Protocol combining GnRH agonists and GnRH antagonists for rapid suppression and prevention of gonadal damage during cytotoxic therapy

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Summary

Purpose of investigation: Infertility represents one of the main sequelae of cytotoxic therapy given for various malignant diseases. Because dividing cells are more sensitive to cytotoxic effects than are cells at rest, it has been hypothesized that inhibition of the pituitary-gonadal axis may facilitate the preservation of future gonadal function. The aim of our study was to find a quick, reliable and economic way to suppress the pituitary-gonadal axis by combining GnRH-agonists with GnRH-antagonists in order to preserve future gonadal function.

Methods: A combination of D-Trp6-GnRH-a (3.75 mg) and cetrorelix (3 mg) was used to achieve a quick downregulation in six postmenarchal young women (aged 15.4 ± 0.7) years with haematological malignancies before the onset of cytotoxic chemotherapy.

Results: The combination of D-Trp6-GnRH-a and GnRH-antagonist cetrorelix induced a reliable and long-lasting suppression of gonadotrophin secretion within 96 hours in all patients allowing cytotoxic therapy to be started without any delay.

Conclusions: The combination of GnRH-agonist and GnRH-antagonist enables a rapid, reliable and cost-effective suppression of the pituitary-gonadal axis to be achieved. Future gonadal function of treated patients will be monitored.

Key words: Chemotherapy; GnRH-analogues; Gonadotoxicity, Fertility.

Introduction

The development of new protocols for therapy of cancer has significantly improved the survival of pediatric, adolescent and reproductive age patients who are then often faced with iatrogenic ovarian failure and its consequences. It has been estimated that 1:900 people aged 15-44 in the U.S. and 1:1,000 people in Great Britain have a history of childhood cancer [1]. Gonadal damage induced by cytotoxic therapy affects both gametogenesis and sex steroidogenesis. While hormonal defects can nowadays be easily dealt with, resulting infertility is one of the major factors influencing the quality of life in adult survivors.

While other options for future fertility protection (translocation of the ovaries away of the radiation fields, embryo- and oocyte freezing, biopsy and cryoconservation of ovarian tissue) are either not entirely successful [1], impractical or not perfected as yet, prior or concomitant treatment with GnRH analogue may be a promising approach for prevention of chemotherapy-induced gonadal damage [2].

Because dividing cells are more sensitive to cytotoxic effects than are the cells at rest, it has been hypothesized that inhibition of the pituitary-gonadal axis and induction of a prepubertal hormonal state may facilitate the preservation of future gonadal function in postpubertal young women [3-5]. However, in critically ill patients the waiting time of 7-14 days until a complete down-regulation after GnRH agonist application is achieved could be often too long. The aim of our study was to introduce a protocol combining GnRH antagonist and agonists to achieve reliable, faster and cost-effective pituitary-ovarian desensitization.

Material and Methods

A prospective clinical protocol was undertaken in six postpubertal young women with haematological malignancies (acute lymphoblastic leukaemia and acute myeloid leukaemia) aged 14.6-16.5 years (15.4 ± 0.7) in whom gonadotoxic therapy was planned. Individual data are described in Table 1. The protocol was approved by the institutional ethical committee.

After informed consent an injection of depot D-Trp6-GnRH-a (Decapeptyl depot 3.75 mg, Ferring) was administered and followed by two injections of 3 mg GnRH antagonist cetrorelix (Cetrotide 3mg, Asta Medica) according to the protocol (Figure

Table 1. — *Characteristics of the patients at the time of gonadal suppression with GnRH antagonist and agonist therapy (before the onset of gonadotoxic therapy)..*

Patient No.	Diagnosis	Age at menarche (yrs)	Age at therapy (yrs)
1	ALL	11.8	12.6
2	AML	12.9	13.8
3	ALL	13.5	14.6
4	ALL	11.0	16.5
5	ALL	9.0	15.4
6	ALL	14.3	15.0

ALL: acute lymphoblastic leukaemia. AML: acute myeloid leukaemia.

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1). Monthly injections of depot D-Trp6-GnRH-a followed as long as necessary during the entire time of cytotoxic therapy. A hormonal profile of serum FSH, LH (immunoasssay, MEIA, AxSym, Abbott) and 17-beta-estradiol (chemiluminiscent method, Architect, Abbott) was evaluated before starting the GnRH-a co-treatment and in regular intervals during the whole treatment course. The LH levels during the combined agonist-antagonist protocol were compared to the gonadotrophin hormonal profile obtained after injection of D-Trp6-GnRH-agonist only (Figure 3) as already published [6, 7].

Statistical methods were mainly descriptive (mean, standard deviation, absolute and relative frequency). The relation between two variables was measured by Spearman's correlation coefficient and paired comparisons were done by the Wilcoxon signed-rank test.

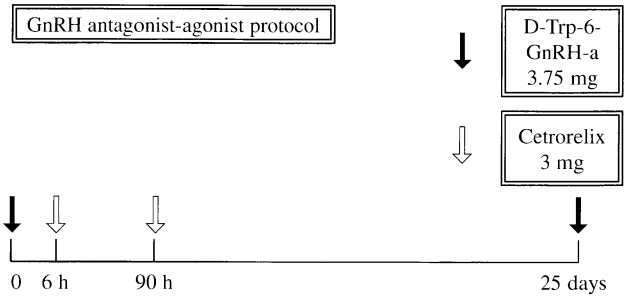

Figure 1. — GnRH antagonist-agonist protocol for rapid onset of pituitary-gonadal suppression.

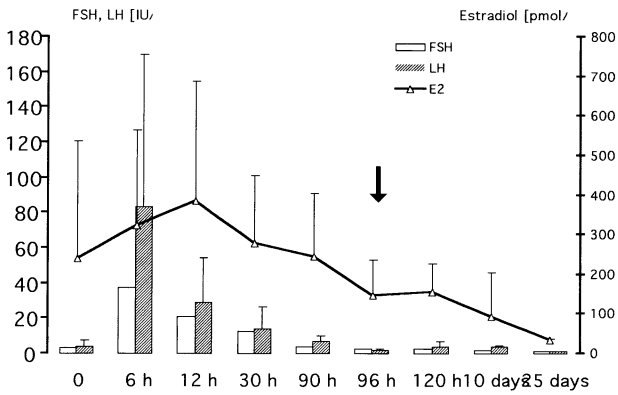

Figure 2. — FSH, LH and E2 serum levels in the GnRH antagonist-agonist protocol.

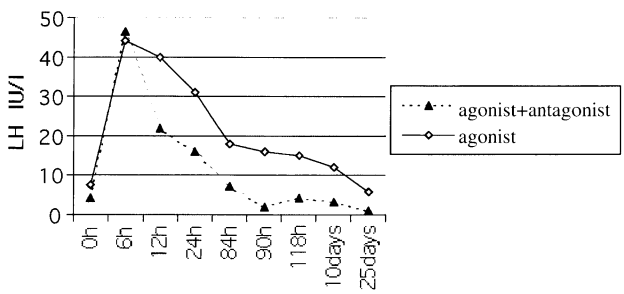

Figure 3. — Serum LH response after D-Trp6 only and after the combination with 3 mg Cetrorelix.

Results

A combination of long-acting GnRH analogue D-Trp6 and subsequent administration of two injections of 3 mg GnRH antagonist cetrorelix three days apart was able to suppress the pituitary-gonadal axis in all treated subjects within 96 hours (Figure 2) below 2I U/l (as indicated by the arrow). Both gonadotrophins and 17-beta estradiol remained suppressed during the entire course of therapy and were controlled by injections of D-Trp6-GnRH-a. The onset of suppression was not influenced by the day of the menstrual cycle when the agonist-antagonist protocol was started. Gonadotrophins were supressed more profoundly and more rapidly than estradiol levels ($p < 0.05$) which should not adversely influence the protective effect of pituitary down-regulation on the fate of germ cells during and after the cytotoxic therapy.

Discussion

In contrast to the continual process of spermatogenesis in males, the female is born with the maximum number of gametes that she will ever have. Out of approximately one million at the time of birth there will be only 300,000 left by the time of puberty and because of the continuous process of atresia there are practically no gametes left by menopause [6]. Histologic studies have found ovarian atrophy and marked loss of primordial follicles as a direct effect of chemotherapy on human ovarian tissue [7]. However, we understand today that this effect is not an "all or none" phenomenon; it should rather be viewed as a syndrome with different degrees (mild to severe) of gonadal damage.

Our current knowledge derives from experiments in rats, where the uptake of tritiated thymidine was significantly reduced as a marker of reduced mitotic activity after administration of GnRH analogues [8]. Morover, the protective effect of GnRH–a against cyclophosphamide-induced ovarian failure has been documented in rat models. In these experiments activated cyclophosphamide generated a decrease in progesterone and prostaglandine E production and diminished cell survival when incubated in vitro with rat granulosa cells. It seems therefore that the damage of granulosa cells is crucial in the process of gonadal damage during cytotoxic therapy [9]. Attaya [10] showed in a prospective experiment in female Rhesus monkeys that GnRH-a may protect the ovary from cyclophosphamide damage. In this experiment 65% of the total primordial follicles were lost in the control group compared to only 29% in the GnRH-a co-treatment group [10]. In humans, Blumenfeld [11] in a prospective protocol treated 18 cycling women with lymphoma aged 15-40 years. This group was compared to a matched control group of 18 women (aged 17-40). While over 93% of the surviving patients in the GnRH-a and chemotherapy group resumed spontaneous ovulation and menses, less than 40% of the women in the control group of chemotherapy without GnRH co-treatment resumed normal ovarian cyclic activity.

However, the waiting period between starting the GnRH-a and administration of gonadotoxic therapy becomes a very sensitive issue. While dealing mostly with critically ill patients, there is pressure to start the chemotherapy as soon as possible after the diagnosis has been established. After application of GnRH agonist there is a one to two weeks flare-up period before desensitization and down-regulation is achieved during which stimulated ovaries may be even more vulnerable to the cytotoxic substances due to increased gonadotrophic stimulation and folliculogenesis. This may also be the reason for the discrepancy between the results of different clinical studies as a short waiting interval may possibly increase the sensitivity of the gonad to the cytotoxic effects [3, 8].

Our protocol combining the desensitization and down-regulation effects of GnRH agonists together with competitive receptor blockage by GnRH antagonists allowed us to suppress the pituitary-gonadal axis within 96 hours. In the literature there is very limited information about the hormonal background on the day when cytotoxic therapy was started in reported patients. According to our knowledge it is the rapid suppression of gonadotrophin levels that turns gonads into the quiescent pre-pubertal stage. Therefore this reliable, quick and cost-effective suppression protocol does not delay the start of effective chemotherapeutic treatment.

However, in spite of some ethical problems double-blind randomized trials are needed to definitely confirm the effectiveness of GnRH-analogue co-treatment in the prevention of chemotherapeutic damage in humans.

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