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The somatotropic axis in postmenopausal women during six month of transdermal continuous 17β-estradiol administration combined with oral medroxyprogesterone

Zmiany aktywności osi somatotropowej w trakcie sześciomiesięcznej przezskórnej podaży 17β-estradiolu wraz z doustną podażą medroksyprogesteronu u kobiet po menopauzie

Milewicz Tomasz[1], Krzysiek Józef[1], Rogatko Iwona[2], Sztefko Krystyna[2], Stochmal Ewa[3], Hubalewska-Dydejczyk Alicja[3], Jach Robert[4], Radowicki Stanisław[5]

[1] Klinika Endokrynologii Ginekologicznej Collegium Medicum Uniwersytetu Jagiellońskiego w Krakowie, Polska
[2] Zakład Biochemii Klinicznej Uniwersyteckiego Szpitala Dziecięcego w Krakowie, Polska
[3] Katedra i Klinika Endokrynologii Collegium Medicum Uniwersytetu Jagiellońskiego w Krakowie, Polska
[4] Katedra Ginekologii i Położnictwa Collegium Medicum Uniwersytetu Jagiellońskiego w Krakowie, Polska
[5] Klinika Endokrynologii Ginekologicznej Akademii Medycznej w Warszawie, Polska

Abstract

Aim: *The evaluation of the influence of continuous transdermal estradiol supplementation combined with oral medroxyprogesterone on the somatotropic axis in postmenopausal women.*

Material and methods: *25 women completed the study. Group A – 13 women received transdermal 17β-estradiol (Oesclim 50 - Fournier-Solvay) combined with oral 5 mg daily medroxyprogesterone (Gestomikron - Adamed). Group B - 12 women without treatment. Basic plasma FSH, estradiol, glucose, insulin, SHBG, hGH, total and free IGF-I, IGFBP-1 as well as IGFBP-3 were measured initially and at the 12th and 24th week of the study.*

Results: *The mean plasma FSH level was reduced and mean plasma estradiol level was increased in group A during estradiol supplementation. Mean plasma level of free IGF-I and free to total IGF-I ratio were increased in group A during 24 weeks of hormone therapy. In the control group (group B) there was the significant increase in mean plasma IGFBP-3 level. Other parameters showed no significant changes in the control group.*

Conclusion: *The administration of transdermal 17β-estradiol combined with oral medroxyprogesterone increases the IGF-I bioavailability. However this influence do not exceed the physiologial level of IGF-I bioavailability.*

Key words: **estradiol** / **medroxyprogesterone actate** / **hGH** / **IGF-I** / **IGFBP-3** / / **postmenopause** /

Adres do korespondencji:
Tomasz Milewicz
Klinika Endokrynologii Ginekologicznej, Collegium Medicum Uniwersytetu Jagiellońskiego,
ul. Kopernika 23, 31-501 Kraków, Polska
tel./fax: +48 12 424 85 60
e-mail: milewicz@interia.eu

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Streszczenie

Cel pracy: *Celem pracy była ocena wpływu łącznej podaży 17β-estradiolu przezskórnie wraz z doustną podażą octanu medroksyprogesteronu na stężenia hormonów osi somatotropowej u kobiet po menopauzie.*

Materiał i metody: *25 kobiet wyraziło zgodę i ukończyło badanie. Grupa A - 13 kobiet, które otrzymywały przezskórnie 17β-estradiol (Oesclim 50 - Fournier-Solvay) wraz z doustną podażą 5 mg octanu medroksyprogesteronu dziennie (Gestomikron - Adamed). Grupa B - 12 kobiet, które nie otrzymywały suplementacji hormonalnej. W chwili rozpoczęcia badania, 12 i 24 tygodniu jego trwania dokonano pomiarów w surowicy w warunkach podstawowych stężeń FSH, estradiolu, glukozy, insuliny, SHBG, hGH, całkowitego i wolnego IGF-I, IGFBP-1 oraz IGFBP-3.*

Wyniki: *W trakcie stosowania łącznej ciągłej podaży estradiolu i octanu medroksyprogesteronu w grupie A stwierdzono spadek stężenia FSH i wzrost stężenia estradiolu. Po 24 tygodniach suplementacji hormonalnej doszło do wzrostu stężenia w surowicy wolnego IGF-I oraz wskaźnika wolnego do całkowitego IGF-I. W grupie B (kontrolnej) obserwowano istotny statystycznie wzrost stężenia IGFBP-3. Stężenia pozostałych parametrów w tej grupie nie uległy istotnym statystycznie zmianom.*

Wniosek: *Zastosowanie łącznej podaży przezskórnej 17β-estradiolu wraz z doustną podażą octanu medroksyprogesteronu doprowadziło do wzrostu biodostępności IGF-I, jednakowoż wzrost ten nie przekroczył stężeń fizjologicznych*

Słowa kluczowe: **estradiol** / **octan medroksyprogesteronu** / **hGH** / **IGF-I** / **IGFBP-3** / / **postmenopauza** /

Introduction

Circulating insulin-like growth factor-I (IGF-I) is mainly produced by the liver under GH stimulation and is influenced by nutrition and insulin. IGF-I bioavailability is regulated by interactions with specific binding proteins (IGFBPs) [1]. The somatotropic axis is also affected by hormonal milieu [2, 3]. A number of experiments indicate that sex steroids exert either stimulatory or inhibitory action on the somatotropic axis [3, 4]. Evidence that estradiol is involved in the regulation of human Growth Hormone (hGH) - Insulin-like Growth Factor-I (IGF-I) - Insulin-like Growth Factor Binding Protein-3 (IGFBP-3) system is provided not only by the observation that mean hGH level is higher in women than men and that the fall in hGH as well as IGF-I levels with aging is correlated with estradiol level but also by the fact that estradiol has a direct effect on IGF-I synthesis and release independent of hGH. Estradiol plays also a permissive role in pituitary hGH release [5-7]. The latter role of estradiol is based in its influence on both pituitary increase in IGF-I mRNA as well as on regulation of pituitary somatostatin concentration [8,9]. Estrogens and IGF-I have synergistic effect on cell proliferation [10]. IGF-I augments the function of estrogen receptor [10]. Total IGF-I serum level correlated directly to free sex hormones and inversely to SHBG level in postmenopausal women [11]. Gunter et al conducted a case cohort study of relation between free IGF-I and endometrial cancer based on data from Women Health Initiative Study and found an inverse association between plasma level of free IGF-I and endometrial carcinoma [12]. In another study of the same group of WHI postmenopausal women they found a positive association between endogenous estradiol level, free IGF-I and risk of colorectal cancer in postmenopausal women [13].

IGF-I is also regarded as auto/paracrine mediator of estradiol action [14]. Estradiol exerts different effects on serum IGF-1 levels depending on its origin, route of administration and dose [2]. Different kinds of hormone therapy (HT) have different effect on the IGF-I system, depending on route of administration, oestrogen dose, basal IGF-I values and type of progestin [1]. Oral estrogens due to their hepatocellular effect decrease the liver IGF-I synthesis and therefore reduces its circulating level [10]. The reduced level increases mean diurnal serum GH level through reduced feedback inhibition [15]. The progestins endowed with androgenic effects-the 19-nortestosterone derivatives and, to a lesser extent, medroxyprogesterone acetate (MPA)-tend to reverse the IGF-I decrease induced by oral oestrogens [1, 16]. There are conflicting reports on the effect of transdermal 17β-estradiol administration on the plasma level of total and free IGF-I. Cardim et al, Stomati et al and Bellantoni et al found no effect of transdermal estradiol therapy on the plasma levels of total and free IGF-I as well as IGFBP-1 [17-19]. Weissberger et al applied transdermal 0.1 mg/24 hours estradiol patches and restored estradiol plasma level to midfollicular one [15]. That resulted in increase of serum total IGF-I level. However, neither oral nor transdermal estrogen administration completely reverses the age related reductions in spontaneous or GHRH-stimulated GH and IGF-I secretion [19].

IGFBP-3 plasma level was higher in postmenopausal women compared to premenopausal ones. This lowers the bioavailability of IGF-I in older females [20]. Oral estrogen administration causes a two to three-fold increase in IGFBP-1 levels and reduction in IGFBP-3 levels. Androgenic progestins oppose the IGFBP-1 increase as well as IGFBP-3 decrease induced by oral oestrogens [1, 16, 17]. The transdermal estradiol administration reduced the plasma level of IGFBP-3 in postmenopausal women older than 62 years of age [19].

In circulation most of IGF-I binds to IGFBP-3, that inhibits the action of IGF-I. The remaining free IGF-I can cross the endothelial barrier and interact with receptors. That is why measuring free IGF-I is more useful than assessment of total IGF-I in evaluation of proliferative influence of IGF-I on neoplastic lesions [21]. Data on the effect of estrogen therapy (ET) and different progestins on the level of free IGF-I are scant and inconsistent [1].

Aim

Therefore it seems interesting to evaluate the influence of continuous transdermal estradiol supplementation combined with oral medroxyprogesterone on the somatotropic axis in postmenopausal women.

Material and methods

The study protocol was approved by the Jagiellonian University Ethical Board. Thirty four postmenopausal (having plasma FSH>30U/l and 17β-estradiol <50ng/l) were recruited for the study and gave the informed consent to participate. None of them had received any form of HT prior to the beginning of the study. Of 34 postmenopausal women assigned to both groups (A and the control group) the study was completed by 25 participants (mean age 51.4±6.5 years) (13 in group A and 12 in group B).The women with diabetes mellitus, thyroid diseases and adrenal glands' disorders as well as with contraindication to hormone replacement therapy were excluded by careful history taking and physical examination and evaluating the glucose fasting level, thyroid hormones, pap smear, mammography and plasma cortisol. Each woman become postmenopausal after 45 yeas of age. The women were randomly assigned to one of the study groups. Women of group A (N=13) were treated with transdermal supplementation of 17β-estradiol (Oesclim 50, Fournier-Solvay) at a dose of 0.05 mg/24 hours combined with oral medroxyprogesterone acetate at a daily dose of 5 mg (Gestomikron - Adamed) as a continuous therapy. Group B (N=12) consisted of women who did not receive HT treatment (control group). The patients were examined before the treatment and twice during the period of 24 weeks of HT in 12 weeks intervals. During each visit, special attention was paid to physical and psychological well-being of each patient, as well as to the characteristic menopausal symptoms. Clinical characteristics of the study groups is presented in table I.

The basal, fasting plasma total insulin-like growth factor-I (IGF-I), free IGF-I, insulin-like growth factor binding protein-1 and 3 (IGFBP-1, IGFBP-3), human growth hormone (hGH) glucose, insulin, estradiol, follicle stimulating hormone (FSH) and sex hormone binding globuline (SHBG) levels were measured before the treatment (examination I) and after 12 weeks (examination II) and 24 weeks (examination III) of HT. At the same time intervals, all the parameters were measured in the control group. All sample tubes were kept and transported in the ice box, than centrifuged as soon as possible at 4^0C (refrigerated centrifuge, Heraeus, Germany). Plasma was stored frozen at -70^0C until the determination of the hormones, not later then after 3 months of storage. Frozen samples were thawed slowly only once and incubated for 30 minutes at 4^0C prior the analysis. Plasma glucose level was determined on the day of blood sampling. Plasma glucose was measured by the enzymatic method (Vitros dry chemistry analyzer, Johnson and Johnson). Plasma hormone levels were measured by using commercial Abbot MEIA kits (sensitivity: 1ng/ml for estradiolu cross reactivity with LH, TSH, hCG 0,039%; sensitivity for FSH 0.5mIU/ml interference 5% with bilirubin and hemoglobin, cross reactivity with hCG 0.016%). IGF-I plasma level was measured by RIA Biosource Europe kits (sensitivity 0.25ng/ml; intersample and interkit variability was 4.1% and 9.3% resp.; cross reactivity with insulin, hGH and IGF-II 0.001%, 0.01% and 0.2% resp.).

Free IGF-I plasma levels were measured by means of DRG USA ELISA kits with sensitivity of 0.015ng/ml and no cross reactivity with free IGF-II, insulin, IGFBP-1, IGFBP-3. IGFBP-1 and IGFBP-3 plasma levels were measured by RIA kits (Diagnostic System Laboratories, USA). Sensitivity for both ones was 0.5ng/ml and specifity was 100% for IGFBP-1 and 99% for IGFBP-3.

Descriptive statistics, including mean values, median and SE, were calculated for all studied variables. The data obtained in two visits / groups were compared by means of Welch's t-test of equality of expected values and F-test of equality of variances. Significance level was set at $p<0.05$. Statistical calculations were performed using Statistica 3.1 software.

Results

At the beginning of the study there were no statistically significant differences between group A and B. (Table I).

Table I. The initial, clinical characteristics of patients with normal glucose metabolism who received transdermal 17β-estradiol supplementation combined with oral medroxyprogesterone (group A) and control group without treatment (group B).

Parametr	Group A n=13	Group B n=12	p
Age [lat]	51.4±7.8	47.1±7.8	NS
BMI	23.6±2.6	24.4±4.6	NS
WHR	0.78±0.05	0.8±0.05	NS
FSH [U/l]	111.8 ±38.9	98.0±31.3	NS
Estradiol [ng/l]	12.2±11.5	14.1±7.9	NS
SHBG [mg/l]	54.0±36.7	50.4±27.9	NS
Glucose fasting [mmol/l]	4.8±0.7	4.7±0.6	NS
Insulin fasting [mU/l]	5.7±2.3	6.7±2.0	NS

The administration of continuous transdermal 17β-estradiol combined with oral medroxyprogesterone reduced the mean FSH level and increased mean estradiol level already after 12 weeks. This change remained for the next 12 weeks. No such changes were seen in the control group. (Table II). Mean plasma level of free IGF-I increased after 12 weeks of HT. The increased level of free IGF-I remained for the next 12 weeks of treatment. This change increased also the free/total IGF-I ratio after 12 weeks of HT. The ratio dropped after next 12 weeks but still was higher than the initial value ($p<0.05$). The mean plasma levels of IGFBP-3, IGFBP-1 and total IGF-I as well as the IGFBP-3/IGF-I ratio were not influenced by the HT. However in the control group the increase in the mean plasma level of IGFBP-3 was found after 24 weeks of observation. (Table III).

Discussion

Our study showed that continuous transdermal administration of 17β-estradiol combined with oral medroxyprogesterone increased the free IGF-I plasma level and showed no influence on mean plasma levels of total IGF-I and IGFBP-1.

Table II. The influence of transdermal 17β-estradiol supplementation combined with oral medroxyprogesterone (group A) on parameters of clinical characteristics compared to the influence of 6 months' observation of the control group in patients with normal glucose metabolism.

		GROUP n = 13	A			GROUP B n = 12	(controls)	
Parametr	**Prior to HT**	**3rd month of HT**	**6th month of HT**	**p**	**Initial visit**	**3rd month**	**6th month**	**p**
BMI	23.6±2.6	24.7±3.6	25.2±3.5	NS	24.4±4.6	24.6±4.3	24.9±5.0	NS
WHR	0.78±0.05	0.79±0.05	0.80±0.05	NS	0.80±0.05	0.80±0.06	0.80±0.08	NS
FSH [U/l]	111.8±38.9	76.8±32.8	73.0±36.4	<0.05	98.0±31.3	83.6±20.9	101.2±51.3	NS
Estradiol [ng/l]	12.2±11.5	40.7 ± 34.9	52.2 ± 50.4	<0.05	14.1±7.9	18.8±8.1	18.5±7.7	NS
Fasting glucose [mmol/l]	4.8±0.7	4.7±0.7	5.0±0.6	NS	4.7±1.1	4.9±0.9	4.8±0.5	NS
Fasting insulin [mU/l]	5.7±2.3	6.2±2.2	9.1±5.4	NS	6.7±2.0	6.0±3.2	8.4±2.7	NS
SHBG [mg/l]	50.8±32.6	42.9±23.7	48.9±36.1	NS	50.4±27.9	82.8±80.4	65.7±65.4	NS

Table III. The influence of transdermal 17β-estradiol supplementation combined with oral medroxyprogesterone (group A) on parameters of hGH-IGF-I-IGFBP axis compared to the influence of 6 months' observation of the control group in patients with normal glucose metabolism.

		GROUP n = 13	A			GROUP B n = 12	(controls)	
Parametr	**Prior to HT**	**3rd month of HT**	**6th month of HT**	**p**	**Initial visit**	**3rd month**	**6th month**	**p**
hGH (mg/l)	3.1±2.3	2.8±2.4	2.1±1.3	NS	2.7±2.6	3.6±3.5	5.9±5.8	NS
IGF-I total (ug/l)	214.9±60.9	168.4±67.9	276.8±166.2	<0.05	203.1±41.9	222.4±59.4	232.6±80.7	NS
free IGF-I (ug/l)	2.2±1.6	5.3±2.2	6.1±2.4	<0.001	3.6±3.0	5.1±4.6	4.1±2.3	NS
IGFBP-3 (mg/l)	4.3±1.3	4.4±0.5	4.5±0.8	NS	3.8±0.5	4.5±0.4	4.9±1.0	<0.005
IGFBP-1 (ug/l)	36.6±15.5	29.6±17.2	37.9±21.0	NS	36.9±13.9	48.4±23.5	43.2±21.9	NS
free/total IGF-I [%]	1.0±1.0	3.6±1.8	2.0±1.0	<0.05	2.0±1.0	2.5±1.6	3.0±2.0	NS
IGFBP-3/IGF-I [%]	2.14±0.9	2.6±1.0	2.1±0.5	NS	1.8±0.6	2.2±0.7	2,6±1.5	NS

The hormone therapy prevented the increase in plasma level of IGFBP-3. However it should be mentioned that the limited number of patients included into the study reduces the strength of the conclusion. Stomati et al found no statistically significant change of plasma level of total IGF-I level during transdermal 17β-estradiol administration combined with oral medroxyprogesterone [18]. Stanosz et al in their recent study of 75 postmenopausal women with osteopenia found the increase in total IGF-I after 12 weeks of transdermal estradiol therapy combined with progesterone given to subgroup of 25 women [22]. Bellantoni et al apllied the transdermal estradiol supplementation with 100µg patches for 6 weeks and also found no influence on plasma IGF-I levels [19]. However the therapy reduced the IGFBP-3 plasma level [19]. Weissberger et al used the same therapy as Bellantoni group but observed the increase in mean total IGF-I level [15]. Similar results were obtained by Słowinska-Srzednicka et al [23]. Our results seem similar to those obtained by Cardim et al [17]. They found the increase in plasma level of free IGF-I and no influence on the plasma total IGF-I and IGFBP-1 levels during six cycles of transdermal estradiol administration with norethisterone acetate [17]. Helle et al compared plasma levels of IGF-I, free IGF-I, IGF binding proteins -1 and -3 in 114 postmenopausal and 39 premenopausal women and found lower levels of total and free IGF-I as well as higher levels of IGFBP-3 in postmenopausal women [20]. That finding may explain the increase in IGFBP-3 during the 24 weeks of observation in group B.

The hormone therapy applied in our study increased the free to total IGF-I ratio from 0.82%±0.41 to 2.0%±0.6. The ratio did not exceed that found by Kawai et al who reported the free/total IGF-I ratio raging from 0.95 to 2.02%[24]. However Skjaerbaek et al reported lower free/total IGF-I ratio (0.66%±0.05%) than that found by Kawai et al [25].

Conclusion

The continuous transdermal 17β-estradiol administration combined with oral medroxyprogesterone increased the bioavailability of plasma IGF-I

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