Nagoya J. Med. Sci. **58.** 35 ~ 39, 1995

SERUM INHIBIN LEVELS IN NORMAL MEN AND MEN WITH IDIOPATHIC INFERTILITY

MASANORI YAMAMOTO, SATOSHI KATSUNO, KEISUKE YOKOI, HATSUKI HIBI, and KOJI MIYAKE

Department of Urology, Nagoya University School of Medicine, Nagoya, Japan

ABSTRACT

In vitro studies have shown that the Sertoli cell is the primary source of inhibin in the male. To assess the regulation of inhibin production we measured serum inhibin, FSH, LH and testosterone in 21 normal men and 104 men with various testicular disorders resulting in infertility. The infertile men were subdivided into groups on the basis of their mean sperm count, FSH and LH levels. The mean serum concentrations of inhibin in the normal men were 602 ± 29 U/L and were significantly decreased in those groups with severe oligozoospermia ($p < 0.01$) or azoospermia ($p < 0.01$; $p < 0.001$). The FSH concentrations correlated inversely with serum inhibin concentrations ($p < 0.001$) in azoospermic men. Azoospermic men with high FSH had significantly lower inhibin and testosterone levels when compared with normal men ($p < 0.01$; $p < 0.001$). Serum FSH concentrations were significantly increased in azoospermic men ($p < 0.001$). Our present results of serum concentrations of inhibin correlating inversely with those of FSH levels suggest that measurement of inhibin may be a useful circulating marker of Sertoli cell function.

Key words: Inhibin, Idiopathic infertility, Correlation of inhibin with FSH

INTRODUCTION

Inhibin is a gonadal glycoprotein hormone involved in the feedback regulation of the secretion of the pituitary gonadotropins, particularly follicle stimulating hormone (FSH). Inhibin was first purified from bovine ovarian follicular fluid.[1] Studies of inhibin have shown that the Sertoli cell is the site of production of inhibin in the male[2] and that the levels increase by stimulation of FSH.[3] Plymate et al. investigated serum inhibin and FSH levels in normal men and patients with varicoceles.[4] They reported that serum inhibin correlated with FSH in a negative fashion when the reproductive system is normal.[4] However, there have been very few reports on the diagnostic value of inhibin in idiopathic male infertility. The measurement of inhibin may provide the circulating marker of Sertoli cell function. In order to investigate this possibility and to study the correlation of inhibin with FSH levels we examined levels of these hormones in normal men and men with idiopathic infertility.

MATERIALS AND METHODS

Subjects

The 21 men forming the normal group in this study had normal histories and physical examinations; normal serum FSH, Luteinizing hormone (LH) and testosterone (T) levels; and normal semen analyses. They were aged between 25 and 37 years.

Correspondence: Masanori Yamamoto, M.D. Department of Urology, Nagoya University School of Medicine 65, Tsurumai-cho, Showa-ku, Nagoya 466 Japan

Accepted for Publication in December 13, 1994.

The men with testicular dysfunctions were aged between 23 and 43 years and underwent evaluation for infertility. Testicular volume was examined using a punched-out orchiometer and the men were asked to provide three semen samples. Patients with azoospermia and testicular volumes of less than 5 ml underwent leucocyte karyotyping. All men provided a blood sample for the measurement of FSH, LH, testosterone and inhibin levels. All patients except those with Klinefelter's syndrome were diagnosed as having idiopathic male infertility. Patients were classified as follows: (1) normal, (2) mild oligozoospermia (10 to 20 million/ml), (3) moderate oligozoospermia (5 to 10 million/ml), (4) severe oligozoospermia (less than 5 million/ml), (5) azoospermia with high FSH and normal LH, (6) azoospermia with high FSH and high LH, and (7) patients with Klinefelter's syndrome diagnosed by an abnormal sex chromosomal complement of 47 XXY. There were no patients with azoospermia with normal FSH and LH. There were only three patients with azoospermia with normal FSH and high LH. Therefore, these men were excluded from the present study.

Hormone assays

Inhibin

Measurements were made using two heterologous double-antibody radioimmunoassays, details of which have been published previously.[5] These assays used purified iodinated 31 kDa bovine inhibin as a tracer and the antisera were raised against 31 kDa bovine inhibin. The standard used in the assay was a serum pool derived from women undergoing ovarian hyperstimulation with human menopausal gonadotrophin as part of an in vitro fertilization program. This pool was calibrated against partially purified human follicular fluid inhibin standard by radioimmunoassay.[5] Normal range for inhibin was 280 to 970 U/L.

FSH and LH

Serum concentrations of FSH and LH were determined using reagents provided by the WHO Matched Reagent Program. The interassay variations were 6.4% and 7.1% and the intra-assay variations were 3.2% and 4.5% for FSH and LH, respectively. The normal range for FSH was 2.9 to 8.2 IU/L and for LH, 1.8 to 5.2 IU/L.

Testosterone

A solid-phase assay incorporating ^{125}I-labelled testosterone was employed. The normal range for testosterone was 2.7 to 10.7 ng/mL.

Statistical analyses

Values were expressed as mean ± s.d. Since heterogeneity of variance was observed between groups of unequal size with some of the parameters investigated, comparisons between normal and other groups were assessed by analysis of variance followed by Tukey's multiple range test. These analyses were performed using the SYSTAT microcomputer statistical package.

RESULTS

Normal men

The mean concentrations for all parameters measured are given in Table 1. The serum inhibin concentrations ranged from 280 to 970 U/l in the 21 normal men. There were no significant correlations between serum inhibin concentrations and any parameters measured.

Men with oligozoospermia

As the mean sperm count decreased in the categories of mild, moderate and severe oligozoospermia (Table 1), there was a significant increase in FSH concentrations ($p < 0.001$) and a significant decrease in testicular volume and serum testosterone concentrations ($p < 0.001$).

Table 1. Data Concerning Patients Admitted to This Study

Group	n	Age (years)	Total testis vol. (mL)	Sperm count (million/mL)	Inhibin (IU/L)	FSH (IU/L)	LH (IU/L)	Testosterone (ng/mL)
(1) Normal	21	31.2±5.2	42.9±10.1	48.9±31.2	602±29	4.5±1.2	3.2±0.9	6.1±1.2
Oligozoospermia								
(2) Mild	14	32.2±6.1	38.1±12.1	15.2±2.9	598±35	5.8±2.2	3.8±1.2	6.2±1.1
(3) Moderate	20	33.1±3.8	37.6±7.8	7.3±1.5	526±68	5.7±1.8	4.9±1.8	5.8±2.1
(4) Severe	19	28.1±2.9	34.2±9.1[b]	1.8±1.4	321±21[b]	12.3±1.6[b]	5.6±1.1	4.2±0.8[b]
Azoospermia								
(5) High FSH and Normal LH	23	34.5±6.1	28.1±5.1[c]	0	215±31[b]	18.5±2.1[c]	3.2±2.2	4.0±0.9[b]
(6) High FSH and High LH	21	35.1±7.2	20.5±3.1[c]	0	217±18[c]	22.3±1.9[c]	8.2±1.3[c]	2.5±1.2[c]
(7) Klinefelter's Syndrome	7	38.1±2.9	5.8±1.2[c]	0	195±14[c]	32.5±1.1[c]	18.1±3.1[c]	1.2±0.6[c]

[a] Oligozoospermia is defined as a sperm count of less than 20 million/mL, mild oligozoospermia 10–20 million/mL, moderate 5–10 million/mL and severe <5 million/mL.

[b] $p<0.01$

[c] $p<0.001$ Compared with normal

Serum inhibin levels were slightly decreased in the patients with moderate oligozoospermia who had normal FSH and LH levels, although this difference did not reach statistical significance. Serum inhibin concentrations were significantly decreased in the group of severe oligozoospermia ($p<0.01$). Serum FSH concentrations were significantly increased in this group ($p<0.01$). Serum inhibin levels correlated inversely with serum FSH levels in this group.

Azoospermia

Strong positive correlations were found between FSH and LH levels ($p<0.01$), which were also negatively correlated with total testis volume ($p<0.001$), sperm count ($p<0.05$) and serum testosterone levels ($p<0.001$). The FSH concentrations correlated inversely with serum inhibin concentrations ($p<0.001$). Azoospermic men with high FSH had significantly lower inhibin and testosterone levels when compared with normal men ($P<0.01$; $P<0.001$). Serum FSH concentrations were significantly increased in azoospermic men ($p<0.001$). Similar findings were observed in the patients with Klinefelter's syndrome.

DISCUSSION

The present results show that the serum inhibin levels in the men with severe oligozoospermia or azoospermia correlated inversely with FSH levels. These results are reasonable since inhibin is the substance involved in the specific negative feedback control of FSH.[6] The present data confirm the observation that, in a series of 52 men with severe seminiferous tubule damage resulting from chemotherapy, those men with decreased inhibin levels have significantly higher FSH levels.[7] On the contrary, Tsatsoulis et al. reported that serum inhibin levels were in the normal range in men treated with chemotherapy for Hodgkin's disease despite the presence of azoospermia and elevated levels of FSH.[8] As to the failure of inhibin to fall in association with

elevated FSH, they proposed the concept that FSH secretion is controlled both by inhibin and testosterone. Their possible explanation for the lack of an inverse relationship between FSH and inhibin is as follows: Declining testosterone causes an increase in FSH levels which in turn could stimulate the Sertoli cells to further increases in inhibin levels.[8] With further testicular damage, the capacity of the Sertoli cells to maintain this increased inhibin secretion may become compromised and inhibin levels decline to below the normal range.[8] On the other hand, de Kretser demonstrated that serum inhibin level did not decline to below normal level in the patients with remarkably increased FSH levels like those in Klinefelter's syndrome.[9] Therefore, results concerning the relationship between serum inhibin and FSH levels are conflicting.

It is evident from the results of this study that increased FSH concentrations are associated with decreased serum inhibin or testosterone concentrations. Our previous work also demonstrated that testosterone production was significantly reduced in infertile men who have an increased serum FSH, but normal LH levels.[10] Therefore, dual control of FSH by inhibin and testosterone may be plausible and might explain our present results and Tsatsoulis's paradoxical findings. Other investigators provide the evidence in support of the hypothesis that serum FSH secretion is regulated by the negative feedback from two testicular endocrine factors: testosterone and inhibin.[11]

Recently, Esch et al. isolated a new protein, FSH-suppressing protein, which has the capacity to suppress FSH in vitro.[12] This protein is structurally and immunologically unrelated to inhibin but has 10% to 30% of the capacity of inhibin to suppress FSH.[12] More recently, Burger et al. have demonstrated that LH, as well as FSH, plays a role in the regulation of inhibin based on the results of gonadotropin therapy for hypogonadotropic hypogonadal patients.[13] Therefore, the relationship between inhibin and gonadotropin, especially FSH, is still complex. Although we did not investigate the histological analysis of testicular biopsy specimens in this study, future morphological studies will be required to determine whether those men with high FSH and low inhibin levels have a significant reduction of Sertoli cell mass or abnormal pathological changes of Sertoli cells.

One of the major hopes of the research on inhibin is that it will provide a new approach to fertility regulation in the male. In the belief that inhibin is a specific suppressor of FSH secretion, it is hoped that selective suppression of spermatogenesis might be achievable without concomitant interference with the LH-testosterone axis and hence with preserving virility and potency. Because inhibin also suppresses FSH in the female, it might be an agent for the inhibition of ovulation. Whether it will be of practical value in these fields depends on the availability of sufficient amounts of inhibin to allow testing in humans.

In the present study serum inhibin levels were slightly decreased in the patients with moderate oligozoospermia who had normal FSH and LH levels, although this difference was not statistically significant.

In conclusion, we demonstrated that low serum inhibin levels were significantly associated with high FSH levels in the men with seminiferous tubular damage. Although testicular histological investigation of the Sertoli cell is needed, on the basis of our data, it is likely that the measurement of basal inhibin may be a useful marker of Sertoli cell function.

REFERENCES

1) Robertson, D.M., Foulds, L.M., Leversha, L., Morgan, F.J., Hearn, M.T.W., Burger, H.G., Wettenhall, R.E.H. and de Kretser, D.M.: Isolation of inhibin from bovine follicular fluid. *Biochem. Biophys. Res. Commun.*, 126, 220–226 (1985).

2) Le Gac, F. and de Kretser, D.M.: Inhibin production by Sertoli cells. *Mol. Cell, Endocrinol.*, 28, 487–498 (1982).
3) Toebosch, A.M.W., Robertson, D.M., Trapman, J., Klaassen, P., de Paus, R.A., de Jong, F.H. and Grootegoed, J.A.: Effects of FSH and IGF, on immature Sertoli cells: Inhibin a and 13 subunits mRNA levels and inhibin secretion. *Mol. Cell. Endocrinol.*, 55, 101–105 (1988).
4) Plymate, S.R., Paulsen, C.A. and McLachlan, R.I.: Relationship of serum inhibin levels to serum follicle stimulating hormone and sperm production in normal men and men with varicoceles. *J. Clin. Endocrinol. Metabol.*, 74, 859–864 (1992).
5) McLachlan, R.I., Robertson, D.M., Healy, D.L., Burger, H.G. and de Kretser, D.M.: Circulating immunoreactive inhibin levels during the normal menstrual cycle. *J. Clin. Endocrinol. Metabol.*, 65, 954–961 (1987).
6) de Kretser, D.M., Burger, H.G., Fortune, D., Hudson, B., Long,A., Paulsen, C.A. and Taft, H.P.: Hormonal, histological and genetic studies on male infertility. *J. Clin. Endocrinol. Metabol.*, 35, 392–401 (1972).
7) Brennemann, W., Stoffel, W.B., Bidlingmaier, F. and Klingmuller, D.: Immunoreactive plasma inhibin levels in men after polyvalent chemotherapy of germinal cell cancer. *Acta. Endocrinol. Copenh.*, 126, 224–228 (1992).
8) Tsatsoulis, A., Shalet, S.M., Robertson, W.R., Morris, l.D., Burger, H.G. and de Kretser, D.M.: Plasma inhibin levels in men with chemotherapy induced severe damage to the seminiferous epithelium. *Clin. Endocrinol.*, 29, 659–665 (1988).
9) de Kretser, D.M., McLachlan, R.I., Robertson, D.M. and Burger, H.G.: Serum inhibin levels in normal men and men with testicular disorders. *J. Endocrinol.*, 120, 517–523 (1989).
10) Yamamoto, M., Tsuji Y., Hibi, H. and Miyake K.: Possible Leydig cell dysfunction in idiopathically infertile men with selective elevations of serum follicle-stimulating hormone. *Jap. J. Fertil. Steril.*, 39, 127–131 (1994).
11) de Jong, F.H. and Robertson, D.M.: Inhibin 1985: Update on action and purification. *Mol. Cell. Endocrinol.*, 42, 95–103 (1985).
12) Esch, F.S., Shimasaki, S., Mercado, M., Cooksey, K., Ling, N., Ying, S., Ueno, N. and Guillemin, R.: Structural characterization of follistatin: A novel follicle stimulating hormone release-inhibiting polypeptide from the gonad. *Mol. Endocrinol.*, 1, 849–855 (1987).
13) Burger, H.G., Tiu, S.C., Bangah, M.L. and de Kretser, D.M.: Human chorionic gonadotrophin raises serum immunoreactive inhibin levels in men with hypogonadotrophic hypogonadism. *Reprod. Fertil. Dev.*, 2, 137–144 (1990).