



The Effect of Levetiracetam on Closure of the Midline in Early Chicken Embryos

Erken Tavuk Embriyolarında Levetirasetamın Orta Hat Kapanmasına Etkisi

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ABSTRACT

AIM: Genetic predisposition and some environmental factors play an important role in the development of neural tube defects. Levetiracetam is a new drug that has been approved in the treatment of partial seizures. We aimed in this study to determine the effect of levetiracetam on chick embryos.

MATERIAL and METHODS: One hundred and sixty fertile non-pathogenic Super Nick eggs were incubated for 24 hours and were divided into four groups of 40 eggs each. Levetiracetam was administered via the sub-blastodermic route. The eggs were incubated for another 24 hours. All eggs were opened at the 48th hour, and the embryos were evaluated morphologically and histopathologically.

RESULTS: The effects of levetiracetam on the embryo were correlated with the dose of levetiracetam. In the light of the results, it was determined that the use of increasing doses of levetiracetam led to defects of midline closure in early chicken embryos.

CONCLUSION: Levetiracetam, a new antiepileptic drug that is effective especially on calcium ion concentration, leads to defects in midline closure in embryos in a dose-dependent manner. Further studies are needed to show the mechanism of embryonic damage and the mechanisms of its teratogenous effects associated with genetic and environmental factors.

KEYWORDS: Levetiracetam, Calcium, Neural tube defect, Chicken embryo

ÖZ

AMAÇ: Genetik yatkınlık ve bazı çevresel faktörler nöral tüp gelişiminde önemli rol oynar. Levetirasetam, parsiyel epilepsilerin tedavisinde etkinliği kanıtlanmış yeni bir antiepileptik ilaçtır. Çalışmada yeni bir antiepileptik ilaç olan levetirasetamın civciv embriyolarında orta hat kapanmasına etkisi ve nöral tüp defekti oluşturma mekanizmasının incelenmesi amaçlandı.

YÖNTEM ve GEREÇLER: 160 fertil nonpatojenik süper Nick cinsi yumurta, 24 saatlik inkübasyon sonrası her birinde 40 yumurta olacak şekilde gruplandırıldı. Levetirasetam, subblastodermik olarak uygulandı. Yumurtalar 24 saat daha inkübe edildi. Tüm yumurtalar 48. saate açılarak, embriyolar morfolojik ve histopatolojik olarak değerlendirildi.

BULGULAR: Çalışma sonucunda veriler değerlendirildiğinde, levetirasetam dozu ile embriyo etkilenimi arasında bir korelasyon olduğu sonucuna varıldı. Bu bulgular eşliğinde levetirasetam kullanımının, artan konsantrasyonlarda erken tavuk embriyolarında orta hat kapanma kusurlarına yol açtığı sonucuna varıldı.

SONUÇ: Yeni bir antiepileptik olan ve etkisini daha çok kalsiyum iyonu üzerinden gösteren levetirasetam, doz bağımlı olarak embriyolarda orta hat kapanma kusurlarına neden olmaktadır. Yapılacak daha ileri çalışmalarla, embriyonik hasarlanmanın mekanizması ile genetik ve çevresel etmenlere bağlı teratojen etkiler arasındaki mekanizmaları ortaya koymak gerekmektedir.

ANAHTAR SÖZCÜKLER: Levetirasetam, Kalsiyum, Nöral tüp defekti, Civciv embriyosu

INTRODUCTION

Genetic predisposition and some environmental factors play an important role in the development of neural tube defects (NTDs), which occur in approximately 6/10000 newborns (18). The most frequent NTDs are anencephaly and spina bifida (11). In newborns, the incidence of birth defect is 3-5%, and NTDs account for 7% of newborn mortality-associated birth defects (3).

Levetiracetam is a new drug that has been approved in the treatment of partial seizures. Although the mechanism of its effect is uncertain, it is thought that levetiracetam binds to some selective membranes in the central nervous system. In contrast to the other antiepileptics, there is no certain evidence of the teratogenicity of levetiracetam in pregnant women.

We aimed in this study to determine the effect of levetiracetam on chick embryos.

MATERIAL and METHODS

The study was conducted in Ankara University, Department of Neurosurgery-Neuroembryology Laboratory. One hundred and sixty fertile non-pathogenic Super Nick eggs were received from the Institute of Akyurt Poultry Husbandry. All eggs were incubated for 24 hours, weighed (overall weight 65±2 g), and kept at a temperature of 37.8±2°C and 65-75% humidity in an incubator that rotated the eggs every two hours. The eggs were opened using window procedure after 24-hour incubation, and were divided into four groups of 40 eggs each. Under sterile conditions, levetiracetam was diluted in water and prepared in the selected dosages. Levetiracetam was administered via the sub-blastodermic route in a volume of 20 µL in Groups 1, 2, and 3 by Hamilton microinjector (60 mg, 180 mg, and 360 mg/ml in Groups 1, 2 and 3, respectively). Group 4 served as the control group and was administered 20 µL 0.9% NaCl via the sub-blastodermic route.

The eggs were covered with sterile drapes after injection. Then, the eggs were rotated 180° and incubated for another 24 hours. All eggs were opened at the 48th hour, and the embryos were evaluated morphologically and histopathologically.

Eggs were opened using a new method and evaluated under the Nikon ZMS-20 light microscope using the Hamburger-Hamilton chick embryology classification system. Embryos were classified as with defect, normal or undeveloped.

Analysis of all findings was performed using the Statistical Package for the Social Sciences (SPSS) 17.0 program. Results were expressed as number and percentage. Fisher's chi-square test was performed to determine any differences between the groups.

RESULTS

In our study, levetiracetam was administered in Groups 1, 2 and 3 via sub-blastodermic route as 60, 180, and 360 mg/ml, respectively, in 20µL. The Control group was administered 20µL 0.9% NaCl also via sub-blastodermic route.

In Group 1, 1 embryo (2.5%) had NTD, 3 embryos (7.5%) were undeveloped, and 36 embryos (90%) were intact. In Group 2, 5 embryos (12.5%) had NTD, 5 embryos (12.5%) were undeveloped, and 30 embryos (75%) were intact. In Group 3, 9 embryos (22.5%) had NTD, 7 embryos (17.5%) were undeveloped, and 24 embryos (60%) were intact.

In Group 4, no embryo (0%) had NTD, 2 embryos (5%) were undeveloped, and 38 embryos (95%) were intact (Table I) (Figures 1A, B; 2A, B).

There was no significant difference in NTDs between Groups 1 and 4 (p>0.05). There was a significant difference in NTDs between Groups 2 and 4 (p<0.05) and between Groups 3 and 4 (p<0.05). After undeveloped embryo was accepted as abnormal, there was no significant difference in abnormal embryos between Groups 1 and 4 (p>0.05). There was a significant difference in abnormal embryos between Groups 2 and 4 (p<0.05) and between Groups 3 and 4 (p<0.05). According to all findings, the new antiepileptic drug, levetiracetam, may cause NTDs and abnormality in early chick embryos in a dose-dependent manner.

DISCUSSION

Neural tube defect (NTD) has become a serious research issue with regard to its predictability and its being a detriment to public health. We aimed to contribute to the literature regarding the effect of levetiracetam on midline closure in early chick embryos. Based on our results, we found that levetiracetam caused NTD in early chick embryos in parallel with increasing dosages. We tried to shed further light on the uncertain mechanism of levetiracetam as being through ion concentration changes in intracellular and extracellular regions. As mentioned above, the etiology of midline closure defect can be divided into major groups as genetic or environmental (maternal age, folic acid deficiency, etc.) factors. Neurulation in high vertebrates consists of two steps as primary and secondary (10, 17). Stages in primary neurulation are: 1. Development of neural plaque by thickening ectoderm, 2. Remodelling of neural plaque, 3. Bending of neural plaque, and 4. Closure of neural cleft. Closure of the caudal eminence, designated as secondary neurulation, is seen after closure of the caudal neuropore (18). From the beginning to the end of neurulation, microtubules and microfilament structures play important roles. Calcium ion has an essential role in this process (5,14,15). Temporarily increased intracellular calcium provides for changing skeletal structure and neural motility. Contraction of elements occurring in skeletal structures depends on calcium ion. Thus, calcium (Ca) ion plays a key role in changing skeletal structure and contraction (9).

Since its approval for clinical use in 2002, levetiracetam has become a widely used antiepileptic drug that is effective in partial and generalized epilepsy syndromes (4). Serum levels of levetiracetam are very similar to corresponding levetiracetam levels (5.95–17 mg/ml) found in the brain tissue of individual patients (13). The mechanism of levetiracetam is thought to relate to its reduction of the intraneural calcium concentration. Application of levetiracetam to hippocampal cell cultures resulted in a 50% decrease in intraneural calcium concentration in 5 minutes (1). Lynch et al. showed

Table I: Distribution of Levetiracetam Dosages in Different Groups

Embryos	1.2mg	3.6mg	7.2mg	Control
Undeveloped	3 (7.5%)	5 (12.5%)	7 (17.5%)	2 (5%)
Neural tube defect	1 (2.5%)	5 (12.5%)	9 (22.5%)	0 (0%)
Intact	36 (90%)	30 (75%)	24 (60%)	38 (95%)



Figure 1:
A) Normal appearance of post-incubation 2-day chick embryo.
B) The neural tube (lower arrow) and notochord (upper arrow) (x200).



Figure 2:
A) Appearance of post-incubation 2-day chick embryo with neural tube defect.
B) Defective neural tube (upper arrow) and notochord (lower arrow) (x200).

that levetiracetam specifically binds to the synaptic vesicle protein SV2A, whereas it does not bind to its two isoforms SV2B or SV2C (8). When levetiracetam was acutely applied at different concentrations to neocortical, hippocampal CA1 neuron cell preparations, high-voltage (HVA) Ca currents were inhibited by an average of 18–40% of HVA channels especially N-type channel (2,7,12). Thus, levetiracetam contributes to the antiepileptic effect by reducing intraneural calcium. In contrast to our study, Guvenc et al. incubated 45 chicken embryos, neural tubes of 41 were closed and the embryos displayed normal development (6). In two studies in chick embryos, Tureci et al. reported that the new-generation anti-epileptic medication levetiracetam and the standard anti-epileptic medication valproic acid were compared in terms of teratogenicity by studying embryonic development in 360 fertile White-Leghorn chicken eggs (conception day 0) (16). It is found that levetiracetam may cause severe developmental abnormalities, and is likely not safe for use in pregnant women (16); and Ozer et al. reported that 4.5 µL levetiracetam in 40 chick embryos found that delay in the closure of the neural tube (10).

In conclusion, levetiracetam, a new antiepileptic drug that is effective especially on calcium ion concentration, leads to defects in midline closure in embryos in a dose-dependent manner. Further studies are needed to show the mechanism of embryonic damage and the mechanisms of its teratogenous effects associated with genetic and environmental factors and to minimize the rate of congenital defects.

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