A Combination of a GnRH Antagonist and Agonist for Fertility Preservation in an Adolescent Female Murine Model

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Abstract

Recent studies have suggested that GnRH agonists (GnRHags) protect ovarian function following chemotherapy. Here, we study the effect of a combination of GnRH antagonist (GnRHan) and GnRHag for gonadal protection from gonadotoxic chemotherapy in adolescent female rats. Cycling Sprague Dawley rats were treated at adolescent age. Thirty female rats were randomized to 5 treatment groups ($n = 6/\text{group}$): (1) placebo, (2) cyclophosphamide (CPA) alone, (3) GnRHan followed by GnRHag with placebo, (4) GnRHan followed by GnRHag with CPA, and (5) GnRHag with CPA. The main outcome measure was live birth rate (LBR), and secondary measures included rat weight, ovarian volume, and follicles. Group 2 had decreased LBR compared to all other groups. Group 4 and 5 had LBR similar to placebo. There was no difference in the ovarian volume. The CPA-alone group had decreased number of antral follicles compared to control. These studies demonstrate that the combination of GnRHan and GnRHag and GnRHag alone preserved fertility in female adolescent rats following gonadotoxic chemotherapy treatment. The addition of a GnRHan to a GnRHag does not confer a greater protective effect.

Keywords

fertility preservation, GnRH suppression, adolescents (AYA)

Introduction

Approximately 40,000 adolescents and young women are diagnosed with cancer each year. Due to advances in treatment, their survival rate exceeds 80%, but chemotherapy can have long-term effects on ovarian function.¹ This is important because ovarian function affects bone health, quality of life, and fertility. Agents most likely to affect ovarian function include alkylating agents like cyclophosphamide (CPA), which is commonly used for adolescent chemotherapy. Due to the complex nature of cancer treatments with multiple protocols, it is difficult to predict an individual's ovarian risk during treatment for their cancer. Currently, the options for fertility preservation include embryo and oocyte cryopreservation, ovarian tissue cryopreservation, and GnRH suppression.² Oocyte cryopreservation is being used by more young single women, but it is an expensive and time-sensitive option.³

Recent studies have suggested that GnRH agonists (GnRHag) protect ovarian function following chemotherapy.^{4,5} The theory, albeit controversial, is that inhibition of the pituitary ovarian axis may decrease early follicular development and prevent the gonadotoxic effects of the chemotherapy. A theoretical concern with the GnRHag is the “flare effect.”⁶

This initial increase in gonadotropins with a GnRHag has the potential to promote folliculogenesis and increase the ovary's vulnerability to the chemotherapy. To obviate the potential adverse “flare effect,” we studied the effect of adding a GnRH antagonist (GnRHan) to a GnRHag on live birth rates (LBRs) in a chemotherapy-treated adolescent rat model.⁷

Material and Methods

Animals

This study was approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio. At 5 weeks of age, 30 Sprague Dawley rats

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were examined for estrous cyclicity. Starting at the age of 6 weeks, cycling rats were randomized to 5 different treatment groups—(1) placebo, (2) CPA alone, (3) GnRHan followed by GnRHag with placebo, (4) GnRHan followed by GnRHag with CPA, and (5) GnRHag with CPA. Cyclophosphamide (6 mg/kg; Sigma-Aldrich, St. Louis, Missouri) was administered intraperitoneally (IP) daily for 3 weeks as previously described.^{8,9} The placebo group received the same volume of phosphate-buffered saline IP. The GnRHan, cetrorelix acetate (25 µg/d IP; Sigma Aldrich), and leuprolide acetate (2.5 µg subcutaneously; Sigma Aldrich) were administered daily in respective groups. The GnRHag was begun on the third day of GnRHan treatment when used in combination or 3 days prior to start of the chemotherapy when used alone. One week after treatment completion, cyclicity was assessed and rats were mated during the second estrous cycle. Pups were counted on the day of birth.

The primary outcome was LBR. Secondary measures included estrous cyclicity, fertility, number of follicles, and corpus luteum. Weights were recorded weekly during and after treatment. Pregnancy rates were recorded. At 7 days of life, the number and weight of the offspring were documented. Ovaries were removed 7 days postpartum.

Tissue Processing

The ovaries were fixed in paraffin. Four random samples were taken from 5-µm serial sections and stained with hematoxylin and eosin. The number of primordial–primary, preantral, antral, and corpus luteum were assessed by blinded examiners as previously described.⁸

Statistical Analysis

Results were analyzed with the software Prism version 6.0 (GraphPad, La Jolla, California). Data were normally distributed and therefore expressed as mean $\pm$ standard error. Live birth rate differences between individual groups were calculated by χ^2 . Statistical analysis was performed by Student *t* test or analysis of variance where appropriate. A *P* < .05 was considered statistically significant.

Results

Chemotherapy-treated rats experienced a statistically significant decrease in weight during and after completion of treatment. One week after treatment, all CPA-treated groups recovered to weights similar to groups not receiving CPA (Figure 1). The CPA group had a decreased LBR compared to all other groups. There was no difference in LBR in placebo compared to the combined GnRHan and GnRHag group with placebo. The combined GnRHan and GnRHag group receiving CPA had increased LBR compared to CPA alone. The GnRHag alone receiving CPA also had increased LBR compared to CPA alone and was not different than placebo. There was no difference in LBR in the combined GnRHan and GnRHag group receiving CPA and the GnRHag with CPA (Figure 2).

Figure 1. Change in rat weight over treatment course: The groups that received cyclophosphamide (CPA) had significant decrease in weight during treatment and end of treatment compared to groups without chemotherapy (*P* = .001). This difference was not seen 1 week after treatment completion.

Figure 2. Live birth rate after treatment: Significant difference between chemo alone and placebo (**P* = .02), GnRHan/GnRHag with placebo ($\dagger P$ = .008), GnRHan/GnRHag with chemo (**P* = .02), and GnRHag with chemo (**P* = .02). GnRHag indicates GnRH agonist; GnRHan, GnRH antagonist.

The placebo and combined GnRHan and GnRHag with placebo groups continued with 100% estrous cyclicity. Cyclicity in the CPA group decreased to 66% and in both GnRHag with CPA and combined GnRHan and GnRHag with CPA groups to 83%. Average litter sizes did not differ between groups. The average litter sizes in each group of rats having live births were 13.2 ± 0.7 , 11 ± 2.5 , 14.7 ± 0.7 , 12.8 ± 2.7 , and 12.3 ± 1.6 in the placebo, CPA, combined GnRHan/GnRH ag with placebo, combined GnRHan/GnRHag with CPA, and GnRHag with CPA groups, respectively. There was no difference in the average weight of pups between groups (*P* = .33).

Postpartum ovarian volume and histology are shown in Figure 3A and B. There was no significant difference (*P* = .86) in combined average ovarian volume among groups. There was no difference in the numbers of primordial–primary, preantral, or corpus luteum in postpartum groups. The postpartum CPA group had decreased number of antral follicles compared to control (1.8 vs 4.2; *P* = .02).

Figure 3. A, Postpartum ovarian volumes: There was no difference in the ovarian volumes among treatment groups ($P = .72$). B, Postpartum ovarian follicular histology: There was no difference in the numbers of primordial, preantral, or corpus luteum in postpartum groups. The postpartum cyclophosphamide (CPA) group had decreased number of antral follicles compared to control (1.8 vs 4.2; $P = .02$).

Discussion

Our study confirms the previous findings that GnRH analogs protect against chemotherapy-induced gonadotoxicity in female rats. The addition of a GnRHan to a GnRHag did not improve LBR compared to the GnRHag alone receiving CPA. Our study is novel in that (1) we assess the addition of a GnRHan to obviate the “flare effect” of the GnRHag in murine model examining LBR as the primary end point, and (2) no prior studies have assessed the chemoprotective effect of GnRH analogues in adolescent rats.

Inhibition of the hypothalamic–pituitary–ovarian axis, through GnRH signaling, should diminish folliculogenesis and theoretically decrease susceptibility of the germinal epithelium to gonadotoxic chemotherapy.¹⁰ GnRH agonists have been shown to protect primordial follicles in a murine model.¹¹ GnRH agonists have also been shown to improve implantation rates in chemotherapy-treated female rats.^{12,13} The literature is controversial regarding ovarian protection by GnRHag in humans, but recent studies have supported their use.^{4,5} GnRH agonists create a “flare effect” that increases gonadotropins and stimulates folliculogenesis. In theory, the combination of GnRHan and GnRHag should increase LBR compared to the GnRHag alone due to prevention of the “flare effect.” However, our study showed no additional benefit of the combination of GnRHan/GnRHag compared to GnRHag alone.

Studies using a GnRHan for fertility preservation are limited. Meior et al concluded that the use of cetrorelix decreases the loss of primordial follicles caused by CPA.¹⁴ Danforth et al did not find a protective effect when assessing the primordial follicle population in female mice subjected to a single dose of CPA and GnRHan.¹⁵ In an adult murine model, Lemos et al showed that administration of a GnRHan had a protective effect on fertility of rats undergoing chemotherapy with CPA.⁸ Li et al showed that the combination of GnRHag with a

GnRHan completely prevented the “flare effect” and enhanced protection of the ovarian follicles from cisplatin-induced gonadotoxicity in a murine model.⁷ There have been a few small human studies with the GnRHan alone or the combination with GnRHag.^{16–18} The GnRHan alone showed improvement in the return to menses in 14 of 15 women treated with CPA.¹⁷ In a pilot study of the combination of GnRHan and GnRHag, 8 of 9 young women resumed menses and 2 conceived.¹⁶ In a recent, open-label, randomized controlled trial with 50 women, return of menstruation at 1 year was not different in those receiving GnRHan with GnRHag with chemotherapy as compared to those receiving chemotherapy alone, in the early treatment group. In this study, live births were not assessed.¹⁸

Our study adds the novelty of using adolescent rats, which is an unexplored aspect of translational research. Previous studies confirm the rats are considered sexually mature adolescents at 6 weeks of age.^{19,20} In our study, the LBR was higher than expected in the chemotherapy-alone group when considering prior studies in adult rats.^{9,12} The chemotherapy-treated groups did lose weight compared to placebo, which confirms the systemic effects of CPA. The ovarian weight did not differ in postpartum groups, which is consistent with a prior study.⁸ Also, as in previous studies, the antral follicles were less in the CPA group compared to control, but no difference was seen in the other follicles.^{13,21,22} We showed some chemotherapy-induced gonadotoxicity in our adolescent rat model but not to the same degree reported in adult rats. This observation is consistent with human studies, suggesting ovarian damage in adolescents requires a higher dose of chemotherapy.²³

In conclusion, the combination of GnRHan and GnRHag with CPA preserved fertility compared to CPA alone but was not more protective than the GnRHag alone. More research is needed on the mechanisms of action of the GnRH analogs for fertility preservation, with translational application for fertility preservation in the adolescent population.

Authors' Note

This was presented at the Society for Reproductive Investigation in March 2016 in Montreal, Canada. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. JFK, RDR, RRT, and RSS contributed in study design. JFK, MTS, PAB, CMF, JPV, and RRT contributed to the research. JFK, RDR, RRT, and RSS contributed to data analysis. JFK, RDR, and RSS drafted the manuscript. The material contained in the manuscript has not been published, has not been submitted, or is not being submitted elsewhere for publication. All authors are in agreement to submission of this manuscript.

Declaration of Conflicting Interests

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References

- Barton SE, Najita JS, Ginsburg ES, et al. Infertility, infertility treatment, and achievement of pregnancy in female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study cohort. *Lancet Oncol.* 2013;14(9):873-881.
- Robinson RD, Knudtson JF. Fertility preservation in patients receiving chemotherapy or radiotherapy. *Mo Med.* 2014;111(5):434-438.
- Cakmak H, Rosen MP. Ovarian stimulation in cancer patients. *Fertil Steril.* 2013;99(6):1476-1484.
- Moore HC, Unger JM, Phillips KA, et al. Goserelin for ovarian protection during breast-cancer adjuvant chemotherapy. *N Engl J Med.* 2015;372(10):923-932.
- Lambertini M, Boni L, Michelotti A, et al. Ovarian suppression with triptorelin during adjuvant breast cancer chemotherapy and long-term ovarian function, pregnancies, and disease-free survival: a randomized clinical trial. *JAMA.* 2015;314(24):2632-2640.
- Blumenfeld Z, Evron A. Preserving fertility when choosing chemotherapy regimens—the role of gonadotropin-releasing hormone agonists. *Expert Opin Pharmacother.* 2015;16(7):1009-1020.
- Li X, Kang X, Deng Q, Cai J, Wang Z. Combination of a GnRH agonist with an antagonist prevents flare-up effects and protects primordial ovarian follicles in the rat ovary from cisplatin-induced toxicity: a controlled experimental animal study. *Reprod Biol Endocrinol.* 2013;11:16.
- Lemos CN, Reis FM, Pena GN, Silveira LC, Camargos AF. Assessment of fertility protection and ovarian reserve with GnRH antagonist in rats undergoing chemotherapy with cyclophosphamide. *Reprod Biol Endocrinol.* 2010;8:51.
- Montz FJ, Wolff AJ, Gambone JC. Gonadal protection and fecundity rates in cyclophosphamide-treated rats. *Cancer Res.* 1991;51(8):2124-2126.
- Blumenfeld Z. How to preserve fertility in young women exposed to chemotherapy? The role of GnRH agonist cotreatment in addition to cryopreservation of embryo, oocytes, or ovaries. *Oncologist.* 2007;12(9):1044-1054.
- Ozcelik B, Turkyilmaz C, Ozgun MT, et al. Prevention of paclitaxel and cisplatin induced ovarian damage in rats by a gonadotropin-releasing hormone agonist. *Fertil Steril.* 2010;93(5):1609-1614.
- Ataya K, Ramahi-Ataya A. Reproductive performance of female rats treated with cyclophosphamide and/or LHRH agonist. *Reprod Toxicol.* 1993;7(3):229-235.
- Ataya KM, McKanna JA, Weintraub AM, Clark MR, LeMaire WJ. A luteinizing hormone-releasing hormone agonist for the prevention of chemotherapy-induced ovarian follicular loss in rats. *Cancer Res.* 1985;45(8):3651-3656.
- Meirow D, Assad G, Dor J, Rabinovici J. The GnRH antagonist cetrorelix reduces cyclophosphamide-induced ovarian follicular destruction in mice. *Human reproduction (Oxford, England).* 2004;19(6):1294-1299.
- Danforth DR, Arbogast LK, Friedman CI. Acute depletion of murine primordial follicle reserve by gonadotropin-releasing hormone antagonists. *Fertil Steril.* 2005;83(5):1333-1338.
- Potolog-Nahari C, Fishman A, Cohen I. Protection of ovarian function and fertility using a combination of gonadotropin-releasing hormone (GnRH) agonist and GnRH antagonist during cancer treatment in young females. *Gynecol Endocrinol.* 2007;23(5):290-294.
- Whitehead J, Toledo MG, Stern CJ. A pilot study to assess the use of the gonadotropin antagonist cetrorelix in preserving ovarian function during chemotherapy. *Aust N Z J Obstet Gynaecol.* 2011;51(5):452-454.
- Elgindy EA, El-Haieg DO, Khorshid OM, et al. Gonadotropin suppression to prevent chemotherapy-induced ovarian damage: a randomized controlled trial. *Obstet Gynecol.* 2013;121(1):78-86.
- Tek M, Cayan S, Yilmaz N, Oguz I, Erdem E, Akbay E. The effect of vascular endothelial growth factor on spermatogenesis and apoptosis in experimentally varicocele-induced adolescent rats. *Fertil Steril.* 2009;91(5 suppl):2247-2252.
- Sengupta P. The laboratory rat: relating its age with human's. *Int J Prev Med.* 2013;4(6):624-630.
- Bokser L, Szende B, Schally AV. Protective effects of D-Trp6-luteinising hormone-releasing hormone microcapsules against cyclophosphamide-induced gonadotoxicity in female rats. *Br J Cancer.* 1990;61(6):861-865.
- Jarrell J, Lai EV, Barr R, McMahon A, Belbeck L, O'Connell G. Ovarian toxicity of cyclophosphamide alone and in combination with ovarian irradiation in the rat. *Cancer Res.* 1987;47(9):2340-2343.
- Levine JM, Kelvin JF, Quinn GP, Gracia CR. Infertility in reproductive-age female cancer survivors. *Cancer.* 2015;121(10):1532-1539.