Study of different modalities to prevent gamma-radiation induced ovarian failure in rats

Thesis presented by

YASMEN FOUAD KHALIFA MAHRAN

B. Pharm. Sc., Ain Shams University (2004)
Assistant lecturer of Pharmacology and Toxicology
Faculty of Pharmacy, Ain Shams University

Submitted for the Ph.D degree in Pharmaceutical Science
(Pharmacology and Toxicology)
Supervised by

DR. Ashraf Bahi El-Deen Abdel-Naim
Professor of Pharmacology and Toxicology department, Faculty of Pharmacy, Ain Shams University.

DR. Ebtehal El-Demerdash Zaki
Professor and Head of Pharmacology and Toxicology department, Faculty of Pharmacy, Ain Shams University

DR. Azza Abd El-Fattah Ali
Professor and Head of Pharmacology and Toxicology department, Faculty of Pharmacy, AL-Azhar University (Girls).

DR. Ahmed Shafik Nada
Professor of Physiology, National Center for Radiation Research and Technology.

Faculty of Pharmacy, Ain Shams University, Cairo
(2013 G- 1434 H)

دراسة وسائل مختلفة لمنع فشل التبويض المحدث بواسطة العلاج بالاشعاع في الجرذان.

رسالة مقدمة من الصيدلانية

ياسمين فؤاد خليفة مهران

بكالوريوس علوم صيدلية . جامعة عين شمس (٢٠٠٤)

مدرس مساعد بقسم علم الأدوية والسموم . كلية الصيدلة . جامعة عين شمس

للحصول علي درجة الدكتوراه فى العلوم الصيدلية

(علم الأدوية والسموم)

بإشراف

أ.د/ آ غ ذ . ا ى ك خ م ع ا ح ك غ ل

أستاذ علم الأدوية و السموم

كلية الصيدلة- جامعة عين شمس.

أ.د/ ئ ة غ ك ط خ ا ف خ ؤ س ر ق ى

أستاذ و رئيس قسم علم الأدوية و السموم

كلية الصيدلة –جامعة عين شمس.

آ خ/ غ ب غ ا ح ا ف ت ؤ ج ع ى

أستاذ و رئيس قسم علم الأدوية و السموم

كلية الصيدلة –جامعة الأزهر (بنات).

آ خ/ آ ح ل خ ف ن ي ف م خ ئ

أستاذ بقسم البحوث الدوائية الاشعاعية

هيئة الطاقة الذرية.

كلية الصيدلة . جامعة عين شمس

1434 - ٢٠١٣

CONTENTS

SUBJECT PAGE


بسم الله الرحمن الرحيم
قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا
عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ
صدق الله العظيم
سورة البقرة آية (٣٢)

Abstract

Radiotherapy is one of the most common and effective cancer treatments. However, it has a profound impact on ovarian function leading to premature ovarian failure and loss of fertility. In the hope to preserve fertility, the need for effective radioprotective is intensified. The present study investigated the mechanisms of the potential radioprotective effect of either Tamoxifen (TAM) or Growth hormone (GH) on gamma irradiation-induced ovarian failure in experimental rats and the impact of the insulin like growth factor 1 (IGF-1) and its receptor. Female Sprague-Dawely rats were either exposed to a single γ-irradiation (3.2 Gy, LD20) and/or treated with either TAM (1 mg/kg) or GH (1 mg/kg). Gamma-irradiation produced an array of ovarian dysfunction that was evident by assessment of hormonal changes, follicular development, proliferation marker (PCNA), oxidative stress as well as apoptotic markers four days post irradiation. In addition, IGF-1 / IGF-1R axis expression was assessed using real time RT-PCR and immunolocalization techniques. Furthermore, fertility assessment was performed. Tamoxifen as well as GH significantly enhanced follicular development and restored the Anti-mullerian hormone (AMH) level as compared to the irradiated group. In addition, TAM and GH significantly ameliorated the deleterious effects of irradiation on oxidative stress, PCNA expression and apoptosis. Interestingly, both drugs were shown to enhance the ovarian IGF-1 on both transcription and translation levels. Their radioprotective effect was also confirmed by histopathological examination of ovaries and uteri. Finally, both TAM and GH regained the fertility that was lost after irradiation in terms of fecundity and fecundability. In conclusion, TAM and

Abstract

GH showed a radioprotective effect and rescued the ovarian reserve and fertility through increasing AMH and local IGF1 level and counteracting the oxidative stress mediated apoptosis.

Keywords: Premature ovarian failure, Growth hormone, Tamoxifen, radiotherapy, IGF-1.

ACKNOWLEDGEMENTS

*Firstly, I am greatly thankful to **ALLAH**, who, without his help, this work would never be accomplished and may this work add to our good deeds to gain his kind mercifulness and forgiveness.*

*No words can express my great thanks to **Dr. Ebtehal El-Demerdash Zaki**, Professor and Head of Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University. I am really grateful for her tremendous effort and indispensable help in practical work as well as thesis writing.*

*I wish to express my appreciation and gratitude to **Dr. Ashraf Bahi El-Deen Abdel-Naim,** Professor of Pharmacology and Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, for his kind cooperation and supervision throughout the work.*

*I would like to thank **DR. Azza Abd El-Fattah Ali,** Professor and Head of Pharmacology and Toxicology department, Faculty of Pharmacy, AL-Azhar University (Girls), for valuable comments and guidance during this work.*

*I would like to express my great thanks to **Dr. Ahmed Shafik Nada**, Professor of Physiology, National Center for Radiation Research and Technology, for his valuable supervision and his great effort in accomplishing the part of radiotherapy.*

*I am deeply indebted to **DR. Reem Nabeel Abou El-Naga,** lecturer of Pharmacology and Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, for her continuous help and valuable comments in thesis writing.*

*It is my great pleasure to thank **all members** of Pharmacology and Toxicology Department, Faculty of*

Pharmacy, Ain Shams University and every person in my faculty who supported me and helped me in my way.

*I wish to express my deep thanks to my **friends** who believe in me and keep encouraging me throughout the work.*

*Finally, but of great importance, I wish to express my deep gratefulness to the soul of my **mother** and **father** for their all sacrifices and patience along the way to help me, my beloved **son; Malek** who has been a great source of sacrifices and inspiration, my **husband** and my **brothers** who believe in me and my ability to achieve my ambitions.*

Yasmen Mahran

Radiotherapy has a profound impact on ovarian function leading to severe depletion of the primordial follicle reserve **(Gosden et al., 1997)**, premature amenorrhea and loss of fertility, during or shortly after completion of irradiation **(Stroud et al., 2009).** Since none of the currently available methods for ovarian protection and fertility preservation is ideal and guarantees future fertility **(Gurgan et al., 2008).** Thus, the need for more effective strategy to protect the ovary from the cytotoxic radiotherapy is highly intensified, not only to maintain the oocyte quality but also to preserve the hormone production which supports fertility.

Tamoxifen is usually employed in the chemoprevention and palliative treatment of breast and other cancers **(Vogel, 2011).** Clinically, TAM has also been used to stimulate the ovarian function in subfertile women with success rates similar to that of the standard therapy (clomiphene) **(Steiner et al., 2005; Kafy & Tulandi, 2007).** In addition, **Ting and Petroff (2010)** have recently shown that TAM decreases ovarian follicular loss in chemotherapy- treated female rats; however, the cellular mechanisms have not been investigated. Furthermore, the role of IGF-1 and its receptor (IGF-1R) in follicular development and ovulation are well documented **(Zhou et al., 1997).** Studies have documented that TAM suppresses the hepatic IGF-1 secretion **(Pollak et al., 1992)** and induces endometrial IGF-1 expression through its estrogenic effect **(Roy et al., 2000)**.

Besides, GH is an anabolic hormone with pleiotropic effects on growth and differentiation of cells. Growth hormone action on the ovary is

mediated through increasing the levels of serum and ovarian IGF-1, and by direct, GHR–mediated effects **(Herrington & Carter-Su, 2001).** Clinically**,** women with hypogonadotropic hypogonadism, achieved more oocytes and higher fertilization rate when co-stimulated with GH **(Homburg & Ostergard, 1995; Kucuk et al., 2008)**. Moreover, it was reported that hGH treatment exerted a radioprotective effect via a DNA repairing mechanism **(Gómez-de-Segura et al., 1998; Madrid et al., 2002)**. Accordingly, GH may be promising as a protective agent against radiotherapy-induced gonado-toxicity through its anti-oxidant mechanisms.

Actually, no data has been reported on the radioprotective role of either GH or TAM in ovarian damage induced by γ-irradiation *in vivo* and the exact mechanisms are not defined. Therefore, the present study was designed to explore the modulatory effects of either GH or TAM on γ-radiation induced ovarian dysfunction *in vivo* as well as the possible underlying mechanisms, particularly, its impact on the ovarian reserve, oxidative stress, proliferation, apoptosis and finally the impact of IGF-1/IGF-1R axis.

Examination Board Approval Sheet

YASMEN FOUAD KHALIFA MAHRAN

Study of different modalities to prevent gamma-radiation induced ovarian failure in rats

Submitted to the Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University

Approved by the committee in charge:

1- Dr. Azza Mounir Agha

Professor of Pharmacology and Toxicology and the Dean of Faculty of Pharmacy, Cairo University.

2- Dr. Azza Abd El-Fattah Ali

Professor & Head of Pharmacology and Toxicology department,

Faculty of Pharmacy, Al-Azhar University (Girls).

3- Dr. Ebtehal El-Demerdash Zaki

Professor & Head of Pharmacology and Toxicology department,

Faculty of Pharmacy, Ain Shams University.

4- Dr. Azza Sayed Awad

Professor of Pharmacology and Toxicology, Faculty of Pharmacy,

Al-Azhar University (Girls).

5- Dr. Ahmed Shafik Nada

Professor of Physiology, National Center for Radiation Research and Technology.

Date: 17 / 9 / 2013

1. Ovarian failure

1.1 Ovarian physiology:

Understanding the ovarian dysfunction requires a good understanding of ovarian physiology. The ovarian reserve of follicles is established during fetal life and gradually depleted during follicular development by apoptosis. As a general, apoptosis is an essential component of ovarian function and development **(Hussein, 2005)**. During adult life, a number of primordial follicles start growing during each menstrual cycle. Usually only one follicle will ovulate and the fate of the rest of the follicles is atresia through apoptotic cell death. Ultimately, only 450 follicles will ovulate during a woman's reproductive life. Between puberty and menopause, 250,000 follicles are destined for atresia **(Morita and Tilly, 1999; Vaskivuo and Tapanainen, 2003).**

After ovulation, the dominant follicle, a leading follicle dominates by secreting more estrogen and inhibin to suppress follicle stimulating hormone (FSH) release and then forms the corpus luteum. This action negatively selects the remaining follicles in the cohort, leading to their ultimate loss (negative selection). At the same time, the increased production of local growth factors allows positive selection of the dominant follicle and eventual ovulation (positive selection) **(Mc Gee and Hsueh, 2000)**. The corpus luteum is responsible for the production of progesterone and maintenance of the endometrium during early pregnancy. Also, apoptosis is responsible for corpus luteum regression; luteolysis **(Mc Cracken et al., 1999).**

Follicular atresia is an essential phenomenon in ovarian physiology occurring at all periods of life **(Amsterdam & Selvaraj, 1997)** and can be activated either by intrinsic or extrinsic extracellular pathways **(Quirk et al., 2004).** Although the exact signals, receptors and intracellular signalling pathways leading to apoptosis within granulosa cells are unclear, it is likely that: multiple molecules are involved such as Fas, caspases, tumor necrosis factor (TNF), p53, c-Myc, and endothelins. These molecules include both survival (such as gonadotrophins, IGF-1, interleukin-1b, epidermal growth factor, basic fibroblast growth factor, TGF-a, bcl-2 & bcl-xl) as well as atretogenic factors (TGF-b, interleukin-6, androgens, bax, Fas, p53, TNF and caspases) **(Driancourt et al., 1998; Tilly, 2001; Jiang et al., 2003).** Notably, Gonadotropins and several growth factors are the primary endocrine factors regulating follicular atresia **(Tilly et al., 1992; Chun et al., 1994)**. In ovarian failure, ovarian follicles did not respond to high level of FSH and did not secrete estradiol (E2) and consequently, ovarian follicles are drived to apoptosis **(Yacobi et al., 2004).**

1.2 Premature ovarian failure (POF):

Premature ovarian failure is a heterogenous disorder of multifactorial origin refers to development of amenorrhoea due to cessation of ovarian function before the age of 40 **(Coulam et al., 1986; Goswami & Conway, 2005).** It is characterized by hypoestrogenism and elevated gonadotropin levels. Some other terms have been used such as, premature menopause, premature ovarian dysfunction and primary ovarian insufficiency