The Effects of Postmenopausal Hormonal Therapy on Genetic Damage in Exfoliated Cells from the Uterine Cervix

POSTMENOPOZAL HORMONAL TERAPİNİN UTERİN SERVİKS YÜZEY HÜCRELERİNDE GENETİK HASAR ÜZERİNE ETKİLERİ

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Abstract

Objective: Although postmenopausal hormonal therapy (PHT) may offer considerable benefits for menopausal women, estrogen administration is accepted as a risk factor of human cancers. The aim of this study was to evaluate the possible genotoxic effects of long-term administration of postmenopausal hormonal therapy (PHT) drugs in postmenopausal women by used micronucleus (MN) analysis, a DNA mutation screening test, which is more rapid, economic and sensitive technique.

Material and Methods: A total of 40 postmenopausal women were included in the study: 20 were taking PHT, and a further 20 were nonuser as a control group. Participants in the study group have been using conjugated equine estrogen 0.625 mg/day+ medroxyprogestrone acetate 2.5 mg/ day for 6 months. All women had gynecological examination, at which time cervico-vaginal smears were obtained with an Ayre spatula. The cervical smear slides were scored blind and MN frequency was evaluated as number of micronuclei per 1000 binucleated cells.

Results: Although the evaluation of micronuclei number in cells showed genomic instability in somatic cells, in our study, analysis of the MN data revealed homogeneity among women within each of the two groups (P>0.05).

Conclusion: This may be interpreted as PHT does not cause any microarchitechtural change leading to a malignancy of the cervix. Future studies may validate this finding among larger groups of patients and also with other PHT agents.

Key Words: Hormone replacement therapy, micronucleus tests, mutation

Özet

Amaç: Postmenopozal hormonal terapinin (PHT) menopozlu kadınlar için oldukça faydalı olduğu düşünülmesine rağmen, östrojen alımının insan kanserlerinde bir risk faktörü olduğu kabul edilmektedir. Bu çalışmanın amacı, menopozal kadınlarda uzun süreli PHT ilaçlarının kullanılmasının olası genotoksik etkilerini, hızlı, ekonomik ve duyarlı bir DNA mutasyon tarama yöntemi olan mikronükleus (MN) testi ile araştırmaktır.

Gereç ve Yöntemler: Toplam 40 postmenapozal kadın çalışmaya dahil edildi (20 PHT alan ve 20 kontrol). Uygulama grubundakiler 6 ay süreyle konjuge equine estrojen 0.625 mg/gün+ medroksiprogestron asetat 2.5 mg/ gün kullandı. Tüm kadınlara jinekolojik muayene yapıldı, aynı zamanda Ayre spatula ile serviko-vajinal smearlar alındı. Servikal smear preparatları körleme skorlandı ve MN frekansı 1000 binüklear hücrede mikronükleus sayısı olarak değerlendirildi

Bulgular: Hücrelerde MN sayısının saptanması somatik hücrelerde genomik kararsızlığın göstergesi olsa da, bizim çalışmamızda MN verilerinin analizi her iki gruptaki kadınlar arasında benzer bulundu (P>0.05).

Sonuç: Bu durum, PHT'nin serviksin kanserleşmesine neden olabilecek herhangi bir mikroyapısal değişikliğe yol açmadığı şeklinde yorumlanabilir. Sonraki çalışmalar daha geniş hasta grubu ve diğer PHT ajanları kullanılarak bu verileri doğrulayabilir.

Anahtar Kelimeler: Postmenopozal hormonal terapi, mikronükleus testleri, mutasyon

Turkiye Klinikleri J Gynecol Obst 2007, 17:431-435

Geliş Tarihi/*Received:* 24.05.2007 **Kabul Tarihi/*Accepted:*** 04.10.2007

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Postmenopausal hormonal therapy (PHT) is synthetic estrogen and progesterone (progestin) designed to "replace" a woman's depleting hormone levels. Past research has shown that PHT may also help prevent osteoporosis, heart

diseases, short-term memory loss, depression and other diseases in post-menopausal women. Recent research has found that PHT may not provide these benefits and may pose other risks, including an increased risk of breast and ovarian cancer (with long-term use) and cancer of the uterine lining (in women do not take progestin with estrogen).[1-7]

Genetic mutations causing abnormal cell growth and differentiation are the basis for all cancer. The great majority of genetic defects that cause cancer are due to unknown causes. The hydroxylated estrogens are catechol estrogens that are easily auto-oxidated to semiquinones and, subsequently, quinones, both of which are electrophiles capable of covalently binding to nucleophilic groups on DNA and hence cause mutations.[8,9] A specific conversion of E_2 to the carcinogenic catechol metabolite 4-hydroxyestradiol by a specific cytochrome P450 has been detected in organs of rodents where estrogens induce tumors and in human breast and uterine tissue.[10]

However, there is no clear understanding of the mechanisms through which estrogens cause cancer. Recent studies showed that chromosomal aberrations positively correlate with cancer risk.[11] Structural or numerical chromosomal abnormalities can reliably be assessed by evaluating the frequency of micronuclei in dividing cells.[12-15] Micronucleus (MN) is formed during the metaphase/anaphase transition of mitosis. If the process is disrupted, or the chromosomes are broken or damaged by chemicals or radiation, then the distribution of genetic material between the two daughter nuclei during cell division may be affected and pieces or entire chromosomes may fail to be included in either of the two daughter nuclei. When this occurs, the genetic material that is not incorporated into a new nucleus may form its own "micronucleus" which is clearly visible with a microscope. Thus, in the MN test, animals are treated with a chemical and then the frequency of micronucleated cells is determined at some specified time after treatment. If a treated group of animals shows significantly higher frequencies of micronucleated cells than do the untreated control animals, then the chemical is considered to be capable of inducing structural and/or numerical chromosomal damage.[13]

The micronuclei in exfoliated cells of the cervix have been suggested to represent a marker of their malignant potential.[16,17] Estrogens like 17β-estradiol, estrone, estriol and ethynyl estradiol are effective in causing various types of chromosomal aberrations,[18] however, studies on the mutagenic effects of PHT drugs are limited. Therefore, in this study an attempt has been made to investigate the possible genotoxic effects that can arise from PHT usage in exfoliated cells from cervices of postmenopausal women by MN test. Results are compared with those seen in controls who received no PHT. As far as we know, this is the first study to examine the number of MN in cervical smear of women taking PHT.

Methods

Forty postmenopausal patients (46.2±0.7 years) were included the study. Women were randomized to active PHT (conjugated equine estrogen. 625 mg/day+medroxyprogestrone 2.5 mg/day) nonuser. Participants were using PHT at least for six month or more. Before Papanicolaou smear (PAP) collection, the individuals were asked to complete a questionnaire. The absence of the exposures that could influence cytogenetic parameters was verified. Potential exclusion criteria included work-related exposure to mutagenic agents, anticancer therapy, viral infections, use of a medical treatment for at least 3 months, and previous exposure to diagnostic X ray. The patients had no previous chemotherapy or radiotherapy. None of them had a clinical history of chronic infection, drug use (including contraceptives), cigarette smoking or radiation exposure. All women received a gynecological examination, at which time a PAP smear was obtained with an Ayre spatula.

Uterin cervical epithelium cells were smeared onto the slides and allowed to air-dry. Smears were fixed in 80% methanol. The fuelgen staining technique and fast green counter stain protocol was used. Stained preparations were analysed by an optical microscopy (10 X 100x). MN frequency

was evaluated by the same two microscopists, who did not know the patient identity. For each subject, 1000 cells were examined and evaluated on the basis of the following criteria: a typical shape and size of cervical cells; a well-defined nucleus and clearly defined cytoplasm. Cells containing small pyknotic nuclei or vacuolization of the cytoplasm were determined according to criteria establihed by Stich and Rosin Rosin and Gilbert.[19,20]

Statistical analysis: Data were analyzed using statistical software programme (SPSS for Windows). Data were compared with normal controls using independent samples t-test. Significance was set at the 95% confidence limit. Results were shown as mean±S.D.

Results

The average age was 43.04 and 45.51 years for the PHT patients and the controls, respectively. The mean frequencies of MN were found to be 4.8±2.9 in control group and 5.2±3.7 in PHT group (Table 1). Individual levels of MN for each patient and control group women are reported in Table 2. No statistically significant difference in MN frequency was observed between PHT group and nonuser groups (P<0.05).

Discussion

Although PHT may offer considerable benefits for menopausal women, estrogen administration is accepted as a risk factor of cancers. Estrogen also compromises the DNA repair system and enables accumulation of lesions in the genome essential to estrogen-induced tumorgenesis.[17,21] There are 3 mechanisms considered responsible for the carcinogenicity of estrogens: receptor-mediated hormonal activity, which stimulates cellular proliferation, resulting in more opportunities for accumulation of the genetic damage that leads to carcinogenesis; a cytochrome P450-mediated metabolic activation, which elicits direct genotoxic effects by increasing mutation rates; and the induction of aneuploidy by estrogen.[8-10,22]

Table 1. The frequency of MN in exfoliated cells from the uterine cervix at control and after 6 months of PHT (n=20).

	MN frequency	
	Mean ±SD	p
Control	4.8 ± 2.9	>0.05
PHT	5.2 ± 3.7	

Estrogens have been identified as mitotic poisons, which at high concentration, cause metaphase arrest, abnormal cell division and chromosome aberrations. Micronuclei are intracytoplasmatic inclusion bodies formed from chromatin fragments or whole chromosomes. Their presence in cells is a reflection of chromosomal aberrations during cellular mitosis. Scoring of micronuclei can be performed relatively easily and on different cell types relevant for human biomonitoring: lymphocytes, fibroblasts and exfoliated epithelial cells, without extra in vitro cultivation step. The analysis of sister chromatid exchange (SCE), which is another useful technique to assess the effect of chemical mutagens and carcinogens on chromosomes has been used to some studies. In these studies, a significant increase SCE frequency was reported in postmenapousal patients after 3 months[23] and 6-12 months[24] of PHT treatment.

Because of the increased risks for venous thrombosis and stroke clinicians have become more careful in their screening of patients and in prescribing PHT. Furthermore, its prevalence and its long-term use, concern about the relationship

Table 2. Individual values of MN frequency at control and PHT treatment group.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean±SD
Control	5	6	4	6	7	11	3	4	1	1	3	9	2	6	8	3	9	2	4	2	4.8±2.9
PHT	2	12	1	3	2	6	2	4	4	8	3	1	7	3	11	5	9	6	13	2	5.2±3.7

between PHT and breast cancer continues to be an issue in the minds of both patients and clinicians. More recent studies show that estrogens induce the formation of endogenous DNA adducts both in humans and animals, and suggest that these could ultimately lead to cancer development.[8-10,21]

Our study showed that oral PHT is not associated with an increased MN frequency after 6 months. When compared to control, in response to after PHT treatment values, MN frequencies increased within the 6 months, but these increase were not statistically significant. The non-significant effect of PHT in MN frequency could be attributed to the small sample size and short duration in this study. In addition the without a difference in the frequency of micronuclei observed at the later time may be due to repairs of damaged genetic material, elimination of cells/ chromosomes with damaged genetic material, or inactivation of drug or its metabolites.

To our knowledge, only a few studies investigated the potential genotoxicity of PHT in postmenopausal women: a significant increase in SCE frequency was reported in 17 patients after 3 months of PHT treatment,[23] in 19 postmenopausal women receiving PHT for 6–12 months.[24] A significant increase in the number of MN in cervical epithelial cells has been reported previously in patients with cancer of the uterine cervix,[16,22] however, the relationship between MN and PHT have not been established. In another study, Casella et al. found that the absence of any significant increase frequency of MN in lymphocytes from patients undergoing oral and/or transdermal PHT, sequentially monitored for up to 12 months of therapy.[25] In the present study, we used exfoliated cervical cells which site-specific biomarker of exposure to genotoxic agents and possibly a biomarker of cancer risk whereas the other studies were used cultured peripheral blood lymphocytes. Hence, our results are side specific rather than general aspect of the blood-cell derived biomarkers.

There was no significant effect of PHT on micronuclei frequency in cervical exfoliated cells. This may be interpreted as PHT does not cause any microarchitechtural change leading to a malignancy of the cervix. The non-significant effect of PHT in MN frequency could be attributed to the small sample size and short duration in this study. On the other hand, prolonged and extensive use of PHT in our daily life may be hazardous and also, that drugs users should be aware of multifactorial risk factors (environmental, genetic and life style patterns) that may be responsible for additional DNA damage. Future studies may validate this finding among larger groups of patients and also with other PHT agents.

Acknowledgments: The authors are grateful to Dr. Saim Yoloğlu for his statistical assistances in this study.

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