



Original Investigation

# Does the Anti-Migraine Drug Rizatriptan Affect Early Neural Tube Development in Chick Embryos?

Murat SAYIN<sup>1</sup>, Gulsen Seren GURGEN<sup>2</sup>, Sevgi Sidika SAYIN<sup>3</sup>, Gonul GUVENC<sup>1</sup>, Nurullah YUCEER<sup>1</sup>

<sup>1</sup>Izmir Katip Celebi University, School of Medicine, Department of Neurosurgery, Izmir, Turkey

<sup>2</sup>Celal Bayar University, School of Medicine, Department of Histology and Embryology, Manisa, Turkey

<sup>3</sup>Izmir Bozyaka Education and Research Hospital, Neurology Clinic, Izmir, Turkey

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## ABSTRACT

**AIM:** To investigate the impact of rizatriptan on neural tube development using early chick embryos as a model organism.

**MATERIAL and METHODS:** A total of 36 pathogen-free Leghorn chicken eggs were selected and categorized in three groups: sham, therapeutic, and supra-therapeutic. After 24 hours, the eggs were opened and injected with sterile drugs, and then re-closed using plastic tape. After a period of 72 hours, the eggs were opened and assessed using the Hamburger–Hamilton chick embryology classification method. TUNEL staining was used to identify apoptosis, and hematoxylin–eosin staining was used to investigate neural tube closure.

**RESULTS:** Treatment with rizatriptan significantly slowed down neural tube development. The supra-therapeutic group showed neural tube closure defects.

**CONCLUSION:** Rizatriptan had a negative effect on neural tube closure. Further research is needed to identify a safe and effective drug for treating migraines during pregnancy.

**KEYWORDS:** Chick embryo, Migraine, Neural tube defect, Pregnancy, Rizatriptan

## INTRODUCTION

Migraine headaches are a challenging issue during pregnancy as there is currently no safe treatment available for pregnant women who experience migraine pain. Research suggests that migraine pain affects about 16% to 24% of the population worldwide (9). Of migraine sufferers, about 90% report using one or more drugs for migraine (2,7,10,13). In pregnancy, migraine attacks are known to decrease significantly during the second and third trimesters (19). Drugs such as triptans, aspirin, and paracetamol/acetaminophen are thought to be safe for the treatment of migraines in pregnant women (19). However, there is no research verifying the safety claim of these drugs during pregnancy.

The aim of the current study was thus to investigate the impact of rizatriptan, a serotonin (5-hydroxytryptamine) 1B/1D receptor agonist commonly used in the treatment of migraines, on early neural development using chick embryos as a model system.

## MATERIAL and METHODS

### Experimental Groups

The research was conducted in collaboration with the Histology Department Research Laboratory, Celal Bayar University, School of Medicine. The Republic of Turkey Ministry of Agriculture and Rural Affairs, Bornova Veterinary Control and Research Institute, was responsible for supplying the Leghorn chicken eggs, which were sterile and pathogen free.



Corresponding author: Murat SAYIN

E-mail: smsayin@gmail.com

Thirty-six eggs with a mean weight of  $65 \pm 5$  g (mean  $\pm$  SD) were divided into three groups: sham group (n=12), rizatriptan therapeutic group (n=12), and rizatriptan supra-therapeutic group (n=12).

### Incubation, Injection, and Dissection

The 36 eggs were placed in incubators at a temperature of  $37.5 \pm 0.2^\circ\text{C}$  and 60–80% relative humidity. Every 2 hours, the position of each egg was changed on its axis. After 24 hours of incubation, the eggs were opened using  $\times 4$  optical magnification and assessed using the Hamburger–Hamilton classification stage 9 (6,11). Seventy percent ethanol was used to rinse the eggs, and a piece of plastic tape was used to cover the egg cavity. A small hole was made to inject the drug. After injection of the drug, the eggs were closed using sterile tape. After 3 days, the eggs were opened for extraction and dissection of the embryos using microsurgery and water-floating techniques. All embryos were immersed in 10% formalin solution for 24 hours. After 24 hours, the embryos were investigated under a microscope (CX41-Olympus, Tokyo, Japan) using the Hamburger–Hamilton chick embryology classification system (11).

### Drug Preparation

The rizatriptan dosages were determined according to the weight of the eggs, which was 65 g for each egg. Orally disintegrating 10 mg rizatriptan tablets (Merck & Co, Inc.) were immersed in NaCl solution. A dosage of 0.01 mg/0.01 mL rizatriptan was used for the therapeutic group and 0.04 mg/0.01 mL rizatriptan for the supra-therapeutic group. The drugs were injected into the embryos using 30-gauge syringes.

### Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) Staining

Apoptosis was assessed using an *in situ* apoptosis detection kit (DeadEnd Colorimetric TUNEL system, Promega G7130). A 5  $\mu\text{m}$  section was cut from the paraffin blocks of the prepared samples. Xylene was then used to remove the paraffin, and the sections were re-hydrated and incubated using 20  $\mu\text{g}/\text{mL}$  proteinase K for 10 minutes. Next, the sections were washed with distilled water, and endogenous peroxidase activity was stopped using 3% hydrogen peroxide. The sections were then studied using a light microscope (Olympus BX40, Tokyo, Japan). Two observers blinded to the experimental groups labeled the intensity grading using semi-quantitative measures. The following scale was used for grading purposes: weak (+), moderate (++), and strong (+++). By determining the quantity of TUNEL-positive cells, the average number of apoptotic cells was determined. TUNEL-positive cells were measured erratically as required per case. For each specimen, the total number of TUNEL-positive and TUNEL-negative cells were counted for each specimen. TUNEL-positive cells were expressed as a percentage, as shown in Figure 1. Cells located in the necrosis areas or margins of sections, and those with poor morphology, were not analyzed.

## RESULTS

The neural tube defect results are summarized in Figure

2. As shown by hematoxylin–eosin staining, neural tube closure appeared normal in the 72 hours control group. The neuroectoderm layer was made from pseudostratified epithelium and had the notochord beneath it. Normal positions were observed in the dermomyotome and sclerotome (Figure 3A). In the therapeutic group, neural tube closure was present in most embryos (10 out of 12, 83%). However, the neuroectoderm layers were far apart compared with the control group. This suggests that neural tube closure occurred later. One embryo showed retardation in growth (Figure 3B). In the supra-therapeutic group, the neuroectoderm layers in some embryos (n=3) were farther apart compared with the therapeutic dose group (Figure 3C<sub>1</sub>), whereas in some embryos (n=2), the apical parts of the neuroectoderm layers did not converge and neural tube closure was not observed.

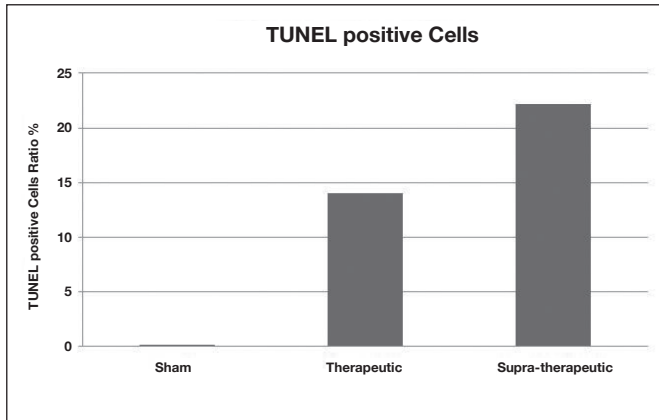
The TUNEL staining results are summarized in Figure 1. TUNEL-positive cells were not observed in the neuroectoderm, dermomyotome, or sclerotome cells of the 72 hours control group (Figure 3A). In the therapeutic group, TUNEL-positive cells were present in the apical end portions of the neuroectoderm and near the basal parts of the notochord. Reactive cells in some sclerotomes were observed (Figure 3B). In the supra-therapeutic group, fewer TUNEL-positive cells were observed in neuroectoderm cells, whereas most TUNEL-positive cells were observed in sclerotome cells (Figure 3C<sub>1</sub>, C<sub>2</sub>).

## DISCUSSION

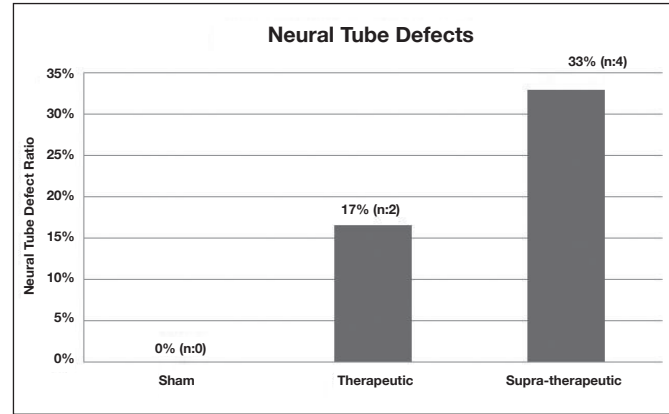
Managing migraines during and post pregnancy is essential as they can have negative impacts on pregnant women and embryos, including dehydration, depression, anxiety, stress, and insomnia (1). Several studies have shown that migraine attacks during pregnancy significantly increased the risk of stroke, pre-eclampsia, and hypertension (1,8,17,20). Pregnant women who experience serious cases of hypertension or pre-eclampsia are at risk of giving birth to premature babies and experiencing placental abruption (3,12,15). Some women who experience menstrual migraines experience relief during pregnancy especially at the second and third trimester (10,16); however, migraine attacks can worsen during the first trimester of pregnancy (21). However, the use of ergotamine and dihydrotamine to treat migraines is restricted during pregnancy because of the uterotonic impacts of these drugs (5). Thus, there remains a pressing need for safe migraine treatment to benefit the health of mothers as well as the infants.

The US Food and Drug Administration (FDA) approved triptan for the treatment of migraines in 1992. Despite its availability, whether it is safe for pregnant women remains unclear. A cohort study of 3,480 pregnant women who suffered migraines reported that 70% used anti-migraine drugs, most frequently non-narcotic analgesics (54%) and triptans (25%) (14). A Swedish registry linkage study demonstrated that the use of eletriptan caused congenital problems in embryos. However, this claim was based on three cases out of 14.

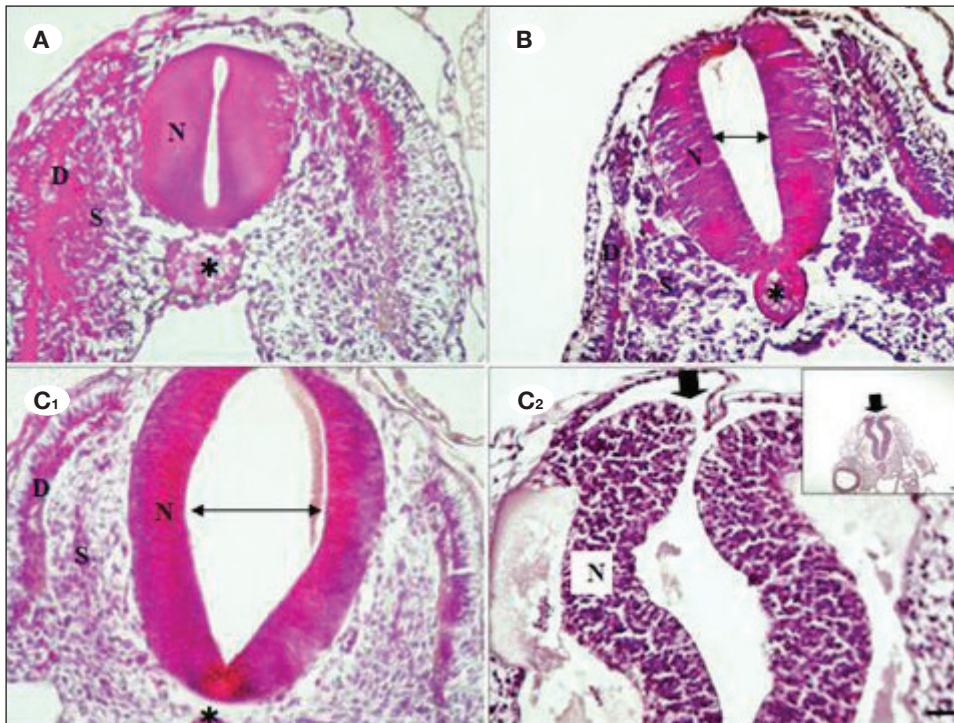
Rizatriptan is a receptor agonist chemically represented as 5-hydroxytryptamine<sub>1B/1D</sub> (5-HT<sub>1B/1D</sub>). Receptors for



**Figure 1:** Bar chart showing TUNEL positive cell ratio. Significantly more apoptotic cells were present in the neural tubes of rizatriptan treated embryos.



**Figure 2:** Total ratio and number of neural tube defects.



**Figure 3:** The 72 hours hematoxylin-eosin dye stained chick embryo. Control group (A), therapeutic group (B), supra-therapeutic group (C1, C2). N: neuroectoderm, D: dermomyotome, S: sclerotome, \*: Notocord. Neuroectoderm is separated in C1 and neural tube closure defect is in C2. (A, B, C figure bar = 50 µm, X400; insert figure bar C.2= 10 µm X100).

rizatriptan are found in the umbilical cord and fetal brains (4). Oxidization of triptans is achieved through phase I metabolic reactions by cytochrome P450 (CYP) enzymes CYP3A4, CYP1A2, and CYP2D6 and monoamine oxidase-A (18). Monoamine oxidases are essential enzymes responsible for metabolizing rizatriptan. Research has identified several mechanisms that support the effectiveness of triptans in the alleviation of migraine attacks. The most common mechanism is the activation of 5-HT receptors, which restrict the blood flow responsible for migraine attacks (13).

Although the general recommendation is that drugs should be avoided during pregnancy because of limited data, relief from migraine attacks is necessary. The orally dissolving tablet form (wafer) of rizatriptan is an effective treatment for migraines and

may be an especially good choice during the first trimester when oral intake may exacerbate nausea. Furthermore, a meta-analysis found that rizatriptan provides the highest pain-free rate at 2 hours (19).

Although there are differences in opinions in terms of the safety of triptans during pregnancy, the existing data suggest they may not be safe (6). If triptans are used during pregnancy, they should be taken under proper medical guidance.

The current study aimed to contribute to the limited literature by investigating the impact of rizatriptan on neural tube development in early chick embryos. The early chick embryo model sufficiently replicates embryonic development in mammals and is thus instrumental for studying the impact of chemicals on embryos during pregnancy.

## ■ CONCLUSION

This study demonstrated that rizatriptan has a negative effect on neural tube closure. Further research is needed to identify a safe and effective drug for treating migraines during pregnancy.

## ■ REFERENCES

- Amundsen S, Nordeng H, Nezvalová-Henriksen K, Stovner LJ, Spigset O: Pharmacological treatment of migraine during pregnancy and breastfeeding. *Nat Rev Neurol* 11:209-219, 2015
- Becker WJ: Acute migraine treatment in adults. *Headache* 55: 778-793, 2015
- Blair EM, Nelson KB: Migraine and preterm birth. *J Perinatol* 31: 434-439, 2011
- Bonnin A, Levitt P: Fetal, maternal, and placental sources of serotonin and new implications for developmental programming of the brain. *Neuroscience* 197: 1-7, 2011
- Boussier MG, Massiou H: Migraine in the reproductive cycle. In: Olesen J, Tfelt-Hansen P, Welch KMA, eds, *The Headaches*. New York: Raven Press, 1991: 413-419
- Cetinkal A, Colak A, Topuz K, Demircan MN, Simsek H, Berber U, Umur AS, Selcuki M, Vatansever HS: The effects of meloxicam on neural tube development in the early stage of chick embryos. *Turk Neurosurg* 20: 111-116, 2010
- Ehi Y, Şahin L, Mutlu MF: Migraine management in pregnancy. *Clin Exp Obstet Gynecol* 43: 661-665, 2016
- Facchinetti F, Allais G, Nappi RE, D'amico R, Marozio L, Bertozzi L, Ornati A, Benedetto C: Migraine is a risk factor for hypertensive disorders in pregnancy: A prospective cohort study. *Cephalalgia* 29: 286-292, 2009
- Frederick IO, Qiu C, Enquobahrie DA, Aurora SK, Peterlin BL, Gelaye B, Williams MA: Lifetime prevalence and correlates of migraine among women in a pacific northwest pregnancy cohort study. *Headache* 54: 675-685, 2014
- Gladstone JP, Eross EJ, Dodick DW: Migraine in special populations: Treatment strategies for children and adolescents, pregnant women, and the elderly. *Postgrad Med* 115: 39-50, 2004
- Hamburger V, Hamilton HL: A series of normal stages in the development of the chick embryo. *Dev Dyn* 88: 49-92, 1951
- Hauth JC, Ewell MG, Levine RJ, Esterlitz JR, Sibai B, Curet LB, Catalano PM, Morris CD; Calcium for Preeclampsia Prevention Study Group: Pregnancy outcomes in healthy nulliparas who developed hypertension. *Obstet Gynecol* 95: 24-28, 2000
- Nappi G, Sandrini G, Sances G: Tolerability of the triptans: Clinical implications. *Drug Saf* 26: 93-107, 2003
- Nezvalová-Henriksen K, Spigset O, Nordeng H: Maternal characteristics and migraine pharmacotherapy during pregnancy: Cross-sectional analysis of data from a large cohort study. *Cephalalgia* 29: 1267-1276, 2009
- Sanchez SE, Williams MA, Pacora PN, Ananth CV, Qiu C, Aurora SK, Sorensen TK: Risk of placental abruption in relation to migraines and headaches. *BMC Womens Health* 10: 30, 2010
- Silberstein SD: Migraine and pregnancy. *Neurol Clin* 15: 209-231, 1997
- Simbar M, Karimian Z, Afrakhteh M, Akbarzadeh A, Kouchaki E: Increased risk of pre-eclampsia (PE) among women with the history of migraine. *Clin Exp Hypertens* 32: 159-165, 2010
- Soldin OP, Dahlin J, O'Mara DM: Triptans in pregnancy. *Ther Drug Monit* 30: 5-9, 2008
- Thorlund K, Mills EJ, Wu P, Ramos E, Chatterjee A, Druyts E, Goadsby PJ: Comparative efficacy of triptans for the abortive treatment of migraine: A multiple treatment comparison meta-analysis. *Cephalalgia* 34: 258-267, 2014
- Williams MA, Peterlin BL, Gelaye B, Enquobahrie DA, Miller RS, Aurora SK: Trimester-specific blood pressure levels and hypertensive disorders among pregnant migraineurs. *Headache* 51: 1468-1482, 2011
- Wright GDS, Patel MK: Focal migraine and pregnancy. *Br Med J (Clin Res Ed)* 293: 1557,1558, 1986