Neurodevelopmental Effects of Teratogens on Chick Embryos: A Model Organism for Human Health

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The chick embryo (*Gallus gallus domesticus*), and its extraembryonic membranes, have been a commonly used model organism in developmental biology due to it being relatively easy to manipulate, inexpensive, and widely available (Coleman, 2008; Davey & Tickle, 2007). Chickens are one of the most valuable model organisms for medical and biological research and have profoundly influenced developmental biology since the 20th century (Yagamata, 2022).

By the time an egg is laid by a hen, development has already begun with a two-layered disc (blastodisc) of cells lying on top of the yolk (Davey & Tickle, 2007). The blastodisc is made up of the area pellucida (AP) and a peripheral, extraembryonic area opaca (AO); these areas are separated by a thin ring called the marginal zone (MGZ) (Lee et al., 2020). After being laid, while incubating, a series of events will determine body axes and define where its organs will develop (Davey & Tickle, 2007). After 15 hours of incubation, before gastrulation, the primitive streak forms from one edge of the AP, providing the first morphological sign of bilateral symmetry (Lee et al., 2020). As the embryo grows and changes shape, distinct structures such as the vascular system, head, trunk, tail, limbs, eyes, brain, and lungs form until, after 21 days of incubation, the chick hatches (Davey & Tickle, 2007).

The embryonic process that forms the brain and spinal cord is referred to as neurulation (Mete et al., 2017). At about 24 hours of development (during gastrulation), epiboly and elongation on the surface of the embryo bring about elongation of the presumptive neural ectoderm cells above the notochord (Mete et al., 2017). As gastrulation comes to an end, the neural ectoderm forms the neural plate, and its edges thicken to form neural folds, which form the neural groove along the midline of the plate (Gilbert, 2000). The neural folds migrate towards the midline and fuse to form the neural tube (Mete et al., 2017). At 33 hours, neurulation of the cephalic region is advanced, while the caudal region still undergoes gastrulation (Gilbert, 2000). Morphogen transcripts from signalling centres determine the axis formation of the neural tube, and at day five of development, the brain is divided into four distinct subsections (Zhou et al., 2015). Complete neurulation is important for the development of the brain and spinal cord (Mete et al., 2017).

Chickens share many morphological, genetic, and biochemical similarities with humans, making them an appropriate model organism to examine teratogenic actions and effects on human development (Wachholz et al., 2021). Teratogens are any agents or factors which cause abnormalities in the development of an embryo and include biological or environmental factors such as medications, recreational drugs, viral infections, chemical and physical agents, and environmental pollutants (Wachholz et al., 2021). The purpose of this paper is to review recent research studying the effects of three very different, known teratogens (Zika virus, cadmium, and alcohol) on chick embryo brain development as a model organism for human brain development.

Zika Virus as a Teratogen

A chick embryo model allows for the assessment of development without sacrificing the animal; by using this model organism, the damage of the Zika virus (ZIKV) can be observed throughout development, which is appealing because there is a need to describe the exact morphological changes causes by ZIKV teratogenesis (Wachholz et al., 2020). ZIKV has been linked to severe neurodevelopmental defects in human fetuses, such as microcephaly (Thawani et al., 2018; Wachholz et al., 2020). To study whether neural progenitors throughout the developing brain are equally susceptible to infection from ZIKV, Thawani et al. (2018) injected ZIKV into the midbrain ventricle of day two chick embryos just after neural tube closure. The timing point at which this study injected ZIKV would be comparable to the fourth week of human gestation. They traced the ZIKV infection and discovered it was consistently found in areas that determined the direction of nervous system development (anterior-posterior and dorsal-ventral) and that it reduced the transcript levels of several important morphogens: SHH, FGF8, and BMP7. Early specification of the central nervous system is determined by signalling centres for SHH, FGF8, and BMP7; these are conserved across vertebrates. For example, SHH acts as a dorsal to ventral signalling centre in the ventral midline (neural plate) of the embryonic neural tube of vertebrate organisms. ZIKV infection led to reduced midbrain size. Overall, their study found that ZIKV targets subpopulations of neural stem cells known to be signalling centres, which led to major alterations in neural patterning that may reflect the neural defects that occur in infected human populations. This study had a few limitations: injecting the virus directly into the embryonic brain does not mimic natural transmission routes, and the extent to which displaced brain tropism can be extended to mammals is, as of now, uncertain. However, it

would be tough to analyze the ZIKV mechanism in mammalian embryos because they are costly and difficult to examine longitudinally (Willard et al., 2017).

Wachholz et al. (2020) conducted a similar study with a less invasive approach to reduce any bias that could result due to excessive embryo manipulation. The researchers noninvasively dripped cell culture media containing the virus onto the chick embryo. This addresses one of the limitations in the study done by Thawani et al. (2018), where they injected the virus straight into the embryo's brain. Wachholz et al. (2020) found similar phenological alterations in neurotropism as Thawani et al. (2018), such as reduced midbrain size. Although Wachholz et al. (2020) did not study the specific developmental signalling occurring in neural development, the physical similarities in the chick embryo after a few days of exposure suggest that the proposed changes in important morphological signals in Thawani et al.'s (2018) study are promising. This research on chick embryos can lead to a nuanced understanding of the ways in which ZIKV influences neurulation, the timing and emergence of morphological abnormalities, and the exact molecular pathways involved in human health conditions (e.g., microcephaly), which could allow for screening of drugs with antiviral potential (Wachholz et al., 2020).

Cadmium as a Teratogen

Cadmium (Cd) is a persistent, bio-accumulative environmental pollutant with understood teratogenic effects (Wachholz et al., 2021; Yamamoto et al., 2012). Cd is a high-priority hazardous substance, yet it ends up in the environment through urban and industrial discharges (Yamamoto et al., 2012). Exposure to Cd during prenatal development has been associated with developmental defects, such as neural tube malformation and apoptosis of cells essential for neurodevelopment (Kmecick et al., 2019). When Yamamoto et al. (2012) started their study, the effects of Cd on early development were fairly unknown. Therefore, they decided to study its effects in chick embryos by injecting a solution containing Cd into the egg, separating its embryonic disc and observing its morphological alterations at 48 and 72 hours of development. These observations were compared to the well-established Hamburger and Hamilton (1951) chick embryo stages (stages 23 and 19, respectively). Embryonic alterations were highest in conditions of higher Cd concentrations and resulted in malformation of the cephalic region. At 48 hours, there were malformations of the forebrain subdivisions. They also noted that Cd is associated with the inhibition of neural signalling pathways that are essential for normal

development, so if neurotoxicity can be reduced or eliminated in early development, it may not have such detrimental effects on the developing embryo.

In a more recent study, Kmecick et al. (2019) aimed to use chick embryos as a model to understand the embryotoxicity of Cd to understand the risks of exposure in humans. Injections occurred between the blastula and the 72-hour stage of chick embryo development to mimic 4-5 weeks of human embryonic development. They note that organogenesis (e.g., neurulation) occurs during this time, making it critical to the future fate of the organism. When Cd was injected early in embryonic development, there was a failure to close the neural tube, and there were major cephalic defects. It is likely that these arise as a result of disruptions in early signalling that specifies cell proliferation and migration, an essential aspect of neural tube development. Research starting at the embryonic level can reveal potential for future research to examine the effects of long-term exposure to Cd, embryotoxicity, and human birth defects (Kmecick et al., 2019).

Alcohol as a Teratogen

Prenatal exposure to alcohol is one of the most preventable causes of neurodevelopmental disability in humans (Flentke & Smith, 2018). In humans, alcohol can pass through placental barriers and reach the fetus, causing developmental abnormalities such as Fetal Alcohol Spectrum Disorder (FASD), which is associated with neural tube defects (Mete et al., 2017). Chick embryos were likely the first model organism used to investigate alcohol teratogenicity, and they continue to be a valuable model organism for researchers to study teratogenic effects (Flentke & Smith, 2018). The molecular pathways that result in neurodevelopmental problems in humans are not well understood; however, alcohol has been linked to the repression of SHH, which impairs neural induction and alters neural crest proliferation (Flentke & Smith, 2018). Early exposure of chick embryos to alcohol (e.g., alcohol being placed directly on the blastodisc) results in apoptosis in the early brain and spinal cord, reduced neuronal migration and extensions, and problems forming the lumen of the neural tube (Flentke & Smith, 2018).

Mete et al. (2017) conducted a study where the embryo was rinsed with 70% ethanol, and developmental samples were examined at embryonic days 3, 5, and 8. At the macroscopic level, they observed deteriorations in brain vesicles and reduced head size. In the alcohol-treated groups of day three embryos, there was a delay in neural tube closure, notochord shrinking, and

reduction in neuroepithelial thickness. For the day seven embryos, there were significant changes in dorsal-ventral orientation, medullary cord maturation, white-grey matter, and other neural structures. The findings from this study demonstrate that applying alcohol before neurulation results in numerous neural tube and developmental defects and that application later also resulted in problems in neural development, perhaps because the cells cannot properly proliferate, migrate, or differentiate. Understanding what happens at certain timing points of chick embryo development after being exposed to alcohol can allow researchers to understand what may be occurring in humans and the mechanisms behind FASD.

Conclusion

This paper gave a general overview of recent research into the effects of teratogens on chick embryo neurodevelopment as a model organism for human conditions. Due to there being such a wide variety of teratogens, an emphasis was placed on examining three teratogens of differing origins: viral infections (ZIKV), environmental pollutants (cadmium), and recreational drugs (alcohol). All of the teratogens showed profound effects on neural development in early chick embryos.

Exposure to ZIKV resulted in changes to morphogens (SHH, FGF8, and BMP7) that are understood to establish the axes of neural development and reduced midbrain size (Thawani et al., 2018; Wachholz et al., 2020). The results of ZIKV teratogenesis led to several anomalies that appear similar to human microcephaly and brain malformation (Wachholz et al., 2020). Injection of Cd into chick embryos resulted in several neural tube defects (inability to close in early embryos) and cephalic malformations (reduced size and difficulty forming cephalic vesicles) (Kmecick et al., 2019; Yamamoto et al., 2012). The toxicity of Cd observed in the cephalic region of chickens may help explain similar malformations found in human conditions such as anencephaly, microcephaly, and holoprosencephaly (Kmecick et al., 2019). The discussion of Cd focused on phenological observations because that is what is available in the current literature, so further research could focus on the occurrence of common molecular initiating effects. Lastly, chick embryo exposure to alcohol resulted in widespread neurodevelopmental and neural tube defects, which could be, in part, due to the downregulation of signalling molecules such as SHH and disruptions in cell migration and proliferation (Flentke & Smith, 2018; Mete et al., 2017).

Because of similarities between human and chick development, researchers can correlate the finding of neural tube alterations in chickens following teratogen exposure with known human congenital malformations (Kmecick et al., 2019). Researchers are still trying to determine the specific molecular mechanisms of teratogens in chick embryos; however, Yagamata (2022) has proposed a project that may make this easier. This project has been coined “Towards tabula gallus” and will involve creating a map of every cell type in a chicken body and chick embryo. This cell atlas would be useful in many realms such as molecular biology, developmental biology, virology, neuroscience and will be beneficial for understanding human health and diseases.

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