Human Reproduction vol.10 no.6 pp.1355–1358, 1995

Inhibition of ovarian-derived prorenin to angiotensin cascade in the treatment of ovarian hyperstimulation syndrome

R.S.Morris[1,3], I.L.Wong[1], E.Kirkman[2], E.Gentschein[1] and R.J.Paulson[1]

[1]Division of Reproductive Endocrinology, University of Southern California School of Medicine, 1240 N. Mission Road Room 1M2, Los Angeles, CA 90033 and [2]Vivarium, University of Southern California School of Medicine, Los Angeles, CA 90033, USA

[3]To whom correspondence should be addressed

The purpose of this experiment was to determine whether use of the angiotensin-converting enzyme (ACE) inhibitor, enalapril, would prevent the occurrence of ovarian hyperstimulation syndrome (OHSS) in the rabbit model. A total of 20 adult female New Zealand white rabbits were studied. All rabbits received 75 IU of human menopausal gonadotrophin s.c. each day for 7 days. On day 8, all rabbits received 2500 IU of human chorionic gonadotrophin (HCG). Ten rabbits were randomly chosen to receive enalapril orally. Five received 1 mg/kg of enalapril and five received 2 mg/kg of enalapril twice daily. The remainder received placebo orally twice daily. On day 10, all rabbits underwent surgical exploration. Total body weight was found to increase significantly in the placebo group (by 293 g, $P < 0.001$) but not in either group receiving enalapril. Haematocrit also increased significantly in the placebo group (by 3%, $P < 0.013$) but not in the enalapril groups. Ovarian weights were highest for the 2 mg/kg enalapril group (5.80 ± 0.52 g), followed by the 1 mg/kg enalapril group (3.64 ± 0.45), and least for the placebo group (2.69 ± 0.17). All 10 placebo rabbits met criteria for severe OHSS whereas only six in the enalapril groups did. We concluded that angiotensin II may play a significant role in the development of weight gain, third space fluid accumulation and intravascular fluid depletion in OHSS. ACE inhibition resulted in a 40% decrease in the incidence of OHSS in the rabbit model.

Key words: angiotensin converting enzyme inhibition/enalapril/ovarian derived prorenin to angiotensin cascade/ovarian hyperstimulation syndrome/rabbit

Introduction

Recent evidence has suggested that altered levels of components of the ovarian derived prorenin to angiotensin cascade (ODPAC) may be responsible for the clinical stigmata of ovarian hyperstimulation syndrome (OHSS). Haning *et al.* (1985) noted that aldosterone is elevated in patients with OHSS and that the concentrations were correlated with plasma renin activity. Navot *et al.* (1987) demonstrated a direct relationship between plasma renin activity and the clinical severity of OHSS in humans. Ong *et al.* (1991) noted that patients with severe OHSS have elevated concentrations of prorenin, renin and aldosterone.

We sought to determine whether the use of angiotensin-converting enzyme (ACE) inhibitors would be successful in preventing some or all of the clinical signs of OHSS. Since the primary manifestations of OHSS are related to increased vascular permeability (third space fluid accumulation, intravascular volume depletion, etc.), we specifically measured the effects on these parameters.

Materials and methods

Experimental subjects

This experiment was approved in advance by the University of Southern California School of Medicine Committee on Animal Care and conformed to the Department of Health Education and Welfare guidelines for the care and use of laboratory animals. We studied 20 reproductively mature, female, New Zealand white rabbits. Each rabbit was housed individually and had free access to food and water. On days 1–7, all rabbits were administered gonadoryl (Mochida Pharmaceutical, Tokyo, Japan), s.c. daily between 06.00 and 07.00 h. Gonadoryl is a gonadotrophin preparation consisting of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the ratio FSH:LH 3.7:1. On day 8, each rabbit received 2500 IU of human chorionic gonadotrophin (HCG) i.m. Ten rabbits were randomly chosen and assigned to receive the ACE inhibitor, enalapril in a dose of 1 mg/kg (EN1) or 2 mg/kg (EN2) orally every 12 h (five rabbits at each dose). The remaining 10 rabbits received a placebo orally every 12 h. Randomization was accomplished by picking rabbit identification numbers out of a hat.

Collections and measurements

All rabbits were weighed daily. On days 1 and 10, blood was collected from the marginal ear vein into a capillary tube and spun for 5 min to determine haematocrit. On day 10, all rabbits underwent surgical exploration of the abdomen and thorax and were evaluated by an investigator masked to the treatment protocol. Each rabbit was assessed for the presence of ascites, pleural, or pericardial effusion and the volume of the fluid, if present, was measured. The ovaries, kidneys, and uterus were carefully excised, blotted dry, and weighed. The diagnosis of severe OHSS was given based on Schenker and

Weinstein's modification of the Rabau classification system for human OHSS (Schenker *et al.*, 1978). Briefly, severe OHSS is distinguished by the presence of enlarged ovaries, abdominal distension, ascites, pleural or pericardial effusion, and haemoconcentration.

Statistics

All data are presented as the mean and SEM. Changes from baseline within each group were determined by paired *t*-test. Differences between the groups were determined by analysis of variance and Fisher's exact test.

Results

Total body weight increased significantly in the rabbits receiving the placebo, whereas weight was unchanged in either of the groups receiving enalapril (Figure 1). The largest differences were observed after HCG administration.

Intravascular volume depletion was estimated by changes in haematocrit. Baseline haematocrit was similar in all groups. However, by day 10, haematocrit in the plaebo group had increased significantly (from 38.4 ± 1.1 to 41.4 ± 1.4%, $P = 0.013$) whereas no change was seen in the enalapril group (from 34.8 ± 1.0 to 35.9 ± 1.3%).

Due to wide variation within the enalapril groups, no significant difference was noted in the mean volume of ascites between enalapril and placebo groups (18.4 ± 5.5 ml versus 21.3 ± 3.35, $P = 0.6$). All placebo rabbits had either pleural or pericardial effusion or both. As with the case for human OHSS, not all placebo rabbits had both types of effusions. Of the enalapril rabbits, six also demonstrated effusions and so no difference was noted in the mean volumes of pleural (0.7 ± 0.3 ml versus 0.4 ± 0.27, $P = 0.538$) or pericardial effusions (0.3 ± 0.2 ml versus 0.6 ± 0.21, $P = 0.22$) between the enalapril groups and the placebo group. Additionally, the small volumes in the affected animals (0–2 ml) were difficult to measure accurately.

All the rabbits had ovaries that were markedly enlarged from the baseline state. The ovaries had multiple follicles and corpora lutea over their surface. Due to the large number and close proximity of the cysts on each ovary, no attempt was made to quantify any difference in the number of these cysts present in the different groups. However, a significant difference was noted in the final ovarian weights in the three groups. A dose–response relationship was seen with the largest ovaries identified in EN2 (5.8 g ± 0.52), followed by EN1 (3.64 g ± 0.45), and the smallest in placebo (2.69 g ± 0.17) ($P < 0.001$). There was no difference noted in the weights of either the kidneys (18.1 ± 1.8 g versus 16.6 ± 0.2) or uteri (9.78 ± 0.46 g versus 11.63 ± 0.97) between the enalapril or control groups.

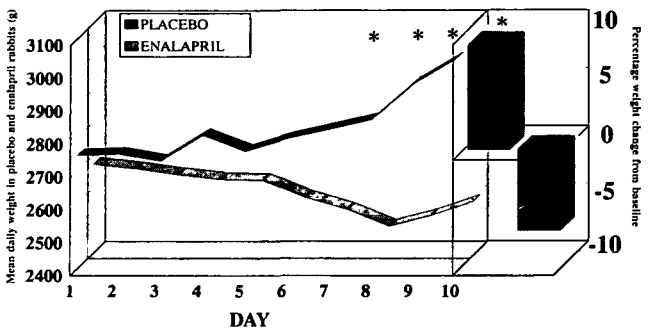

Figure 1. Weight change in rabbits receiving placebo compared with rabbits receiving enalapril ($P < 0.0012$).

Table 1 shows the distribution of rabbits according to diagnosis of severe OHSS as previously stated. All rabbits receiving placebo had increased weight, enlarged ovaries (compared to pre-stimulation), profuse ascites (7–55 ml), elevated haematocrit, and either pleural or pericardial effusion and were diagnosed with severe OHSS, whereas only six rabbits receiving enalapril met all these criteria. Thus a statistically significant reduction of 40% in the incidence of OHSS was seen (Fisher's exact test, $P < 0.05$).

Discussion

The underlying physiological derangement which is primarily responsible for the manifestations of OHSS is increased vascular permeability (Golan *et al.*, 1989). Capillary leakage leads to the commonly observed third space fluid accumulation and weight gain. Simultaneously, decreased circulating blood volume leads to oliguria which may lead to electrolyte abnormalities and renal failure. Hyperviscosity of blood in the circulation, a result of low plasma volume, may predispose to thrombosis and embolization and is reflected by haematocrit (Rizk *et al.*, 1990). In fact, haemoconcentration, as determined by the haematocrit, has been shown to be closely associated with the severity of OHSS (Pride *et al.*, 1990).

Evidence that derangements in ODPAC account for the changes seen in OHSS are threefold. Firstly, ODPAC is driven to high levels by gonadotrophin stimulation, especially HCG (Sealey *et al.* 1985; Navot *et al.*, 1987). Secondly, polycystic ovary syndrome patients, who are known to be at high risk for OHSS, have elevated baseline concentrations of prorenin (Morris *et al.*, 1994). Lastly, angiotensin II has been demonstrated to increase vascular permeability in several different models. Bisset and Lewis (1962) injected angiotensin II s.c. into the guinea pig and under light microscopy noted oedema in the areas of injection. Robertson and Khairallah (1972) isolated rabbit aorta and filled them with Evan's blue dye. They then injected some areas with angiotensin II and some with saline. Only the areas injected with angiotensin II showed leakage of dye. The probable mechanism for this leakage was provided by Morel *et al.* (1990). They determined that pulmonary vascular endothelial cells contracted when angio-

Table I. Diagnosis of severe OHSS according to group ($P < 0.05$)

	OHSS	No OHSS
Enalapril	6	4
Placebo	10	0

OHSS = ovarian hyperstimulation syndrome

tensin II was added. This contraction increases the space between them and allows fluid leakage to occur.

ACE inhibitors have been used to treat other syndromes of increased vascular permeability. For instance, diabetic retinopathy is caused by vascular leakage of the retinal vessels. This retinopathy is also associated with high prorenin levels (Franken *et al.*, 1988). Use of ACE inhibitors has been shown to slow the progression of this disease (Larsen *et al.*, 1990). Investigators have also noted that diabetic nephropathy is improved by treating patients with ACE inhibitors (Ikeda *et al.*, 1989) and these improvements are independent of any change in blood pressure (Stornello *et al.*, 1988). From our data in this experiment, it cannot be concluded with certainty that the beneficial effects of ACE inhibition were due to a reduction in angiotensin II since ACE inhibitors have other effects. Another interesting experiment would be to determine if specific angiotensin II receptor antagonists are also capable of reducing the incidence or severity of OHSS in this model.

In the present experiment we observed that use of ACE inhibition was associated with improvements in vascular permeability. We chose to study the New Zealand white rabbit as it has been used in the past by other investigators to study potential treatments for OHSS and it has proven to be a reproducible model for human OHSS (Knox, 1974; Erlik *et al.*, 1978; Pride *et al.*, 1984). As in those experiments, all the placebo rabbits and some of the enalapril rabbits in the present study developed a clinical and laboratory picture which appears to be similar to human OHSS. Affected animals develop haemoconcentration, enlarged ovaries, and third space fluid accumulation in the peritoneal, pleural, and pericardial spaces.

Enalapril was chosen since it has a long half-life. More than 90% inhibition in ACE activity persists at 12 h, so twice daily dosing is adequate (Garrison and Peach, 1990). Increases in haematocrit, an index of intravascular fluid depletion, were blocked with the use of enalapril. This is further reflected by the lack of weight gain in the treated rabbits. Since vascular volume is maintained, it is possible that secondary complications such as oliguria, electrolyte abnormalities, and thrombosis, which were not directly assessed in the present study, may also show significant improvement.

A paradoxical finding in the present study was the greater size of the ovaries in the rabbits that received enalapril. We had expected that the ovaries in the rabbits with the most severe OHSS would be larger than those with milder degrees or without OHSS. In fact, ovarian size was increased in a dose-dependent fashion in the group that received enalapril. This may be an effect of the enalapril independent of the effects of OHSS. It has been reported that ACE inhibitors are able to alter steroid synthesis within the ovary (Peperell *et al.*, 1993). Such an alteration may cause enlargement of the steroid synthetic apparatus and subsequently enlargement of the ovary. Alternatively, some investigators have shown that angotensin II inhibition may inhibit ovulation (Pellicer *et al.*, 1988). Retention of the oocytes and follicular fluid might also explain why these ovaries were larger.

Since OHSS can be a serious side-effect of ovarian stimulation in humans, it is tempting to speculate that ACE inhibitors may be useful in the treatment of OHSS in humans. Since severe OHSS usually occurs with pregnancy, possible fetal effects must be addressed. There are no well controlled studies of ACE inhibitors in pregnant women and as such they are classified in FDA Pregnancy Category C (Sifton, 1994). Use in the second and third trimester has been associated with fetal anuria and renal failure, oligohydramnios, neonatal neurological complications, and fetal death (Perlman and Volpe, 1989; Sifton, 1994). Since OHSS occurs around the time of implantation, treatment would be discontinued well before these effects would become manifest. Important effects to consider would include teratogenicity and miscarriage. Inadvertent exposure to ACE inhibitors limited to the first trimester of pregnancy in humans has not been reported to affect fetal outcome adversely. No teratogenic effects have been observed in rats or rabbits at 330 and 50 times the recommended maximum human dose respectively (Sifton, 1994).

In conclusion, angiotensin II plays an important role in the development of OHSS in the rabbit model. Angiotensin II probably works by increasing capillary vascular permeability as has been demonstrated in other systems. Inhibition of angiotensin II production prevents vascular leakage and therefore may become an important treatment for OHSS in humans.

Acknowledgements

We wish to thank Joseph Foldi for his assistance in providing anaesthesia.

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Received on July 11, 1994; accepted on March 15, 1995