ANIMAL EXPERIMENTATION

The Role of Intravenous Immunoglobulin in the Prevention of Severe Ovarian Hyperstimulation Syndrome

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Purpose: The role of intravenous immunoglobulin (IVIG) in the prevention of severe ovarian hyperstimulation syndrome (OHSS) was evaluated.

Methods: Ovarian hyperstimulation was induced in eight rabbits using human menopausal gonadotropin/human chorionic gonadotropin (hMG/hCG) after pretreatment with IVIG (IVIG group) or bovine serum albumin (BSA group). Main outcome measures included (1) signs of OHSS, such as the degree of ascites formation and the increase in body weight; and (2) the degree of ovarian stimulation as reflected by serum sex-steroid hormone levels.

Results: A significantly lower ascites response and a tendency toward a decreased change in body weight were observed in the IVIG group compared to the BSA group. Serum estradiol, progesterone, total protein, and ovarian weights were not statistically different between the two groups.

Conclusions: IVIG prevented severe OHSS in a rabbit model, whereas BSA did not. Further studies are justified in an attempt to clarify the role of the immune system and IVIG in the pathophysiology and prevention of severe OHSS.

KEY WORDS: ovarian hyperstimulation syndrome; intravenous immunoglobulin; albumin; prevention; immune system.

INTRODUCTION

Recently indications have accumulated concerning interactions between the immune and the reproductive systems that result from sharing certain cytokines and their receptors (1). Several cytokines were found to be produced in the ovaries or contained in the follicular fluid (FF). Furthermore, cytokines have been suggested to play a role in the prediction and pathogenesis of ovarian hyperstimulation syndrome (OHSS) (2).

Intravenous immunoglobulin (IVIG) has been shown to have an anticytokine nature and to inhibit the secretion of various cytokines (3). If cytokines have a substantive role in the pathogenesis and pathophysiology of OHSS, it may be possible to prevent the development of OHSS with IVIG, which blocks the immune response and assists immunologic homeostasis. The aim of this study was to evaluate the role of IVIG in preventing severe OHSS in a rabbit model.

MATERIALS AND METHODS

Eight sexually mature New Zealand White virgin female rabbits were housed in individual cages and fed a standard diet. The animals were divided into two equal groups. Each group of four rabbits was subjected to pretreatment with serial administration of either bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Missouri) (BSA group) or IVIG (GamImuneN; Bayer, Germany; generously contributed by Promedico, Petah Tikva, Israel) (IVIG group), 0.4 g/kg/day for 5 days (4). On completion of pretreatment, i.m. human menopausal gonadotropin (hMG; Pergonal;

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Table I. Serum E2 and P Levels in the Different Study Groups

Groups	Day 1		Day 6		Day 12	
	E2 ^a	P ^b	E2	P	E2	P
	pg/mL	ng/mL	pg/mL	ng/mL	pg/mL	ng/mL
IVIG	4.87± 2.22	1.02± 0.59	7.35± 3.66	0.35± 0.3	41.75± 27.82	0.57± 0.26
BSA	7.0± 3.84	3.82± 2.76	5.9± 4.40	0.7± 0.42	73.5± 35.41	2.02± 2.26

^a Conversion factor to SI unit, 3.67.^b Conversion factor to SI unit, 3.18.

Teva, Petah Tikva, Israel), 37.5 IU, was administered daily to all animals in the preovulatory state for 6 consecutive days, followed by i.m. human chorionic gonadotropin (hCG; Chorigon; Teva), 2500 IU on day 7.

The hMG dosage used in this experiment was administered to the animals according to previously established data, which have clearly demonstrated that in all animals receiving 37.5 IU hMG, the mean ovarian size reached three to four times that of controls and ascites were found in all animals (5). Furthermore, two reasons led us to choose BSA for the control group versus IVIG instead of placebo. First, BSA is a known and widely used control in experiments assessing the immunologic effect of IVIG (6); and second, confusing evidence has recently emerged regarding the role of human albumin in the prevention of severe OHSS (7).

All animals were autopsied on day 15 after an overdose of sodium pentobarbital (Pental Veterinary; CTS Chemical Industries Ltd., Tel Aviv, Israel). After laparotomy via a midline incision, the degree of ascites was assessed by a single observer (R.O.) using the visual score (8). When there was no ascites, a score of 1 was assigned. The scores for minimal, moderate,

marked, and gross (abdomen obviously distended and ascitic fluid exuding on incising peritoneum) ascites were 2, 3, 4, and 5, respectively. Next, each animal was hung over a container for 15 min so that all ascitic fluid could be drained and quantified. Both ovaries were then excised and weighed. The average ovarian weight was calculated for each animal.

Body weights were recorded on days 1, 6, and 15. Serial blood samples were obtained from a marginal ear vein on days 1, 6, 12, and 15 to measure serum estradiol (E₂), progesterone (P), albumin, and globulin.

The results are expressed as means and SDs. Statistical analysis was performed by Student's *t* test. We accepted significance whenever the *P* value was 0.05 or less, while *P* less than 0.1 was considered to indicate a tendency.

RESULTS

The mean pretreatment body weights of the animals in the IVIG (3720 ± 332 g) and BSA (3977 ± 412 g) groups were comparable. The Δ percentage body weight on day 6 compared with the pretreatment body

Table II. Albumin and globulin levels in the different study groups

Groups	Day 1		Day 6		Day 12		Day 15	
	Albumin	Globulin	Albumin	Globulin	Albumin	Globulin	Albumin	Globulin
	gr%	gr%	gr%	gr%	gr%	gr%	gr%	gr%
IVIG	4.1± 0.24	1.62± 0.17	3.85± 0.13	3.5±* 0.14	3.97± 0.17	2.4± 0.18	4.0± 0.16	2.35± 0.06
BSA	4.0± 0.18	1.52± 0.09	4.25±** 0.13	1.8± 0.16	4.05± 0.17	2.12± 0.33	3.95± 0.17	2.22± 0.23

* *P* < 0.0001.** *P* < 0.005.

weight was not statistically different in the IVIG group ($0.05 \pm 1.05\%$) compared to the BSA group ($-0.05 \pm 0.95\%$). However, a tendency ($P < 0.1$) was observed toward a lower Δ percentage body weight on day 15 compared with that on day 6 in the IVIG group ($0.92 \pm 1.5\%$) compared to the BSA group ($2.67 \pm 0.95\%$).

On gross inspection, the ovaries in both groups contained numerous follicular, hemorrhagic cysts, and corpora lutea (CL). Mean ovarian weights did not differ between the IVIG and the BSA groups (0.81 ± 0.18 and 1.04 ± 0.35 g, respectively).

Serum E_2 and P taken on days 1, 6, and 12 were compared between the two groups (Table I). Moreover, day 1, 12, and 15 serum albumin and globulin levels were also compared between the two groups. However, day 6 (end of pretreatment) serum albumin and globulin were higher in the BSA ($P < 0.005$) and IVIG ($P < 0.0001$) groups, respectively (Table II). No statistically significant differences were observed between the two groups comparing total protein levels on day 12 (day of hCG administration) and day 15.

Ascites formation was less pronounced after hyperstimulation in the IVIG compared to the BSA group (Fig. 1). The mean ascites scores in the IVIG and BSA groups were 1.75 ± 0.96 and 4.5 ± 0.5 , respectively ($P < 0.003$), while the ascitic volumes were 1.52 ± 0.53 and 6.0 ± 1.78 ml, respectively ($P < 0.003$). Of note is that a 0.8-ml effusion was observed in the right pleural cavity of one rabbit in the BSA group.

DISCUSSION

To the best of our knowledge, this is the first report demonstrating a role of IVIG in the prevention of severe OHSS. Because IVIG prevents severe OHSS without affecting ovarian weight and serum E_2 and P levels, it may be suggested that two unrelated mechanisms are responsible for the ovarian response to gonadotropin. One may account for ovarian enlargement and excessive steroidogenesis, while the other accounts for the release of a certain intermediate causing an increase in capillary permeability (5).

Interactions between the immune and the reproductive systems which result from sharing certain lymphohematopoietic cytokines and their receptors are now recognized (1). Furthermore, several cytokines, as well as vascular endothelial growth factor (VEGF), were found to play a role in either the prediction or the pathogenesis of OHSS (9–13). However, whether their presence is an epiphenomenon or directly contributes to the vasomotor instability and deterioration in OHSS

Fig. 1. Mean ascitic fluid volumes and ascites scores of the IVIG and BSA groups. Vertical bars represent 1 SD.

patients is still unclear. The aforementioned observations, together with the established interactions between the immune and the reproductive system, suggest a role of cytokines in the pathogenesis of OHSS. Because IVIG has been shown to have an anticytokine nature and to inhibit the secretion of various cytokines (3), we elected to assess its role in preventing severe OHSS in a rabbit model.

In the present study we have demonstrated a significant effect of IVIG in preventing severe OHSS without affecting morphological and endocrinological signs of ovarian stimulation, as demonstrated by ovarian weight and serum E_2 and P levels, respectively. Furthermore, this preventive effect was also observed after controlling for the oncotic properties of IVIG. These observations may suggest different unrelated ovarian responses to gonadotropin, which account for (i) ovarian enlargement and excessive steroidogenesis, which were comparable between the two groups in the present study; and (ii) the release of a certain intermediate causing an increase in capillary permeability. In the present study, IVIG was shown to prevent increased permeability, by the tendency toward a decreased change in

body weight and a significantly lower level of ascites formation. These suggested different ovarian response mechanisms are also in accordance with, and may further elucidate, the known clinical observation of patients developing severe OHSS, despite a slight to moderate elevation of serum E_2 levels (14,15), and different ovarian sizes. Because IVIG was shown to prevent severe OHSS without affecting ovarian morphology or blood sex-steroid levels and after controlling for its oncotic properties, it may be suggested that IVIG acts through its anticytokine nature by inhibiting cytokines' secretion or their effects.

In summary, different unrelated ovarian response mechanisms to gonadotropins are suggested. A direct action of excessive gonadotropins on ovarian tissue accounts for the ovarian enlargement and increased steroidogenesis, while none of the hitherto reported studies have identified the cause of the third space fluid-shifting phenomenon. However, it was implicated in a rabbit model that a certain intermediate, released by gonadotropin-hyperstimulated ovaries at ovulation, causes this increase in capillary permeability. In the present study, we demonstrated the ability of IVIG, probably by its anticytokine nature, to block the effect of this intermediate, which may be either a cytokine(s) (such as IL-2) or another member(s) of the immune response (2). Further studies are justified in an attempt to clarify the role of the immune system and IVIG in the pathophysiology and prevention of severe OHSS and to find a reliable predictive test for the syndrome.

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