Reaction and the Effectiveness of Injectable Oestrogen Treatment, Initiated with Estradiol, Estradurin, Sintofolin

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The purpose of this study has been to identify the reaction occurring at the level of the vulvovaginal epithelium in ovariectomised female rats, after the administration of injectable oestrogens. We have studied 30 female Wistar white rats, with control group. 15 days after surgery, the hormonal substitution therapy with injectable oestrogens was initiated (Estradiol, Estradurin, Sintofolin), with a dosage of 0.2 mg/rat/day, and after 14 days of treatment, all animals were sacrificed and biopsies at vaginal and vulvar levels were taken from all groups, the samples being then subjected to a histopathological examination. Our study has shown the prompt reaction and increased receptivity of the vulvovaginal epithelium to the administration of oestrogens, after bilateral ovariectomy. Moreover, we have demonstrated that Estradiol treatment yields the promptest response, compared to synthetic oestrogen therapy, such as Estradurin or Sintofolin.

Keywords: menopause, oestrogenic receptors, reaction mechanism, atrophy, vulvovaginal hypertrophic, chemical structure

The lack of oestrogen production that characterizes menopause, which may occur progressively in natural menopause, or suddenly in surgically-induced menopause by bilateral ovariectomy, is often associated with disequilibrium at the level of various organs, influencing quality of life [1,2]. Among them, vulvar-vaginal atrophy is highly prevalent and, contrary to others such as vasomotor symptoms, it is not resolved spontaneously in time [3,4].

The female sex hormone estradiol is the clinical gold standard for the treatment of postmenopausal women suffering from many symptoms, especially vulvovaginal dryness [5,6].

Over the last years, there has been some controversy over hormone replacement therapy, pertaining to various side effects or risks, such as breast cancer, endometrial cancer, ovarian cancer, cerebrovascular accident, or thromboembolic disease [7,8].

Estradiol-treated postmenopausal women still having a uterus require the addition of a progestin to inhibit uterine epithelial cell proliferation and endometrial carcinoma [9]. Compared with estradiol-only therapy, combined oestrogen-progestin therapy enhances mammary epithelial cell proliferation [10]. The Women's Health Initiative study using conjugated equine oestrogens plus medroxyprogesterone acetate reported an enhanced breast cancer risk of the combined hormone therapy arm in comparison with the conjugated equine oestrogens-only arm [11].

Given the role of oestrogens in preventing, causing and exacerbating disease, a good knowledge of how oestrogens are synthesised and metabolised may help in the understanding and treatment of disease [12].

One possible strategy for the development of oestrogen therapies with reduced side effects in postmenopausal women is to improve their tissue selectivity, a concept that has been successfully employed in the development of selective oestrogen receptors modulators. Tissue selectivity of oestrogens is influenced by the cells-specific expression of ER-α and ER-β [13]., as well as by the tissue-specific expression of their co-activators and corepressors [14]. Therefore, ER isotype-selective ligands or ER ligands stimulating specific coactivator interactions could be used to improve tissue selectivity [15].The availability of ligand and its tissue-specific metabolization is another important parameter.

The three most common oestrogens are estrone (E1), estradiol (E2) and estriol (E3). A fourth oestrogen is estetrol (E4). Estradiol is the most potent. Estrone and estradiol are synthesised by the aromatisation of androstenedione and testosterone, respectively. They can also be interconverted by the action of 17β-hydroxysteroid dehydrogenases (17β-HSDs) [16].

Many enzymes catalyse reactions that have an oestrogen as a substrate and/or a product. The reactions catalysed include aromatisation, oxidation, reduction, sulfonation, desulfonation, hydroxylation and methoxylation. The enzymes that catalyse these reactions must all recognise and bind oestrogen but, despite this, they have diverse structures [17]. Figure 1 shows the chemical structure of injectable substances used in this study.

Oestrogens exert their effects in several ways and the effects of estradiol are mediated by two oestrogen receptors, oestrogen receptor α (ER-α) and beta β (ER-β),

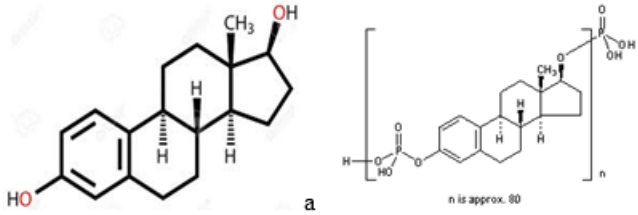

Fig. 1. Structure for: a) Estradiol - $C_{18}H_{24}O_2$; b) Estradurin (polyestradiolphosphate)- $C_{18}H_{24}O_2$; c) Sintofolin (Hexestrol diacetate)- $C_{22}H_{26}O_4$

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which belong to the superfamily of nuclear hormone receptors [1]. In the *classic genome response*, oestrogen binds to specific intracellular oestrogen receptors (ERá and ERβ) that, subsequent to oestrogen binding, dimerise and translocate to the nucleus, where they modulate the transcription of target genes that contain oestrogen-responsive elements in their promoters. However, these same oestrogen receptors have also been shown (a) to bind to other transcription factors, thus influencing the expression of genes that do not contain oestrogen-responsive elements in their promoters, and (b) to engage signal transduction pathways (that may include, but are not limited to, the activation of protein kinases), thus modulating cellular responses to oestrogen. Signal transduction pathways can also be activated by oestrogen binding to cell surface membrane bound receptors [19-2]. A decrease in hormonal activity can be experimentally created through bilateral ovariectomy in female rats [22, 23].

This experimental study aims to assess reaction and reactivity of the vulvovaginal epithelium in ovariectomised female rats, after the administration of injectable oestrogens.

Experimental part

This study has been conducted using a total of 30 female Wistar white rats, with an average weight of 200 g, obtained from the Bio-base of the *Iuliu Hatieganu* University of Medicine and Pharmacy of Cluj-Napoca. Throughout the experiment, the rats were given standard food and water ad libitum, thus maintaining the standard conditions required by the current legislation on the protection of laboratory animals.

The following study groups were created: Group 1 - control group (no surgical intervention, no oestrogenic treatment, premenopausal), comprising 5 subjects; Group 2 - operated, menopausal, with no treatment administered, comprising 5 subjects; Group 3 - operated, Estradiol treatment administered, i.e. a natural oestrogen, with a dosage of 0.2mg/day/rat, for a period of 14 days, comprising 6 subjects; Group 4 - operated, Estradurin treatment administered, i.e. a synthetic oestrogen (polyestradiol phosphate), with a dosage of 0.2 mg/rat, every 7 days, for a period of 14 days, comprising 7 subjects; Group 5 - operated, Sintofolin treatment administered, i.e. a synthetic oestrogen (hexestrol diacetate), with a dosage of 0.2mg/day/rat, for a period of 14 days, comprising 7 subjects.

Estradiol (Biofarm, Bucharest, Romania) was used for group 3, each 1 mL vial of injectable oily liquid containing 2.5 mg estradiol, which was diluted using 9 mL neutralised and sterilised sunflower oil, so that for 0.2 mg of estradiol/rat/day, an amount of 0.8 mL of oily injectable liquid was administered. Estradurin, (Pharmacia & Upjohn Company LLC - a subsidiary of Pfizer Inc., 7000 Portage Road Kalamazoo, MI 49001 United States), a synthetic oestrogen, was used for group 4, each 2 mL vial of injectable distilled water containing 80 mg of powder polyestradiol phosphate, diluted with 38 mL of distilled water, so that for 0.2 mg of estradiol/rat/day, an amount of 0.1 mL injectable liquid was administered. Since Estradurin is a powerful phosphatase inhibitor, and release is particularly slow, ensuring considerable oestrogenic activity for a prolonged period of time, even weeks following the administration of an injection, it was decided to administer it at 7-day intervals. Sintofolin (Terapia S.A, Cluj Napoca, Romania) a synthetic oestrogen, was used for group 5, each 2 mL vial of injectable oily liquid containing 5 mg of hexestrol diacetate, which was diluted with 8 mL of neutralised and sterilised sunflower oil, so that 0.4 mL of injectable oily liquid was administered for 0.2 mg/rat/day. Of the total number of 30 female rats included in the study, bilateral ovariectomy was performed on 25 of them. 15 days after surgery, menopause was considered to be installed. To confirm this, estradiol was determined 15 days after surgery, with the possibility of comparing estradiol hormonal levels pre and post-surgery.

The bilateral ovariectomy in female Wistar rats was conducted in accordance with the techniques described by Waynforth et al [24]. 15 days after surgery, with menopause confirmed in all groups, the administration of various injectable hormonal oestrogen treatments was initiated, over a 14-day period. The final stage of the study involved the scarification of all animals, 15 days after treatment, using the cervical dislocation method. Biopsies at the vaginal and vulvar levels were taken from all groups, and the samples were subjected to a histopathological examination, so as to obtain the necessary results and data for attaining the objective of this experimental study.

The aforementioned protocol utilised as part of this experimental study is based on approval no. 116/06.03.2015 of the Ethics Committee of the Iuliu Hatieganu University of Medicine and Pharmacy of Cluj-Napoca.

Statistical analysis

The first step in this analysis was a descriptive evaluation of the variables utilised, at both the parametric (through mean and standard deviation) and the graphic levels. Taking into account that all pertain to the quantitative area (scale), the level of normalcy in their distribution was assessed, in order to establish the type of tests that will be applied (parametric or non-parametric). The final decision was taken on the basis of the Shapiro-Wilk test.

The combination of Estradiol values pre and post-surgery was performed with the help of the Student (t) test, in its Paired Samples variant. The comparison of the thickness of the vagina and vulva, in the case of the 5 groups of rats included in the study, was performed based on the ANOVA method (analysis of variance in a simple format). The standard level of significance considered was 5%, but when the results proved to be significant at 1% level, this was clearly explained.

Results and discussions

Surgically-induced menopause was demonstrated by determining post-surgery estradiol, with the occurrence of statistically significant differences between the groups studied (fig.2).

There were statistically significant differences in thickness of the vaginal epithelium within the groups treated with injectable oestrogens, with a statistical index $p = 0.000 < 0.05$ (fig .3), therefore the manner of treating the rats has a significant influence on the thickness of the vagina.

There were statistically significant differences with respect to thickness of the vulva within the groups treated with injectable oestrogens, with a statistical index $p = 0.000 < 0.05$. (fig .4); therefore, the manner of treating the rats has a significant influence on the thickness of the vulva.

Moreover, differences were noticed with regard to the thickness of the vaginal and vulvar epithelium only in the groups that had been treated with injectable oestrogens, which implies that the manner in which the rats are treated significantly influences the thickness of the vaginal and vulvar epithelium (fig.5).

Atrophy at the level of the vulva and vagina was calculated on the basis of 3-point measurements, of morphometry at this level; hence, there is a real

morphological quantification, with statistically significant differences between the groups. Inducing menopause through bilateral ovariectomy in female rats and the subsequent use of hormone replacement therapy is a well-known experimental model [25, 26].

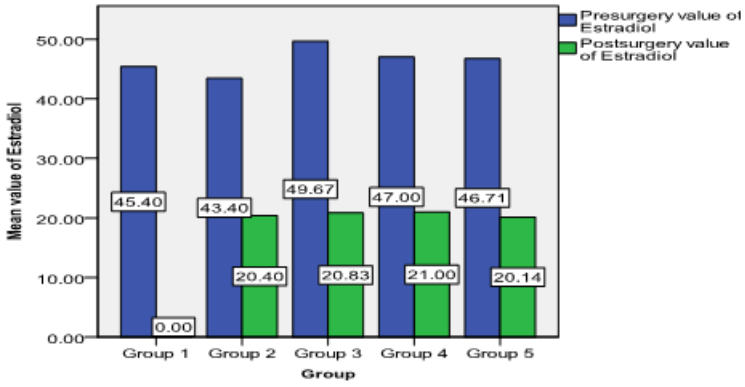

Fig.2. Comparing pre/post-surgery estradiol values

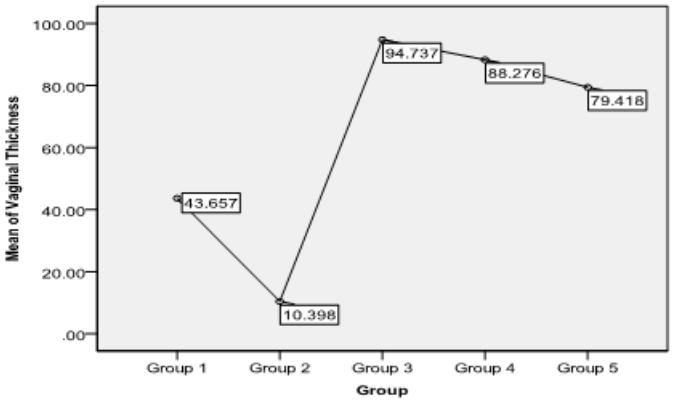

Fig.3. Differences in thickness of vaginal epithelium

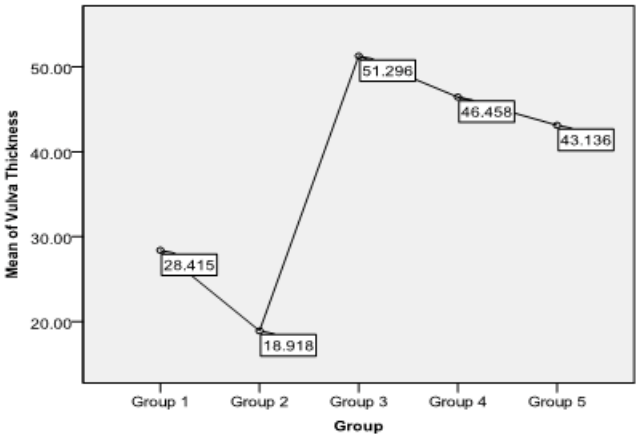

Fig .4. Differences in thickness of vulva

Due to the fact that at the level of the vulva and vagina, the number of oestrogenic receptors is particularly high, these tissues will have a rapid, prompt reaction and increased receptivity to the action of oestrogens, both natural and synthetic [27, 28].

In this study, with morphometry, we have proved in group 2 the presence of evident atrophy at the level of the vulvovaginal epithelium. Alternatively, in groups 3, 4 and 5, treated with injectable oestrogens (Estradiol, Estradurin, Sintofolin), we have encountered marked hypertrophy, with the increase in thickness of the vulvovaginal epithelium. Furthermore, there have been statistically significant differences among the groups treated with oestrogens with respect to the effectiveness of the oestrogen used and the prompt reaction of the epithelium to the action of the latter.

Based on our findings, we can state that the most powerful effects on the proliferation of the vaginal and vulvar epithelium have been encountered in the group treated with Estradiol, and to a lesser extent in the group treated with Sintofolin. Of the synthetic oestrogens, the most powerful effect was in the case of Estradurin therapy. This study has aimed to prove and indeed shown that injectable oestrogen treatment over a period of 14 consecutive days has triggered a prompt response of the vulvovaginal epithelium, marked by vulvovaginal hypertrophy. This is due to the large number of oestrogenic receptors at this level, thus causing a rapid reaction and increased receptivity of the epithelium to the action of oestrogens, results which are in keeping with those obtained by Kangas et al or Basha et al. [29, 30].

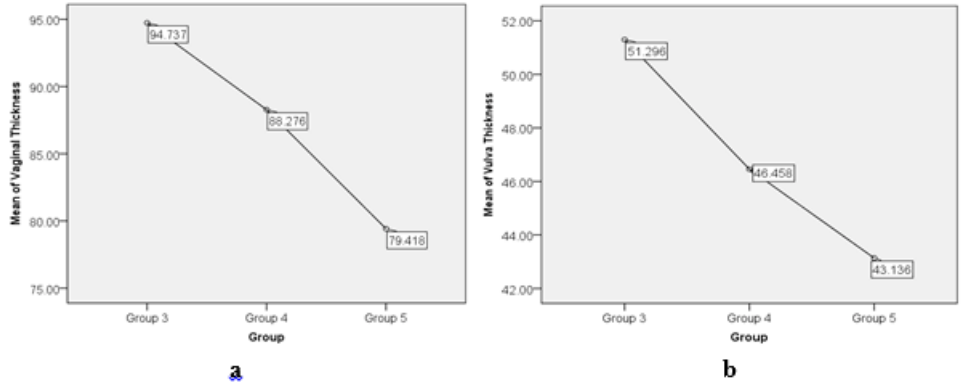

Fig.5. Differences in thickness of vaginal (a) and vulvar epithelium (b)

Conclusions

Our study shows that the reaction and receptivity of the vulvovaginal epithelium during menopause after the administration of injectable oestrogens, is prompt and swift, marked by the hypertrophy of the vulvovaginal epithelium. Moreover, we have demonstrated that Estradion therapy triggers the promptest response, compared to Estradurin or Sintofolin therapy.

All three types of oestrogens, injected over a 14-day period, have resulted in the regression of vulvovaginal atrophy, with the induction of hypertrophy at both the vaginal and the vulvar levels, due to the prompt reaction and increased receptivity of the vulvovaginal epithelium.

References

1. DIBONAVENTURA M., MOFFATT M., BUSHMAKIN A.G., KUMAR M., BOBULA J., United States and Western Europe., **24**, nr.**9**, 2015, p.713.
2. PALMA F., VOLPE A., VILLA P., CAGNACCI A., Maturitas., **83**, 2016, p.40.
3. NAPPI R.E., PALACIOS S., Climacteric.,**17**, nr.**1**, 2014, p.3.
4. PORTMAN D.J., Gass M.L., Maturitas., **79**, nr. **3**, 2014, p.349.
5. ABDI F., MOBEDI H., MOSAFFA N., DOLATIAN M., RAMEZANI T.F., Arch. Iran. Med., **19**, nr.**2**, 2016, p.141.
6. BARRETT-CONNOR E., STRYNKEL C., Int. J. Epidemiol., **30**, 2001, p.423.
7. PREDNA L., HABANOVA M., SLAVIKOVA E., WYKA J., Rocz. Panstw. Zakl. Hig., **66** nr.**3**, 2015, p.269.
8. MORCH L.S., KJAER S.K., KEIDING N., LOKKEGAARD E., Lidegaard O., Int. J. Cancer., **138**, nr.**6**, 2016, p.1506.
9. OTTO C., KANTNER I., NUBBEMEYER R., SCHKOLDOW J., FUCHS I., KRAHL E., VONK R., SCHULER C., FRITZEMEIER K.H., ERBEN R.G, Endocrinol., **153**, 2012, p.1725.
10. HOFSETH L.J., RAAFAT A.M., OSUCH J.R., PATHAK D.R., SLOMSKI C.A., HASLAM S.Z., J. Clin. Endocrinol. Metab., **84**, nr.**12**, 1999, p.4559.
11. ROSSOUW J.E., ANDERSON G.L., PRENTICE R.L., LACROIX A.Z., KOOPERBERG C., STEFANICK M.L., JACKSON R.D., BERESFORD S.A., HOWARD B.V., JOHNSON K.C., KOTCHEN J.M., Ockene J., JAMA., **288**, nr.**3**, 2002, p.321.
12. MARK P. T. , BARRY V. L. P., J. Steroid Biochem. Mol. Biol., **137**, 2013 p.27.
13. ENMARK E., GUSTAFSSON J.A., J. Intern. Med., **246**, nr.**2**, 1999, p.133.
14. SMITH C.L., O'MALLEY B.W., Endocr. Rev., **25**, nr.**1**, 2004, p.45.
15. HILLISCH A., PETERS O., KOSEMUND D., MÜLLER G., WALTER A., SCHNEIDER B., REDDERSEN G., ELGER W., FRITZEMEIER K.H., Mol. Endocrinol., **18**, nr.**7**, 2004, p.1599.
16. MILLER W.L., AUCHUS R.J., Endocrine. Research, **32**, 2011, p.81.
17. SAFE S., KIM K., J. Mol. Endocrinol., **41**, 2008, p.263.
18. SILBERSTEIN S. D., MERRIAM G. R., Cephalalgia, **20**, 2000, p.148.
19. MARINO M., GALLUZZO P., ASCENZI P., Current Genomics, **7**, 2006, p.497.
20. SAFE S., KIM K., J. Mol.Endocrinol., **41**, 2008, p.263.
21. MAGGIOLINI M., PICARD D., J. Endocrinol., **204**, 2010, p.105.
22. LÓPEZ-BELMONTE J., NIETRO C., ESTEVEZ J., DELGADO J. L., Moscosodel Prado J., Maturitas., **72**, 2012, p.353.
23. SANTOS M., FLORENCIO-SILVA R., TEIXEIRA C. P., DA SILVA SASSO R. G., SOUZA M. D., SIMOES S. R., SIMOES M. J., FERRAZ CARBONEL A., Climacteric., **19**, nr.**1**, 2016, p.77.
24. NEVALAINEN T., BERGE E., GALLIX P., JILGE B., MELLONI E., THOMANN P., WAYNFORTH B., VAN ZUTPHEN L. F., Lab. Anim., **33**, nr.**1**, 1999, p.1.
25. LAMAS A.Z., CALIMAN I.F., DALPIAZ P.L., DE MELO A. F. JR., ABREU G. R., LEMOS E. M., GOUVEA S. A., BISSOLI N. S., Life Sci., **124**, nr.**1**, 2015, p.101.
26. SACCO S. M., JIANG J. M., THOMPSON L. U., WARD W. E., J. Med. Food., 15, nr.9, 2012, p.846.
27. ACCONCIA F., KUMAR R., Cancer. Lett., **238**, nr.**1**, 2006, p.1.
28. van HAAFTEN M., DONKER G. H., SIE-GO D. M., HASPELS A. A., THIJSSEN J. H., Gynecol. Endocrinol., **11**, nr. **3**, 1997, p.175.
29. KANGAS L., HARKONEN P., VAANÄNEN K., KESKITALO J., EIGELIENE N., Horm. Metab. Res., **46**, nr. **5**, 2014, p.328.
30. BASHA M. E., CHANG S., BURROWS L. J., LASSMANN J., WEIN A. J., MORELAND R. S., CHACKO S. J., Sex Med., **10**, nr.**5**, 2013, p.1219

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