Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication of the ovulation induction for infertility, especially in patients for whom methods using exogenous gonadotrophins are attempted. Although the severe form of OHSS is rare, it is potentially life-threatening (Golan et al., 1989). It is widely accepted that the major clinical components of this syndrome are marked enlargement of the ovaries containing luteal cysts and haemorrhagic cysts along with the shifting of fluid to the third space including the peritoneal cavity (Gergely et al., 1976). Polishuk et al. (1969) reported that fluid shift from the intravascular compartment into the peritoneal cavity was due to increased capillary permeability induced by a substance released from the ovaries. Mediators causally related to the phenomenon of OHSS in humans or animals have been suggested by several investigators. However, the pathophysiology of this syndrome is still controversial, and specific therapeutic management is not available.

Previous observations have revealed elevated plasma concentrations of oestradiol, progesterone, prolactin and testosterone during the clinical phase of this syndrome (Yuen et al., 1979). It is also well recognized that OHSS is usually seen several days after the injection of human chorionic gonadotrophin (HCG), and that its severe form is frequently associated with a conceptual cycle (Golan et al., 1989). We therefore postulated that progesterone released from the corpus luteum by HCG stimulation was related to the pathophysiology of OHSS. Accordingly, the present study was designed to determine the relationship of progesterone to the pathophysiology of OHSS in an animal model.

Materials and methods

Animals

Immature female Wistar rats aged 22 days, weighing between 42.0 and 48.0 g, obtained from Kyudo Co. (Kumamoto, Japan) were used throughout the study. All animals studied were kept in our laboratory, fed on a 12-hour light-dark regimen (lights 7:00–19:00), and allowed free access to water and a standard diet.

All procedures performed in this study were approved by the Animal Care and Use Committee of the Kumamoto University School of Medicine.

Production of hyperstimulated manifestations in rats

Eighteen rats were divided into three groups.

Group I
Six rats were given a subcutaneous injection of 10 IU of equine chorionic gonadotrophin (eCG; Teikoku Hormone Manufactory Co., Tokyo, Japan) in 0.2 ml of 0.9% saline at 8:00–8:30 a.m. for 4 consecutive days, and were given 30 IU human chorionic gonadotrophin (HCG; Mochida Pharmaceutical Corp., Tokyo, Japan) in 0.2 ml of saline at 8:00–8:30 a.m. on the fifth day (26th day of life).

Group II
Six rats were given 10 IU eCG at the time indicated above on the 24th day of life, and were given 10 IU HCG 48 h later (26th day of life).

Control group
As a control, six rats were given the same dosage of 0.9% saline for 5 consecutive days, from the 22nd to 26th day of life.

The ovarian weights of all rats were determined, expressed as the

Role of progesterone in capillary permeability in hyperstimulated rats

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Abstract: Ovarian enlargement, associated with the severe form of ovarian hyperstimulation syndrome (OHSS), was investigated in immature rats. A total of 96 female Wistar rats aged 22 days were given 10 IU of equine chorionic gonadotrophin daily for 4 consecutive days, and given 30 IU of human chorionic gonadotrophin on the fifth day to produce hyperstimulated manifestations. On the sixth day, groups of 12 rats each received RU486 at a dose of 0, 1, 2.5, 5, 10, 15 or 20 mg/kg (groups 1–7), or RU486 at 5 mg/kg combined with 6α-methyl-17α-hydroxy-progesterone acetate at 10 mg/kg (group 8). On the 7th day, the ovarian weight and capillary permeability of all rats were determined. Capillary permeability was evaluated from the Evans blue dye (EB) content in the ovaries and the EB level in peritoneal irrigated fluid at 30 min after the intravenous injection of EB. The peritoneal fluid EB level was significantly lower in groups 3, 4, and 5 than in the vehicle group. However, the peritoneal EB level in group 7 was higher than in the vehicle group, although not significantly. These findings demonstrated that RU486 has two divergent effects on capillary permeability, depending on the dose administered. In group 8, on the other hand, the peritoneal EB level and ovarian EB content were significantly higher than the corresponding values in group 4, respectively, suggesting that progesterone has a role in capillary permeability and ovarian enlargement. These results imply that progesterone may contribute, at least in part, to the pathophysiology of OHSS in this experimental model.

Key words: ovarian hyperstimulation syndrome/progesterone/ru486

Introduction

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication of the ovulation induction for infertility, especially in patients for whom methods using exogenous gonadotrophins are attempted. Although the severe form of OHSS is rare, it is potentially life-threatening (Golan et al., 1989). It is widely accepted that the major clinical components of this syndrome are marked enlargement of the ovaries containing luteal cysts and haemorrhagic cysts along with the shifting of fluid to the third space including the peritoneal cavity (Gergely et al., 1976). Polishuk et al. (1969) reported that fluid shift from the intravascular compartment into the peritoneal cavity was due to increased capillary permeability induced by a substance released from the ovaries. Mediators causally related to the phenomenon of OHSS in humans or animals have been suggested by several investigators. However, the pathophysiology of this syndrome is still controversial, and specific therapeutic management is not available.

Previous observations have revealed elevated plasma concentrations of oestradiol, progesterone, prolactin and testosterone during the clinical phase of this syndrome (Yuen et al., 1979). It is also well recognized that OHSS is usually seen several days after the injection of human chorionic gonadotrophin (HCG), and that its severe form is frequently associated with a conceptual cycle (Golan et al., 1989). We therefore postulated that progesterone released from the corpus luteum by HCG stimulation was related to the pathophysiology of OHSS. Accordingly, the present study was designed to determine the relationship of progesterone to the pathophysiology of OHSS in an animal model.
Effects of equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (HCG) on ovarian weight, peritoneal Evans blue (EB) dye content, ovarian EB content and serum concentration of oestradiol and progesterone at 48 h after the HCG injection. EB was injected intravenously at 30 min before the evaluation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ovarian weight (mg)</th>
<th>Peritoneal EB (µg)</th>
<th>Ovarian EB content (ng/mg)</th>
<th>Serum oestradiol (pg/ml)</th>
<th>Serum progesterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n = 6)</td>
<td>308.91 ± 23.33</td>
<td>d 8.98 ± 1.39</td>
<td>d 58.56 ± 2.83</td>
<td>d 52.05 ± 9.38</td>
<td>c 279.60 ± 22.02</td>
</tr>
<tr>
<td>II (n = 6)</td>
<td>113.65 ± 9.02</td>
<td>c 1.63 ± 0.62</td>
<td>c 27.96 ± 5.93</td>
<td>c 28.49 ± 3.97</td>
<td>c 52.42 ± 11.18</td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>36.37 ± 2.90</td>
<td>e 0.47 ± 0.08</td>
<td>e 24.95 ± 2.62</td>
<td>e 19.36 ± 4.12</td>
<td>d 3.70 ± 0.72</td>
</tr>
</tbody>
</table>

Values are expressed as the means ± SEM. Those which are significantly different are connected by rules: *P < 0.05; **P < 0.03; ***P < 0.001 respectively. Group I (n = 6): received 10 IU of eCG for 4 consecutive days starting on the 22nd day of life followed by 30 IU of HCG on the fifth day (26th day of life). Group II (n = 6): received a single injection of 10 IU of eCG on the 24th day of life followed by 10 IU of HCG 48 h later (on the 26th day of life). Control group (n = 6): received normal saline instead of eCG/HCG.

Measurement of plasma adrenocorticotrophic hormone (ACTH) concentrations

Another group of 42 immature female rats was prepared for the measurement of plasma ACTH concentration, which was employed to detect the anti-glucocorticoid effect of RU486 in the hyperstimulated rats.

Statistical analyses

All data are presented as the mean ± SEM. Statistical analysis was performed by one-factor analysis of variance (ANOVA) followed by Fisher’s protected least significant difference test for multiple comparisons. The level of significance was set at P = 0.05.

Results

Production of hyperstimulated manifestations in rats (Table I)

At 48 h after the last injection, the ovarian weights in group I (308.91 ± 23.33 mg) were significantly greater than those in group II and the control group (113.65 ± 9.02, 36.37 ± 2.90 mg; P < 0.0001, respectively). The EB level of the peritoneal cavity in group I (8.98 ± 1.39 µg) was significantly higher than that in group II and the control group (1.63 ± 0.08 µg).
Progesterone acetate. Progesterone acetate.

In parentheses over each bar. Significantly different \( P < 0.0001 \).

In the rats receiving 2.5 or 5 mg/kg of RU486, the ovarian weight was significantly lower than that in rats receiving vehicle alone \((198.21 \pm 14.69, 241.64 \pm 14.83 \text{ versus } 292.63 \pm 13.89 \text{ mg}; P < 0.005, P < 0.05, \text{ respectively})\) (Figure 2). The ovarian EB content in the rats receiving 2.5, 5 or 10 mg/kg of RU486 was significantly lower than that in the rats receiving the vehicle alone \((47.95 \pm 2.62 \text{ versus } 14.83 \pm 3.15 \text{ ng/mg wet weight tissue}; P < 0.005)\) (Figure 2).

The peritoneal irrigated fluid EB level in the rats receiving progesterone concomitantly with RU486 was significantly higher than in the rats receiving the same dose of RU486 alone \((6.97 \pm 0.54 \text{ versus } 2.01 \pm 0.31 \text{ µg}; P < 0.03)\) (Figure 1). In addition, a similar result was obtained for the ovarian EB content \((57.82 \pm 5.07 \text{ versus } 46.83 \pm 1.59 \text{ ng/mg}; P < 0.03)\) (Figure 2). These results indicated that the decrease in capillary permeability induced by RU486 injection was reversed by the concomitant administration of progesterone.

**Analysis of peripheral oestradiol, progesterone and ACTH concentrations**

There were no significant differences in the serum concentrations of oestradiol and progesterone among any of the groups (Figure 3a and 3b, respectively).

The plasma ACTH level in the rats receiving 20 mg/kg of RU486 was significantly higher than that in the rats receiving 0, 1, 5 or 10 mg/kg \((2.88 \pm 0.78 \text{ versus } 1.44 \pm 0.18, 1.25 \pm 0.06, 1.46 \pm 0.12 \text{ and } 1.46 \pm 0.12 \text{ ng/ml}; P < 0.01, \text{ respectively})\) (Figure 4). This augmentation of the plasma ACTH level seemed to reflect the existence of an anti-glucocorticoid effect of RU486 in this situation.
Figure 4. Alterations in the plasma concentration of ACTH by administration of RU486 in hyperstimulated rats. Values are expressed as the means ± SEM. Control rats were unstimulated animals receiving normal saline and ethanol, instead of eCG/HCG or RU486 respectively. # = significantly higher than in rats receiving 0–10 mg of RU486 (#1, #2, #3, #4). Numbers of animals are shown in parentheses over each bar.

RU486 is a synthetic steroid hormone that binds to the progesterone receptor and acts as a progesterone antagonist (Tanaka et al., 1993). RU486 is reported to have a long half-life and a high affinity for progesterone and cortisol receptors, while it does not bind to the oestrogen receptor (Speroff et al., 1994). The administration of the lower doses (2.5, 5 and 10 mg/kg) of RU486 decreased the capillary permeability in the present study. This may have resulted from progesterone receptor blockade by lower concentrations of RU486. In contrast, the administration of the high dose (20 mg/kg) of RU486 augmented the capillary permeability, although not significantly. This was probably due to the anti-glucocorticoid effect of excess RU486 (Speroff et al., 1994). In fact, an increase in the plasma ACTH level was observed by the administration of 20 mg/kg of RU486 in the present study, which might reflect the anti-glucocorticoid effect of this agent (Kettel et al., 1996). Our finding that the ovarian weight decreased following the administration of RU486 is inconsistent with the report on rats by Schoot et al. (1987). This decrease in ovarian weight may have resulted from a decline of capillary permeability in the ovarian stroma. The maximal inhibitory effect of RU486 on the peritoneal irrigated fluid EB level was observed at 2.5–5 mg/kg. The administration of progesterone at the dosage of 10 mg/kg concomitantly with 5 mg/kg of RU486 reversed the decline in capillary permeability. This result suggests that progesterone may influence capillary permeability in our OHSS experimental model. On the other hand, there were no significant changes of the serum concentrations of ovarian steroid hormones in the present study. An inhibitory effect of RU486 on progesterone synthesis has been reported in the immature rat (Tanaka et al., 1993), pregnant rats (Kawano et al., 1988), and humans (Spitz et al., 1994), while Telleria et al. (1994) reported that there was an increase in serum progesterone concentration after the administration.
of RU486 in pro-oestrous rats. These discrepancies may be due to the difference of the time of its administration.

Bergqvist et al. (1993) demonstrated the existence of progesterone receptors in the peripheral veins and suggested that this implied hormonal control of vascular function. Moreover, Higuchi et al. (1995) detected the expression of progesterone receptors in the human pelvic peritoneum by means of reverse transcription–polymerase chain reaction (RT-PCR). These reports provide support for the possibility that progesterone may have a modulatory role on peritoneal capillary permeability.

Yuen et al. (1979) reported elevated plasma concentrations of progesterone, in addition to oestradiol, during the clinical phase of OHSS. Moreover, cultured human granulosa cells obtained from women at risk of OHSS showed an increased capacity for the synthesis of steroid hormones, including progesterone (Leya et al., 1992). The widely-known fact that severe OHSS occurs frequently in association with pregnancy suggests an involvement of progesterone in the pathophysiology of this syndrome.

Lyons et al. (1994) and Morris et al. (1995a) reported that OHSS can be induced by endogenous or exogenous HCG. Indeed, the use of progesterone instead of HCG for luteal phase support is one practical method for attempting to reduce the incidence of severe OHSS. However, HCG administration may induce a higher local progesterone concentration, especially in enlarged ovaries with multiple corpora lutea, rather than the administration of progesterone itself, and such a local increase of progesterone may play a role as the stimulatory mediator for ovarian hyperstimulated manifestations. On the other hand, the use of gonadotrophin-releasing hormone analogues (GnRHa) to induce ovulation is reported to eliminate effectively the risk for developing OHSS, compared to the use of HCG (Lewit et al., 1996). This finding may provide support for the hypothesis that the protracted luteinizing hormone (LH)-like activity rendered by HCG, which has a longer half-life than LH, with prolonged stimulation of the corpus luteum may be involved in the pathophysiology of OHSS (Kol et al., 1996). The sustained local level of progesterone derived from these HCG-stimulated ovaries may conceivably contribute to the clinical features of this syndrome.

In clinical situations, RU486 is difficult to use as a therapeutic candidate for OHSS patients, because of its abortive effects (Thonneau et al., 1995), except for the cases in which elective cryopreservation of all embryos are performed in in vitro fertilization (IVF) cycles. However, low-dose RU486 administration was recently suggested to be effective in delaying the appearance of the endometrial implantation window which might have been advanced by the ovulation induction (Paulson et al., 1997). Therefore, the administration of this agent set at a critical dose presents a possibility for its use without its abortive side-effect in the luteal phase. To our knowledge, the therapeutic management using RU486 for OHSS patients has not been reported previously.

Polishuk and Schenker (1969) found no ovarian enlargement or ascites formation when exogenous progesterone was given to experimental animals. We also obtained similar results by administration of progesterone in immature female rats (data not shown). Therefore, it is conceivable that progesterone does not cause the increase in capillary permeability by itself but rather modulates the increased permeability caused by factor(s) through the ovulatory process initiated by gonadotrophins. In fact, markedly elevated progesterone levels are often seen in clinical situations, whereas many of these individuals do not develop into the clinical scenario of OHSS. Further studies are needed to clarify the interactions of several mediators in the pathophysiology of clinical OHSS development.

Several investigators reported that the ovarian-derived protein to angiotensin cascade (ODPAC) may be related to the pathogenesis of OHSS. Navot et al. (1987) demonstrated a direct correlation between plasma renin activity and the severity of OHSS. Morris et al. (1995b) found that angiotensin converting enzyme inhibition caused a decrease in the incidence of OHSS in a rabbit model. On the other hand, Morris et al. (1995c) demonstrated that the administration of an angiotensin converting enzyme inhibitor appeared to lower the serum progesterone concentration in human stimulated cycles. These data are not inconsistent with the hypothesis that the ODPAC may stimulate the production of progesterone which then induces OHSS.

Vascular endothelial growth factor (VEGF) may contribute to the development of OHSS (McCleure et al., 1994; Neulen et al., 1995). Charnock-Jones et al. (1993) observed that steroids are probably involved in VEGF regulation in human endometrium. In addition, Torry et al. (1996) demonstrated temporal fluctuations of VEGF mRNA expression in the human endometrium and its increase in the secretory phase. These findings indicate the possibility that progesterone might participate in the aetiology of OHSS through its modulatory action on VEGF in ovaries and peritoneum. Whether progesterone-dependent enhancement of VEGF activity may occur in our experimental model is now under investigation.

Several inflammatory cytokines have been suggested to be involved in the pathogenesis of OHSS. Orvieto et al. (1995) reported elevated intrafollicular interleukin (IL)-2 concentrations in IVF cycle patients who developed OHSS, and high concentrations of IL-6 (Friedlander et al., 1993; Abramov et al., 1996) as well as IL-8 (Abramov et al., 1996) were observed in the ascitic fluid of severe OHSS patients. On the other hand, since the serum concentrations of IL-6 in gonadotrophin-stimulated cycles were significantly higher than in the natural cycles, gonadotrophin may have a stimulatory role on the IL-6 production (Loret de Mola et al., 1996). An immunohistochemical study revealed that IL-6 was strongly localized in human endometrial glandular and epithelial cells during the secretory phase compared to the proliferative phase (Tabibzadeh et al., 1995), supporting the contention that progesterone may have an ability to stimulate IL-6 production at least in endometrium. Based on these findings, the possibility exists that progesterone may stimulate the production of those cytokines which relate to the clinical phase of OHSS. Further studies are needed to elucidate the exact mechanisms of the interactions between these cytokines and progesterone.

In conclusion, the alterations in capillary permeability mediated by gonadotrophins through luteal function may be influ-
enced by progesterone; i.e., progesterone may, at least in part, have a role in the pathophysiology of OHSS.

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References


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