A THESIS

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Exposure to Copper (II) Chloride Induces the Behavioral and Endocrine Changes in Zebrafish

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Abstract

The aim of this study is to identify copper (II) chloride ($CuCl_2$) effect in zebrafish. Zebrafish were exposed to different concentrations or time for measurements of median lethal concentration (LC_{50}) value. We performed measurement of whole-body cortisol level and behavioral parameters using the open field test (OFT) or the novel tank test (NTT) to evaluate stress responses. Zebrafish exposed to $CuCl_2$ solution at concentration of 1.5 - 150 μg/l or vehicle for 1 hour before behavioral tests or colleting samples for whole-body cortisol. In results, LC_{50} were 30.3, 25.3 and 14.8 μg/l in 24, 48 or 96 hours, respectively. As a result of NTT, distance moved, mobile duration and velocity were significantly decreased by the exposures to $CuCl_2$ compared with control group ($p<0.05$). On the other hand, turn angle was significantly increased by the exposure at concentration of 150 μg/l of $CuCl_2$ compared with control group ($p <0.05$). In addition, in OFT, distance moved, mobile duration and velocity were significantly decreased by the exposures at all concentrations of $CuCl_2$ compared with control group ($p <0.05$). On the other hand, meandering and turn angle was significantly increased by the exposure to $CuCl_2$ compared with control group ($p <0.05$). Whole-body cortisol levels were significantly increased by the exposure to $CuCl_2$ compared with control group ($p <0.05$). In conclusion, these results of study suggest that the exposure of $CuCl_2$ induces the toxic response or the stress response on lethality, behavioral changes and whole-body cortisol level in zebrafish.

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I. Introduction

Organisms in the aquatic environment are exposed to contamination of various organisms or minerals [6]. Globally, aquatic ecosystems are continuously polluted from toxic factors by commercial or industrial activities, such as aquaculture [24], and chronic or acute exposures of anthropogenic pollutants might lead to negative consequences to aquatic organisms [5].

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Heavy metals generally refer to metals with high molecular weight, high density and high atomic number. Some heavy metals are used as essential nutrients such as iron, cobalt, zinc and copper, and partial heavy metals such as ruthenium, silver and indium are relatively harmless. However, most heavy metals are known to be highly toxic. Interestingly, many studies demonstrated that excessive exposures to heavy metals induced some adverse actions to ecosystems as well as organisms [49]. Copper is a metal commonly used in industry and in life, such as copper pipes. Copper pipes are strong enough to withstand thermal deformation because they have good stretch ability, are cheap, and have excellent corrosion resistance, so they are frequently used to transport water, which transports water to homes and industrial sites. Copper pipes with these various advantages can also cause problems. Even if the quality of raw water or treated water meets environmental standards, secondary water contamination due to internal corrosion of water treatment pipe or network during transportation may occur [2, 25].

Copper, which is an essential micronutrient, exists in the form of Cu^{+} or Cu^{2+} in vivo as a structural cofactor of a variety of catalytic and structural enzymes which have a part of energy metabolism, mitochondrial respiration, and antioxidant defense [36]. The crucial role of copper is through the direct interaction of amino acid residues and polypeptide chains with activation of structural change catalysis and protein-protein interactions [18, 31].

Nowadays, concerns are growing about copper exposure in various environments, whereas the related research is insufficient. Therefore, we performed analysis of median lethal concentration, whole-body cortisol and behavioral pharmacological to investigate toxicity to copper.

II. Materials and Methods

1. Animals

We used 5-6 month-old wild-type zebrafish purchased from World-fish aquarium (Jeju-si, Republic of Korea). All fish were acclimated for at least two weeks in the experimental room and maintained in constant temperature (26 ± 1℃) tanks with aerated water. Fish were kept on a 14–10 hour light/dark cycle (lights on from 07:00-21:00 hour) and fed two times a day with TetraMin (Tetra, Germany) commercial flakes. During experiment, zebrafish were not fed. Animal treatment and maintenance were conducted in accordance with the Principles of Laboratory Animal Care (NIH Publication No. 85-23, 8th edition).

2. Measurement of Median lethal concentration (LC_{50})

The estimates of median lethal concentration of $CuCl_2$ were conducted according to the OECD Guidelines for the Testing of Chemicals Test No. 203: Fish, Acute Toxicity Test [29]. Zebrafish were randomly selected and were put into the 1-liter beaker filled with vehicle or $CuCl_2$ solutions (1 g fish/300 ml). We conducted pilot studies to determine a valid concentration or exposure time. The concentrations increased in geometric ratio of 1.4. Vehicle or $CuCl_2$ solutions were replaced with fresh ones every 24 hours. Mortality after 24, 48 and 96 hours exposure were recorded and median lethal concentrations of $CuCl_2$ (LC_{50}) were computed by probit calculations to get regression line of each time.

3. Drug treatment

We did drug treatment before behavioral test and measurement of whole-body cortisol level. Copper chloride (Sigma-Aldrich, St. Louis, MO, USA) dissolved in distilled water and treated at 4 concentrations of 0, 1.5, 15 and 150 μg/l for 1 hour. Zebrafish were randomly selected and were placed in a each concentarion $CuCl_2$ solution.

4. Novel tank test (NTT)

To assess the effect of $CuCl_2$ exposure on zebrafish behavior, NTT was performed according to the method of Robinson *et al* [27] between 11:00 and 15:00 hour. The zebrafish (n= 8-10 in each group) were put to the tank (15 cm height × 28 cm top × 23 cm bottom × 7 cm width) maximally filled with water and divided into two equal virtual horizontal portions. Zebrafish behaviors were recorded with subsequent automated analysis of generated traces by Ethovision XT 8.5 software (Noldus IT, Wageningen, Netherlands) from the side view for 6 minutes to calculate the distance moved, velocity, mobile duration, turn angle and duration in top.

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5. Open Field test (OFT)

To assess the effect of $CuCl_2$ exposure on zebrafish behavior, OFT was performed according to the method of Cachat *et al* [10] between 11:00 and 15:00 hour. The zebrafish (n= 8-10 in each group) were put to the white plastic cylinder (22 cm bottom × 24 cm top × 20.5 cm height) filled with 4litter of water and divided into two portions. Zebrafish behaviors were recorded with subsequent automated analysis of generated traces by Ethovision XT 8.5 software (Noldus IT, Wageningen, Netherlands) from the side view for 6 minutes to calculate the distance moved, velocity, mobile duration, turn angle and meandering movement.

6. Measurement of whole-body cortisol level

Extraction of cortisol was using the method of Barcellos *et al* [20]. Zebrafish were sacrificed by tricaine (Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 150 mg/l to obtain the body fluid. After the skin moistness of the zebrafish was dried, it was put into a prepared cryo tube with 2 ml of 0.1 M phosphate buffered saline (PBS) for homogenization. The 5 ml of diethyl ether was put into a cryo tube, then voltexing for 1 minute 3 times. After which, Samples were centrifuged (Hanil, Korea) at 4,000 g for 15 minutes and were rapidly cooled for 45 seconds by liquid nitrogen to move supernatant to the test tube. The test tubes containing a sample were evaporated by vacuum evaporator (CVE-2000, EYELA, Japan) to remove diethyl ether from the sample. After the evaporation of diethyl ether, 1 ml of 0.1 M PBS was added to the test tube, and the content was moved to a 1.7 ml tube. The tube was then stored at -20℃ until it was submitted for cortisol measurement.

Whole-body cortisol level was measured using a cortisol assay kit (R&D system, Minneapolis, MN, USA). To analyze the ELISA plate, the absorbance was measured at 450 nm using a microplate reader (Molecular Device, San Jose, CA USA). The absorbance value was converted to cortisol concentrations based on 4-parameter sigmoid minus. Whole-body cortisol was expressed the ratio of concentration to weight of each fish.

7. Statistical analysis

Values are expressed as the means ± S.E.M. Data were analyzed by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test for multiple comparisons. Statistical significance was set at $p<0.05$.

III. Results

1. $CuCl_2$ time-dependently increases mortality of zebrafish

Mortality after 24, 48 and 96 hours exposure were recorded and median lethal concentrations of $CuCl_2$ (LC_{50}) were computed by Biostat LE program to get regression line of each time. The LC_{50} values of $CuCl_2$ were 30.3, 25.3 or 14.8 μg/l in the 24, 48 or 96 hours, respectively (Fig. 1).

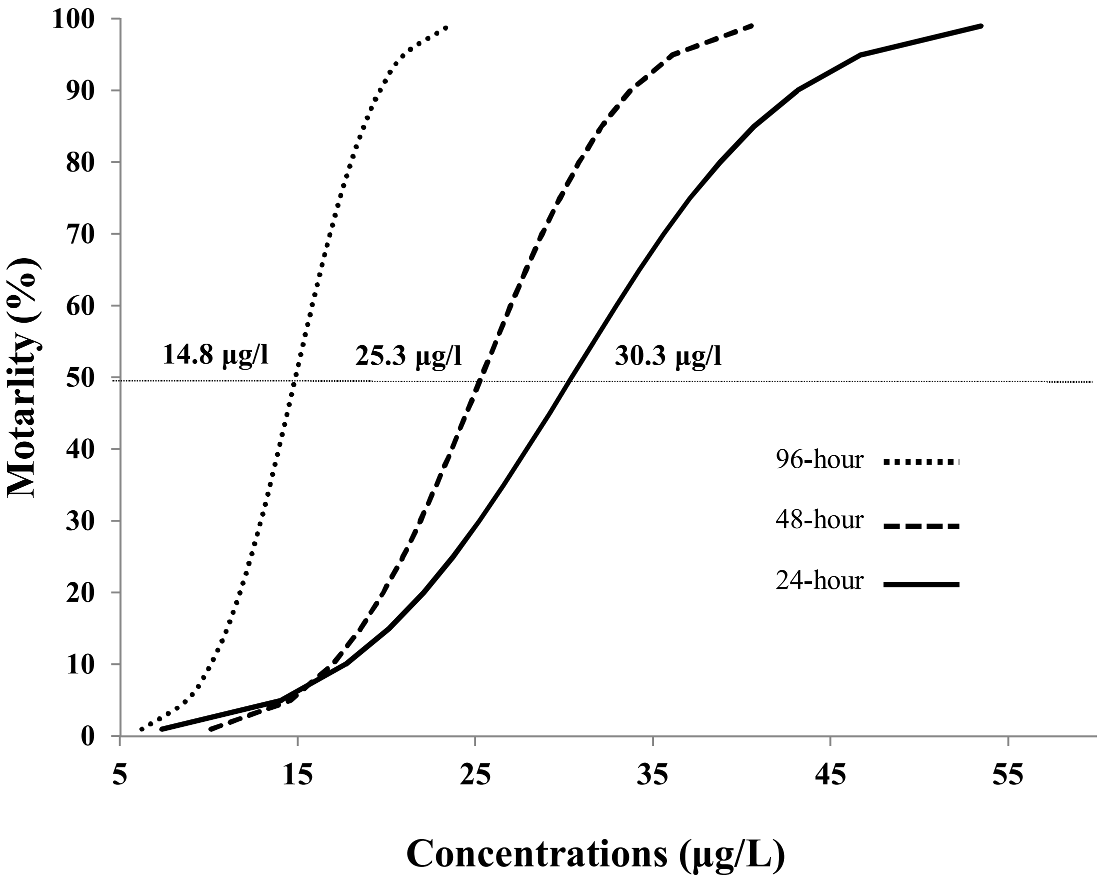


Figure 1. Probit analysis for the median lethal concentrations value of $CuCl_2$ in zebrafish. The graph shows the probit values of observed and expected percentage of mortality.

2. $CuCl_2$ induces anxiety-like behaviors on novel tank test (NTT) in zebrafish

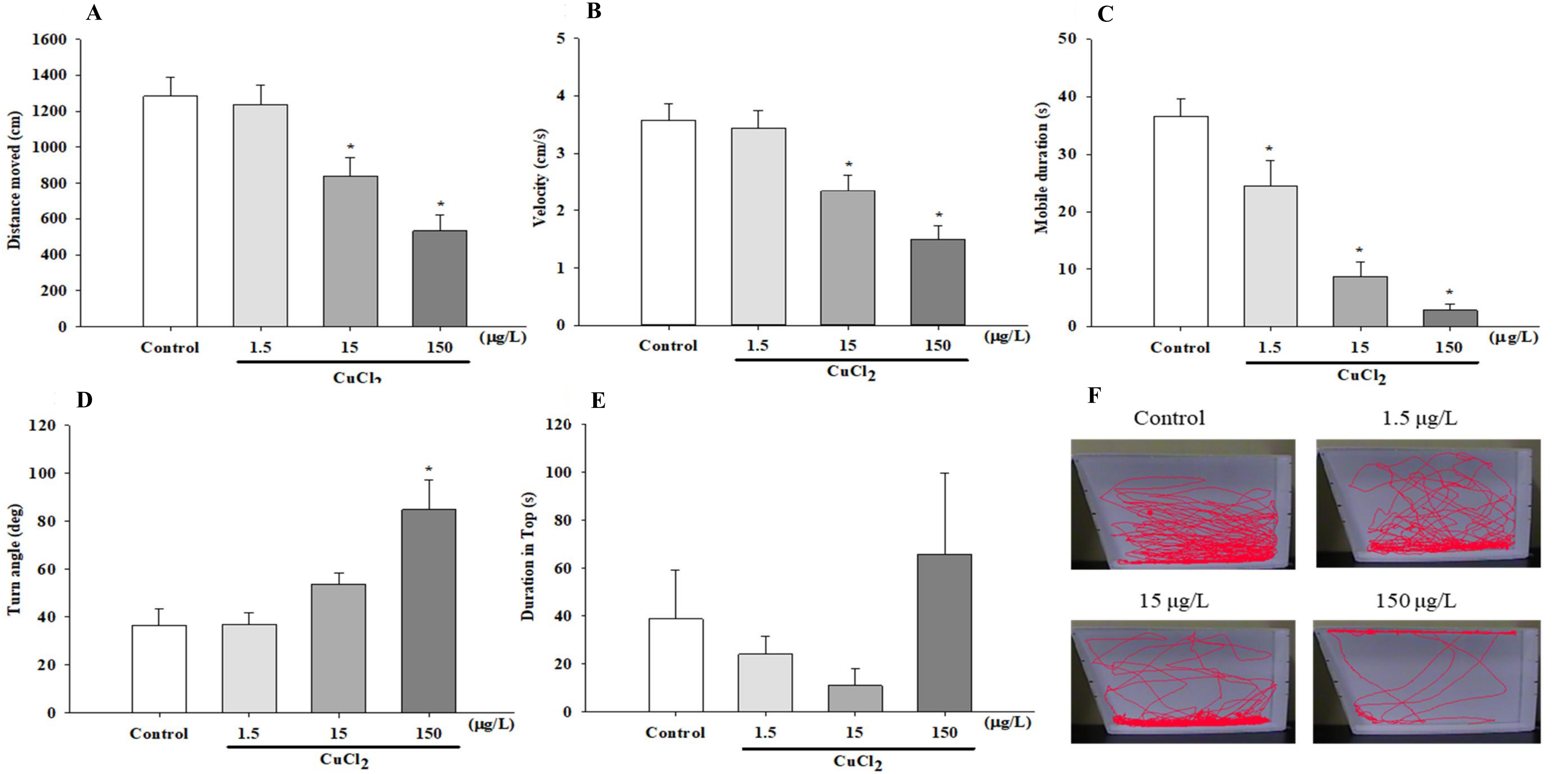


Figure 2. $CuCl_2$ increases anxiety on the novel tank test in zebrafish. (A) Distance moved, (B) velocity, (C) mobile duration, (D) turn angle and (E) duration in top after exposure of $CuCl_2$ (1.5, 15 or 150 μg/l) for 1 hour. Each bar represents mean ± S.E.M. of 8-10 animals. P values for the group comparisons were obtained by one-way ANOVA followed by Student-Newman-Keuls test (*$p<0.05$ vs. control). Visual data were analyzed through Ethovision XT 8.5 software (F).

2.1. Distance moved

As a result of measuring the distance moved for 6 minutes in the NTT, the distance moved values were respectively 1,282.9 ± 105.5, 1,236.2 ± 109.8, 840.9 ± 98.7 or 530.8 ± 89.3 cm at the concentrations of control group, 1.5, 15 or 150 μg/l of $CuCl_2$. In the $CuCl_2$-treated groups at the concentrations of 15 and 150 μg/l, the distance moved values significantly decreased compared with control group, but not significantly decreased at the concentration of 1.5 μg/l of $CuCl_2$ compared with control group ($p<0.05$; Fig. 2A).

2.2. Velocity

As a result of measuring the velocity for 6 minutes in the NTT, the velocity values were respectively 3.6 ± 0.3, 3.4 ± 0.3, 2.3 ± 0.3 or 1.5 ± 0.3 cm/s at the concentrations of control group, 1.5, 15 or 150 μg/l. In the $CuCl_2$-treated groups at the concentrations of 15 and 150 μg/l, the velocity values significantly decreased compared with control group, but not significantly increased at the concentration of 1.5 μg/l of $CuCl_2$ compared with control group ($p<0.05$; Fig. 2B).

2.3. Mobile duration

As a result of measuring the mobile duration for 6 minutes in the NTT, the mobile duration values were respectively 36.6 ± 3.0, 24.5 ± 4.4, 8.7 ± 2.5 or 2.8 ± 1.1 seconds at the concentrations of the control group, 1.5, 15 or 150 μg/l of $CuCl_2$. In the $CuCl_2$-treated groups at the concentrations of 1.5 – 150 μg/l, the mobile duration values significantly decreased compared with control group ($p<0.05$; Fig. 2C).

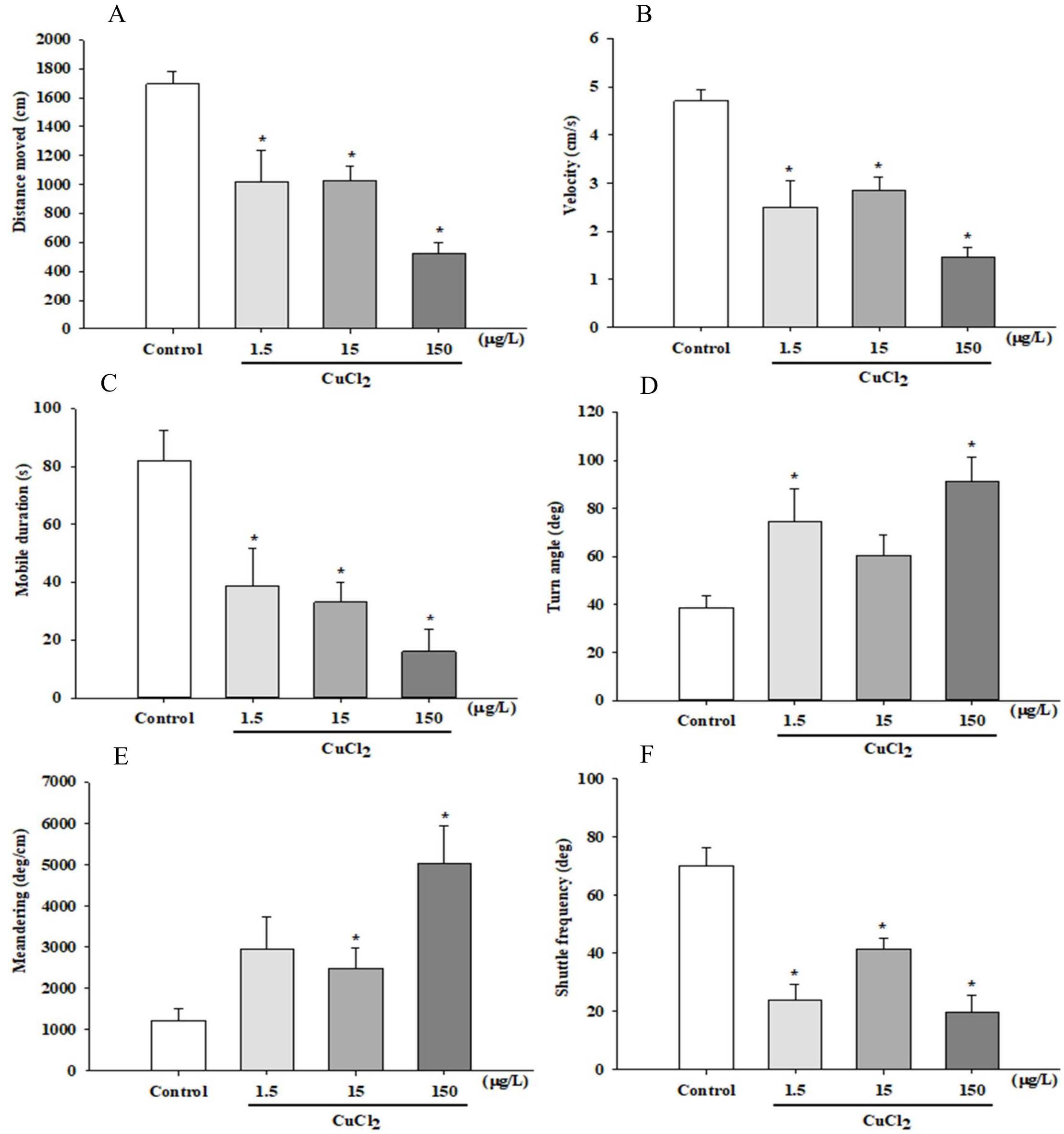
2.4. Turn angle

As a result of measuring the turn angle for 6 minutes in the NTT, the turn angle values were respectively 36.5 ± 6.6, 36.7 ± 5.1, 53.7 ± 4.7 or 84.9 ± 12.2 deg at the concentration of the control group, 1.5, 15 or 150 μg/l of $CuCl_2$. In the $CuCl_2$-treated groups at the concentration of 150 μg/l, the turn angle values significantly increased compared with control group, but not significantly increased at the concentrations of 1.5 and 15 μg/l of $CuCl_2$ compared with control group ($p<0.05$; Fig. 2D).

2.5. Duration at the top

As a result of measuring the duration at the top for 6 minutes in the NTT, the duration at the top values were respectively 38.8 ± 20.6, 24.0 ± 7.6, 10.9 ± 7.0 or 65.9 ± 33.7 seconds at the concentrations of the control group, 1.5, 15 or 150 μg/l of $CuCl_2$. In the $CuCl_2$-treated groups at the concentrations of 1.5 - 150 μg/l, the duration at the top values did not significantly change compared with control group (Fig. 2E).

3. $CuCl_2$ induces anxiety-like behaviors on Open Field test (OFT) in zebrafish



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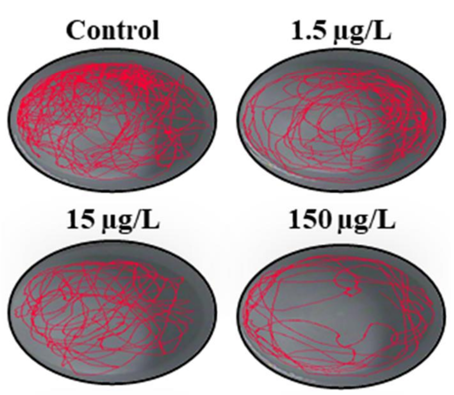

Figure 3. $CuCl_2$ increases anxiety on the open field test in zebrafish. (A) Distance moved, (B) velocity, (C) mobile duration, (D) turn angle, (E) Meandering and (F) shuttle frequency after exposure of $CuCl_2$ (1.5, 15 or 150 μg/l) for 1 hour. Each bar represents mean ± S.E.M. of 8-10 animals. P values for the group comparisons were obtained by one-way ANOVA followed by Student-Newman-Keuls test (*p<0.05 vs. control). Visual data were analyzed through Ethovision XT 8.5 software (G).

3.1. Distance moved

As a result of measuring the distance moved for 6 minutes in the OFT, the distance moved values were respectively 1,694.1 ± 87.6, 1,022.7 ± 212.0, 1,028.4 ± 98.8 or 526.4 ± 72.7 cm at the concentrations of the control group, 1.5, 15 or 150 μg/l of $CuCl_2$. In the $CuCl_2$-treated groups at the concentrations of 1.5 – 150 μg/l, the distance moved values significantly decreased compared with control group (p<0.05; Fig. 3A).

3.2. Velocity

As a result of measuring the velocity for 6 minutes in the OFT, the velocity values were respectively 4.7 ± 0.2, 2.5 ± 0.5, 2.9 ± 0.3 or 1.5 ± 0.2 cm/s at the concentrations of the control group, 1.5, 15 or 150 μg/l of $CuCl_2$. In the $CuCl_2$-treated groups at the concentrations of 1.5 – 150 μg/l, the velocity values significantly decreased compared with control group (p<0.05; Fig. 3B).

3.3. Mobile duration

As a result of measuring the mobile duration for 6 minutes in the OFT, the mobile duration values were respectively 82.0 ± 10.7, 38.8 ± 12.9, 33.2 ± 6.9 or 16.1 ± 7.7 seconds at the concentration of the control group, 1.5, 15 or 150 μg/l of $CuCl_2$. In the $CuCl_2$-treated groups at the concentrations of 1.5 – 150 μg/l, the mobile duration values significantly decreased compared with control group (p<0.05; Fig. 3C).

3.4. Turn angle

As a result of measuring the turn angle for 6 minutes in the OFT, the turn angle values were respectively 38.8 ± 4.9, 74.8 ± 13.4, 60.5 ± 8.5 or 91.3 ± 10.1 deg at the concentration of the control group, 1.5, 15 or 150 μg/l of $CuCl_2$. In the $CuCl_2$-treated groups at concentrations of 1.5 and 150 μg/l, the turn angle values significantly increased compared with control group, but not significantly increased at the concentration of 15 μg/l of $CuCl_2$ compared with control group (p<0.05; Fig. 3D).

3.5. Meandering

As a result of measuring the meandering movement for 6 minutes in the OFT, the meandering movement values were respectively 1,227.1 ± 268.5, 2,959.8 ± 773.0, 2,481.7 ± 494.4 or 5,039.1 ± 895.2 deg/cm at the concentration of the control group, 1.5, 15 or 150 μg/l of $CuCl_2$. In the $CuCl_2$-treated groups at concentrations of 1.5 and 150 μg/l, the meandering movement values significantly increased compared with control group, but not significantly increased at the concentration of 15 μg/l of $CuCl_2$ compared with control group (p<0.05; Fig. 3E).

3.6. Shuttle frequency

As a result of measuring the shuttle frequency which is the number of time zebrafish move from wall to the central arena, the shuttle frequency values were respectively 70.3 ± 6.1, 23.8 ± 5.6, 41.4 ± 3.8 or 19. 8 ± 5.68 deg at the concentration of the control group, 1.5, 15 or 150 μg/l of $CuCl_2$. In the $CuCl_2$-treated groups at the concentrations of 1.5 – 150 μg/l, the shuttle frequency values significantly decreased compared with control group (p<0.05; Fig. 3F).

4. Effect of $CuCl_2$ on Whole-body cortisol

The whole-body cortisol levels collected from control and three treatment groups are showed in the Fig. 4. Whole-body cortisol level of control was 30.63 ± 4.14 ng/g. Whole-body cortisol level of three treatment groups were respectively 53.3 ± 2.4, 55.0 ± 6.0 or 41.2 ± 8.0 ng/g at the concentrations of the 1.5, 15 or 150 μg/l. In the $CuCl_2$-treated groups at the concentrations of 1.5 and 15 μg/l, the whole-body cortisol level values significantly increased compared with control group, but not significantly increased at the concentration of 150 μg/l of $CuCl_2$ compared with control group (p<0.05).

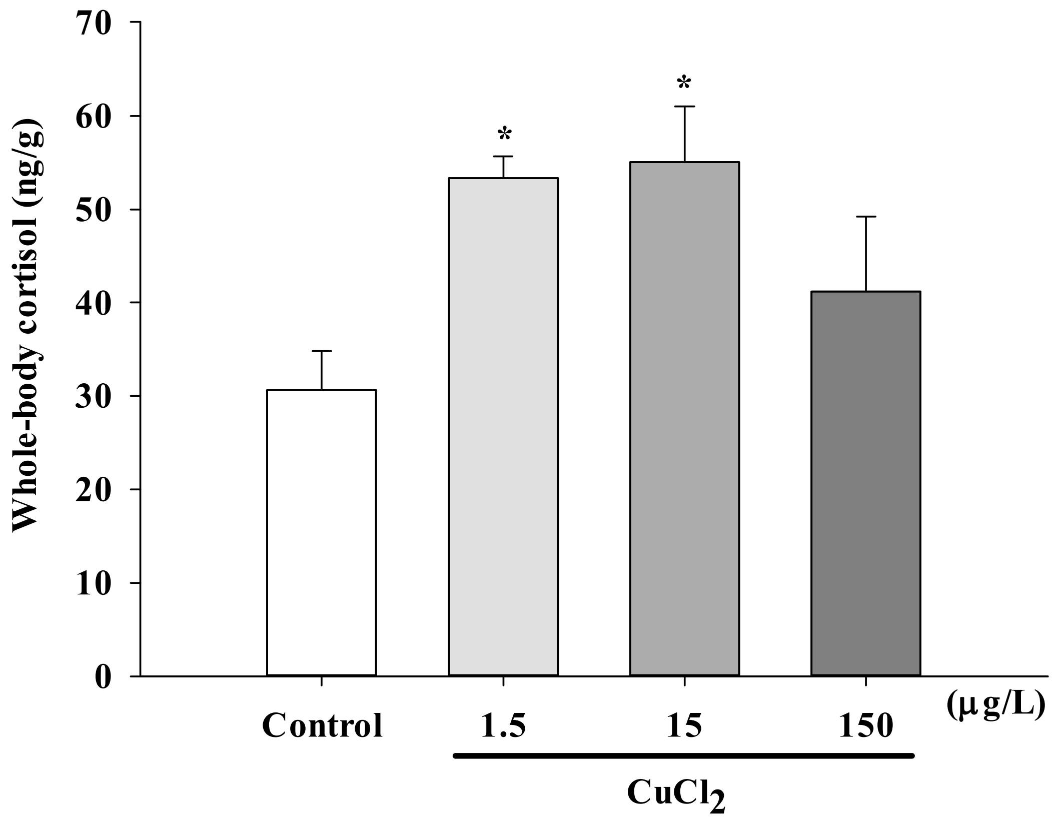

Figure 4. $CuCl_2$ increases whole-body cortisol level. Zebrafish were exposed by $CuCl_2$ (1.5, 15 or 150 μg/l) for 1 hour. Each bar represents the mean ± S.E.M. of the whole-body cortisol level of 3-4 animals. P values for the group comparisons were obtained by one-way ANOVA followed by Student-Newman-Keuls test (*p<0.05 vs. control)

IV. Discussion

Copper, which is an essential micronutrient, exists in the form of Cu^{+} or Cu^{2+} in vivo as a structural cofactor of a variety of catalytic and structural enzymes which have a part of energy metabolism, mitochondrial respiration, and antioxidant defense [36]. Although copper is also an essential micronutrient on the aquatic organisms, it should be approached with further cautions because aquatic organisms are 10 - 100 times more susceptible to the toxic effects of copper than are mammals [39]. We performed measurement of median lethal concentration, whole-body cortisol level and behavioral tests to investigate toxicity of copper in zebrafish. As a result of the above-mentioned experiment, The LC_{50} values of $CuCl_2$ were 30.3, 25.3 or 14.8 μg/l in the 24, 48 or 96 hours, respectively (Fig. 1), and the related mobility parameters including distance moved, mobile duration and velocity are significantly decreased compared with control group ($p<0.05$; Fig. 2 and Fig. 3). Interestingly, whole-body cortisol levels were significantly increased by exposures at concentrations of 1.5 and 15 μg/l of $CuCl_2$ compared with control group, but not in 150 μg/l of $CuCl_2$ ($p<0.05$; Fig. 4).

In this study, 48h-LC_{50} value of $CuCl_2$ was 25.3 μg/l though 48h-LC_{50} value of $CuCl_2$ was 130 μg/l in the study of Griffitt *et al.* [22]. Many studies demonstrated that hardness of water affected the mortality by toxicities of heavy metals in zebrafish [4]. Pascoe *et al.* demonstrated that cadmium is less toxic in hard water than in soft water in toxicity tests with rainbow trout [32]. Moreover, Pourkhabbaz *et al.* reported that the toxicity of Co and Ni increased with decreasing water hardness in *Capoeta fusca* [1]. Actually, in low hardness of water, toxicities of heavy metals were revealed more strongly. Therefore, we assume that lower LC_{50} value was observed because of using distilled water (hardness: 3.4×10^{-4} mmol/l) as solvent. In addition, LC_{50} values of $CuCl_2$ decreased time-dependently. It might be considered that the toxic effect is severe by extension of exposure time to $CuCl_2$.

Exposures to environmental threats induce some abnormal behaviors that reflect the condition of animals and their response for escape [26]. When they are exposed to unaccustomed or a potentially perilous environments, anxious reaction is not only natural response but also essential response to survive [11, 15]. Behavioral tests are used to measurement of various condition such as smell relation behavior [28], anxiogenic [47], addiction [16], social behavior [17, 43], sleep [16], memory [48] and toxic responses [13, 44] in zebrafish.

NTT is one of zebrafish vertically behavioral test that is an experiment to observe whether zebrafish is staying on the bottom to make steady state or behaving vertically for exploration, or observing information about mobile ability. NTT is very useful to observe behavior parameters, such as distance traveled, turn angle, swimming speed and duration at the top [9, 41]. Pharmacological agents could change behavior parameters, for example buspirone [8], fluoxetine [21] and diazepam [45] which are anxiolytic drugs are increasing duration in top. On the other hand, anxiogenic drugs or induction of stress tend to decreasing duration at top, mobility and increasing erratic movements [46]. In our NTT experiment, we observed that copper exposure can lead to behavioral anxiety or decreased motor performance in zebrafish adult. As a result of the NTT for 6 minutes, the distance moved, mobile duration and velocity were significantly decreased, on the other hand the turn angle values were significantly increased. Unfortunately, however duration at the top which is a typical anxiety-related parameter was not significant change in contrast to other parameters in the NTT experiment. This result is thought to be attributable to aquatic surface respiration because copper toxic to the gills, and substances that are toxic to the gills can cause aquatic surface respiration [1, 3, 33].

OFT is an experimental method to observe the movement of experimental animals in the open space and OFT is using to confirm the basic motility and anxiety on various experimental animals [23, 38, 42]. In addition, OFT is more suitable to observe horizontal movement than NTT. In the OFT, stress-induced zebrafish causes abnormal swim pattern such as decreased distance moved, mobile duration, velocity and shuttle frequency or increased turn angle and meandering movements [10, 35]. We have observed that decreased distance moved, velocity and mobile duration and increased turn angle in the OFT as result of NTT. In addition, exposure of adult zebrafish to $CuCl_2$ increased meandering movement and decreased shuttle frequency in the OFT. Two behavioral tests of OFT and NTT showed that exposure of adult zebrafish to $CuCl_2$ induces anxiety-related behavior, except for duration at the top.

Many studies mentioned an important relation between stress hormone and behaviors [14, 30, 34]. Cortisol has been used as a primary indicator of stress response in fish as human being [7, 40]. In this study, exposure of zebrafish adult to the $CuCl_2$ has significantly whole-body cortisol level changed, but at the highest concentration of $CuCl_2$ was not. Similar shaped curves have been reported after exposure of heavy metal. In previous study, the effects of exposure of *Oncorhynchus mykiss* to $Cd(NO_3)_2$ were examined in vitro. When *Oncorhynchus mykiss* were exposed to $Cd(NO_3)_2$, cortisol increased at less time (15 or 30 minute), while at increased time cortisol returned to the level before exposure to heavy metals [37]. The other study, the effects of lead nitrate on steroid profiles of *Heteropneustes fossilis* were examined in vivo and vitro. Cortisol elicited a concentration-dependent response of Pb^{2+} ion at low concentrations (1 or 0.1 μg/ml) were stimulatory, while the high concentrations were inhibitory. Based on these results, the authors suggested that Pb^{2+} ion could cause endocrine disruption [12]. In addition, adrenotoxic response have been observed in the reports on $CuSO_4$, previously. Gagnon *et al.* demonstrated that adrenocortical cells treated with $CuSO_4$ were inhibited the cortisol secretion in rainbow trout. According to authors, Cu^{2+} ion has a potential adrenotoxicity and impairs the secretory capacity of the adrenocortical cells [19]. In our experiment, as in the above mentioned heavy metal experiment including copper, it indicates that the highest concentration of $CuCl_2$ may inhibit the secretion of cortisol. In other words, excessive $CuCl_2$ exposure can cause acute adrenal fatigue syndrome. Further research is required to explain the effect of $CuCl_2$ concentration on cortisol secretion in detail.

In conclusion, the present study provides some evidences for the toxicity of $CuCl_2$. According to measurement of LC_{50} value, $CuCl_2$ might have more toxic effect on organism concentration- and time-dependently, and behavioral tests have shown that exposure of zebrafish to $CuCl_2$ was inducing anxiety-related behavior. Additionally, excessive $CuCl_2$ exposure may inhibit cortisol secretion similar to acute adrenal fatigue syndrome. Taken together, this study suggests that overexposure of $CuCl_2$ to adult zebrafish can cause abnormal condition.

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Abstract in Korean

$CuCl_2$ 노출에 의해 유도되는 제브라피시의 행동과 내분비계의 변화

본 연구의 목표는 $CuCl_2$ 가 지브라피시에 미치는 영향을 조사하는 것이다. $CuCl_2$ 에 대한 지브라피시의 반수치사농도를 조사하기 위하여 각기 다른 시간에 따른 농도를 설정하여 노출시켰다. 본 연구에서는 스트레스 반응의 증가를 보기 위하여 whole-body cortisol level 측정과 행동약리학적 변화를 측정하기 위해서 open field test 와 novel tank test 를 실시하였다. Whole-body cortisol 의 sample 을 수집하거나 행동실험을 실시하기 전에 $CuCl_2$ 또는 vehicle 을 1 시간 동안 처리하였다. $CuCl_2$ 의 반수치사농도는 24 시간, 48 시간, 96 시간에서 각각 30.3, 25.3, 14.8 µg/l 으로 측정되었다. $CuCl_2$ 에 노출된 지브라피시의 novel tank test 의 결과는 총 이동거리, 움직이는 시간 그리고 속도는 유의성이 있게 감소한 반면, turn angle 은 최고농도에서 유의성이 있게 증가하였다(p <0.05). 다른 행동실험인 open field test 의 결과에서도 총 이동거리, 움직이는 시간 속도가 $CuCl_2$ 의 노출에 의해서 감소하였으며, turn angle 과 meandering 이 유의하게 증가하였다(p <0.05). $CuCl_2$ 에 노출된 지브라피시의 whole-body cortisol 은 1.5 or 15 µg/l 의 농도에서는 유의하게 증가하였으나, 150 µg/l 의 농도에서는 유의성 있는 변화를 발견하지 못하였다(p<0.05). 본 연구의 결과는 $CuCl_2$ 의 노출이 지브라피시에서 치사율 증가, 행동 및 내분비의 변화 등을 포함하는 독성 반응을 야기하는 것을 나타낸다.