The Effect of Diazepam on The Development of 
Neural Tube Defects in Early Chick Embryos

Erken Cevciv Embriyosu Nöral Tüp Gelişim Defektlerine 
Diazepamın Etkisi

ÖNDER GÜNEY, MEHMET SELÇUKI, AĞAHAN ÜNLÜ, CELAL BAĞDATOĞLU

Department of Neurosurgery (ÖG), Selçuk University, School Of Medicine, Konya, Turkey,
Department of Neurosurgery (MS, AÜ, CB), Ankara University, School of Medicine, Ankara, Turkey

Abstract: This study reveals the effects of diazepam on 
the development of neural tube defects in the chick based 
on light microscopy and histopathological study. Thirty 
fertile Hubbard Broil eggs were divided into two equal 
groups. Group 1 embryos (n:15) at Stage 8 (the four-somite 
stage) of development were explanted and grown for 18 
hours in nutrient medium (thin albumin). Group 2 
embryos (n:15) at Stage 8 (four-somite stage) of 
development were explanted and grown for 18 hours in 
nutrient medium containing 400 µg/ml Diazepam. After 
the incubation period, 86.6% of the control embryos (Group 
1) had intact neural tubes, and 80% of the Group 2 embryos 
showed neural tube defects. The results of this study 
suggest that Diazepam causes neural tube defects.

Key Words: Diazepam, early chick embryo, neural tube 
defect

Özet: Bu çalışmada, civcivde nöral tüp defektlerinin 
gelişimine diazepamın etkileri histopatolojik çalışma ve 
ışık mikroskopu kullanılarak araştırıldı. 30 adet fertil 
Hubbard Broil cinsi yumurta eşit iki gruba bölündü. Grup 
1 embriyolar (n:15) gelişmenin 8. evresinde (dört-somit 
evresi) elde edildi ve besleyici ortamda (ince albümin) 18 
saat gelişirdi. Grup 2 embriyolar (n:15) gelişmenin 8. 
Evresinde (dört-somit evresi) elde edildi ve 400 µg/ml 
diazepam içeren besleyici ortamda gelişirdi. Kontrol 
grubu embriyoların (grup 1) % 86.6’sında nöral tüp 
intaktti. Grup 2 embriyoların % 80’inde nöral tüp defekti 
gözlemdi. Bu çalışmanın sonucunda diazepamın nöral tüp 
defektlerine neden olduğu desteklenmiştir.

Anahtar Kelimeler: Diazepam, erken civciv embriyosu, 
nöral tüp defekti

INTRODUCTION

Knowledge of normal embryonic and fetal 
natural tube development is of great importance in 
understanding the pathogenesis of neural tube 
defects, especially those of the lumbosacral region. 
Three distinct phases of caudal neural tube 
development are described in the literature, namely 
caudal neuropore closure, secondary neurulation, 
and retrogressive differentiation (2, 3, 15, 22, 23, 24, 
25).

Diazepam has been widely used for the 
treatment of anxiety and muscle spasm in humans. 
The discovery that neurons of the central nervous 
system (CNS) possess large numbers of specific cell 
surface receptors for benzodiazepines, the group of 
compounds that includes Diazepam, has prompted 
studies on the potential teratogenic effects of this 
agent in the CNS (6, 16, 26, 27). Several studies have 
shown that Diazepam selectively inhibits neural tube 
closure in the chick (11, 19). The research done to 
date suggests that the biomechanical basis of
Diazepam-induced neural tube closure defects is a general inhibition of the contractile activity of microfilament bundles (1, 18, 20, 28). Here we report that exposure to 400 μg/ml Diazepam significantly increases the incidence of neural tube defects in early chick embryos.

**MATERIALS and METHODS**

We prepared a stock solution of Diazepam (5 mg/ml) in avian Ringer's solution (adjusted to pH 7.2 using 10% NaHCO3), immediately before use. The amounts of stock solution added to the nutrient medium (thin albumin) were such that the final concentration of Diazepam was 400 μg/ml. This concentration is known to selectively inhibit neural tube closure in chick embryos explanted at Stage 8, which is just prior to neural tube closure in the midbrain and the anterior portion of the hindbrain (5, 11).

Thirty fertile Hubbard Broil eggs were incubated at 37.5°C until the embryos reached Stage 8 of development (4). The eggs were then divided into two equal groups, and embryos were explanted using New's technique (21). Half of the embryos were grown for 18 hours on medium that contained Diazepam, and the other half on medium free of the drug. After the incubation period, the chicks neural tube development was examined under a light microscope. The embryos were assigned to one of the following three categories based on gross morphology: 1) no development 2) abnormal 3) normal (Figure 1).

Table I: Effect of Diazepam on the development of chick embryos explanted at Stages 8 and cultured for 18 hours.

<table>
<thead>
<tr>
<th>Group</th>
<th>No development</th>
<th>Abnormal</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>

Some embryos were fixed in 10% formaldehde, stained with Delafield's hematoxylin and kept as whole mounts. These were reexamined to assess the extent of gross malformations. Others were embedded in paraffin, serially sectioned at 7 μm, and stained with Delafield's hematoxylin and eosin and examined under light microscopy.

**RESULTS**

Chick embryos were explanted at Stage 8 of development and grown for 18 hours on medium with or without Diazepam.

Incubation allowed all of the embryos to advance to Stage 8 of development. At the time of explantation, each embryo had four somite pairs, and the neural folds in the future midbrain and a portion of the hindbrain had already made contact.

After 18 more hours of incubation postexplantation 13 embryos (86.6%) of the control series exhibited characteristic of Stage 12 development. Several features define this stage: head turning to left side, anterior neuropore closed, telencephalon identifiable, primary optic vesicles and optic stalk well-established, auditory pit deep but wide open, heart slightly S-shaped and head-fold
After the same incubation time, 12 embryos (80%) of the experimental series (Group 2) showed neural tube defects (Figure 3, 5). The defects were such that the neural folds showed no signs of contact throughout the neuroepithelium. Table I summarizes the developmental effects of Diazepam treatment on chick neural tube development.

**DISCUSSION**

Numerous chemical agents, such as cytochalasins, caffeine, ionophore A23187, papaverine, and local anesthetics are known to cause neural tube defects in the chick (7, 8, 9, 10, 11, 13, 14, 17).

Lee et al. (10) showed that exposure to 500 µg/ml caffeine significantly increased the incidence of neural tube defects in explanted early chick embryos of the development stage at treatment. Lee et al. (12) suggested that local anesthetics inhibited elevation of the chick neural folds by disrupting the organization and calcium-dependent function of microfilaments in neuroepithelial cells.

Studies have also been done on the process that causes these defects. Nagele et al. (20) used morphometry to investigate the biomechanical basis of Diazepam (400 µg/ml) - induced neural tube defects in chick embryos that were explanted at Stage 8 of development and cultured for 6 hours. Nearly 80% of these embryos showed neural tube closure defects, and the effects were most pronounced in the midbrain region, where neural folds were often retracted. These authors were able to show that Diazepam selectively inhibited neural tube closure in the chick. Electron microscopy of the adversely affected neuroepithelium revealed alterations in the organization and substructure of microfilament bundles situated at the apical ends of neuroepithelial cells.

Although the precise nature of the drug's effects on microfilament bundles is uncertain, Diazepam has been shown to specifically inhibit the synthesis and accumulation of myosin in cultured muscle and nonmuscle cells (1, 18, 20, 28). It seems plausible that the biomechanical basis for Diazepam-induced neural tube closure defects is general inhibition of the contractile activity of microfilament bundles. The drug's effect on myosin likely interferes with the contractile activity of apical microfilament bundles and with apical constriction of developing neuroepithelial cells. The fact that microfilament
bundles, as well as myosin-rich regions of these bundles, are less conspicuous in Diazepam-treated neuroepithelial cells supports this idea (17).

Our study showed that treatment with 400 μg/ml Diazepam significantly increased the incidence of neural tube defects in explanted early chick embryos. Overall results indicate that neural tube defects associated with exposure to Diazepam are due largely to a general inhibition of the contractile activity of apical microfilament bundles in neuroepithelial cells.

Correspondence: Önder Güney
Selçuk Üniversitesi Tip Fakültesi
Nöroşirüj ABD
Konya - Türkiye

REFERENCES


Güney: Diazepam And Neural Tube Defect