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

Erken Civciv Embriyosu Nöral Tüp Gelişim Defektlerine Diazepamın Etkisi

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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 wer 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 geliştirildi. Grup 2 embriyolar (n:15) gelişmenin 8. Evresinde (dört-somit evresi) elde edildi ve 400 µg/ ml diazepam içeren besleyici ortamda geliştirildi. Kontrol grubu embriyoların (grup 1) % 86.6'sında nöral tüp intaktti. Grup 2 embriyoların % 80'ninde nöral tüp defekti gözlendi. 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 neural 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.

	No development	Abnormal	Normal
Group I	1	1	13
Group II	2	12	1

Some embryos were fixed in 10% formaldehyde, 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.

Figure 1: View of a normal chick embryo at 48 hrs in nutrient medium.

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

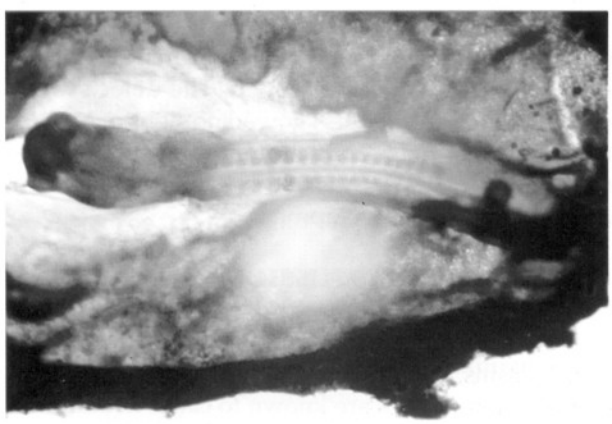

Figure 2: View of a chick embryo explanted at Stage 8 of development and cultured for 18 hrs in nutrient medium (thin albumin).

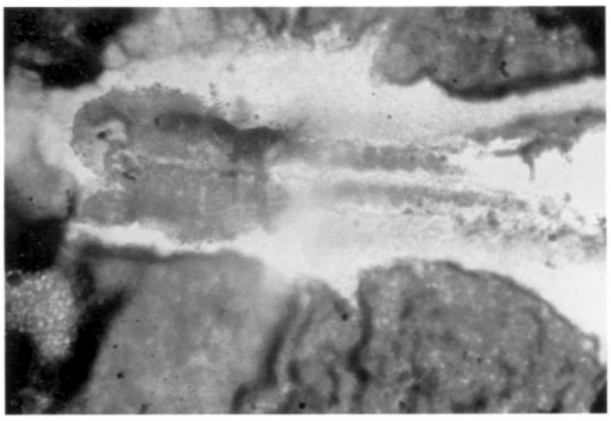

Figure 3: View of an embryoexplanted at Stage 8 ant cultured for 18 hrs in medium containing 400 µg/ ml Diazepam.

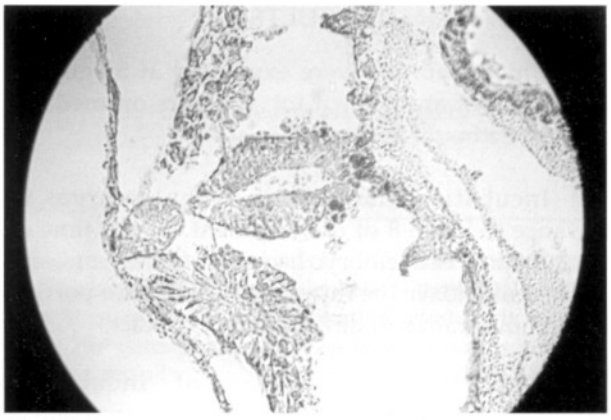

Figure 4: Transverse section through the spinal cord region of a control I embryo. The neural tube is closed.

amnion covers the entire forebrain region. The neural tube was closed in these embryos (Figure 2, 4).

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

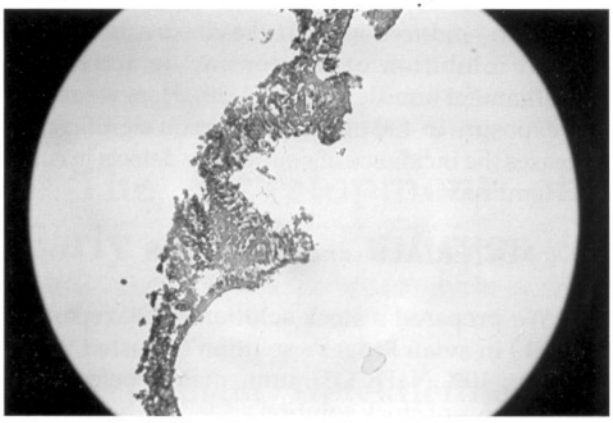

Figure 5: Transverse section through the spinal cord region of a Diazepam -treated embryo. The neural tube is open.

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 distrupting 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.

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