when used in the second and third trimesters (Briggs et al., 1998). The aetiology of the defects is probably related to fetal hypotension and decreased renal blood flow. It can be speculated that the renin–angiotensin system (RAS) may have a role in the development of OHSS. Administration of enalapril results in angiotensin I-converting enzyme inhibition and finally to the suppression of RAS. In this study, we investigated whether the administration of enalapril had a beneficial effect by reducing the severity of OHSS.

Ovarian secretion of renin and the active precursors of angiotensin II has been an interesting topic of reproductive medicine in recent years. The system, known as ovarian derived pro-renin angiotensin cycle (ODPAC), has roles in steroid hormone synthesis, folliculogenesis, oocyte maturation and corpus luteum formation (Morris and Paulson, 1994). Detection of interleukin-6 (IL-6) RNA production during neovascularization and angiogenesis in follicular growth
period implied some relationships between IL-6 and ovarian function (Motro et al., 1990). IL-6 may have an important role in reproductive angiogenesis. Angiotensin II may have a role in the neovascularization. High pro-renin concentrations in ovarian follicular fluid suggest that angiotensin II, which is synthesized from renin, may have a role in OHSS aetiopathogenesis (Frederick et al., 1984). Both IL-6 and angiotensin II concentrations are found to be increased in the synovial fluids of patients with rheumatoid arthritis (Frederick et al., 1984). It has been speculated that such relationships may be present in the ovary. Pro-renin is an inactive form of renin. Plasma pro-renin increases twice in mid-cycle (Sealey et al., 1985). A possible source of pro-renin is the mature ovarian follicle. Pro-renin levels in ovarian follicular fluid are 10 times higher than in plasma (Glorioso et al., 1986). Plasma pro-renin increases at the time of ovulation, remains higher during the luteal phase and decreases before menstrual bleeding. Ovarian pro-renin secretion is regulated by pituitary LH concentrations (Sealey et al., 1985). Plasma pro-renin increases after the appearance of HCG in plasma following conception (Itskovitz et al., 1987). RAS may be activated in OHSS. However, Haning et al. (1985) reported that elevations of plasma renin and aldosterone concentrations are secondary to the physiological changes induced by OHSS (Haning et al., 1985).

Materials and methods

Sexually mature New Zealand rabbits were used in the study. The rabbits were caged individually at 27°C, and fed a standard diet. The study was approved by the ethics committee of our institution. The rabbits were divided into two groups, each comprising 10 rabbits. Basal weights of rabbits in follicular phase were measured in each group. All blood samples were taken from the marginal ear vein. The blood was centrifuged at 1600 g for 10 min before the serum was removed and stored at −70°C until analysis.

Basal serum concentrations of IL-6, renin, aldosterone, oestradiol, progesterone and prolactin were measured before gonadotrophin stimulation in all rabbits. For gonadotrophin stimulation, 75 IU FSH (Pergonal®; Serono, Rome, Italy) were given i.m. between 0900 and 1000 h in the pre-ovulatory period for 7 consecutive days and i.m. injections of 2500 IU HCG (Profasi HP®; Serono) were given to all the rabbits on day 7. Oral enalapril tablets (Enapril®, Ibrahim Ethem, Turkey) were given additionally with a dose of 2 mg/kg, twice daily between days 0 and 9 to 10 of the rabbits (group 1, enalapril group). Ten rabbits undergoing ovarian stimulation did not receive enalapril (group 2, control group).

Body weights of the rabbits were measured on day 9 in both groups. Blood samples were also taken on day 9 for measuring IL-6, renin, aldosterone, oestradiol, progesterone and prolactin concentrations. After centrifugation as described above, serum samples were kept at −70°C until analysis. Thereafter, all rabbits were killed by pentobarbital overdose on day 9 and the peritoneal, pleural and pericardial cavities were opened immediately. Since the quantities of pericardial and pleural fluids were so small, these fluids were not taken into account for analysis. The amount of peritoneal fluid was measured, ovaries were excised carefully and immediately. The weights of both ovaries were recorded.

Serum IL-6 was measured by enzyme-linked immunosorbent assay (ELISA) method (Bender Medsystems, Vienna, Austria). Intra- and interassay coefficients of variation (CV) for IL-6 at 4 pg/ml were 4.5 and 8.3% respectively. Serum renin was measured by double-side immunoradiometric assay using two antibodies (Diagnostics System Laboratories, Inclusion, TX, USA). Intra- and interassay CV for renin at 10 pg/ml were 4.3 and 3% respectively. Oestriadiol was measured by chemiluminescent technique and a competitive inhibition method. Intra- and interassay CV for oestradiol at the level of 771 pg/ml were 8.1 and 8.7% respectively. Progesterone was measured by the same technique as oestradiol. Intra- and interassay CV for progesterone at concentrations of 48.7 pg/ml were 5 and 7.8% respectively. Prolactin was measured by two-sided sandwich immunossay with chemilumino metric technology by using two antibodies. Intra- and inter assay CV for prolactin at 2.7 ng/ml were 2.5 and 3.6% respectively. Oestradiol, progesterone and prolactin assays were performed with an ACS analyser (The Chiron Diagnostics ACS, East Walpole, USA). Aldosterone concentrations were measured by radioimmunosassay (DSL Inc., Webster, USA). Intra-assay CV for aldosterone at 50.4 and 254.6 pg/ml were 7 and 36% respectively, while the interassay CV for 50.6 and 257.6 pg/ml were 10.4 and 7.3% respectively.

Wilcoxon’s rank sum test was used for the comparisons within the same group and Mann–Whitney U-test was used to compare the groups. Correlation regression analysis was also used. Probability lower than 0.05 was considered statistically significant.

Results

Serum IL-6 concentrations increased significantly on day 9 compared with basal concentrations in both groups (P < 0.05). Basal and day 9 IL-6 concentrations did not significantly differ between the two groups (Table I). Renin increased significantly at day 9 compared with basal concentrations in both groups (P < 0.05), but there was no significant difference in basal renin concentrations between the two groups. At day 9, renin for group 2 was significantly higher than that of group 1 (P < 0.05) (Table I). Basal aldosterone did not significantly differ between the two groups. At day 9, aldosterone decreased significantly in group 1 and increased significantly in group 2 compared with basal concentrations (P < 0.05). Aldosterone concentrations of group 2 were significantly higher than group 1 at day 9 (P < 0.05). Prolactin concentrations obtained on day 9 did not differ from those of basal values in both groups (Table II). On the contrary, oestradiol and progesterone concentrations obtained on day 9 were significantly higher compared with basal concentrations in both groups (P < 0.05). Both groups showed a significant increase in body weight of −7% between day 0 (basal) and day 9 (Table III). However, there was no difference in basal or day 9 body weights between the two groups. Total ovarian weight of group 1 was significantly higher than that of group 2 (P < 0.05). We found

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal concentrations (pg/ml)</th>
<th>Day 9 concentrations (pg/ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (enalapril)</td>
<td>IL-6 2.26 ± 0.44</td>
<td>3.44 ± 0.72</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>renin 2.09 ± 0.40</td>
<td>2.91 ± 0.58</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>aldosterone 369 ± 79.36</td>
<td>40.5 ± 13.97</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>IL-6 2.19 ± 0.47</td>
<td>3.71 ± 0.79</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>renin 2.21 ± 0.36</td>
<td>3.55 ± 0.55</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>aldosterone 308.9 ± 89.86</td>
<td>566.5 ± 102.52</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Table I. Basal and Day 9 serum IL-6, renin and aldosterone concentrations for both groups (mean ± SD)
Table II. Basal and day 9 serum oestradiol, progesterone and prolactin concentrations for both groups (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal concentrations</th>
<th>Day 9 concentrations</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oestradiol pg/ml</td>
<td>2580 ± 432</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Progesterone ng/ml</td>
<td>0.67 ± 0.18</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Prolactin ng/ml</td>
<td>0.24 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>Oestradiol pg/ml</td>
<td>2697 ± 234</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Progesterone ng/ml</td>
<td>0.67 ± 0.28</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Prolactin ng/ml</td>
<td>0.21 ± 0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table III. Basal and day 9 body weights, weight gain, ovarian weights and peritoneal fluid amounts in all rabbits (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal body weight (g)</td>
<td>1698 ± 182a</td>
<td>1808 ± 202b</td>
<td>NS</td>
</tr>
<tr>
<td>Day 9 body weight (g)</td>
<td>1816 ± 139a</td>
<td>1947 ± 234b</td>
<td>NS</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>118 ± 65</td>
<td>139 ± 44</td>
<td>NS</td>
</tr>
<tr>
<td>Ovarian weight (g)</td>
<td>0.903 ± 0.035</td>
<td>0.740 ± 0.181</td>
<td>0.021</td>
</tr>
<tr>
<td>Peritoneal fluid amount (ml)</td>
<td>2.70 ± 2.01</td>
<td>3.92 ± 3.62</td>
<td>NS</td>
</tr>
</tbody>
</table>

aBasal versus day 9 body weight in group 1, P = 0.036.
bBasal versus day 9 body weight in group 2, P = 0.005.

no significant difference in terms of peritoneal fluid amounts between the two groups (P > 0.05) (Table III).

There was a significant positive correlation between total ovarian weight and body weight gain in both groups (group 1, r = 0.881; group 2, r = 0.754, both P < 0.05). In group 2, a positive correlation was observed between the amount of peritoneal fluid and body weight gain (r = 0.669, P < 0.05). There were positive correlations between IL-6 and progesterone in group 1 (r = 0.658, P < 0.05); IL-6 and renin in group 1 (r = 0.689, P < 0.05) and in group 2 (r = 0.689, P < 0.05) at day 9. We observed a negative correlation between day 9 aldosterone and oestradiol concentrations in group 2 (r = -0.653, P < 0.05). Relationships between other parameters were not statistically significant.

Discussion

OHSS may be an inevitable result of ovulation induction. The main phenomenon underlying this syndrome is the increased permeability of the vascular system (Golan et al., 1989). Vascular endothelial growth factor (VEGF) increases the capillary permeability and may be responsible for the development of OHSS. In fact, high serum concentrations of VEGF were found in patients who developed severe OHSS (Krasnow et al., 1996). We have investigated the importance of IL-6 and RAS in the aetiopathogenesis of OHSS and the role of enalapril in the prevention of OHSS, with the intention of finding new methodologies for the treatment of OHSS.

In our study, both oestradiol and progesterone concentrations increased after gonadotrophin induction in both groups. The ovaries looked multifollicular and increased in size following stimulation by gonadotrophins. Increased amounts of peritoneal fluid were found in both groups. IL-6 increased significantly on day 9 of stimulation compared with basal, in both groups. The increase in IL-6 has been reported in previous studies of OHSS (Buyalos et al., 1992; Tsigirgottis and Craft, 1994).

Loret de Mola et al. (1996) found that serum and ascites IL-6 concentrations were increased significantly in patients with OHSS compared with normal post-ovulatory women and they proposed that IL-6 does not have a predictive value because an increment of IL-6 occurs in women both with or without OHSS (Loret de Mola et al., 1996). Increased concentrations of both IL-6 and angiotensin II in synovial fluids of patients with rheumatoid arthritis were reported (Frederick et al., 1984). Positive correlation between IL-6 and renin may suggest a possible relationship between IL-6 and RAS. We found significantly increased IL-6 and renin in rabbits with OHSS. Therefore IL-6 and RAS may play a role in OHSS pathogenesis. Administration of enalapril suppressed aldosterone, but IL-6 concentrations remained high. It may be speculated that IL-6 has a role in the aetiology of OHSS independently from aldosterone concentrations.

We could not find any positive correlation between IL-6 and amount of peritoneal fluid in both groups. OHSS parameters may be affected by other factors (Simon et al., 1999). There was no significant correlation between basal and day 9 oestradiol and IL-6 concentrations. It is known that oestradiol is a basic marker of OHSS, although OHSS can develop without an abnormal increase in oestradiol (Meirow et al., 1996). We think that a positive correlation between IL-6 and oestradiol may be absent in OHSS. IL-6 concentrations increase in both serum and ascitic fluid in patients with OHSS (Aboulghar et al., 1999). However decreased IL-6 in peritoneal fluids of OHSS patients have been reported (Chen et al., 1999).

We found that renin levels increased significantly compared with basal concentrations in both groups. There was no significant correlation between day 9 renin concentration and OHSS parameters such as peritoneal fluid, oestradiol, ovarian weight and weight gain in both groups. Increased plasma renin in patients with OHSS was reported in a previous study (Ong et al., 1999). Positive correlation between plasma renin activity and degree of OHSS was also reported in a study by Navot et al. (Navot et al., 1987). Van de Vrie et al. reported high renin and pro-renin concentrations in plasma and peritoneal fluid of a case with severe OHSS (Van de Vrie et al., 1997). Yoshimura et al. (1994) suggested that gonadotrophins stimulate renin activity and increase angiotensin II production (Yoshimura et al., 1994).

Morris et al. (1995) reported that ACE inhibition by enalapril resulted in a decrease of the incidence of OHSS in the rabbit model (Morris et al., 1995). They proposed that angiotensin II may cause weight gain, fluid accumulation in the third space and decreased intravascular volume. Ovarian weight was significantly higher in the enalapril group than the control group (Morris et al., 1995). They thought that it was a paradoxical finding. We also found higher ovarian weight in the enalapril group compared with controls. In our study, the higher ovarian weight of the enalapril group may be attributed to the lack of beneficial effects of enalapril in preventing the development of OHSS.

Plasma oestradiol concentrations were found to be higher
in rabbits with OHSS despite the administration of enalapril (Morris et al., 1995). We also found that oestradiol concentrations were higher in the enalapril group but the difference was not statistically significant. Sahin et al. (1997) developed OHSS in a rabbit model and they reported that oestradiol was significantly decreased when cilazapril was given (Sahin et al., 1997). This result contradicts the findings of Morris et al. (1995), and ours. Pucell et al. (1987) found that angiotensin II stimulates oestradiol secretion but not progesterone in vitro (Pucell et al., 1987). Enalapril administration did not seem to alter progesterone or oestradiol production in our study. Sahin et al. (1997) reported that there was no difference in ovarian weight between the cilazapril group and controls. On the other hand, we found increased ovarian weight in the group given enalapril. We suggest that further studies are needed to understand the relationship between ovarian weight and ACE inhibitors. Our study, conducted to prevent development of OHSS by ACE inhibitor, showed that there is no significant difference between enalapril and control groups in terms of body weight gain and peritoneal fluid accumulation. Peritoneal fluid amount was lower in the ACE inhibitor group, but the difference was not statistically significant. Sahin et al. (1997) also found no difference in terms of weight gain (Sahin et al., 1997).

Our findings suggest that the ACE inhibitor enalapril does not prevent ascites formation. Morris et al. (1995) investigated the effect of enalapril on the severity of OHSS in a rabbit model. For this purpose, they observed basic criteria of OHSS such as weight gain, increased ovarian size, ascites, haematocrit, and pleural and pericardial effusion in rabbits (Morris et al., 1995). They reported that severe OHSS developed in six of the 10 rabbits (60%) in the enalapril group. However, all 10 rabbits (100%) in the control group had severe OHSS (Morris et al., 1995). Therefore they reported that ACE inhibition resulted in 40% reduction in the severity of OHSS (Morris et al., 1995). We explored pericardial and pleural fluids during laparotomy and thoracotomy and found that these fluids were present in such small amounts that they could not accurately be measured. For this reason, we did not take into account pericardial or pleural fluids. Ascites did not develop in two of the 10 rabbits (20%) in the enalapril group, compared to one of the 10 rabbits (10%) in the control group in our study. Therefore, we could not find any beneficial effect of ACE inhibitor use on the severity of OHSS. Furthermore, we demonstrated weight gain and an increment in ovarian weight in rabbits which had no ascites.

In our study, there was no significant difference in basal aldosterone concentrations between the two groups. Day 9 aldosterone was significantly suppressed by ACE inhibition (enalapril), but was increased significantly in the control group. The suppressed aldosterone concentrations in the enalapril group could not prevent OHSS development. As we mentioned before, there was no significant difference in the mean oestradiol concentrations between the two groups. Enalapril did not show an effect on either oestradiol or progesterone secretion.

There were no significant correlations between basal body weight and peritoneal fluids in both groups. The wide range in peritoneal fluid amount within the same group suggested that each rabbit’s response to HMG might be different. In regard to peritoneal fluid and body weight gain, we found no difference between the two groups. Therefore, ACE inhibition did not seem to reduce the severity of OHSS in our study.

In conclusion, we found that serum renin and IL-6 concentrations increased in OHSS. There was a positive correlation between IL-6 and renin in both ACE inhibitor and control groups. The ACE inhibitor enalapril could not prevent ascites formation, although aldosterone concentrations were suppressed. Ovarian RAS may not be the sole factor in ascites formation. There may be other factors such as VEGF contributing to the pathogenesis of ascites formation. Enalapril appears not to be of benefit in reducing the severity of OHSS.

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References


Enalapril in the prevention of OHSS


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