Preservation of Fertility and Ovarian Function and Minimization of Chemotherapy-Induced Gonadotoxicity in Young Women by GnRH-a

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Improved long-term survival in young women with lymphoma and leukemia has increased attention to the preservation of their future fertility. We have attempted to minimize the gonadotoxic effect of chemotherapy by cotreatment with a GnRH agonist analog, inducing a temporary prepubertal milieu. Our prospective clinical case series includes 92 women with lymphoma, aged 15–40 years, 10 with leukemia and 18 undergoing chemotherapy treatments for nonmalignant autoimmune diseases. Depot D-TRP6-GnRH-a was injected monthly from before the initiation of chemotherapy until its conclusion, for up to 6 months. We used 82 similarly treated patients with lymphoma not given GnRH-a as a comparison group. All but five of the surviving evaluable patients with GnRH-a/chemotherapy cotreatment resumed spontaneous ovulation and menses or conceived, whereas 53% of the patients in the comparison group experienced premature ovarian failure (P<01). Mechanisms to explain this apparent chemoprotective effect are discussed, and the work of other investigators in this area is reviewed. [J Natl Cancer Inst Monogr 2005;34:40–3]

As survival rates for young cancer patients continue to improve, protection against iatrogenic infertility caused by chemotherapy with or without radiotherapy assumes higher priority (1–4). Premature ovarian failure (POF) is a common long-term consequence of chemotherapy (1,5–9) and radiotherapy (5,7). The risk of gonadal damage is usually dose and age related. Alkyating agents are the most common chemotherapeutic agents associated with gonadal damage (10).

Because dividing cells are known to be more sensitive to the cytotoxic effects of alkylating agents than are cells at rest, it has been suggested that inhibition of the pituitary–gonadal axis would reduce the rate of spermatogenesis and oogenesis and thereby render the germinal epithelium less susceptible to the effects of chemotherapy (1,2,5,11–14). Although several investigators have demonstrated that GnRH-a can inhibit chemotherapy-induced ovarian follicular depletion in the rat (15,16), uncertainty remained about human application (1,14,16,17). No protection from ovarian damage, caused by irradiation to rats, by the GnRH agonist was observed (18).

Chemotherapy for lymphoma can lead to infertility in girls and women. Approximately half to three-quarters of women receiving the Cyclophosphamide, Oncovin, Procarbazine, Prednisone/ Adriamycin, Bleomycin, Vinblastine, Dacarbazine [COPP/ABVD] regimen of chemotherapy for lymphoma exhibit hypergonadotropic amenorrhea and ovarian failure (19–22). In contrast, a long-term follow-up of 240 children 15 years of age or younger who were treated by MOPP for Hodgkin disease showed that only 13% of the girls suffered ovarian failure (17,21). Chapman et al. (23) found that of their female patients treated for Hodgkin disease, 69% developed POF if they were younger than 29 years of age, whereas 96% of those over 30 years of age developed POF. Because ovarian function has been preserved in most long-term survivors treated prepubertally for lymphoma (21,24), but only in a minority of similarly treated adult patients (19), it is clinically logical to temporarily create a prepubertal milieu in women of reproductive age before and during the chemotherapeutic insult (1,11,17).

Ataya et al. (16) demonstrated in female Rhesus monkeys that GnRH-a may protect the ovary from cyclophosphamide-induced gonadal damage. Administration of GnRH-a in parallel with cyclophosphamide significantly decreased the daily rate of follicular decline and the total number of follicles lost during the chemotherapeutic insult, as compared to administration of cyclophosphamide alone, without GnRH-a (16). This preliminary experience in Rhesus monkeys is in keeping with our own early clinical results (1,11,17). Only five of the 75 evaluable surviving women in our study group of lymphoma patients became menopausal after the GnRH-a/chemotherapy cotreatment (<7%), compared to more than half (44 of 82) of the patients in the chemotherapy-alone (control) group (Table 1) (1,11,17). Ninety-three percent of survivors of the chemotherapy (with or without radiotherapy) who received the GnRH-a cotreatment resumed ovulatory menses (>93%), compared to 47% of women who had similar cancer treatment without hormonal protection. Moreover, half of these women (44 of 82) had POF and hypergonadotropic amenorrhea (1,11,17). Neither age at chemotherapy administration or after follow-up nor the dosages of the various cytotoxic drugs were significantly different between the study and control groups (11,17). The only significant difference between the two groups was the incidence of POF and hypergonadotropic amenorrhea (53% vs. 7%; P<.05; Table 1) (1,2,11,17).

However, one should be very cautious about drawing definitive conclusions from these promising—but still preliminary—data, as our study was neither randomized nor double-blinded (1,11,17). The comparison group included retrospective historical controls or patients who were treated concurrently with the study group but who were referred after having already been exposed to gonadotoxic chemotherapy (1,11,17).

It is encouraging that similar results regarding the protective effect of GnRH-a against chemotherapy-associated...
Inhibiton Measurements

We have found temporary increases in follicle-stimulating hormone (FSH) levels in about one-third of the young women who ultimately resumed ovarian cyclic function, indicating reversible ovarian damage (1,17). Longitudinal individual follow-up measurements of inhibin A immunonconcentrations in the study group have shown a decrease during treatment with chemotherapy and GnRH-a, with a subsequent return to normal levels within a few months of completion in the patients who resumed ovarian function, but not in those who became permanently menopausal. The mean ± standard error inhibin A concentrations were 40.73 ± 10 pg/mL before starting GnRH-a/chemotherapy treatment, decreasing to 4.77 ± 1.4 and 1.83 ± 0.5 pg/mL at 1–3 and 4–6 months of the treatment protocol, respectively (32). The mean (± standard error) inhibin A concentrations increased to 26.5 ± 20 pg/mL at 2 months after the protocol, 96.4 ± 47.6 at 6 months, 69.4 ± 17 at 1 year, and 177 ± 134.7 pg/mL at 2 years after treatment (32). The mean inhibin A concentration in the patients who developed POF after chemotherapy was below 4 pg/mL, compared to 300 ± 200 pg/mL in women undergoing hMG/hCG superovulation and 170 ± 50 in those patients who spontaneously conceived after the GnRH-a/chemotherapy cotreatment protocol (32).

Whereas inhibin A is secreted mainly at and after ovulation, and therefore is a marker of the luteal phase, the concentration of inhibin B is higher in the follicular phase. Because we are interested in predicting the return of ovarian function after completion of the chemotherapy/GnRH-a cotreatment, before we know whether the patients will resume spontaneous menstruation, it is important to measure both inhibins A and B to reflect the function and reserve of ovarian granulosa cells. Not unexpectedly, the levels of inhibin A and inhibin B were low, which is compatible with menopausal levels in those women who experienced hypergonadotropic amenorrhea, whereas those who subsequently resumed cyclic ovarian function had ir-inhibin A and ir-inhibin B concentrations within normal levels (1,32). Because most women who have not conceived are young, unmarried women who were not yet interested in fertility, it is premature to draw any conclusions at this time about whether the level of ir-inhibin A and ir-inhibin B after chemotherapy is predictive of future fertility. The addition of anti-Mullerian hormone may possibly improve our ability to predict the future resumption of ovarian function after the gonadotoxic insult.

Mechanisms of Chemoprotection

Several hypotheses may explain the mechanism by which GnRH-a minimizes chemotherapy-associated gonadotoxicity. First, the hypogonadotropic state generated by the GnRH-a simulates the prepubertal hormonal milieu. If the alkylating agents increase the rate of apoptosis of the nonresting follicles and subsequently decrease the secretion of sex steroids and inhibins produced by these follicles (11), the resultant decrease in plasma concentrations of sex steroids and inhibins subsequently creates negative feedback to the hypothalamus and pituitary, resulting in an increase in FSH secretion, which may bring about an increased recruitment of preantral follicles to begin maturation and to be exposed to the gonadotoxic effect of the alkylating agents, ending in an increased rate of follicular apoptosis and degeneration. This vicious cycle may be interrupted by GnRH-a administration.
Supporting the detrimental effect of high gonadotropin concentrations on primordial and primary follicles, transgenic mice with increased luteinizing hormone (LH) concentrations that have a number of follicles similar to that of wild-type controls at birth suffer a significant premature loss of their primordial and primary follicles within several weeks (33). Moreover, primordial and primary follicles express mRNA for FSH and LH receptors (34–36) in keeping with the notion that they may be gonadotropin dependent.

A second possible explanatory mechanism for the effect of GnRH-a in decreasing chemotherapy-associated gonadotoxicity is that the hypoestrogenic state may decrease utero-ovarian perfusion (11,30), resulting in a decreased total cumulative exposure of the ovaries to the chemotherapeutic insult.

Third, not only rodent gonads but also primate and human gonads contain GnRH-a receptors (1,19,22,25). In an ovarian carcinoma cell line, Grundker and Emons (37) have shown that activating GnRH-I and GnRH-II receptors decreases apoptosis. It is possible that GnRH-a directly affects the oocyte cumulus complex, the granulosa cell, or possibly another ovarian compartment. Additional studies are obviously necessary to answer this important question.

Fourth, it is possible that GnRH-a may up-regulate an intragonadal antiapoptotic molecule such as sphingosine-1-phosphate (S-1-P). Recently, Morita et al. (38) have identified several molecules that are required for chemotherapy-induced oocyte apoptosis. Although much of their work has relied on gene knockout mice, these researchers have identified a small lipid factor in the proapoptotic second messenger complex—S-1-P—as a potent protective molecule in vitro. In mice, in vivo S-1-P pretreatment resulted in a dramatic dose-dependent protection of oocytes from radiotherapy-associated gonadotoxicity (38,39). Whether GnRH-a adjuvant cotreatment protects the ovary directly or is associated with an intraovarian increase in S-1-P is a question of tremendous scientific interest and clinical impact that awaits further investigation.

Most recently Tilly’s group (40) has presented revolutionary data indicating that mouse ovaries possess mitotically active germ cells that continuously replenish the pool of immature follicles. One may speculate that GnRH-a protects the undifferentiated germline stem cells, which ultimately generate de novo primordial follicles.

**Conclusions**

Because cryopreserving ovarian tissue or unfertilized oocytes has not yet generated acceptable success rates, it is important to educate young women facing cancer treatment about the option of having GnRH-a cotreatment. Furthermore, combining the various modalities for a specific patient may increase the odds of preservation of future fertility. There is no contraindication to ovarian biopsy for cryopreservation combined with GnRH-a administration or to adding follicular aspiration for in vitro fertilization and embryo freezing if the patient has a spouse or partner. Until we have more answers to our scientific questions, withholding information on all options may violate the ancient dictum primum non nocere.

**References**


NOTES

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