HISTOLOGICAL STUDY of THE MAMMARY GLAND in FEMALE OOPHORECTOMIZED ALBINO RATS RECEIVING ESTROGEN HORMONE VERSUS ISOFLAVONE with THE USE of TAMOXIFEN as an ANTIDOTE

Thesis

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Contents

List of abbreviations

AD	Alzheimer's Disease
AR	Androgen Receptor
Bad	Bcl-2-associated death promoter
Bak	Brassinosteroid- associated receptor kinase
Bax	Bcl-2-associated X protein
Bcl-2	B-cell leukemia/lymphoma 2
Bok	Bcl-2 related ovarian killer
CNS	Central Nervous System
DNA	Deoxyribonucleic acid
E2	Estradiol
E	Estrogen
ECM	Extracellular Matrix
Ed	Embryonic Day
EGF	Epidermal Growth Factor
EPT	Estro- Progestive Therapy
ER	Estrogen Receptor
ER+	Estrogen Receptor positive
ER^-	Estrogen Receptor-negative
ERα	Estrogen Receptor alpha

ERA	Estrogen Replacement and Atherosclerosis Trial
ERβ	Estrogen Receptor beta
FDA	Food and Drug Administration
FSH	Follicular Stimulating Hormone
GH	Growth Hormone
HDL	High Density Lipoprotein
HERS	Heart and Estrogen/Progestin Replacement Study
HRT	Human Replacement Therapy
HT	Hormonal Therapy
IGF-1	Insulin-like Growth Factor-1
IL	Interleukin
IU	International Unit
LDL	Low density lipoproteins
LH	Luteinizing Hormone
MMP	Matrix Metalloproteinase
mRNA	Messenger Ribonucleic Acid
P4	Progesterone
PR	Progesterone Receptor
PRL	Prolactin
PTEN	Phosphatase and Tensin homolog deleted in chromosome Ten

RXR α	Retinoid X receptor alpha
SERM	Selective Estrogen Receptor Modulator
TDLU	Terminal Duct Lobular Unit
TDUs	Terminal Ductal units
TEB_s	Terminal End Buds
TGF-β	Transforming growth factor beta
TK	Tyrosine Kinase
TNFα	Tumor Necrosis Factor Alpha
WHI	Women's Health Initiative
WHIMS	Women's Health Initiative Memory Study

Introduction

The mammary gland is a dynamic organ, the structure of which changes throughout the female reproductive life. Two cellular compartments constitute the gland: the epithelium and the surrounding stroma ***(Daniel and Silberstein, 1987)***.

Histologically, the rat mammary stroma is different from the human stroma. The rat mammary gland contains a large amount of fat with small amounts of interspersed fibrous connective tissue ***(Topper and Freeman, 1980).***

It has been well established that almost all aspects of mammary biology are under hormonal regulation. Among the various hormones and growth factors implicated, the minimal requirements for mammary epithelial cell proliferation are Estrogen, Progesterone, Prolactin and Growth Hormone ***(Parmar and Cunha, 2004).***

Administration of Estradiol (E2) to oophorectomized adult rats leads to an increase in DNA synthesis in the mammary epithelial cells. The site of the mitotic activity resides almost exclusively in the terminal end buds that result in ductal growth. The biological effects of Estrogens are

mediated through Estrogen Receptors (ER), and the detection of ER in both ductal epithelial cells and stromal cells provides additional support for the action of Estrogen on mammary gland development ***(Parmar and Cunha, 2004).***

In order to reduce menopause symptoms, Hormone Therapy (HT) has been used, predominantly represented by Estrogens alone or in combination with Progesterone. However, HT also has negative aspects, such as increased relative risk of hormone-dependent cancers for example endometrial and breast cancer, as well as the increased risk of thromboembolism ***(Manson et al., 2013).***

Due to complications of Replacement Therapy, some authors are investigating new drugs that can offer benefits similar to Estrogen during menopause, including isoflavones, which are a group of phytoestrogens whose molecular structure has biochemical similarity with the 17 β- estradiol. These isoflavones may also bind to Estrogen receptors. Functionally, they may have estrogenic or anti-estrogenic activity depending on the target organs involved ***(Somjen et al., 2003).***

Introduction

Estrogen therapy is not only directed at the early symptoms (hot flushes) but also aims to arrest certain well-known menopausal conditions such as osteoporosis and skin atrophy ***(Chedraui et al., 2008).***The effects of Estrogen are particularly obvious in prevention of skin aging and promoting wound healing ***(Emmerson and Hardman, 2011).***

Relatively long-term, systemic treatment with isoflavone showed comparable efficacy to Estrogen in reversing some molecular, histological and functional changes of the skin associated with oophorectomy in aged rats. It might be an effective alternative therapy for the management of age-related skin changes in postmenopausal women ***(Messina and Wood, 2008).***

Regarding Tamoxifen; it is a selective estrogen receptor modulator. It is a mixed Estrogen agonist/antagonist that has been shown to prevent osteoporosis and breast cancer in women ***(Kim et al, 2002).***The drug is also administered as a prophylaxis to individuals who are at high risk for developing cancer breast***(Morrow and Jordan, 2000).***

Side effects associated with Tamoxifen therapy include menopause-like symptoms such as hot flushes, joint pain, sleep

disorders and depression, which may be reduced by the use of hormone replacement therapy (HRT) ***(Chiechi, 2003)*** which, at the same time, is associated with an increased risk for mammary carcinogenesis.

Aim of the Work

The aim of this work is to evaluate the histological changes of the mammary gland and the overlying skin in oophorectomized rats, to compare between the effects of hormonal (estradiol) and non-hormonal (soy-derived isoflavones) replacement therapy and to examine the outcome of Tamoxifen combination with both regimens.

Aim of the work

Mammary gland

The mammary gland is the only organ that undergoes most of its development postnatally. During embryogenesis, a rudimentary ductal system develops that grows isometrically with the rest of the body during the first weeks of life. At the onset of puberty, the ducts extend from the nipple area into a pad of fatty connective tissue that lies under the skin. The tips of the ducts enlarge to form club-shaped structures called terminal end buds (TEBs), which contain highly proliferative cells ***(Daniel and Silberstein, 1987).***

Once the ducts have penetrated the fat pad through dichotomous branching, the complexity of the milk duct system increases with repeated estrous cycles through the growth of side branches. Ductal side branching becomes more extensive during pregnancy. Subsequently, alveoli bud off the ducts and differentiate to become sites of milk production ***(Brisken, 2002).***

Development of the mammary gland in human

The epithelial/mesenchymal interactions that will give rise to the glandular tissue of the mammary gland, in both

sexes, can first be seen at about the fifth or sixth week, when two ventral bands of thickened ectoderm, the mammary ridges or milk lines, extend from the axilla to the inguinal region. Usually, invagination of the thoracic mammary bud occurs by day 49, and the remaining mammary line involutes ***(Howard and Gusterson, 2000).***

The thoracic ectodermal ingrowths branch into 15–20 solid buds of ectoderm which will become the lactiferous ducts and their associated lobes of alveoli in the fully formed gland. They are surrounded by somatopleuric mesenchyme which forms the connective tissue, fat and vasculature which is invaded by the mammary nerves ***(Sakakura et al., 1982).***

Continued cell proliferation, elongation and further branching produce the alveoli and define the duct system. Nipple formation begins at day 56, primitive ducts (mammary sprouts) develop at day 84 and canalization occurs at about the 150th day. During the last 2 months of gestation the ducts become canalized; the epidermis at the point of original development of the gland forms a small mammary pit, into which the lactiferous tubules open ***(Ellis et al., 1993).***

Development of the mammary gland in rats

A- Beginning

In rats, mammary gland development begins at embryonic day (Ed) 10 and proceeds from placode to bud stage by Ed14 similarly in both sexes. However, as a result of androgen receptor (AR) activation in mesenchymal cells of male embryos at Ed14, the connection of the bud to the surface epidermis is lost. Consequently, male rats have either no ductal system or only a very rudimentary system, and no nipples ***(Cowin and Wysolmerski, 2010).***

B- Specification

Specification of the secondary mammary mesenchyme also begins at around Ed14 and gives rise to the fat pad, which supports the development of mammary glands throughout postnatal life ***(Sakakura et al., 1982).*** At Ed16, the distal tip of the bud invades the fat pad precursor tissue, and the first bifurcation of the primary sprout is detected at late Ed16 or early Ed17 ***(Cowin and Wysolmerski, 2010).***

By birth, a ductal tree with 10–15 branches has formed. Postnatal growth of the mammary gland is proportional to the