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ORIGINAL ARTICLE

Effect of ondansetron application on neural tube development in 48-hour chick embryos

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Abstract

The study aims to show that ondansetron, which is used safely in pregnant women, can cause serious side effects. Neural tube defects are among the most common congenital malformations of the central nervous system. It is known that genetic predisposition, environmental factors, and some drugs play an important role in the development of neural tube defects. Ondansetron is a selective 5-hydroxytryptamine-3 receptor antagonist used in the treatment of cancer, nausea, and vomiting during pregnancy and after anesthesia. In the literature studies, it was not found that developmental anomalies were observed. Seventy-five free specific pathogen eggs were incubated for 32 hours and divided into five groups of 15 eggs each, including a control group. Ondansetron was administered to these five groups by sub-blastoderm route in 4 different doses with a Hamilton microinjector. At 48 hours of incubation, the embryos were dissected and examined morphologically and histopathologically. At the end of the study, a significant dose-dependent decrease was observed in crown-rump lengths, somite numbers, and mean the number of silver-dyed nucleolar regulatory regions (AgNOR) and total AgNOR / nuclear area ratios. Statistically significant differences were observed between the experimental groups in terms of neural tube closure ($p < 0.05$). Ondansetron has been shown to affect neuronal development and vertebral growth in chicken embryos depending on increasing doses.

Keywords: *Chicken embryo, ondansetron, mRNA, neural tube defect*

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Introduction

Ondansetron is a fast-acting and safe antiemetic drug. It is often used after chemotherapy, in the first trimester of pregnancy, and due to nausea and vomiting associated with anesthesia. It exerts its pharmacological action as a 5-hydroxytryptamine receptor antagonist. It has a superior efficacy, safety, and pharmacoeconomic profile compared to other antiemetic groups. There are many different usage forms; which makes it a useful candidate for the treatment of women with persistent vomiting [1]. The most common side effects of pregnancy are nausea and vomiting [2]. It is most common in the first trimester, where organogenesis is most frequently affected.

Ondansetron is one of the most commonly used drugs for the treatment of pregnancy nausea and vomiting [3]. Ondansetron stands out because of its less sedation and superior antiemetic properties [4, 5]. Ondansetron use has been increasing, but unsafe data are still available [1].

Today, many different congenital malformations are encountered. Many factors are effective in the etiology. Environmental factors, genetic causes, and drug use during pregnancy are most common. Although the pregnancy categories of the new generation drugs used during pregnancy are certain, it is not known exactly whether they cause malformation due to their low use [6, 7]. It is the chick embryo that is most similar in development to the human embryo [7]. Animal studies on the use of ondansetron in pregnant women are not sufficient, and there is no information about neural tube defects in human use. Therefore, this study was designed to overcome this information gap in the literature.

Materials and Methods

This study was conducted after obtaining ethics committee approval (AKUHADYEK-49533702/45). 75 SPF eggs were divided into 5 groups. When creating groups: group 1; control group (No drug injection), group 2; ondansetron 0.08 mg / kg, group 3; ondansetron 0.16 mg / kg, group 4; ondansetron 0.32 mg / kg and group 5; ondansetron was determined to be 0.64 mg / kg.

All eggs were incubated at 37±0.5°C and turned automatically. Eggs removed from the incubator at the 32nd hour of the study were perforated in a sterile manner and appropriate doses of drugs were injected. The eggs, the holes of which were resealed, were placed in the incubator and removed from the device at the end of the 48th hour. Embryos removed with appropriate hand tools were examined with a light microscope. At this stage, the fore and aft length of the embryos in all groups, the number of somites, and whether the neural tubes were closed or not were determined. Embryos were then followed up histologically. Embryos passed through appropriate alcohol series were embedded in paraffin. Then, sections with a thickness of 5 μm were cut and subjected to AgNOR and Hematoxylin-Eosin staining [Figure 1].

The average AgNOR number and Total AgNOR region / Nuclear region (TAA / TNA) ratio were calculated for each nucleus [Figure 2]. mRNA threshold cycle values were calculated for genetic analyses.

Statistical analysis

While NT closures were analyzed with the chi-square test, Kruskal-Wallis tests were used for the analysis of other data. $p<0.001$ was considered significant. REST 2009 V2.0.13 software was used for genetic data analysis. Analysis of histological findings was done with IBM SPSS 22.0 software.

Results

In our study, neurological development retardation was investigated on embryos of 48-hour chicken eggs of ondansetron, which was given in four separate doses.

In the control group, the eggs in group 1 were not injected with ondansetron and the neural tube was closed in all 10 embryos and no developmental delay was observed. When the embryos were evaluated macroscopically under the light microscope, the mean crown-rump length was found to be 678.86±103.60. The mean number of somites was found to be 16.6±2.8. The mean TAA/NA ratio was 0.35 ±0.07, while the mean of AgNOR number was 17.5±1.8 (Table 1).

Group 2; The neural tube was found to be open in 7 of 10 embryos. The mean crown-rump length of the embryos was 650±98.46. The mean number of somites was found to be 15.5±2.4. When cell proliferation was evaluated by the AgNOR staining method in histological sections of embryos, the mean TAA/NA ratio was 0.30±0.05, while the average AgNOR number was 16.2±1.6 (Table 1).

Group 3; It was observed that the neural tube was open in 8 out of 10 embryos. The mean crown-rump length was found to be 609.81±80.54. The mean number of somites was found to be 13.9±2.2. The mean TAA/NA ratio was 0.28±0.03, while the average AgNOR number was 15.6±0.9 (Table 1).

Group 4; It was observed that the neural tube was open in 8 out of 10 embryos. The mean crown-rump length was 606.18±62.24. The mean number of somites was found to be 13.2±2.0. When the histological sections of the embryos were evaluated, the mean of the TAA/NA ratio was 0.25 ±0.02, while the average of the AgNOR number was 14.8±1.4 (Table 1).

Group 5; It was observed that the neural tube was open in 9 out of 10 embryos. The mean crown-rump length was 588.04±48.86. The mean number of somites was found to be 12.9±1.6. When the histological sections of the embryos were evaluated, the mean of the TAA/NA ratio was 0.24 ± 0.02, while the average of the AgNOR number was 14.3 ±1.0 (Table 1).

When NT closures were compared, significant differences were found between the groups ($p < 0.05$). While NT closure increased in a dose-dependent manner, all other parameters decreased in a dose-dependent manner. A dose-dependent neural tube defect was observed. As a result of the study, it was determined that fore and aft length, somite numbers, TAA / NA ratios, and AgNOR number averages decreased depending on the dose (Table 1).

A significant decrease was observed between group 1 and group 5 in the crown-rump length comparison (p=0.008). When the somite counts were compared, a significant decrease was observed between group 1, group 2, and group 5 (p=0.005). When AgNOR numbers were compared, a significant decrease was observed between group 1 and group 5 (p=0.015).

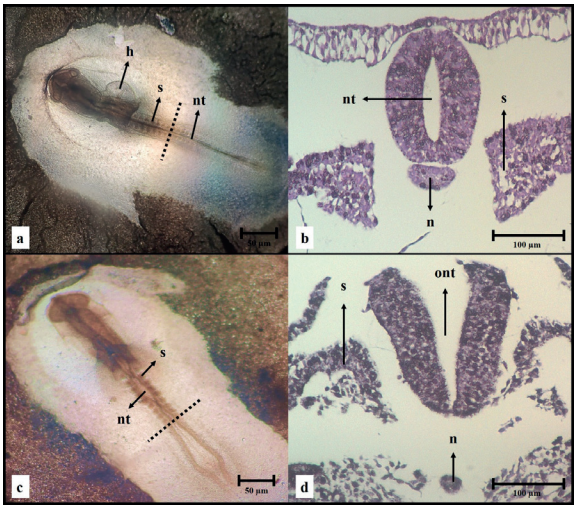

Figure 1. Effects of ondansetron on chick embryo development. Light microscope image of chick embryo in control (a), tissues stained with Hematoxylin and Eosin staining (H&E) in control (b), light microscope image of chick embryo with open neural tube (c), tissues stained with H&E in open neural tube (d), h, heart; n, notochord; nt, neural tube; ont, open neural tube; s, somite.

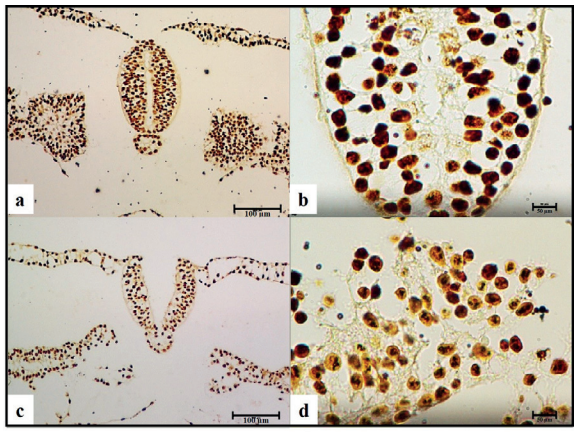

Figure 2. Counting nucleoli by determining random areas in tissues stained with AgNOR Staining Method. NT is closed (a-b), NT is open (c-d).

Table 1. Statistical analyses embryonic development in control and experimental groups (48-hour embryos)

Parameters	Group 1, Control	Group 2, Ondansetron 0.08 mg/kg	Group 3, Ondansetron 0.16 mg/kg	Group 4, Ondansetron 0.32 mg/kg	Group 5, Ondansetron 0.64 mg/kg	Significant P Values
Somite number	16.6±2.8	15.5±2.4	13.9±2.2	13.2±2.0	12.9±1.6	G5-G1 (P=0.005) G5-G2 (P=0.005)
Crown-rump length, mm	678.86±103.60	650±98.46	609.81±80.54	606.18±62.24	588.04±48.86	G5-G1 (P=0.008)
AgNor number	17.5±1.8	16.2±1.6	15.6±0.9	14.8±1.4	14.3 ±1.0	G5-G1 (P=0.015)
TAA/NA rate	0.35 ±0.07	0.30±0.05	0.28±0.03	0.25 ±0.02	0.24 ± 0.02	G5-G1 (P=0.002) G4-G1 (P=0.002) G3-G1 (P=0.002)
Open NT/closed NT	0/10	7/3	8/2	8/2	9/1	
Stage of embryo	13	12	11	11	11	

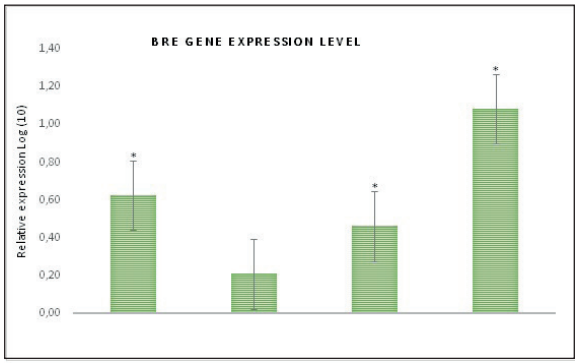

Figure 3. Relative mRNA expression of BRE in chick embryos exposed to different doses of ondansetron, given as fold regulation levels. GAPDH is the reference gene for normalization. * Represents the significance of $p < 0.05$.

When the TAA/NA ratios were compared, a statistically significant decrease was observed between the group and group 3, group 4, and group 5 ($p=0.002$).

BRE gene expression was observed to increase in embryos at each dose of ondansetron (group 2, 3, 4, 5) compared to the control group (group 1) (4.21; 1.61; 2.89; 12.11 fold regulation value, respectively). This increase was found to be statistically significant between groups 2, 4, and 5 when compared with the control group ($p < 0,05$). In addition to this result, the BRE gene expression was found to be increased in all embryos exposed to ondansetron in terms of mRNA levels compared to control (3.92; regulation value folds; $p<0.05$) [Figure 3].

Discussion

Today, ondansetron, which is used by many branches in the treatment of persistent nausea and vomiting, is a fast-acting and reliable 5-HT 3 receptor antagonist. The mechanism of action in controlling vomiting and nausea is not fully known. The effect of ondansetron in controlling nausea and vomiting caused by cytotoxic chemotherapy and radiotherapy is likely due to blocking 5-HT3 receptors in neurons in both the peripheral and central nervous systems.

According to the US Food and Drug Administration drug classification, the pregnancy category of ondansetron is designated as B. Ondansetron multiple hepatic cytochrome P-450 enzymes are metabolized by CYP3A4, CYP2D6, and CYP1A2. There is still timidity in terms of usage of ondansetron in human pregnancy.

Ondansetron is the most commonly used prescription oral antiemetic drug in pregnancy in the United States and was ranked as the fifth most commonly used oral drug in pregnancy in the Slone Epidemiological Center Birth Defects Study [3]. Compared to alternatives, ondansetron has been shown to have fewer side effects with less sedation and superior antiemetic effects [4, 5]. Regarding hyperemesis gravidarum treatment, half of the women receiving treatment in some emergency departments receive intravenous ondansetron [9].

Ondansetron crosses the placental barrier, therefore, concerns have been raised that its use in the first trimester of pregnancy may increase the risk of major congenital malformations. In this context, the findings from a meta-analysis of 6 cohorts and 2 case-control studies show that early pregnancy exposure to ondansetron increases the risk of increased heart defects and orofacial defects [10].

While many studies have shown ondansetron to be an effective and safe treatment in the treatment of hyperemesis gravidarum, there is still some concern about adverse effects on the fetus in a small number of studies [11].

Animal study models are very limited regarding whether there is a relationship between ondansetron and neural tube development. The first of the studies conducted on pregnant women was carried out by Anderka et al, and the increased risk of cleft palate associated with the use of ondansetron in the first trimester was found in women exposed to ondansetron [12].

Pasternak et al. compared the pregnant women who were prescribed and not prescribed ondansetron in the first trimester and reported that there was no significant major birth defect and cleft palate was not observed in women exposed to ondansetron (36 / 1,233) and those who were not exposed (141 / 4,932) [13].

In 2014, Danielson, like Pasternak and others, did not find a significant risk of malformation [14]. However, they found an increased risk of cardiovascular defects.

In their study, Damkier et al showed that ondansetron used in the first trimester of pregnancy did not increase the risk of cardiac malformation and that the oral cleft palate risk was only three for every 10,000 liveborn children exposed to ondansetron [15].

No teratogenicity has been reported regarding intravenous ondansetron therapy for hyperemesis gravidarum. When intravenously administered ondansetron and metoclopramide were compared in the treatment of hyperemesis gravidarum, it was reported that both had a generally preferred profile

in terms of side effects and showed similar clinical efficacy [4].

Animal studies on the use of ondansetron in pregnant women are not sufficient, and there is no information about neural tube defects in human use. Many models have been developed to show NT defects using chicken embryo models [16, 17]. The reason for choosing the chicken embryo model is that the first 48 hours of development are highly similar to the development of the mammalian spine. Therefore, different parameters were measured by using different doses of ondansetron in this model. The first parameter to be looked at was NT on or off, and the results showed that the use of ondansetron caused NT to remain open, depending on the dose [Table 1]. Nucleolar regulatory regions (NORs) are parts of metaphase chromosomes from which ribosomal genes are created. Throughout the interphase, NORs are linked to a large number of regulatory proteins and contain parts of the operative subunits of the nucleolus. They are where RNA organization and ribosomal gene transcription occur. NORs contain extremely acidophilic proteins, so NORs are seen sensitively and selectively by silver nitrate staining methods at the light microscope level (AgNOR). Changes in AgNOR protein levels also indicate the metabolic effects of cells. The AgNOR parameter, which was initially applied as a malignancy parameter, is useful for evaluating the prognosis of cancer [18, 19]. Our results showed that the average AgNOR number decreased in a dose-dependent situation (Table 1).

Another parameter examined was the average embryonic stages. The average embryonic stage was 13 in the control group and 11 in group 5. These results are similar to other studies [7, 20].

In our study, it was observed that the average crown-rump length and the number of somites decreased depending on the dose. This decrease was very significantly between the control group and group 5.

BRE gene is mainly detected in NT, nerve crest cells, somites, and neurites at the early embryonic development stage [21]. It has been suggested that when the BRE mRNA level decreases, somite development also decreases [20, 21]. Differently in our study, although BRE mRNA levels showed a dose-dependent increase, it was observed that somite counts decreased as the dose increased. In our study, the BRE gene was found to be significantly higher in group 5 compared to the control group and the second group. When the BRE mRNA was examined, no significant difference was observed between group 2 and the control group. BRE expression is affected by the high dosage.

Conclusion

Our study has shown that ondansetron has a direct teratogenic effect on the process of NT formation in chick embryos in a dose-dependent manner. These changes cannot be directly attributed to the human embryo. We interpreted our results according to genetic and histopathological findings. Studies with improved technical materials and larger sample sizes will be valuable in showing the possible dose-dependent toxic effects of ondansetron in the prenatal period. Findings obtained from all studies have shown that potential risks are less after the period of organogenesis and short-term treatment is always preferred. The present findings cannot be conclusive evidence of the use of ondansetron, but require caution in pregnancy use. When deciding on the use of ondansetron, it should be kept in mind that severe nausea and vomiting during pregnancy can cause significant physical and psychological morbidity to a degree suggesting termination of pregnancy in some women.

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Conflict of interest

"The authors declared that they have no competing interests to disclose."

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