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Original Research

Inhibin B level changes during the follicular phase in rats with unilateral ovarian torsion

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Summary

Objective: To investigate the association between duration of ovarian torsion and levels of serum inhibin B in an animal model. *Materials and Methods:* An animal model prospective study was conducted in a tertiary referral hospital and chemical pathology institute. Nineteen female Lewis rats were divided to four groups. In the first group, the control group, laparotomies alone were performed. In groups 2-4 laparotomies and right ovarian torsion for 720 degrees with fixation of the adnexa to the parietal peritoneum were performed. Blood samples were taken from the rat groups at 12, 24, and 36 hours after laparotomy. Inhibin B levels were measured by ELISA. *Results:* Serum mean ± SD inhibin B level in the control rats during the beginning of the metesterus, 12, 24, and 36 hours later were 108.4 ± 43.6, 129.5 ± 49.3, 99.07 ± 60.50, and 91.58 ± 27.74 pg/ml, respectively. Inhibin B levels in the control group increased 12 hours after the beginning of the metesterus, whereas in the study group, a decline in inhibin B levels was found at the same time. This difference was significant ($p = 0.05$). Inhibin B levels at 24 and 36 hours from the beginning of the metestrus did not show statistical difference. *Conclusions:* Unilateral ovarian torsion in rats changed the pattern of inhibin B secretion at the beginning of the metesterus and was associated with a significant decrease in inhibin B serum levels. This observation, following acute ovarian vascular occlusion, is probably due the effects of ischemia on hormonal regulation during the early follicular phase.

Key words: Ovarian torsion; Inhibin B; Follicular changes; Animal model; Rats.

Introduction

Adnexal or ovarian torsion is a surgical emergency among women who present to the ER with acute abdominal pain [1]. Torsion of the ovary can occur with or without concomitant torsion of the fallopian tube. Torsion severs the blood supply to the ovary and, if not promptly treated, can damage the viability of the ovary. This condition is more common among premenarchal girls and women of reproductive age [1]. The high prevalence of ovarian torsion in the reproductive age and the desire of these women to maintain fertility requires emergency surgical detorsion.

The current diagnosis of ovarian torsion is based on clinical presentation of acute abdominal pain, nausea, vomiting, and sonographic demonstration of an ovarian mass. Women presenting with acute abdominal pain associated with a high suspicion of ovarian torsion are usually treated with urgent laparoscopy. In the present authors' experience, during laparoscopy, most of these women (56%) will not be found to have ovarian torsion [2]. Currently no medical or laboratory tests are available to enable us to separate between those patients who need emergency laparoscopy for urgent detorsion and those who can be cared for conservatively.

Cohen *et al.* [3] showed a relationship between ovarian torsion and elevated IL–6. They also showed that TNF-α levels are not elevated following ovarian torsion [3]. Ozkan *et al.* [4] demonstrated a connection between gonadal torsion and reduced serum inhibin B levels. They demonstrated that unilateral testicular torsion and detorsion caused contralateral testicular damage and a reduction in the secretion of inhibin B [4].

Inhibin B is secreted in the female from the ovary during the follicular phase. The present authors did not find in the medical literature any reports regarding a possible connection between ovarian torsion and changes in inhibin B secretion. The aim of this study was to investigate the relationship between ovarian torsion during the early follicular phase and changes in levels of serum inhibin B in rats.

Materials and Methods

Nineteen female Lewis rats were housed in cages of five animals each. They were housed in constant temperature of 24°C. The rats were in their reproductive age, 12.5-week-old, with a mean weight of 180 grams and a day/night cycle of 12 hour/12 hour. The rats had free access to water and standard rat chow.

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Table 1. — *Mean (± SD) serum inhibin B levels (pg/ml) at different time points during the first 36 hour of metestrus in an investigational ovarian torsion rat model.*

Hours in metestrus	0	12	24	36
Control group	108.3 (43.6)	129.5 (49.3)	99.1 (60.5)	91.6 (27.7)
Group 2 (12 hours after torsion)	128.6 (44.3)	78.5 (22.7)		
Group 3 (24 hours after torsion)	91.5 (34.2)		112.0 (62.6)	
Group 4 (36 hours after torsion)	84.4 (62.5)			122.9 (26.0)

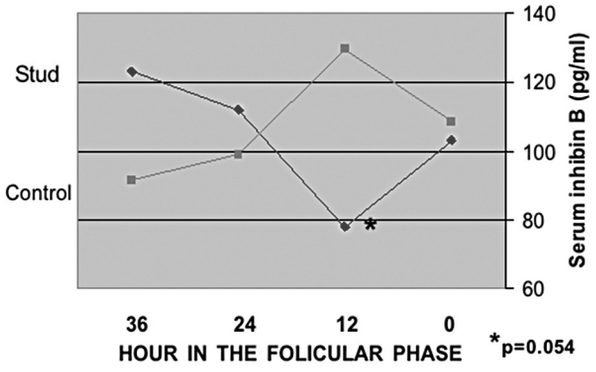

Figure 1. — Mean serum inhibin B levels after ovarian torsion versus control rats during the first 36 hour of the estrus cycle.

These rats have a menstrual cycle of 96 hours. For each rat the phase of her cycle was checked by vaginal smear. All experiments were done in the first day of the cycle – the beginning of the metestrus. For 17 rats the timing at the metestrus was established. For two rats the authors could not establish the metestrus phase. One rat died during the experiment.

The animals were divided into four groups. Under ether anesthesia and sterile conditions, laparotomy and wound closure were performed in all animals. Basal serum inhibin B was taken in the beginning of the metestrus in all animals. Group 1, the control group, included three animals. This group was designed to study the normal inhibin B level in the estrus phase. Blood samples for serum inhibin B were taken 12, 24, and 36 hours after laparotomy from all control animals. Blood samples for serum inhibin B levels were taken from group 2 (five animals), group 3 (four animals) and group 4 (four animals), respectively, after 12, 24, and 36 hours of ovarian torsion from the beginning of metestrus.

For all rats in groups 2-4, at the beginning of metestrus, ovarian torsion of 720° was performed via laparotomy with fixation of the ovary to the parietal peritoneum. The rats were anesthetized with an ether solution. All operations were preformed under sterile conditions. After each surgery the incision was surgically closed.

Serum inhibin B levels were measured by an enzymatically amplified two-site immunoassay (inhibin B Elisa). The sensitivity of the assay was 7 pg/mL and the interassay CV ranged from 6 to 8%.

The statistical analysis was performed using Student's *t*-test for continuous variables. Statistical significance was defined as $p < 0.05$.

Results

The experimental model was-well tolerated by most animals. One rat died, the only animal to expire during the experiment. The mean serum inhibin B levels in the control group during the first 36 hours of estrus and the inhibin B levels in the study groups are described in Table 1. The mean ± SD serum inhibin B level of all study groups at the beginning of the metestrus was 103.6 ± 48.5 pg/ml. This value was not statistically different from that observed for the control group.

The initial levels of serum inhibin B in study group 2 were not different from that of the control group. The serum inhibin B levels at 12 hours of torsion were lower then the initial levels and reached statistical significance ($p = 0.05$). When comparing between the initial inhibin B levels and the levels after 24 and 36 hours of torsion, no statistical significance could be demonstrated. The results of the study groups at each time were compared to the control group and they did not reach statistical significance.

Figure 1 shows the levels of serum inhibin B through the first 36 hours of the metestrus in the control and study groups. The inhibin B secretion in the study groups showed an opposite pattern compared with that of the control group.

Discussion

Inhibin B is a marker for ovarian reserve and is secreted from the ovary mainly in the follicular phase. Serum inhibin B levels show a peak rise in the early follicular phase and a smaller elevation at the time of ovulation [5]. The inhibin B secretion in the rat behaves similar to what has been observed in humans [6]. The present authors observed in their control rats an elevation in serum inhibin B levels from the beginning of metesterus to a peak after 12 hours, and then a decrease to the baseline level after 24-36 hours. This pattern is similar to the pattern of serum inhibin B observed in the early follicular phase in other studies on female rats. It also resembles the serum inhibin B pattern in women. In contrast to the control group, group 2 of the study showed a decline in inhibin B levels in the first 12 hours of torsion of one of the ovaries (Figure 1). Moreover, 24 hours after torsion the serum inhibin B levels returned to the initial levels and after 36 hours there was only a slight elevation of the inhibin B levels. Therefore, the authors

found an almost significant decrease in the secretion of inhibin B in rats after torsion of one ovary.

This unexpected decline in inhibin B secretion may be caused by the severance of the vascular flow from the ovary which secrets it. The present authors hypothesize that this decline leads to a hormonal cycle which is influenced by the changes in inhibin B. Furthermore, FSH secretion from the hypophysis is responsible for secretion of inhibin B from the ovary. Inhibin B has a negative feedback effect on the secretion of FSH from the hypophysis. The decline in inhibin B levels can lead to an elevation in FSH secretion. In turn, higher FSH levels may enhance the secretion of inhibin B from the contralateral ovary. This cycle can cause a gradual elevation in serum inhibin B levels.

The present study shows that ovarian torsion in the early metesterus is associated with a decline in serum inhibin B levels. The influence of torsion on serum inhibin B levels in the follicular phase has not been studied till now. The present study provides the first demonstration that ovarian torsion changes serum inhibin B levels.

This experiment is one of limited size and tested only for serum inhibin B. However, one advantage of the present study is that all ovarian torsions were performed at the same time during the cycle of the rat, at the time of highest secretion of inhibin B. The timing of ovarian torsion reduced possible physiological inhibin B changes as a factor in this study.

Further research is clearly needed to support the present study hypothesis. A larger study should simultaneously examine the changes in FSH and E2 levels' secretion following ovarian torsion in an attempt to prove the present author' theory on this endocrinological cycle. A decline in estrogen levels with a concurrent decline in inhibin B levels and a subsequent rise in FSH secretion will lend support to this hypothesis. Future research should include a comparison of inhibin B levels before ovarian torsion and a long time after torsion, as a marker of ovarian reserve remaining after the insult.

Conclusion

Ovarian torsion at the beginning of the metestrus of the rat correlates with a decline in serum Inhibin B levels. This change may be caused by occlusion of ovarian blood flow, with secondary elevation in serum inhibin B levels due to a negative feedback stimulation of FSH secretion from the hypophysis. The present authors conclude that measurement of serum inhibin B levels could possibly serve in the future as a clinical tool for the preoperative diagnosis of ovarian torsion. However, at present, this hormonal response observed in the rat model deserves further study in women with ovarian torsion.

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