CLINICAL STUDY

Relationships between FSH, inhibin B, anti-Mullerian hormone, and testosterone during long-term treatment with the GnRH-agonist histrelin in patients with prostate cancer

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Abstract

Objectives: Medical castration with long-acting GnRH-agonist (GnRHa) is a well-established treatment for metastatic prostate cancer. Our aim was to explore the relationships between FSH, inhibin B, anti-Mullerian hormone (AMH), and testosterone during treatment with an implant releasing GnRHa.

Design: Analysis of hormone levels in frozen serum samples.

Methods: Ten patients aged 77 ± 7 (means $\pm$ s.e.m.) years with prostate cancer were treated with the GnRHa histrelin for at least a year. Two weeks prior to insertion and for 3–4 months following removal the patients were treated with the antiandrogen flutamide. Serum inhibin B, FSH, testosterone, and AMH levels were measured retrospectively.

Results: FSH, inhibin B, and testosterone increased during antiandrogen administration and levels fell after implant insertion. Four weeks post insertion, FSH gradually increased while inhibin B and testosterone remained fully suppressed. AMH levels did not change during antiandrogen treatment, but increased following implant insertion and remained elevated for the duration of implant use. Following removal, FSH and testosterone increased, inhibin B remained low, while AMH decreased.

Conclusions: The secondary increase in FSH following initial suppression with the implant is probably related to impaired inhibin B secretion. The lack of inhibin B response to the secondary increase in FSH suggests that long-term exposure of Sertoli-cells to GnRHa impairs their function. This effect appears to be selective since unlike inhibin B, AMH increased. In the absence of testosterone, FSH has a role in AMH regulation.

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Introduction

Medical castration with long-acting GnRH-agonists (GnRHa) is well established in the treatment of metastatic prostate cancer (1). Long-term GnRHa administration suppressed LH and testosterone completely, while FSH levels were only transiently suppressed (2). Recently, hydrogel implant containing 50 mg of the GnRHa histrelin has been shown to completely suppress LH and testosterone for at least a year (3–5). There is no clear explanation for this pattern of FSH release. It could be due to a differential sensitivity of the pituitary gonadotroph to the GnRHa or that FSH synthesis and secretion is desensitized to a lesser degree than LH. Alternatively, this could be related to the effect of other factors on the pituitary gonadotroph.

In addition to GnRH and sex steroids, FSH secretion is also regulated by gonadal peptides; the principal one in males is inhibin B (see (6) for a review). Inhibin B is a member of the transforming growth factor (TGF)- β

superfamily, heterodimer that is comprised of disulfide-linked α and β subunits. FSH stimulates the secretion of inhibin B with negative feedback of inhibin B on FSH expression. Inhibin B is a useful serum marker of Sertoli-cell (SC) function; many studies have shown a significant correlation between serum inhibin B and sperm concentration and a significant negative correlation between FSH and inhibin B (7). Anti-Mullerian hormone (AMH), another member of the TGF- β superfamily is also a Sertoli cell-specific product, (see (8) for a review). This gonadal glycoprotein is responsible for the regression of the Mullerian duct (uterus and fallopian tubes) in the male fetus. Serum AMH declines substantially during puberty, as an early sign of local testicular testosterone activity and spermatogenic development. In the rat, AMH negatively modulates Leydig cell differentiation and testosterone synthesis through its receptors on Leydig cells (9). AMH concentration in seminal plasma in azoospermia is lower than in normal men (10), suggesting that AMH is also a marker of SC function.

The aim of this study was to explore the mechanism(s) of the restoration of FSH secretion after long-term GnRHa treatment in patients with prostate cancer. We examined the pattern of serum inhibin B, FSH, and AMH levels and their mutual relationships during the use of the histrelin implant in patients with prostate cancer.

Patients and methods

Patients

Ten patients aged 77 ± 7 years (means $\pm$ S.E.M.) with histologically proven prostate cancer whose prostate specific antigen (PSA) ranged from 30 to 110 ng/ml with a Gleason score from 5 to 8 were evaluated retrospectively. No patient had evidence of bone metastasis. Full clinical details of the patients have been previously described (3).

The patients were treated with a hydrogel implant (Vantas) releasing the GnRHa histrelin (ImBzl)-D-His6,Pro9-Net GnRH) for at least a year. Prior to insertion, the patients were treated for 2 weeks with the antiandrogen flutamide (250 mg three times daily). This was continued for up to 3 months following implant insertion and was recommenced on the day of implant removal. Blood samples were taken every 1–4 weeks throughout treatment until 3 months following implant removal. The study was approved by the Internal Review Board of Shaare Zedek Medical Center and all patients signed informed consent.

Methods

Serum samples were frozen in aliquots at -20°C . LH, testosterone, and PSA have been previously reported (4). Serum inhibin B and AMH were measured using highly sensitive two-site ELISAs (Diagnostic Systems Laboratories, Webster, TX, USA). The assay sensitivities were 15 pg/ml and 0.025 ng/ml respectively. These determinations were performed on previously unfrozen serum aliquots. Serum FSH was measured using Immulite 2000 (Diagnostic Product Corporation, Los Angeles, CA, USA); the assay sensitivity was 0.1 IU/l. The previously reported serum testosterone levels were measured by a solid phase ^{125}I RIA (Diagnostic Product Corporation); assay sensitivity was 0.2 ng/ml.

Data were analyzed by the Mann–Whitney and Wilcoxon signed-rank tests. Results are presented as means $\pm$ S.E.M. $P < 0.05$ was considered statistically significant.

Results

During the 2 weeks of antiandrogen treatment, there was a significant increase ($P < 0.05$) in FSH and inhibin B (Fig. 1, upper panel). Following implant insertion,

Figure 1 Means $\pm$ S.E.M. serum levels of FSH and inhibin B (upper panel) and anti-Müllerian hormone (AMH) and testosterone (lower panel) in patients with prostate cancer treated with histrelin implant. Flutamide treatment began 2 weeks before implant insertion and was continued for up to 3 months. The arrowhead indicates implant insertion. Testosterone levels have been previously reported (4). FSH, testosterone, and inhibin B were measured in 10 and AMH in seven men.

FSH decreased until 2–4 weeks post insertion ($P < 0.005$) and then gradually increased to pre-treatment levels. In contrast, inhibin B continued to decrease throughout the time that the implant was in place. At 12 months the levels were 41.5 ± 14.3 pg/ml ($P < 0.004$).

AMH levels did not change during antiandrogen treatment (Fig. 1, lower panel). One month following implant insertion, AMH levels increased from 2.3 ± 0.8 to 7.2 ± 2.5 ng/ml ($P < 0.02$) and remained stable for the duration of implant use. As has been previously published, testosterone increased during flutamide treatment and decreased to the lower limit of assay sensitivity by 1 month and remained at this level for the duration of implant use (3).

Following implant removal, FSH levels gradually increased (Fig. 2, upper panel, $P < 0.04$). Inhibin B levels did not change significantly and remained partially suppressed; AMH decreased (Fig. 2, lower panel) from 8.3 ± 2.3 to 0.6 ± 0.1 ng/ml. As has been published, LH and testosterone levels increased following implant removal (5).

Discussion

The relationships between inhibin B, FSH, testosterone, and AMH levels have been evaluated during the use of the histrelin implant in patients with prostate cancer. The antiandrogen flutamide inhibits testosterone binding to its intracellular receptor, and is associated with an increase in LH and testosterone (11). Both FSH and inhibin B increased 2 weeks after commencement of flutamide. Following implant insertion, LH and testosterone were suppressed completely for the

Figure 2 Means $\pm$ S.E.M. serum levels of FSH and inhibin B (upper panel) and anti-Müllerian hormone (AMH) and testosterone (lower panel) in patients with prostate cancer following histrelin implant removal and flutamide treatment. Arrowhead indicates implant removal. Testosterone levels have been previously reported (4). FSH, testosterone and inhibin B were measured in seven and AMH in five men.

duration of the implant use. FSH suppressed transiently until 4 weeks and then there was a secondary rise. Inhibin B also decreased following implant insertion and remained low for the duration of implant use. These observations suggest that the impaired inhibin B secretion during implant use results in deficient negative feedback on FSH secretion and explains the secondary FSH rise. Prolonged suppression of both LH and FSH was shown in young infertile men who were treated with GnRHa for 2 months as a part of fertility treatment (12). However, in contrast with our patients, in these men inhibin B was not suppressed. This observation supports our hypothesis.

Intact SCs should respond to the increase in FSH by secreting inhibin B, producing a new steady state. It is possible that the long-term exposure of SCs to GnRHa or to testosterone deficiency may damage these cells. To evaluate this, another marker of SC function, AMH was measured. The fact that AMH gradually increased following implant insertion, argues against total SC damage. This is supported by the observation that in contrast to full LH and testosterone suppression, inhibin B levels were only partially suppressed. Nevertheless, severe SC morphological abnormalities (including tubular atrophy, SC degeneration, and interstitial fibrosis) have been observed during prolonged other GnRHa treatment (13, 14). There is still controversy as regards the reversibility of these changes (5, 13, 15). Our study shows specific SC dysfunction during prolonged GnRHa treatment as shown by the increase in AMH and partial suppression of inhibin B. This implies dissociation between inhibin B and AMH secretion following long-term exposure to GnRHa. Huhtaniemi *et al.* (14) observed a reduction in testicular FSH receptors by 80% without a change in LH receptors

following 6 months of GnRHa treatment. This may explain our findings, since in contrast to AMH, inhibin B secretion is FSH dependent.

The defect in inhibin B secretion could be related to testosterone deficiency. Controversies exist regarding the effect of FSH alone in patients with hypogonadotropic hypogonadism: neither human chorionic gonadotrophin (hCG) nor FSH alone but only their combination was able to increase serum inhibin B levels (16). However, long-term (≥ 1 year) FSH treatment did increase inhibin B serum levels. This probably represents proliferation of immature Sertoli cells (17). Further increase in inhibin B with induction of spermatogenesis occurred following hCG co-treatment. In young men with acquired hypogonadotropic hypogonadism, LH alone was unable to increase inhibin B although FSH treatment and the combination did increase inhibin B levels (18). The persistent suppression of inhibin B following implant removal despite the rise in LH, FSH, and testosterone argues against a major role of these hormones in the inhibin B suppression. Further evidence implying that the control of inhibin B secretion is independent of testosterone comes from observations made in subjects with complete androgen insensitivity syndrome (AIS), where LH and testosterone are high but inhibin B and FSH are normal (19). GnRH receptors have been detected in Leydig but not in SC (20). Thus, GnRHa itself is unlikely to be responsible for the persistent inhibin B suppression.

An alternative explanation for the discrepancy between inhibin B and AMH is their distinct regulation. While the expression of inhibin B β -subunit is dependent on the coexistence of spermatogenesis (21, 22), AMH expression is downregulated by meiotic germ cells (23). In the men, in our study, suppression of spermatogenesis due to the lack of testosterone could stimulate AMH and inhibit inhibin B expression. Spermatogenesis was not analyzed in this group of elderly men with prostate cancer; neither could we measure seminal fluid AMH concentrations. Seminal fluid AMH concentrations are significantly higher than that observed in serum ((24) for review). In normal men both inhibin B and AMH serum levels are rough markers of Sertoli cell function (25, 26); however, seminal AMH concentration is the only significant marker of the existence of spermatozoa when intracytoplasmic sperm injection is considered (10, 26).

In this study, AMH levels increased following implant insertion, in parallel with the increase in FSH and decrease in testosterone; following implant removal there was a decrease in AMH in association with a further increase in FSH and increase in testosterone levels. The AMH suppression was unrelated to the antiandrogen administration since AMH did not change during flutamide administration. Nevertheless, the molecular mechanism underlying the repressive effect of testosterone on AMH expression is not clear since no androgen response element was found in the regulatory

regions of the AMH gene (see (27) for review); non-genomic or androgen-receptor independent pathways may also exist. Our results suggest that both testosterone and FSH have a role in modulating AMH secretion and this is supported by previous studies. In untreated hypogonadotropic hypogonadal men, FSH induces a gradual increase in AMH levels; when hCG was added, AMH secretion was suppressed (28). In patients treated with monthly injections of the GnRHa, triptorelin, for prostate cancer, a modest increase of serum AMH was observed (29). In patients with AIS, AMH levels are high (19). During pubertal development, AMH falls in association with the increase in testosterone (see (8) for review). These observations support the notion that FSH stimulates AMH secretion in the absence of testosterone. In the presence of testosterone, its suppressive effect overcomes the stimulating effect of FSH on AMH production by SCs. Our results suggest that the same mechanisms exist in elderly men and that the inhibitory effect of testosterone on AMH expression is reversible many years after its physiological occurrence at puberty.

Physiologic role(s) of AMH in adult men is less obvious than during testicular differentiation (see (24) for review). AMH type II receptors have been detected in Sertoli cells, suggesting an autocrine effect, which was first suggested by the inhibition of aromatase activity by AMH in cultured rodent Sertoli cells. Paracrine effects of AMH on Leydig cells and adult germ cell were found, as AMH directly inhibited Leydig cell differentiation and steroidogenesis and might be involved in sperm motility.

It is concluded that the secondary increase in FSH following initial suppression during prolonged GnRHa treatment is related to impaired inhibin B secretion. The absent inhibin B response to the secondary FSH increase suggests that long-term exposure of SC to GnRHa or to testosterone deficiency partially impairs their function. This appears to be selective since unlike inhibin B, AMH levels increased. The inverse relationship between testosterone and AMH suggests the presence of a negative feedback loop in which testosterone inhibits AMH secretion. AMH increases when testosterone levels are suppressed. It appears that FSH has a role in AMH regulation in the absence of testosterone.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- 1 Catalona WJ. Management of cancer of the prostate. *New England Journal of Medicine* 1994 **331** 996–1004.

- 2 Santen RJ, Demers LM, Max DT, Smith J, Stein BS & Glode LM. Long term effects of administration of a gonadotropin-releasing hormone superagonist analog in men with prostatic carcinoma. *Journal of Clinical Endocrinology and Metabolism* 1984 **58** 397–400.
- 3 Chertin B, Spitz IM, Lindenberg T, Algur N, Zer T, Kuzma P, Young AJ, Catane R & Farkas A. An implant releasing the gonadotropin hormone-releasing hormone agonist histrelin maintains medical castration for up to 30 months in metastatic prostate cancer. *Journal of Urology* 2000 **163** 838–844.
- 4 Schlegel PN & Histrelin Study Group. Efficacy and safety of histrelin subdermal implant in patients with advanced prostate cancer. *Journal of Urology* 2006 **175** 1353–1358.
- 5 Fridmans A, Chertin B, Koulikov D, Lindenberg T, Gelber H, Leiter C, Farkas A & Spitz IM. Reversibility of androgen deprivation therapy in patients with prostate cancer. *Journal of Urology* 2005 **173** 784–789.
- 6 de Kretser DM, Buzzard JJ, Okuma Y, O'Connor AE, Hayashi T, Lin SY, Morrison JR, Loveland KL & Hedger MP. The role of activin, follistatin and inhibin in testicular physiology. *Molecular and Cellular Endocrinology* 2004 **225** 57–64.
- 7 Jensen TK, Andersson AM, Højlund NH, Scheike T, Kolstad H, Giwercman A, Henriksen TB, Ernst E, Bonde JP, Olsen J, McNeilly A, Groome NP & Skakkebaek NE. Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 4059–4063.
- 8 Josso N, Picard JY, Rey R & di Clemente N. Testicular anti-Müllerian hormone: history, genetics, regulation and clinical applications. *Pediatric Endocrinology Reviews* 2006 **3** 347–358.
- 9 Racine C, Rey R, Forest MG, Louis F, Ferre A, Huhtaniemi I, Josso N & di Clemente N. Receptors for anti-Müllerian hormone on Leydig cells are responsible for its effects on steroidogenesis and cell differentiation. *PNAS* 1998 **95** 594–599.
- 10 Fujisawa M, Yamasaki T, Okada H & Kamidono S. The significance of anti-Müllerian hormone concentration in seminal plasma for spermatogenesis. *Human Reproduction* 2002 **17** 968–970.
- 11 Hellman L, Bradlow HL, Freed S, Levin J, Rosenfeld RS, Whitmore WF & Zumoff B. The effect of flutamide on testosterone metabolism and the plasma levels of androgens and gonadotropins. *Journal of Clinical Endocrinology and Metabolism* 1997 **45** 1224–1229.
- 12 Foresta C, Bettella A, Spolaore D, Merico M, Rossato M & Ferlin A. Suppression of the high endogenous levels of plasma FSH in infertile men are associated with improved Sertoli cell function as reflected by elevated levels of plasma inhibin B. *Human Reproduction* 2004 **19** 1431–1437.
- 13 Hadziselimovic F, Senn E & Bandhauer K. Effect of treatment with chronic gonadotropin releasing hormone agonist on human testis. *Journal of Urology* 1987 **38** 1048–1050.
- 14 Huhtaniemi I, Nikula H, Parvinen M & Rannikko S. Histological and functional changes of the testis tissue during GnRH agonist treatment of prostatic cancer. *American Journal of Clinical Oncology* 1988 **11** S11–S15.
- 15 Murthy V, Norman AR, Barbachano Y, Parker CC & Dearnaley DP. Long-term effects of a short course of neoadjuvant luteinizing hormone-releasing hormone analogue and radical radiotherapy on the hormonal profile in patients with localized prostate cancer. *BJU International* 2007 **99** 1380–1382.
- 16 Giagulli VA & Carbone D. Hormonal control of inhibin B in men. *Journal of Endocrinological Investigation* 2006 **29** 706–713.
- 17 Raivio T, Wikström AM & Dunkel L. Treatment of gonadotropin-deficient boys with recombinant human FSH: long-term observation and outcome. *European Journal of Endocrinology* 2007 **156** 105–111.
- 18 Young J, Couzinat B, Chanson P, Brailly S, Loumaye E & Schaison G. Effects of human recombinant luteinizing hormone and follicle-stimulating hormone in patients with acquired

- hypogonadotropic hypogonadism: Study of Sertoli and Leydig cell secretions and interactions. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 3239–3244.
- 19 Lahlou N & Roger M. Inhibin B in pubertal development and pubertal disorders. *Seminars in Reproductive Medicine* 2004 **22** 165–175.
- 20 Yao B & Kawaminami M. Stimulation of annexin a5 expression by gonadotropin releasing hormone (GnRH) in the Leydig cells of rats. *Journal of Reproduction and Development* 2008 **54** 259–264.
- 21 Andersson AM, Müller J & Skakkebaek NE. Different roles of prepubertal and postpubertal germ cells and Sertoli cells in the regulation of serum inhibin B levels. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 4451–4458.
- 22 Fujisawa M, Yamazaki T, Fujioka H & Takenaka A. Differential regulation of inhibin subunits by germ cells in human testes. *Archives of Andrology* 2004 **50** 339–345.
- 23 Rey R, Lukas-Croisier C, Lasala C & Bedecarrás P. AMH/MIS: what we know already about the gene, the protein and its regulation. *Molecular and Cellular Endocrinology* 2003 **211** 21–31.
- 24 Teixeira J, Maheswaran S & Donahoe PK. Müllerian inhibiting substance: an instructive developmental hormone with diagnostic and possible therapeutic applications. *Endocrine Reviews* 2001 **22** 657–674.
- 25 Appasamy M, Muttukrishna S, Pizzey AR, Ozturk O, Groome NP, Serhal P & Jauniaux E. Relationship between male reproductive hormones, sperm DNA damage and markers of oxidative stress in infertility. *Reproductive Biomedicine Online* 2007 **14** 159–165.
- 26 Rey R. Assessment of seminiferous tubule function (anti-Müllerian hormone). *Baillière's Best Practice & Research. Clinical Endocrinology & Metabolism* 2000 **14** 399–408.
- 27 Lasala C, Carré-Eusèbe D, Picard JY & Rey R. Subcellular and molecular mechanisms regulating anti-Müllerian hormone gene expression in mammalian and nonmammalian species. *DNA and Cell Biology* 2004 **23** 572–585.
- 28 Young J, Chanson P, Salenave S, Noel M, Brailly S, O'Flaherty M, Schaison G & Rey R. Testicular anti-Müllerian hormone secretion is stimulated by recombinant human FSH in patients with congenital hypogonadotropic hypogonadism. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 724–728.
- 29 Young J, Rey R, Couzinet B, Chanson P, Josso N & Schaison G. Anti-Müllerian hormone in patients with hypogonadotropic hypogonadism. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 2696–2699.

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