Alexandria Journal of Medicine (2016) **xxx**, xxx–xxx

HOSTED BY

ELSEVIER

Alexandria University Faculty of Medicine

Alexandria Journal of Medicine

http://www.elsevier.com/locate/ajme

ORIGINAL ARTICLE

New treatment paradigm of combined raloxifene and conjugated estrogen for postmenopausal symptoms in VCD-induced menopausal rats

Marwa N. Emam *, **Rehab E. Abo El Gheit**

Physiology Department, Faculty of Medicine, Tanta University, Egypt

Received 20 February 2016; revised 25 May 2016; accepted 3 June 2016

KEYWORDS

Raloxifene;
Estrogen;
Osteoporosis;
Vulvar/vaginal atrophy

Abstract *Introduction:* The decreased ovarian estrogen production that occurs at menopause, results in osteoporosis and climacteric manifestations, and decreases women's quality of life. The hormone replacement therapy (HRT) is the primary treatment options but has been associated with increased oncogenic potential. The tissue selective estrogen complex (TSEC) is a novel therapy, partnering a selective estrogen receptor modulator (SERM) with one or more estrogens.

Aim: Our study was done to evaluate the potential relative estrogenic agonist activities of a SERM, raloxifene (RLX), when dosed alone and its antagonist activities when paired with conjugated estrogen (CE), as a TSEC and its potential use for the postmenopausal osteoporosis, vulvar/vaginal atrophy (VVA) in VCD induced menopausal rat model.

Material and methods: Female rats were dosed daily with 4-vinylcyclohexene diepoxide (VCD) (80 mg/kg/d, IP) for 15 days to induce ovarian failure, followed by one month free drug. VCD injected rats received 12 weeks of RLX, CE, or combined RLX/CE with 17β-estradiol (E2), vehicle treated groups used as positive and negative controls, respectively. The bone turnover markers (BTM) were measured. The uterotropic activity was assessed by the uterine index and peroxidase assay. Vaginal wet weight (wt.) and glycogen were measured to evaluate the vaginotropic effects. Uterine and vaginal (ER) protein levels were assayed.

Results: Our findings showed that the appropriate RLX/CE dose combination exhibits significant bone sparing with minimal vaginal stimulation and neutral uterine effect.

Conclusion: We can conclude that appropriate RLX/CE combination could effectively be a promising alternative for the prevention of postmenopausal osteoporosis and VVA with no oncogenic risk.

1. Introduction

The menopausal transition is characterized by gradual ovarian follicle depletion, with dramatic decrease in the

* Corresponding author. Tel.: +20 1224089068; fax: +20 1276640441.
E-mail addresses: maroo_drmaroo@yahoo.com (M.N. Emam), extrasystole.2003@gmail.com (R.E. Abo El Gheit).

Peer review under responsibility of Alexandria University Faculty of Medicine.

http://dx.doi.org/10.1016/j.ajme.2016.06.002

Please cite this article in press as: Emam MN, Abo El Gheit RE New treatment paradigm of combined raloxifene and conjugated estrogen for postmenopausal symptoms in VCD-induced menopausal rats, *Alex J Med* (2016), http://dx.doi.org/10.1016/j.ajme.2016.06.002

ovarian-derived estradiol, results in multiple undesirable side effects including vasomotor instability, VVA, mood changes and a substantial increase in bone turnover, eventually osteoporosis and bone fractures with increased risk of cardiovascular and cerebrovascular complications.[1]

Under menopausal hypoestrogenism, the tissues of the vulva and the lining of the vagina become thinner, drier, and less elastic, a condition known as VVA. Unlike vasomotor symptoms, VVA typically worsen without treatment and can significantly impact the quality of life.[2]

Unlike the ovariectomy (OVX) model, which parallels with surgically-induced menopause in humans, VCD induced ovarian failure rat model provides a more physiologically relevant model, that resembles the natural and transitional menopause, in which there is a gradual rather than abrupt decline of sex hormones.[3]

Although hormone replacement therapy (HRT) with exogenous estrogen may effectively offset the menopausal symptoms, some women are susceptible to hormone-dependent neoplasias.[4]

CE is the most widely used estrogen for HT, comprised of estrone sulfate and at least ten other hormones. Estradiol is the most potent and natural human form of estrogen in premenopausal women, comprised of 17β-estradiol. The estrone (ER) binding potency is approximately $\frac{2}{3}$ the affinity of estradiol to the ERα and about $\frac{1}{3}$ the potency of estradiol for ERβ. Additionally, estrone and estradiol likely have differential potencies for membrane mediated actions.[4]

Continued efforts to provide women with efficacious menopausal therapies have generated interest in the development of SERMs. Similar to estrogens, SERMs have been shown to bind to ERs with high affinity, despite lacking the estrogen steroid moiety and to regulate transcriptional events in a variety of target tissues.[5]

Whereas estrogens typically exhibit ER agonist effects in all tissues, SERMs demonstrate mixed functional activity (ER agonist/antagonist) depending on the target tissue. Unfortunately, they do not provide relief from climacteric symptoms.[5]

RLX is a second generation SERM. It is the only SERM actually approved internationally by the Food and Drug Administration (FDA) for the prevention and treatment of postmenopausal osteoporosis, with added benefit of preventing breast cancer.[6]

Currently RLX is undergoing clinical investigation for the management of postmenopausal conditions associated with estrogen deficiency, as the most attractive SERMs with acceptable safety and tolerability.[7]

To date, no SERM alone has been able to achieve an ideal balance of ER agonist and antagonist activity for an optimal menopausal therapy that would act as an ER agonist by preserving the positive estrogenic effects on bone, CNS and lipid metabolism, while acting as an ER antagonist by maintaining breast and endometrial safety. However, it may be possible to achieve optimal results based on the blended tissue-selective activities of a SERM and estrogens in a novel approach termed (TSEC).[8]

The goal of the present study was to investigate the effects of combined RLX/CE, as TSEC, on the bone and reproductive organs in VCD-treated rats, after selection of the appropriate dose combination depending upon uterine weight.

2. Material and methods

Three-months-old albino female rats (weight: 250 ± 10 g) were purchased from the Faculty of Science, Tanta University. The rats were acclimatized to the controlled laboratory conditions for one week and appropriately housed, four per cage, at a constant temperature of 22 ± 2 °C and 12 h artificial light/-dark cycle. All rats had free access to food pellets and water throughout the study period. All the animal experiments were conducted according to the guidelines established by the Research Advisory Ethical Committee of Faculty of Medicine, Tanta University, Egypt.

All experimental animals were injected with VCD, except control normal group. All treatments (obtained from Sigma, St Louis, MO, USA) were started, one month after induction of ovarian failure, continued for 12 week and were administered orally (by gavage) in a vehicle of 2%Tween 80/0.5% methylcellulose with the exception of E2, which was delivered I.P., dissolved in distilled water with 1% dimethyl sulfoxide (DMSO) and 0.1% Tween 20.[1]

2.1. Chemical induction of ovarian failure

After acclimation, the occupational chemical 4-vinylcyclohexene diepoxide (VCD) (96% purity, Sigma–Aldrich, St Louis, MO) was administered at a dose of (80 mg/kg/day, IP; 5 times per week; for 15 days) dissolved in sesame oil (2.5 mL/kg), followed by a drug-free period of 30 days. The control group dosed with sesame oil (2.5 mL/kg). Body weights were checked twice weekly throughout the period of induction to adjust the VCD dose. VCD specifically causes gradual apoptotic cell death of primordial and primary follicles resulting in induced ovarian failure without evidence of systemic toxicity, resulting in an endocrine state that closely mimics the natural modeling the transition to menopause in women.[9]

2.2. Vaginal smear

After the injection period was completed, the estrous cycles were monitored daily by obtaining vaginal smears and evaluating samples microscopically using a standard light microscope at 100× magnitude to ensure loss of cycling and validating ovarian failure. The rats are considered acyclic with persistent diestrus phase, which is dominated by leukocytes, while control animals showed regular estrous cycles.[9]

2.3. Experiment I: determination of minimally efficacious CE dose

An initial study was conducted on, *80 female albino rats*, to determine the appropriate CE dose to elicit an increase in uterine wet wt., a surrogate measure for an estrogenic stimulatory response. The VCD treated rats were randomly assigned into seven groups, 10 rats each, dosed with vehicle, 0.05, 0.5, 2.5, 5 or 10 mg/kg/d of CE and E2 (5 μg/kg/d) was included as a positive control, for 2 weeks. The minimal officious CE dose would then be used in the second experiment.

Please cite this article in press as: Emam MN, Abo El Gheit RE New treatment paradigm of combined raloxifene and conjugated estrogen for postmenopausal symptoms in VCD-induced menopausal rats, *Alex J Med* (2016), http://dx.doi.org/10.1016/j.ajme.2016.06.002