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Dose-Dependent Developmental Toxicity of Cisplatin in Zebrafish (*Danio rerio*) Embryos

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Abstract

Cisplatin is a widely used chemotherapeutic agent in treating a variety of cancers. However, it can cause off-target toxic side effects in healthy tissues. Despite extensive research on cisplatin, there are still knowledge gaps surrounding how it affects embryonic development. Using zebrafish (*Danio rerio*) embryos as a model organism, we investigated the developmental effects of cisplatin exposure. Microscopic evaluation revealed a dose-dependent increase in developmental toxicity. Higher concentrations of cisplatin resulted in an increased incidence of spinal deformities, darkened yolk sacs, yolk sac edema, and pericardial edema indicating that cisplatin exposure disrupts normal embryonic development in zebrafish.

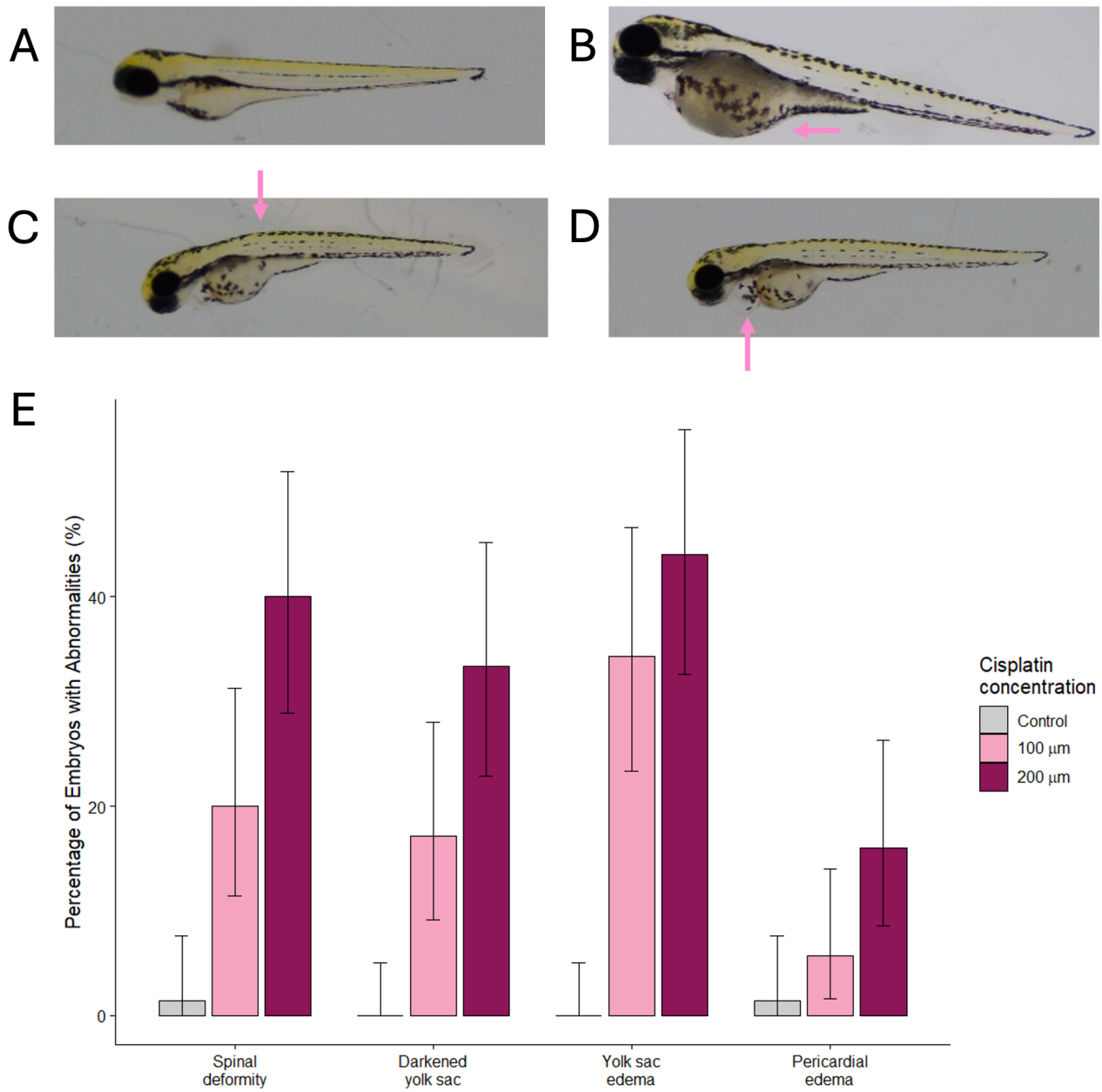

Figure 1. Morphological Abnormalities in Zebrafish Embryos Following Cisplatin Exposure:

Representative bright-field images of zebrafish embryos at 72 hours post-fertilization (hpf) showing (A) a morphologically normal control embryo, (B) an embryo exhibiting yolk sac edema and a darkened yolk, (C) an embryo exhibiting a spinal deformity, and (D) an embryo exhibiting pericardial edema. Images are representative of embryos observed across treatment groups. Arrows indicate the morphological abnormalities shown in the representative images. (E) Percentage of embryos exhibiting spinal deformities, darkened yolk sacs, yolk sac edema, or pericardial edema following exposure to 0, 100, or 200 µM cisplatin from 3-72 hpf. Number of biological replicates (n) and total embryos used per treatment group: 0 µM (n = 5, 121), 100 µM (n = 3, 82), 200 µM (n=4, 92).

Description

Cisplatin is a platinum-based chemotherapeutic used to treat various types of cancer. The drug exerts its cytotoxic effects by forming DNA adducts through binding guanine and adenine bases. As a result, it disrupts DNA replication and transcription, leading to cell cycle arrest and apoptosis (Brown et al., 2019). However, cisplatin can also cause off-target effects in healthy tissues, including developmental toxicity. Previous studies in zebrafish embryos have demonstrated that cisplatin exposure impairs normal development and reduces hatching success (Hung et al., 2019). Additionally, treatment with cisplatin in other model organisms, including mice and rats, has been shown to produce stage-dependent developmental toxicity (Keller et al., 1983). As the incidence of cancer continues to increase, the use of chemotherapeutic drugs such as cisplatin is also expected to rise. Therefore, understanding the developmental effects of cisplatin is

increasingly important because the drug can remain in the body for weeks, years, or even permanently in some organs following treatment (Breglio et al., 2017). The free form of cisplatin is also known to cross the placental barrier, potentially affecting maternal health and fetal development (Zemlickis et al., 1994). In addition, cisplatin is excreted in the urine, allowing it to enter aquatic environments where it may exert toxic effects on developing organisms (Turner et al., 2015).

Embryos are highly sensitive to toxic compounds because of their rapid cell proliferation and differentiation. Disruptions during early embryonic development can lead to morphological abnormalities, impaired organ formation, and lethality (Timme-Laragy et al., 2018). Zebrafish (*Danio rerio*) are an excellent model organism to examine development due to their genetic similarity to humans, rapid development, transparent embryos, and large clutch sizes (Hill et al., 2005).

Although cisplatin toxicity has previously been examined in zebrafish (Karas et al., 2019; Ceobanu et al., 2026), its effects on distinct morphological abnormalities during early embryonic development have not been fully characterized. In this study, we evaluated multiple developmental phenotypes following cisplatin exposure from 3 to 72 hpf and compared them to untreated control embryos (Figure 1A). We looked for spinal deformities, yolk sac edema, darkened yolk, and pericardial edema, and quantified the incidence of each phenotype across different cisplatin concentrations. These morphological abnormalities may reflect disruptions in different developmental processes and organ systems. Embryos exhibiting yolk sac edema and darkened yolk (Figure 1B) may indicate disruptions in osmoregulation and nutrient metabolism. The yolk sac serves as the primary source of nutrients during early zebrafish development, and abnormalities in this structure may indicate impaired yolk absorption and utilization, which may compromise growth and organ development (Fraher et al., 2018).

Beyond these yolk sac abnormalities, embryos exhibiting spinal deformities (Figure 1C) suggest that cisplatin may be interfering with cell proliferation, differentiation, apoptosis, or developmental signaling pathways involved in body axis and nervous system development. Skeletal and body curvature abnormalities are widely recognized indicators of developmental toxicity in zebrafish embryos and can impair mobility, balance, feeding, and survival after hatching (Li et al., 2022).

In addition to these spinal abnormalities, embryos exhibiting pericardial edema (Figure 1D) likely reflect disruptions in osmoregulation and cardiac function. The heart is one of the first functional organs in an embryo, and abnormalities in this structure suggest that cisplatin may impair cardiovascular development, heart function, or circulation, which can compromise delivery of oxygen and nutrients throughout the body, impacting the development of other organ systems (Kemmler et al., 2021).

Collectively, these abnormalities suggest that cisplatin disrupts multiple developmental processes including fluid homeostasis, nutrient utilization, nervous system formation, and cardiovascular development. Together, these findings indicate broad developmental toxicity rather than damage limited to a single tissue or organ system.

Through microscopy and statistical analysis, our results demonstrate that cisplatin exposure increases the incidence of developmental abnormalities in zebrafish embryos (Figure 1E). No mortality attributable to cisplatin treatment was observed during the exposure period. Therefore, no embryos or larvae were excluded from the analysis of developmental abnormalities due to cisplatin-related mortality prior to the 72 hpf assessment. Fisher's exact test revealed a significant overall effect of cisplatin treatment group on the incidence of all four abnormalities (spinal deformity: $p < 0.001$; darkened yolk sac: $p < 0.001$; yolk sac edema: $p < 0.001$; pericardial edema $p < 0.001$). Compared with controls, embryos exposed to 100 µM and 200 µM cisplatin exhibited significantly increased incidences of spinal deformities (20% and 40%), darkened yolk sacs (17% and 33%), yolk sac edema (34% and 44%), and pericardial edema (6% and 16%) relative to control. Direct comparison between the 100 µM and 200 µM groups showed a significantly higher incidence of spinal deformities and darkened yolk sacs at 200 µM relative to 100 µM (both $p < 0.001$), while yolk sac edema and pericardial edema did not differ significantly between the two treatment concentrations ($p = 0.240$ and 0.127, respectively). These findings indicate that cisplatin disrupts normal embryonic development in a largely dose-dependent manner and support the use of zebrafish embryos as a vertebrate model for evaluating developmental toxicity. Future studies should investigate lower, environmentally relevant concentrations of cisplatin to better understand its ecological and developmental impacts.

Methods

Zebrafish Breeding and Embryo Selection:

AB wild-type strain zebrafish (*Danio rerio*) were used. Embryos were obtained by natural spawning and raised at 28.5°C on a 14:10 h light/dark cycle in egg water 30 mg/L instant ocean in deionized water. Fertilized embryos were collected the following morning and screened under a stereomicroscope. Healthy embryos were staged according to hours post-fertilization (hpf), and unfertilized or fungus-contaminated embryos were removed.

Embryo Treatment:

Healthy embryos at 3 hpf were randomly assigned to treatment groups. 30 embryos were placed into each 100 mm petri dish containing 20 mL of egg water. Embryos were exposed to cisplatin at final concentrations of 0, 100, or 200 µM from 3 to 72 hpf. All embryos were maintained at 28.5°C under a 12 h light/12 h dark cycle throughout the exposure period.

Microscopy:

Embryos were examined using a stereomicroscope at 24, 48, and 72 hpf. Developmental abnormalities, including spinal deformities, cardiac abnormalities, developmental delay, edema, and other morphological defects, were documented. Fungus-contaminated embryos were removed immediately to prevent contamination of the remaining embryos. At 72 hpf, embryos were anesthetized with 0.016% tricaine (MS-222) and imaged using the NIC imaging system. Embryos that failed to hatch were manually dechorionated. Following imaging, embryos were euthanized and disposed of according to Belmont University laboratory safety protocols.

Statistical Analysis:

All statistical analyses were performed using R (version 4.6.1; R Core Team, 2026). Due to zero-count cells present in the control group, Fisher's exact test was used to compare the incidence of each developmental abnormality across treatment groups. For each abnormality, Fisher's exact test assessed the overall treatment effect, followed by pairwise Fisher's exact tests with Holm-adjusted p-values. A value of $p < 0.05$ was considered statistically significant.

Reagents

Strain	Genotype	Available from
AB	Wild-type Danio rerio (AB)	Zebrafish International Resource Center (ZIRC)

Reagent	Source	Description
Cisplatin	Cayman Chemical	Cis-Diammineplatinum (II) dichloride
Tricaine (MS-222)	MilliporeSigma	Ethyl 3-aminobenzoate methanesulfonate

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