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Efects of hormone replacement and tamoxifen on depression in ovariectomized rats

Wpływ hormonalnej terapii zastępczej oraz tamoxifenu na depresję u szczurów poddanych usunięciu jajników

Terek Mustafa Cosan[1,2], Kanit Lutfiye[2], Doğan Yusuf Hakan[2], Gözen Oğuz[2], Zeybek Burak[1], Keser Aysegul[2], Ozsener Serdar[1], Pogun Sakire[2]

[1] Department of Obstetrics and Gynecology, Ege University Faculty of Medicine, Izmir, Turkey
[2] Department of Physiology, Ege University Faculty of Medicine, Izmir, Turkey

Abstract

Objectives: *To determine the effects of tamoxifen and hormone replacement therapy in order to assess their role in depressive behavior.*

Material and Methods: *Different protocols of hormone replacement therapies were administered to surgically ovariectomized rats. Intact rats were used for tamoxifen experiments. Properly assigned control groups were used and cognitive processes were studied on animal models of surgical menopause using the Porsolt Forced Swim Test and locomotor activity experiments.*

Results: *In the tamoxifen experiments, an interaction between treatment and days did not reach statistical significance, but indicated a trend in this direction [F(1,26)=3.557, p=0.071]. The number of repeated movements significantly decreased after the Porsolt test (F(1,44) = 8.483, P<0.006) in the hormone replacement experiments. In the tamoxifen experiments, the number of repeated movements significantly decreased after the Porsolt test (F(1,26) = 74.410, P<0.001).*

Conclusions: *While sequential hormone replacement is found to be protective against depression, tamoxifen seems to augment behavioral despair.*

Key words: **tamoxifen** / **hormone replacement** / **depression** / **menopause** /

Corresponding author:
Burak Zeybek
Department of Obstetrics and Gynecology, Ege University Faculty of Medicine
Bornova, Izmir, 35100 Turkey
tel./fax: +90 232 343 0711
e-mail: bzeybek@yahoo.com

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Streszczenie

Cel: *Określenie wpływu tamoxifenu oraz hormonalnej terapii zastępczej celem oceny ich roli w zachowaniach depresyjnych.*

Materiał i metody: *Różne protokoły hormonalnej terapii zastępczej zastosowano u szczurów poddanych chirurgicznemu usunięciu jajników. Tamoxifen podawano szczurom, których nie operowano. Wykorzystano odpowiednio dobraną grupę kontrolną. Zbadano procesy poznawcze na modelach zwierzęcych z chirurgiczną menopauzą przy pomocy testu Porsolt Forced Swim oraz doświadczeń aktywności ruchowej.*

Wyniki: *W doświadczeniach z tamoxifenem, związek pomiędzy terapią a dniami nie osiągnął statystycznej istotności, ale wykazał trend w tym kierunku [F(1,26)=3,557,p=0,071]. Liczba powtórzeń ruchów istotnie zmniejszyła się po teście Porsolta [F(1,44)=8,483,p<0,006] w doświadczeniach z hormonalną terapią zastępczą. W grupie leczonej tamoxifenem liczba powtórzeń ruchów istotnie zmniejszyła się po teście Porsolta [F(1,26)=74,410, p<0,001].*

Wnioski: *Wydaje się, że sekwencyjna terapia hormonalna pełni ochronną funkcję przed depresją, natomiast tamoxifen nasila zachowania depresyjne.*

Słowa kluczowe: **tamoxifen** / **hormonalna terapia zastępcza** / **depresja** / **menopauza** /

Introduction

In the postmenopausal period many body systems are affected by reduced estrogen levels and have a negative impact on the quality of life. Cognitive defects and mood disorders, including a spectrum of symptoms from mild mood changes to severe, major depression, are among these problems. Because the lifetime prevalence of depression in women is twice as high as in men, gonadal hormones may play a role in this gender disparity [1].

Experimental and clinical studies provide evidence for the antidepressant and cognitive effects of estrogens [2-7]. An increase in sensory perception, decreased seizure threshold, reduced acute phase inflammatory response, increased in cerebral blood flow, augmented cerebral blood glucose utilization, enhanced mood effects, and reduced formation of β-amyloid have been shown [8].

Selective estrogen receptor modulators (SERMs) are synthetic, non-hormonal compounds acting as estrogen agonists on tissues such as bone and brain, while functioning as estrogen antagonists on other tissues, including the breast and the uterus [9, 10]. Recent studies [11, 12], as well as a study conducted in our department [13], have indicated that raloxifene has a neuroprotective effect on the central nervous system. Tamoxifen is used as an adjuvant therapy for breast cancer and there are studies on clinical cognitive effects on postmenopausal women [14, 15], but there is very limited data about the effect of tamoxifen on mood disorders.

Objectives

The objective of the present study was to determine the effects of tamoxifen and hormone replacement therapy with different hormone regimens to assess their role in depressive behavior.

Material and methods

Experimental Animals. Adult sexually mature female Sprague Dawley rats (190-250 g) were used for the experiments. Rats were housed (3–4 rats/cage) in standard plastic cages with food and water provided *ad libitum* during the habituation period for 2 weeks. Animals were maintained on 12:12 h light-dark cycle (lights on 07:00–19:00). Manipulations of the rats were performed under the rules of the Institutional Animal Ethics Committee of Ege University (Izmir, Turkey), in compliance with the European Communities Council Directive (2003/003) and guided by the 'International Guiding Principles for Biomedical Research Involving Animals' developed by the Council for International Organizations of Medical Sciences (NIH). All the animals were handled before the tests.

Surgical procedure: Postmenopausal status was achieved by bilateral surgical ovariectomy. Rats were anaesthetized by thiopental sodium (Pentotal; 40mg/kg, intraperitoneal). The ovaries were exteriorized through small bilateral flank incisions (1 cm each), the junctions between the fallopian tubes and the ovaries were ligated, the sides of the ovaries were cut, and the ovaries were removed. The horns were returned to the abdominal cavity through the openings and the skin incisions were closed with sutures. During the recovery period (1 week) the animals were housed singly in the cages. Following recovery from the surgery, the rats were kept under standard colony conditions. After 21 days the rats were randomly allocated to study and control groups.

Hormone/Drugs. All hormone/drug treatments were applied for 12 days intraperitoneally at the same time each day. Tamoxifen (5 mg/kg; Sigma, St. Louis, MO, USA) was dissolved in 10% DMSO. The vehicle (10% DMSO) was used for control injections. Medroxyprogesterone (2.5 mg/kg) and 17-β estradiol (50 mg/kg) were dissolved in sesame oil. Progesterone, tamoxifen, and sesame oil injections were done at 08:30-09:00. Estrogen injections were done at 16:00-17:00. The rat menstrual cycle is 4 days and for the sequential hormone replacement progesterone injections were not done for 2 days. Drug treatment was continued for additional 4 days in the Porsolt Forced Swim Test (FST) and spontaneous locomotor activity (total, 18 days).

Experimental groups

- **Tamoxifen experiments**: 10 control (10% DMSO) and 10 tamoxifen injections for each Porsolt FST and locomotor activity experiment.
- **Hormone replacement experiments**:
  - Control group (n=10),
  - Ovariectomy and sesame oil injections (n=10),
  - Ovariectomy and continuous combined estrogen and progesterone injections (n=10),
  - Ovariectomy and intermittent (sequential) estrogen and progesterone injections (n=10).

Porsolt FST. This procedure consists of exposing an animal to a situation of inescapable stress, in which the rat is forced to swim. The rats were placed individually in a cylinder (height, 50 cm; diameter, 30 cm); the level of water did not allow the animal to lean on the floor or arise by the border. The temperature of the water was maintained at 25ºC. Animals were subjected to two trials during which they were forced to swim in a plexiglass cylinder filled with water and from which they could not escape. The first trial lasted 15 minutes. After 15 minutes of forced swim each animal was dried with paper towels, then returned to their cages. After 24 hours, a second trial was performed that lasted 6 minutes. The first 1-minute scores were excluded to allow the rats adaptation period to the experiments. During tests, the total freeze, swim duration, and struggling were measured. Videotapes of the test sessions were scored by an experimenter blind to the treatment for complete immobility time (freeze duration), swim duration, and vigorous struggle time.

Struggle was defined as follows: attempts to jump out of the tank; attempts to climb the walls; and attempts to dive into the tank. A rat was considered to be immobile when the rat floated or made only small movements necessary to keep the nose above the water. Statistical analysis was performed for the first 5 (6-1) minutes on days 1 and 2. The results of the 1st day 15 minutes were analyzed separately.

Locomotor activity experiments. The locomotor activity of each group of animals was tested in 30x30x30 transparent plexiglass cages. The cages were comprised of infrared light sources, photocells, and a computer unit. Locomotor activity cages were placed in a silent room and a constant tone of sound was given to mask the background noise. The cross-over of the animal and block of infrared source through the different corners of the cage were regarded as cross-over movements and blocking the same light source more than once by moving on the same side of the cage as repeated movements. Repeated movements, such as rearing, grooming, and extremity movements, indicate stereotypical behavior. The locomotor activities of the animals were recorded 1 day before and after the Porsolt FST for a time period of 30 minutes.

Statistics. The data were analyzed using SPSS (version 8.0) to perform one-way analysis of variance (ANOVA) with multiple comparison test of Bonferroni. Differences between the groups were evaluated by the *post hoc* Duncan test. Effects with degrees of freedom (df) values exceeding unity in the numerator were set to unity in a conservative Geisser-Greenhouse correction procedure; the level of rejecting the null hypothesis was 0.05.

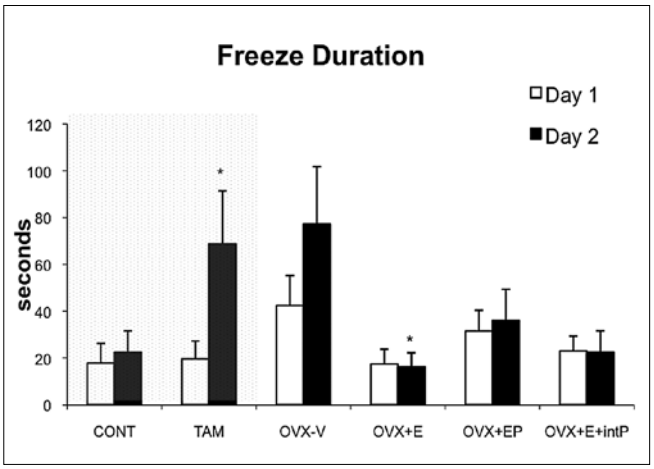

Figure 1. Freeze duration results of the Porsolt Forced Swim Test

Cont: Intact control animals TAM: Tamoxifen OVX-V: Ovariectomy and vehicle, OVX-E: Ovariectomy and estrogen, OVX-EP: Ovariectomy and combined estrogen and progesterone, OVX-EintP: Ovariectomy and intermittent (sequential) progesterone. Different from OVX-V or Control (Day 2): *p<0.05

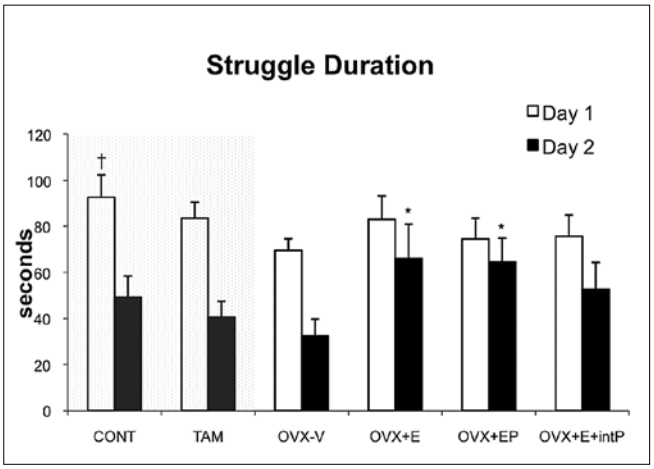

Figure 2. Struggle duration results of the Porsolt Forced Swim Test

Cont: Intact control animals TAM: Tamoxifen OVX-V: Ovariectomy and vehicle, OVX-E: Ovariectomy and estrogen, OVX-EP: Ovariectomy and combined estrogen and progesterone, OVX-EintP: Ovariectomy and intermittent (sequential) progesterone. Different from OVX-V (Day 2): *p<0.05. Different from control (Day 1): †p<0.05.

Result

Porsolt FST. In hormone replacement experiments, treatment emerged as a significant factor [F(4,63) = 3.207, p=0.018]. One-way analysis of variance revealed that the groups were different on the 2nd day [F(4,63) = 2.678, p = 0.040] and the *post hoc* test of Bonferroni indicated significant differences between the ovariectomized and estrogen-treated ovariectomized groups, suggesting estrogen replacement to be effective in reducing freeze duration. In swim duration, only days emerged as a significant factor [F(4,63) = 5.487, p = 0.022], but no other group differences were observed. In struggling duration, days emerged as a significant effect [F(4,63) = 28.140, p = 0.001] since the rats struggled less during the 2nd day of testing compared to the 1st day, indicating behavioral despair.

In the tamoxifen experiments, days also emerged as a significant factor [F(1,26) = 5.165, p = 0.032]. An interaction between treatment and days did not reach statistical significance, but indicated a trend in this direction [F(1,26) = 3.557, p = 0.071] as the increase in the freeze duration was much higher on day 2 compared to intact rats. Along the same lines, on the 2nd day there was a trend in the tamoxifen-treated group for a higher freeze duration compared to intact rats, although the level of significance was < 0.05 (p=0.068). In swim duration, no significant differences were observed. In struggling duration, days emerged as a significant effect [F(1,26) = 40.554, p = 0.001] because the rats struggled less during the 2nd day of testing compared to the 1st day, indicating behavioral despair (Figures 1 and 2).

Locomotor activity experiments. The number of repeated movements significantly decreased after the Porsolt FST (F(1,44) = 8.483, P<0.006) in the hormone replacement experiments. Hormone replacement also emerged as a significant factor (F(1,44) = 18.567, P<0.001). The sequential estrogen-progesterone replacement group had a significantly higher number of repeated movements and combined the estrogen-progesterone replacement group had a significantly lower number of repeated movements (p<0.05).

The number of cage crosses significantly decreased after the Porsolt FST (F(1,44) = 31,116, P<0.001). Hormone replacement also emerged as a significant factor (F(1,44) = 17.868, P<0.001). The combined estrogen-progesterone replacement group had a significantly lower number of repeated movements than the vehicle and estrogen-treated ovariectomized groups (p<0.05).

In the tamoxifen experiments, the number of repeated movements significantly decreased after the Porsolt FST (F(1,26) = 74.410, P<0.001). Tamoxifen treatment also emerged as a significant factor (F(1,26) = 5.969, P<0.022). On day 1, the tamoxifen-treated group had a higher score than the control one based on the Student's t-test (p = 0,013)

In the tamoxifen experiments, the number of cage crosses significantly decreased after the Porsolt FST (F(1,26) = 70.304, P<0.001). Tamoxifen treatment had no significant effect on the number of cage crosses.

Discussion

The Porsolt FST is an animal model of depression and is used to evaluate new anti-depressive drugs and chemical changes in the brain. The duration of immobility indicates the degree of depression and constitutes one of the important parameters of the experiment. The increase in the duration of immobility on day 2 was an anticipated finding. Ovariectomized rats without hormone replacement showed significantly higher durations of immobility. However, the increase in struggle time showed an anti-depressive behavior of the animal and was expected to be shorter on day 2 of the experiment. On day 2 ovariectomized rats without estrogen replacement were significantly different from all three hormone replacement groups. Overall, the results suggest that estrogen prevents behavioral despair, and is more effective when applied alone, rather than in combination with progesterone.

Studies in the literature support the converse effect of these two hormones on the mood. While estrogen has been shown to increase frontal cerebral blood flow, increase degradation of the catabolic serotonin enzyme MAO, affect the regulation of free tryptophan available in the brain, increase density of 5HT binding sites in areas that control mood and cognition, progesterone has been shown to decrease MAO and COMT enzyme activity, act as an anti-estrogen, and down-regulate estrogen receptors [16, 17]. The overall outcome appears to be dose-dependent, and while synthetic progestin in estrogen-primed postmenopausal women washes out the benefit of estrogen, a larger dose of estrogen can have a protective effect.

In a meta-analysis of 26 studies, Zweifel and O'Brien [18] evaluated the effects of hormones on mood in peri-and post-menopausal women; overall effect sizes of 0.68 for estrogen and progesterone and 0.69 for unopposed estrogen are shown. The effect size was larger among perimenopausal women (1.81 vs. 0.90, respectively) and more robust if the duration of treatment was >8 months.

Although there are many studies about HRT on mood and behavior, there is limited data about SERMs on this issue. In a prospective clinical trial of 257 women with breast cancer, 15% of women treated with tamoxifen had symptoms of depression compared with 3% in controls, 6-12 months following treatment [14]. In a more recent multicenter study of 11,064 women treated for 5 years, no difference existed between the tamoxifen and placebo groups with respect to depression and overall quality of life [19]. In a recent study of our laboratories we investigated the effects of raloxifene on cognition and depression in rat experimental models. Raloxifene decreased immobilization (p<0.001) and increased struggling (p<0.001) in the Porsolt FST suggesting the prevention of despair. Raloxifene, as a SERM, acts as an antidepressant in ovariectomized rats and has a positive impact on memory despite the negligible effects on spatial learning [13]. In the present study, intact rats having tamoxifen showed a significantly higher duration of immobility. These results suggest a trend for tamoxifen treatment to augment behavioral despair.

Although the number of repeated movements significantly decreased after the Porsolt FST in locomotor activity experiments, the tamoxifen group had a significantly higher number of repeated movements. The sequential estrogen-progesterone replacement group had a significantly higher number of repeated movements and the combined estrogen-progesterone replacement group had a significantly lower number of repeated movements. The number of cage crosses significantly decreased after the Porsolt FST, with the tamoxifen group showing a significantly higher number of repeated movements. The combined estrogen-progesterone replacement group had a significantly lower number of repeated movements than the vehicle and estrogen-treated ovariectomized groups. The combined and sequential hormone replacements had significantly different results in locomotor activities. This may be due to the longer duration of progesterone use which antagonized estrogen activity in the combined HRT group.

Conclusion

In conclusion, although evidence is accumulating, many questions regarding the factors that play a role in the cognitive neuroprotection afforded by HRT and tamoxifen remain without answers. While studies suggest that HRT is neuroprotective, the protective effect of tamoxifen is a matter of debate. More studies with larger patient populations are needed to find the remaining answers.

DECLARATION OF INTEREST

The authors declare no conflict of interests.

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