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EFFECTOS TOXICOLÓGICOS DEL TALIO EN EL MODELO EXPERIMENTAL ELEGANOS DE CAERNOHABDITIS

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INTRODUCTION

Thallium is a trace metal widely distributed throughout the Earth, both in the continental and oceanic crust (MARTIN et al., 2018) and that for many years was misused in the composition of depilatory creams and magnetic resonance imaging contrast. Today it is applied optical lenses and semiconductor production, X-ray and ultra-red detectors, and in electronic manufacture, due to its ease of conducting current with low energy dispersion through heat. Recently, a thallium reserve was found in the city of Barreiras, in the western region of Bahia, which raised several questions regarding environmental and toxicological impacts. Due to the similarity to potassium ions, thallium can be captured in cells through potassium pump (TATSI et al., 2015; PRYTULAK et al., 2017; MARTIN et al., 2018).

The exposure, both through inhalation and ingestion, leads to mitochondrial changes and damage to the nervous system resulting in several symptoms, such as gastroenteritis, polyneuropathy, and alopecia (LIU et al., 2019). However, toxic mechanisms are not yet completely elucidated. In this perspective, the experimental model *Caenorhabditis elegans* (*C. elegans*) has been very useful in neurotoxicological analyses, since it has a fast life cycle (about three days), low maintenance cost, easy manipulation, and large offspring (300 eggs per adult). In addition, its transparency enables the visualization of cholinergic, gabaergic, dopaminergic, and serotonergic neurons by fluorescent labels. The neurotransmitters are related to movement,

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egg-laying, and pharyngeal pumping behaviors. Besides, the worm genome has about 60-80% of similarities to the mammals, preserving similar signaling pathways and metabolism. Thus, to highlight the mechanisms on neuronal effects and resulting behaviors, we analyzed the metal thallium in various concentrations in the experimental model *Caenorhabditis elegans*.

METHODOLOGY

Maintenance

Wild-type worms (N2) were acquired at the Caenorhabditis Genetics Center (CGC University of Minnesota) and kept under standard culture conditions in plates containing nematode growth medium (NGM), nutritionally supplied with *Escherichia coli* (*E. coli*) strain OP50 (BRENNER, 1974). Adult worms were used to obtain eggs, which lead to a synchronized population of L1 (first larval phase) for the experiments.

Thallium acetate exposure and survival (CI₅₀)

The L1 larval stage worms were exposed to different concentrations of thallium acetate (50 - 1000 μ M) for 1 h. After exposure, the worms were washed and centrifuged three times to discontinue treatment. Subsequently, they were transferred to NGM plates inoculated with *E. coli*/OP50 for further assays. To determine survival, worms were exposed as described above and live nematodes were counted 24 after acute exposure. The lethality was assessed by normalizing the data against the control. Four experiments were performed to determine the lethal concentration.

Larval development

The worms were monitored from the L1 larval stage to adulthood to assess larval development after thallium acetate exposure. During the observations, the worms were kept at 20°C $\pm$ 2. The larvae were classified as L1, L2, L3, L4, and gravid adults according to the size, vulva development, and presence of eggs.

Behavioral activity

The worms in the larval stage of L4 were observed regarding their feeding behavior. The pharyngeal pumping was evaluated by monitoring worms on food. The contractions of the posterior pharyngeal bulb individually counted for 10 seconds, in triplicate, and the mean normalized to one minute (WANG; ROURKE; RUVKUN, 2009). The defecation of each worm was obtained by counting the intervals between the defecation cycles. For each worm, three defecation intervals were measured, and the mean of these intervals was used for further

comparisons between the groups (WANG; ROURKE; RUVKUN, 2009).

Reproductive profile was assessed by determining the number of eggs in gravid animals (day 1 of adult) (SCHAFER; KENYON, 1995; WANG; ROURKE; RUVKUN, 2009). The number of eggs (egg production) in the uterus was determined by counting after individual lysis of the worms with bleaching solution. The experiments were performed at a temperature of $20^{\circ}\text{C} \pm 2$ and at least four times at different times.

Statistical analyses

The ImageJ program (NIH) was used for image analysis and densitometry. Statistical and graphical analyses were performed using GraphPad Prism 5® software. The significance of the difference between the means was verified through Student's t-test or analysis of variance (ANOVA) of one way followed by Bonferroni's test or two-way ANOVA when appropriate (GraphPad Scientific, San Diego, CA, USA).

RESULTS

Survival (CI_{50})

After treating *C. elegans* with thallium acetate at different concentrations, the mortality rate was determined to determine the lethal concentration in 50% of the treated population. It was observed that there were no significant changes in the 50 μM concentration. From the concentration of 100 μM to 1000 μM , it was noted that there is a gradual and significant decrease in the number of surviving worms. The LC_{50} was determined at 222.708 μM within 72 hours of treatment (**Figure 1**).

Figure 1: The values are displayed as % survival of total worms (mean $\pm$ SEM) ($n = 4$ independent experiments, with 10 nematodes classified by group). The worms were treated with thallium acetate solutions

in concentrations ranging from 50 μM - 1000 μM for 1 hour. After three washes in autoclaved distilled water, the worms were transferred to NGM plates sown with *E. coli* OP50 and survival to adulthood was assessed. **** The statistical significance ($p < 0.0001$) in comparison to the ANOVA control group followed by a Bonferroni multiple comparison test was indicated.

The lethal dose of thallium ranges from 10 to 15 mg kg^{-1} for humans and chronic exposure can occur in the range 0.1 to 100 mg kg^{-1} (Li et al., 2018). With these data, their high toxicity could be compared by other toxic metals, such as arsenic, mercury, cadmium, lead and copper. Thus, it was found through experiments that Ti^+ has high toxicity in low concentrations.

DEVELOPMENT

The exposure of *C. elegans* to thallium acetate affected its development. It was observed a significant delay in growth in parallel to the increase in thallium acetate concentration. After the treatment all the surviving worms were in stage L1 (Figure 2A), 24 hours after the treatment it is observed that there are still worms in stage L1 larval in at 100 μM thallium acetate, it can be seen that there was a delay in the development of the animal (Figure 2B). Also, 48 h after, it was observed worm in stage L1 in worms exposed to 250 μM (Figure 2C) and after 72h to 1000 μM thallium acetate (Figure 2D). It was not possible to observe many animals in concentrations of 500 μM and 1000 μM since the high toxicity which killed most of the worms. These data suggest that acute exposure to thallium acetate in *C. elegans* is capable of impairing the development of the animals, with, therefore, delay in larval growth in all stages.

Figure 2: **Larval development of *C. elegans* nematode exposed to Tl^+ .** The worms were treated with different concentrations of larval stage thallium acetate solution and observed 1h (A) 24h (B) 48h (C) and 72h (D) after treatment. Values are displayed as the mean $\pm$ SEM ($n = 4$ independent experiments, with 10 nematodes classified by group in the behavioral test).

Studies show that the neurotoxic mechanisms of this metal can induce the formation of reactive oxygen species (ROS) and low production of cellular energy by mitochondria. Increased ROS generation and concomitant disruption of low energy production may block cell cycle progression. Tl^+ is also associated with inhibition of deoxyribonucleic acid replication and increased oxidative stress by inducing lipid peroxidation, which in turn leads to cellular apoptosis. This mechanism may slow the development of the worm gradually at non-lethal concentrations.

Pharyngeal pumping and defecation cycle

No significant differences were found in the pharyngeal beating behavior of the worms in all tested concentrations compared to the control group. A significant difference was observed defecation cycle at a concentration of 200 μM thallium acetate (** $p < 0.01$) suggesting toxicity. No significant differences were found in the pharyngeal beating behavior of the worms in all tested concentrations compared to the control group. A significant difference was observed defecation cycle at a concentration of 200 μM thallium acetate (** $p < 0.01$) suggesting toxicity. No significant differences were found in the pharyngeal beating behavior of the worms in all tested concentrations compared to the control group. A significant difference was observed defecation cycle at a concentration of 200 μM thallium acetate (** $p < 0.01$) suggesting toxicity.

Figure 3: Evaluation of pharyngeal beating behavior in wild worms after being exposed to thallium acetate solution for 1 hour. The worms were observed for pharyngeal beating per minute. Treatment with thallium acetate solution (50 μ M - 1000 μ M) started at the larval stage L1 and lasted 1 hour. After 48 hours, in the larval stage L4, the behaviors were determined. No significant differences were observed in the treated worms in relation to control. Values are displayed as mean $\pm$ SEM (n = 4 independent experiments, with 10 nematodes classified per group in the behavior assay).

Figure 4: Duration of the defecation cycle in adult *C. elegans* worms after 1 hour of exposure to thallium acetate solution. Nematoids were evaluated in the larval stage L4 after exposure to Tl^{+} in NGM plates sown with *E.coli* OP50. ** $p < 0.01$ indicates statistical significance compared to the control group by means of ANOVA followed by the Bonferroni Multiple Comparison Test. Values are displayed as mean $\pm$ SEM (n = 4 independent experiments, with 10 nematodes classified per group in the behavior test).

Reproduction

There was a significant reduction in egg production with treatment from 100 μ M thallium acetate (**** $p < 0.0001$, figure 5). According to Mahoney et al. (2006), this reduction may indirectly indicate a rupture in the cholinergic system, so there may be damage to the nervous system. The results show the developmental delay also observed in worms exposed to thallium acetate. The 500 μ M and 1000 μ M groups had a high rate of dead animals or larval delay, so there were not enough worms for analysis, justifying the gap as zero egg production. Considering that the reproductive system is closely linked to longevity in *C. elegans* (WANG, D.; XING, 2008), we found that the shelf life was also reduced in worms exposed to Tl^{+} when compared to controls. According to the literature, Tl^{+} increased lipid peroxidation production, reduced shelf life and also affected reproduction. Notably, exposure to Tl^{+} caused a reduction in the amount of eggs produced, indicating that cholinergic neurons may be affected by this metal.

Figure 5: Evaluation of the reproductive behavior of *C. elegans* after exposure to thallium acetate for 1 hour. The production of eggs from worms in adult larval stage was observed after 72 hours of treatment with different concentrations of thallium acetate. The results represent the mean number of eggs inside the worms. ****The results were statistically different compared to the ANOVA control group followed by the Bonferroni test. The values are displayed as mean $\pm$ SEM (n = 4 independent experiments, with 10 nematodes classified per group in the behavior test).

CONCLUSION

The thallium acetate induced toxicity in *C. elegans*, which was evidenced by lethality, developmental impairment, and behavioral changes in the worm. The impacts are possibly related to neurotoxicity, and the next step of the present study will be investigating thallium acetate mechanisms behind the phenotypes observed.

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