Abstract. Levetiracetam is a new-generation antiepileptic drug initially approved as an adjunctive treatment for patients with refractory partial seizures and is now also used as a monotherapy. The aim of this study was to evaluate the genotoxic effects of levetiracetam exposure during pregnancy on rat offsprings. In this study, we used the newborn pups of rats exposed to levetiracetam during pregnancy. Thirty Sprague-Dawley rats were divided into three groups. The mother rats of groups 1 and 2 were treated with different doses of levetiracetam (25 mg/kg/d and 50 mg/kg/d) from gestational days 1 to 18 during pregnancy. Group 3 (control group) was not treated with any drug. In vivo sister chromatid exchange (SCE) induction and in vivo micronucleus formation were assessed. As a result of this study, levetiracetam exposure did not alter SCE frequencies or the mean of number of micronuclei in the prenatal period (p>0.05). Levetiracetam did not cause miscarriage during pregnancy in mother rats. The present study highlighted fetal safety after prenatal exposure to levetiracetam.

Epilepsy is one of the most common neurological disorders during pregnancy, often necessitating the use of antiepileptic drugs (AEDs). Use of several AEDs during pregnancy has been associated with increased risk of major congenital malformations (MCM), and developmental delay. Epidemiological investigations indicate that 0.3-0.4% of the general population of women have epilepsy and pregnancies of epileptic women constitute about 1% of all pregnancies (1, 2). Newborns of mothers exposed to antiepileptic drugs are at increased risk for major congenital malformations and foetal death (1, 2). The human foetus is exposed to a variety of environmental agents and drugs that cross the placenta and can induce DNA damage (3). Included among these agents are AEDs, the majority of which are able to penetrate the placenta (5-6). Pregnancy of a woman with epilepsy is a high-risk pregnancy due to the more frequent occurrence of complications and a higher risk for foetal congenital malformations and post-natal developmental anomalies, than observed in the general population (7-12). For many years, the risk of MCMs in children born to women with epilepsy (WWE) treated with AEDs during pregnancy is approximately two- to three-times higher than the 1-2% frequency in the general population (13). In women with proven epilepsy, it may be dangerous to stop or even change the AED regimen during pregnancy. Prescribing AEDs in pregnancy is a challenge to the clinician (13). Neural tube defects and other congenital anomalies were observed more frequently in neonates whose mothers were treated with AEDs in poly-therapy compared to those whose mothers were exposed to monotherapy, at 6% and 3.7%, respectively (14-16). On the other hand, the prevalence of MCM in the offspring of epileptic mothers who did not take antiepileptic drugs during pregnancy was 3.5% (14-16).

Levetiracetam is a new generation AED initially approved as an adjunct treatment for patients with refractory partial seizures, and is now also used as monotherapy (17). A low risk for MCM has been suggested with levetiracetam use in pregnancy (18). There are limited data about the teratogenic effects of levetiracetam. Based on a number of studies, increased seizure risk during pregnancy was suggested for use of lamotrigine and oxcarbazepine, both of which are glucuronidated for elimination (19-20). However, to our knowledge, no study has been published concerning genotoxic effects of levetiracetam use in pregnancy on the foetus.

The sister chromatid exchange (SCE) technique occupies an important place in monitoring and evaluating damage in...
people exposed to different chemical substances, drugs or agents and in directing the treatment of the respective disease (21). No study has been published concerning SCE and micronucleus tests in cultured rat bone marrow of newborns whose mothers were treated for epilepsy by levetiracetam during pregnancy.

The aim of the present study was to evaluate the potential genotoxic effect on the structure of DNA of bone marrow of newborn rats whose mothers were treated for epilepsy by levetiracetam during pregnancy.

Materials and Methods

**Animals.** Sexually mature 12- to 14-week-old female Sprague-Dawley rats (weighing 250-300 g) were obtained from Ondokuz Mayis University Laboratory Animal Research Center (Samsun, Turkey). Animals were housed in a quiet, temperature- and humidity-controlled room (22°C) in a 12-h light/dark cycle, receiving food and water ad libitum. All procedures and protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (22). The study procedure was approved by the Animal Care and Use Committee at Ondokuz Mayis University (approval number 2011.43).

**Experimental design.** Female rats in oestrus were mated with males, and pregnancy was confirmed by the presence of a vaginal plug. Fifteen pregnant female rats were divided into three groups of five and each rat was housed separately. Groups 1 and 2 were treated with different doses of levetiracetam (Abdi Ibrahim, Istanbul, Turkey) (25 and 50 mg/kg/d intragastrically, respectively) by gavage through a polyethylene tube during gestational days. Group 3 (control group) was treated with the same volume of saline (1 ml/kg/d) by the same route. From gestational day 18 until delivery, each presumably pregnant female was checked routinely for difficulties in parturition. The day of parturition was defined as postnatal day (PND) 1. On the 5th day of the postnatal period, the 10 pups in each group (two pups from each pregnant rat) were analyzed.

**Bone marrow SCE assay.** The bone marrow SCE assay was conducted as previously described (22-25). Bromodeoxyuridine (BrdU) (Sigma-Aldrich, USA) was dissolved in distilled water containing activated charcoal (Sigma) (1 ml BrdU solution for every 100 mg charcoal) to a final concentration of BrdU equal to 20 mg/ml. The mixture was magnetically stirred and divided into 2 ml for each rat in the dark at room temperature. The pups were injected i.p. with BrdU-charcoal mixture at final dose of 1 mg BrdU/g body weight. Colchicine (0.3 mg/rat; Sigma) was injected i.p. 22 h after BrdU injection and then pups were sacrificed 2 h after colchicine administration. The femurs were quickly excised, and bone marrow cells were flushed from the femur into hypotonic solutions (0.075 M KCl). The cellular suspension was centrifuged at 161 × g for 10 min, decanted, and the cellular pellet was resuspended in hypotonic solution. Cells were then incubated in 10 ml hypotonic solution at 37°C for 20 min. The cellular suspension was centrifuged at 161 × g for 10 min. The cells were fixed with three changes of ice-cold methanol:acetic acid (3:1). The cellular suspension was then placed onto prechilled microscope slides to obtain metaphase spreads. The slides were allowed to air dry and subsequently stained with the fluorescent-plus-Giemsa technique as described previously (26), with slight modifications. The slides were treated with a solution of 5 μg/ml Hoechst 33258 (Serva, Heidelberg, Germany) in distilled water in the dark for 10 min, then washed with distilled water. The slides were then placed in a glass tray containing 2xSSC (sodium thiosulfate) and exposed to a UV source (256 nm) for 5 min, then washed again in distilled water. Next, the slides were treated in 2xSSC at 60°C for 120 min and then washed with distilled water. Finally, the slides were stained with 5% Giemsa for 6 min (26). The slides were analysed using light microscopy at ×400 magnification. The individual who scored SCE was blind to the treatment. For SCE analysis, at least 20 metaphase spreads were scored for the frequency of SCE per metaphase cell.

**Bone marrow micronucleus assay.** The femurs were quickly excised and bone marrow cells were flushed from the femur into isotonic solution. The bone marrow cells were spread over glass slides. Feulgen reaction following the modified method of Proudlock et al. (27) was used to determine the frequency of micronuclei. The slides were then dried at room temperature. Thereafter, slides were dipped for 10 min in 1 N HCl at 37°C and were rinsed in distilled water for 3 min. The smears were placed in Feulgen dye (Schiff’s reagent) for about 20 min and were transferred to acid sulphate solution for 5 min. Then the slides were rinsed with tap water. The slides were stained with 1% light green about 2 min and fixed in ethanol. Finally, they were dried and mounted for microscopic evaluation. The number of micronuclei per 1,000 cells for each sample was scored and the frequency per 1,000 cells was calculated. Only cells with well-preserved cytoplasm were scored. The incidence of micronucleated cells per 1,000 micronucleated mature erythrocytes per animal was determined. Identification criteria were as described by Proudlock et al. (27).

**Statistical analysis.** Statistical analyses were performed using SPSS version 15.0 (Chicago, USA) for Windows. SCE frequencies exhibited a normal distribution, and it was appropriate to use standard parametric statistical methods for their analysis. Therefore, SCE data were analyzed by one-way ANOVA. In the analysis of micronuclei results, Kruskal–Wallis test was conducted.

Results

Cytogenetic end-points such as in vivo SCE induction and in vivo micronuclei formation in rat bone marrow were used to evaluate the genotoxic effects of levetiracetam. At birth, no gross malformation was observed in any of the three groups. When SCE values for groups treated with levetiracetam at 25 mg/kg/d and 50 mg/kg/d were compared to those of the control group, it was observed that levetiracetam did not significantly alter SCE frequencies in the prenatal period (even at different doses). No statistically significant correlation was found for the mean number of micronuclei and levetiracetam treatment (Table 1). The number of pups born alive was not changed by levetiracetam exposure. Intragastric levetiracetam exposure at 25, and 50 mg/kg/d doses did not affect the gestation duration. It was observed that levetiracetam did not cause miscarriage in mother rats.
Discussion

This study was undertaken to explore the effect of levetiracetam on DNA of bone narrow of newborns of rats whose mothers were treated for epilepsy during pregnancy. SCE and micronuclei tests were used as an indicator of chromosome damage. SCE reflects an interchange between DNA molecules at homologous loci within a replicating chromosome (23). An increased frequency of SCE is an indirect measure of mutation resulting from DNA damage during the synthetic phase of mitosis. Our results showed that levetiracetam exposure at 25 mg/kg/d or 50 mg/kg/d did not affect the SCE frequency or the mean number of micronuclei in pups in the prenatal period (even at different doses). Levetiracetam did not cause miscarriage in mother rats.

There are limited data on the teratogenic effects of levetiracetam in the literature. The teratogenic potential of levetiracetam on organogenesis period was tested by different doses and no significant gross external malformations were observed in mice (27). In Genton et al.’s study, it was determined that different doses of levetiracetam did not have embryotoxic or teratogenic effects during the period of organogenesis in mice (28). In rats, decreased foetal weight and minor skeletal abnormalities were seen at a dose of 3,600 mg/kg/d, and in rabbits an increase in embryo-fetal mortality, an increase in minor foetal skeletal abnormalities and a decrease in foetal weight were observed at doses of 600-1,800 mg/kg/d (28). Hunt et al. analyzed the epilepsy and pregnancy register of 117 pregnancies with exposure to levetiracetam to determine the safety of prenatal exposure to levetiracetam (29). Among these, 39 pregnancies were exposed to levetiracetam monotherapy, and in 78 cases, levetiracetam was combined with at least one other antiepileptic drug. Minor and MCMs were noted only in the poly-therapy group. In another series consisting of 11 pregnancies, 10 women took levetiracetam during pregnancy, and no foetal malformation was detected (28). It was also expressed that levetiracetam at therapeutic dose is unlikely to cause serious adverse effects on developing human and animal embryos.

There are no data on the genotoxic effects of prenatal exposure to levetiracetam on rat offsprings. The present study showed that intragastric levetiracetam exposure at 25 and 50 mg/kg/d doses did not affect the gestation duration. In another study, Ozurek et al. showed that levetiracetam had only a transient impact on reflex maturation and no impact on physical and cognitive function in the offspring of rats exposed to the drug during pregnancy (17). They suggested that levetiracetam may become a promising candidate for the treatment of epileptic women in pregnancy, which our results support (17). It was also shown that the language and motor development of children exposed to levetiracetam in utero were superior in comparison to children exposed to valproate (27, 28). In a manner consistent with our results, it was seen that levetiracetam could be used on women with epilepsy during pregnancy.

In one of the largest studies, increased risk of congenital malformations were found in the offspring of mothers both on valproate monotherapy and polytherapy. However, the offspring of mothers using carbamazepine, oxcarbazepine or phenytoin had no excess risk of congenital malformations when used as monotherapy or combination therapy without valproate (29-32). In addition to minor and major anatomical malformations, the majority of clinical and animal studies suggested that maternal exposure to first-generation AED was associated with cognitive and behavioural difficulties during post-natal life (33). Additionally, it was reported that AED exposure in utero might lead to neuro-behavioural impairments which could occur at dosages lower than those required to produce somatic malformations.

The mechanism underlying the anti-convulsant action of levetiracetam is not exactly known. According to the results of another study, carbamazepine and topiramate alone did not induce neuronal death (34). In a previous study, it was shown that several AEDs significantly increased the frequency of chromosomal aberrations and SCE in vivo (34) and in vitro (12, 35). Hu et al. investigated the frequency of chromosome aberrations and SCE in cultured peripheral blood lymphocytes obtained from 20 epileptic children exposed to long-term therapy (6-52 months) with valproate and reported that the frequency of chromosomal aberrations was higher in the group of valproate-treated children than in the control group, but this difference was not statistically significant (p>0.1) (36). In contrast, the mean number of SCE per cell was significantly higher in the children treated with valproate compared to the controls (p<0.01). Sinues et
also recoded a statistically significant increase in SCE frequency in 37 patients on long-term therapy with carbamazepine, including 23 treated for idiopathic epilepsy and 14 for trigeminal neuralgia, compared to the controls, but the frequency of chromosomal aberrations in this group of patients did not differ significantly from that in the control group (37). Schaumann et al. investigated the frequency of SCE and CA in cultured peripheral blood lymphocytes from nine patient who used long-term carbamazepine therapy (over 18 months) for epilepsy (38). The mean number of SCE per cell (6.9±1.0) did not differ significantly when compared to the controls (6.3±0.5) (p>0.1); the mean number of chromosome aberrations/cell also did not differ from that of the controls. In comparison to the controls, Sardas et al. found a significant increase of SCE frequency in 27 patients who took phenytoin or carbamazepine in monotherapy (35). Kaul and Goyle found a similar result in 30 and 42 patients, respectively, treated for epilepsy with phenytoin (39, 40), but Schumann et al. did not notice any significant difference in SCE frequency between controls and patients on long-term phenytoin/phenobarbital therapy (41). Bellini et al. measured SCE in 15 children, aged 5-10 years, in whom PB therapy was started for treatment of epilepsy (42). The SCE frequency was significantly lower before than after 12-month PB therapy. In our study, no increase in SCE frequency was observed in any of the investigated groups. In the present study, in terms of micronuclei frequency, levetiracetam had no statistically significant effect. Sinues et al. similarly did not observe any significant increase in micronuclei in lymphocytes from 37 patients treated with carbamazepine (37) Witzcaka et al.’s study showed that the mean number of micronuclei was higher in the epileptic drugs-exposed study group overall than in the controls, but the difference was only borderline significant (p=0.07) (12).

Comparing levetiracetam to valproate and to a control group, a recent study reported that children exposed to levetiracetam achieved higher developmental scores at an age less than 24 months compared to children exposed to valproate. The children in the levetiracetam-treated group did not differ from the control children (37). Two studies investigating the neuro-development effects of levetiracetam after in utero exposure similarly suggested that in utero exposure to levetiracetam did not have adverse effects on neuro-development and language skills of children at 3-4 years of age (43, 44). In another recent study, Mawhinney et al. confirmed a low risk for MCM with levetiracetam monotherapy use in pregnancy, in a meaningful number of exposed pregnancies (44). They also suggested that MCM risk is higher when levetiracetam is taken as part of a polytherapy regimen (43). Similarly a recent review suggested a safe foetal profile of levetiracetam, both as monotherapy and in combination with other AEDs. The current evidence suggests that the overall risk of major malformation after first trimester exposure to levetiracetam is within the population baseline risk of 1-3%, with no apparent adverse effects on long-term child development. They also reported that first trimester exposure to levetiracetam monotherapy was not associated with an increased risk for MCM as compared to other AEDs in different cohorts (45).

Our findings confirmed the literature and as a result of our study, it could be assumed that levetiracetam taken by pregnant women with epilepsy does not evoke genotoxic effects. The present study emphasizes on foetal safety after prenatal exposure to levetiracetam. However, further work is required to confirm these findings in different and larger study groups.

Conflicts of Interest

None.

Acknowledgements

We would like to thank Ondokuz Mayis University BAP for supporting our project (PYO-TIP-1901.11).

References


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Received September 12, 2014
Revised October 18, 2014
Accepted October 24, 2014