Neurochirurgie 69 (2023) 101502

Contents lists available at ScienceDirect

Neurochirurgie

journal homepage: www.elsevier.com/locate/neuchi

Original Article

Effects of sodium fluoride on neural tube development in chick embryos

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ARTICLE INFO

Keywords:
Neural tube defect
Early chick embryo
Fluoride
Sodium fluori

ABSTRACT

Objective: Various environmental factors encountered in daily life are associated with the development of neural tube defects. This study aims to investigate the effects of fluoride on neural tube development in chick embryos.
Methods: A total of 60 specific pathogen-free, fertile, zero-day Leghorn-type eggs were used in the study. Group 1 was the control group, in which only saline was administered. Group 2 was the low-dose group, in which 0.003 mg of fluoride was administered, and Group 3 was the high-dose group, in which 0.006 mg of fluoride was administered. After 72 h of incubation, the embryonic disc was evaluated microscopically.
Results: In the control group, the surface ectoderm of all sections was intact, the neural tube was closed, and the neuroepithelium, the basement membrane surrounding the neuroepithelium, the somites, and the notochord displayed standard structure. Neural tube defects were observed in 3 of the chick embryos, that was given low-dose fluoride. In Group 3, which was administered high doses of fluoride, neural tube defects were observed in 4 embryos. It was observed that the development of neural tube defects was no statistically significantly higher in low and high-dose fluoride group compared to the control group.
Conclusion: Low and high-dose fluoride exposure was associated with developing neural tube defects, but there was no statisticaly significance.

1. Introduction

Neural tube defects (NTD) are congenital malformations affecting around 1 in every 1000 pregnancies and have multifactorial etiologies (chromosomal abnormalities, maternal nutritional deficiencies, environmental exposures, etc.). In vertebrates, neurulation consists of primary and secondary neurulation stages. Any disruption during these stages may lead to the development of congenital neural tube defects [1]. They present with a broad spectrum of neurological abnormalities, from anencephaly to sacral agenesis, and can result in severe medical and social problems and even death [1].

Maternal exposure to drugs or additives has been shown to cause spinal dysraphisms [1,2]. Maternal exposure to high doses of fluoride in the intrauterine period is known to cause some anomalies. Adding fluoride to drinking water to prevent tooth decay has been practiced for a long time. For this purpose, the recommended dose without any adverse effects is 1 mg/L [3]. Fluoride can easily pass through the maternal placental barrier, leading to maternal ingestion of high-dose fluoride that can be detected in maternal blood, fetal blood, and amniotic fluid [4–6]. Gupta at al. showed that an association between the exposure to fluoride during the prenatal period and the occurrence of spina bifida occulta, also the postnatal intake of fluoride was rather linked to the occurrence of dental and bone fluorosis in children [5]. However, there are a limited number of studies on this subject. This study aimed to investigate whether there is a relationship between fluoride exposure in chick embryos and the development of neural tube closure defects.

2. Material and method

Specific pathogen-free, fertile, zero-day Leghorn type 60 eggs with a weight of 65 ± 5 g obtained from Bornova Izmir Veterinary Experimental Animals Department were used in the study. The eggs were kept in an incubator for 26 h at a temperature of 37.8° ± 0.2°C and a humidity of 60 ± 5, with the pointy sides facing down. The incubator (Cimuka, CT60 S, Ankara) was used to control humidity and temperature distribution and to change the position of the eggs. Every 90 min, the eggs were turned along their vertical axes.

Abbreviations: NTD, Neural tube defects; NaF, sodiu mfluoride; HH, Hamburger–Hamilton.

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https://doi.org/10.1016/j.neuchi.2023.101502
Received 22 April 2023; Received in revised form 29 August 2023; Accepted 12 September 2023
Available online 21 September 2023

2.1. Incubation and injection

Embryos were processed for sodium fluoride (NaF) injection at Hamburger–Hamilton (HH) Stage 6 (HH6) (24 h). Calculations were made according to the daily fluoride dose for human (0.05 mg/kg) recommended by the National Academy of Sciences Institute of Medicine [7]. When similar studies were examined, it was determined that fluoride was applied as NaF [3,7,8]. A 0.003 mg dose of fluoride was calculated for eggs with an average weight of 60 g. When the molecular mass was added to the sodium, the adult NaF dose was calculated as 0.0066 mg, with the double dose being 0.0132 mg. NaF was dissolved in 0.1 cc saline. Three groups containing 20 eggs each were formed. Group 1 was the control group, which received only 0.1 cc of saline at the end of 24 h; Group 2 was the low-dose group, which received 0.003 mg/0.1 cc of fluoride; and Group 3 was the high-dose group, which received 0.006 mg/0.1 cc of fluoride.

A 5 mm hole was formed in the eggshell close to the blastoderm under sterile conditions for the injection process. Prepared solutions were administered under the embryonic disc. After the holes on the eggs were tightly closed with the help of sterile plaster, they were placed back in the incubator. The eggs were re-incubated until the end of 72 h. At HH stage 20 (at the end of 72 h), embryonic discs were identified, excised, and placed in distilled water heated to 35°C.

2.2. Microscopic examination

For microscopic examination, embryonic tissues were fixed in 10% neutral buffered formalin for 72 h. Four µm-thick sections were taken from paraffin blocks using a microtome (Medite M530). Embryos stained with hematoxylin-eosin were investigated for morphological changes in the neural tube, notochord, and neuroepithelium using a computerized photo-light microscope (Olympus BX51, Tokyo, Japan) with a CCD camera attachment (Olympus DP 72, Tokyo, Japan) to capture images from the sections.

2.3. Statistics

The obtained data were stored and analyzed using MATLAB R2022B Statistics and Machine Learning Toolbox Version 12.4. The Fisher's exact test was used to determine whether there was a significant difference between the embryos treated with 0.003 mg NaF, the embryos treated with 0.006 mg NaF and the control groups. A value of $p < 0.05$ was considered a statistically significant difference between the groups.

3. Results

Examination of the sections of 20 chick embryos in the control group revealed no neural tube defects in the transverse and sagittal planes. In all sections, the surface ectoderm was observed to be intact, the neural tube was closed, and the neuroepithelium, the basement membrane surrounding the neuroepithelium, the somites, and the notochord displayed typical structure (Fig. 1a and b). When 20 chick embryos in the group administered 0.003 mg of fluoride were examined, tube defects were observed in three samples (Fig. 2a and b). Other 17 embryos within the same group were observed to exhibit a similar neural tube structure compared to the control group. Evaluation of the sections of 20 chick embryos in the group administered 0.006 mg of fluoride revealed NTD and abnormal neuroepithelial proliferation towards the lumen in four embryo samples, and neural tube was intact in other 16 embryos (Fig. 3) (Tables 1 and 2).

The values of P was determined: as 0.106/group 3 vs. group1 and 0.2308/group 2 vs group1 (mid-P exact). As a result, there is no significant correlation in the two cases and the high-dose of fluoride has no correlation with the occurrence of NTD. Therefore, the development of neural tube defects is not statistically significantly higher in the high-dose fluoride group compared to the control group.

4. Discussion

Spinal neural tube defects are pathologies that can occur in a wide range from only bone closure defect (spina bifida occulta) to spinal cord closure anomalies (myelomeningocele, meningocele etc.). Neural tube defects are malformations that can affect multiple systems, resulting in neural tissue loss and vertebral anomalies. They often accompany distal bowel, urogenital, cardiac, and skeletal anomalies. Neural tube defects can occur due to many environmental factors. The most influential environmental factors include folate deficiency, air pollution, smoking, maternal drugs (anticonvulsants, etc.), or exposure to food additives [9]. Food additives, preservatives, colorants, and other substances mixed with soil or water through contamination may cause congenital spinal anomalies in early pregnancy [10–12]. Chick embryos are often used in studies investigating spinal cord anomalies caused by exposure to various substances [11,12]. Chicken eggs are preferred in similar studies due to their similarity to human neurulation, ease of follow-up, and

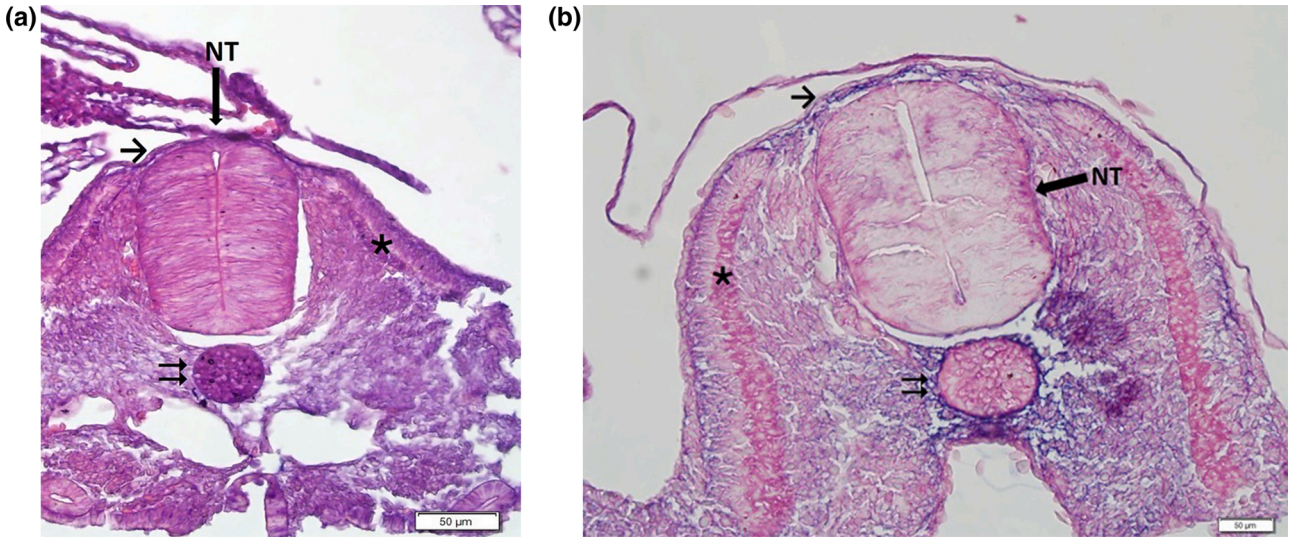

Fig. 1. (a and b): Control group, Neural tube (NT), notochord (⇉), somite (*), and surface ectoderm (→) exposed in the transverse plane under light microscopy (Hematoxylin and eosin, X20 µm).

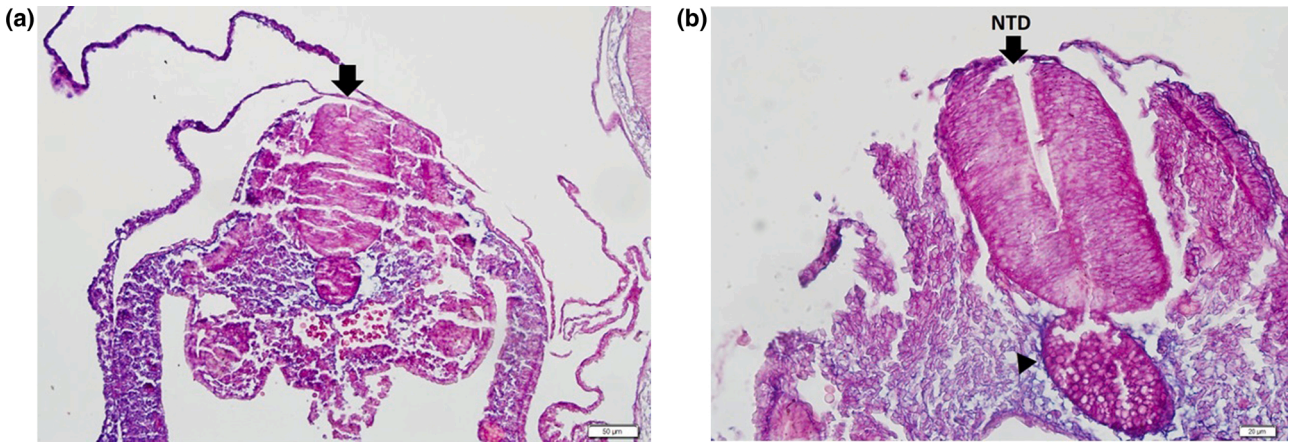

Fig. 2. (a and b): Low-dose fluoride group, neural tube defect (NTD) exposed in the transverse plane under light microscopy (hematoxylin and eosin, X50 μm). Vacuolized notochord in the neural tissue (▾) exposed in the transverse plane (Hematoxylin and eosin, X20 μm).

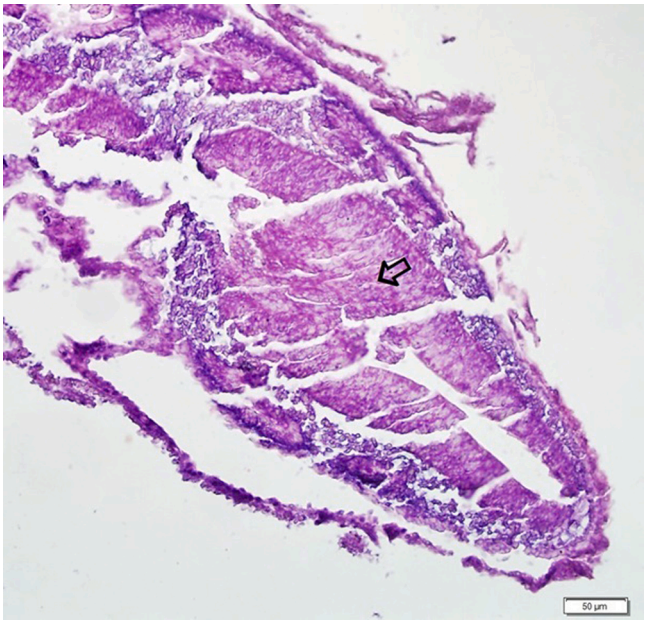

Fig. 3. High-dose fluoride group, neural tube defect (NTD) exposed in the transverse plane under light microscopy (Hematoxylin and eosin, X20 μm). Neuroepithelial proliferation (⇒) exposed in the sagittal plane.

Table 1
Distribution of chick embryos with neural tube defects by groups.

Groups	NTD	Normal tube
Group 1	0	20
Group 2	3	17
Group 3	4	16

Table 2
Statistical evaluation between groups.

Groups	Significance Level	P value
Group 2 vs. Control Group	5%	0.2308
Group 3 vs. Control Grup	5%	0.106

*$p < 0.05$.

rapid embryo development. Therefore, we preferred chick embryos to examine the effects of fluoride on neural tube closure in our study.

Fluoride is a naturally occurring mineral, and it is widely used in daily life. Exposure to higher doses of fluoride is possible in many ways. High doses of fluoride are available in tea extract, grape juice, soy products, toothpaste, and some non-steroidal anti-inflammatory agents, including soil [7]. Although fluoride is an essential natural substance for living organisms, excessive ingestion of fluoride has been shown to cause harmful effects on the brain, bones, teeth, and kidneys [13]. Fluoridation of water, which started in the mid-twentieth century and is still applied in some countries, may increase daily fluoride exposure and create chronic fluoride toxicity [14,15]. This chronic fluoride exposure negatively affects bones, kidneys, brain, and teeth.

Our estimated daily fluoride intake can be as high as 7 mg/L. Daily fluoride is often ingested as sodium fluoride rather than fluoride alone. Even tea alone causes a daily intake of 5−12 mg NaF [16].

Acute fluoride intoxication as a result of high-dose ingestion presents with stomach and intestinal symptoms, which can be confused with gastroenteritis. On the other hand, a condition called bone and dental fluorosis occurs in the event of chronic exposure to high doses of fluoride [8].

In experimental studies conducted to investigate its teratogenic effect, no pathology was observed in pregnant rats (27 mg/kg/day), where fluoride was administered at a dose greater than 300 mg/L. In contrast, visceral and skeletal abnormalities were observed in rat embryos at a dose of 40 mg/kg/day [4].

Our study investigated whether two separate doses of fluoride exposure caused a closure defect in the developing spinal cord. As a result, no statistically significant neural tube development was detected in the embryos that received the daily dose determined by the National Academy of Sciences Institute of Medicine. However, pathological findings were detected in 3 embryos in the group given 0.05 mg/kg/day and 4 in the group given 0.1 mg/kg/day.

In experimental studies, fluoride has been found to exert serious neurotoxic effects on learning and memory due to hippocampal neuron degeneration [17]. Additionally, there are studies showing that it disrupts myelination in cerebellar Purkinje cells and causes oxidative neuronal damage [18]. Maternal ingestion of high fluoride doses may cause permanent brain damage to the developing human embryo. High-dose fluoride ingestion has been shown to disrupt the structural integrity of the blood-brain barrier, causing astrocyte edema, mitochondrial degeneration, and microvascular endothelial cell dysfunction [18]. There are also prominent studies showing neurodevelopmental damage, adverse effects on neurobehavioral performance, and causing low IQ in children [19]. However, the working mechanism of low IQ is not yet fully elucidated. Guan et al. [20] demonstrated that the contents

of phospholipid and ubiquinone were modified in rat brains affected by chronic fluorosis, and these changes in membrane lipids could cause low IQ. In line with these adverse effects on neural tissue, the present study aimed to determine the effects of fluoride on neural tube development and closure.

In their study, Gupta et al. [5] formed two groups of 50 randomly selected children. The study group consisted of children aged 5–12 years, weighing 15–30 kg, who consumed fluoride-rich drinking water (4.5–8.5 mg/L fluoride) and displayed dental and skeletal fluorosis symptoms. The control group consisted of age- and weight-matched children who had 1.5 mg/L or less of fluoride in their drinking water and did not display any fluoride toxicity. Spina bifida occulta was detected in the lumbosacral region in 22 (44%) of 50 children in the study group and 6 (12%) of 50 children in the control group. In our study, a closure defect was observed in the group given low and high dose fluoride, although it was not statistically significant. In other words, our microscopic examination revealed that low and high-dose exposure was no associated with developing spinal dysraphism.

5. Conclusion

Closure defects were observed in groups administered both low and high doses of fluoride and were found to be no statisticaly significant in both chick embryos groups. This recent research was thought us that if we increase the number of our study group, the results of embryos receiving high and low doses of fluoride may be affected. As a result, it was concluded that fluoride should be carefully monitored in daily life and that future studies should be conducted with larger groups.

Conflict of interest

We don't have any conflict of interest and any financial support.

Ethics

The ethics committee of our hospital did not require approval because we used embryos younger than two weeks

Credit author statement

Mazhar Mammadov; Validation, Investigation.

Selin Tural Emon; Conceptualization, Methodology, Validation, Writing - Original Draft, Writing - Review & Editing.

Ezgi Akar; Software, Validation, Investigation, Data Curation, Writing - Original Draft, Supervision.

Dilek Akakin; Resources, Data Curation.

Dila Şener; Resources, Data Curation.

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