

Chelation of Zinc by Diethyldithiocarbamate Acid Affects Taurine System in Retina Rat

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Abstract

Purpose: Examined *in vitro* effects of the intracellular zinc chelator, diethyldithiocarbamate acid (DEDTC), zinc sulphate (ZnSO₄) or the combination of both and *in vivo* effects of DEDTC on retinal taurine system. Qualitative design was used. A total of 20 participants were purposively selected and semi-structured interviews were conducted for data collection. Thematic analysis was utilized for data analysis. The research revealed that some parents do discuss sexual issues and HIV prevention with their adolescents but not a lot. The information that they discuss is mainly abstinence. The challenges that parents encounter during discussions is lack of skills and information to initiate the discussion and also cultural beliefs that do not expect a parent to discuss sexual issues and HIV prevention with their own children.

Methods: Concentrations of DEDTC were 1 to 500 µm and those of ZnSO₄ 10 and 100 µm for *in vitro* experiments. For *in vivo* assays, DEDTC, 1000 mg/kg, dissolved in saline solution (0.9% NaCl) was administered ip and the retinal and heart zinc levels were measured at 1, 6 and 24 h after the injection. Levels of zinc in rat retina and heart were determined in order to establish optimal concentration of DEDTC for further analysis. Zinc was determined by spectrophotometry. Taurine levels determined by high performance liquid chromatography with fluorescence detection.

Results: Intermediate and high concentrations of DEDTC *in vitro* significantly decreased [³H]taurine transport. The incubation of the cells in the presence of DEDTC 40 µm plus zinc, 40 µm and 100 µm, did not affect [³H]taurine transport modified by the chelator. DEDTC *in vivo* administration decreased zinc in 27% and 43% at 24 h in retina and heart, respectively. Capacity of [³H]taurine transport was decreased in 32% and affinity was increased in 42% in retina after ip DEDTC. Taurine levels decreased 62% and 40% at 24 h in retina and heart, respectively.

Conclusion: Optimal concentrations of zinc are necessary for the equilibrium of taurine system in the retina. The study of these molecules in the retina is relevant for understanding the interactions of taurine and zinc in this structure.

Keywords: Central nervous system; Chelator of zinc; Eye; Retina; Taurine; Taurine transport; Zinc

Introduction

Zinc deficiency it is caused by inadequate intake, increased needs or excretion, conditional deficiency of metal or genetic causes [1]. Zinc deficient diets produce retardation of growth in humans and animals [2]. A previous study that described deficiency of zinc in rats [3] in which rats were submitted to an diet intensive and deficient of zinc (1 mg zinc/kg diet) for 3 weeks and caused malformations in heart and lungs, affection the systems: Gastrointestinal, immune, reproductive, hormonal, skeletal and nervous. Subsequently, it was observed that deficiency of zinc during prenatal development in rats causes irreversible alteration of normal development of brain [2,4] spinal cord and eye [4,5]. Defects in the eye include microphthalmia, anophthalmia and dysplasia of photoreceptors (Phot) [5]. Studies in the zinc-deficient diet in cats and dogs cause alteration in electroretinogram [6], malformations in the cones [7] conjunctivitis and

cataracts [8]. At the present chelators of metal ion such as intracellular chelators N,N,N',N'-tetrakis (2-pyridylmethyl), ethylenediamine (TPEN) and diethyldithiocarbamate acid (DEDTC) and extracellular chelators such as diethylenetriaminepentaacetic acid (DTPA), [1,2-bis (oaminophenoxy), ethane-N,N,N',N'-tetraacetic acid] (BAPTA) and its derivatives, 3-diaminoethanetetraacetic acid (EDTA) and ethyleneglycoltetraacetic acid (EGTA) they are used for limiting the available metal ions in cellular medium [9-12].

TPEN for being an intracellular chelator is usually more effective in reducing the zinc and are required lower concentrations to cause the effect as it has been previously shown in studies of taurine transport *ex vivo* [13] and also *in vivo* [14,15]. The extracellular chelator, DTPA, *ex vivo* [13] and *in vivo* decreases taurine transport and localization of taurine and zinc transporters in retina [16].

Taurine has been linked to zinc, not only because there are high levels of both molecules in the retina, but they also play similar properties in this structure, such as neuro-protection, membrane stabilization and modulation of development, perhaps by acting in

parallel or as interacting agents [17]. The concentration of taurine in the retina in order of μM has triggered a series of investigations to understand its role in several patho-physiological states. It also exerts a critical function in cardiovascular diseases, diabetes, migraine, cerebral ischemia and epilepsy [18,19]. In macular degeneration of the retina is a medical condition which may result in blurred or no vision in the center of the visual field and is associated with decreased of the levels of taurine and zinc in the retina [20,21].

To support these findings, it is necessary know the mechanisms of action of taurine and interaction with molecules such as zinc. The balance of zinc, taurine and transporters proteins can contribute to the comprehension of the interaction of both molecules in the retina. However, there are still many unknowns about the mechanisms of the interaction of taurine and zinc in this structure. The objectives of this work is to study effects of the intracellular chelator of zinc, diethylenedithiocarbamate acid (DEDTC) *in vitro* and *in vivo* administered systemically (intraperitoneally, ip) on the transport and levels of taurine in retina and heart of rats.

Materials and Methods

Animal handling

They were used male Sprague-Dawley rats with a weight between 150-200 g from the animal housing at Instituto Venezolano de Investigaciones Científicas. They had a period of adaptation of 48 h in the in the Laboratory with food and water provided ad libitum. The rats were decapitated between 8:00 and 10:00 am and the retinas were extracted. The use of animals (rats) was conducted following the standards of animal bioethics [22] and was approved by the Bioethics Committee for Animal Research of the Institute.

Obtaining rat retinal cells

The retinas were extracted and cells were isolated with 0.25 % of trypsin with Locke buffer (500 μl), composed (in mM) of: 154 sodium chloride (NaCl), 2.7 potassium chloride (KCl), 2.1 dibasic potassium phosphate (KH_2PO_4), 0.95 monobasic potassium phosphate (KH_2PO_4), 2.7 sucrose and 2.5 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), for 10 min at 37°C. Subsequently, the cells were subjected to mechanical disaggregation with Pasteur pipette. The cells were washed with phosphate buffered saline (PBS) at 0.1 M pH 7.4, were centrifuged for 10 min at 2,000 rpm (300 g) and counted in Neubauer chamber. The integrity of membrane was determined by 50% Trypan blue exclusion (>96 %) [13].

Zinc and Intracellular zinc chelator diethylenedithiocarbamate acid *in vitro* on the taurine transport: As described in the previous section, all experiments were performed in duplicates, using 250,000 cells per tube, pre-incubated for 5 min and incubated for 25 s at 37°C. To determine the effect of zinc sulphate (ZnSO_4) and zinc chelator on [^3H] taurine transport, each tube had 40 μl of cell suspension, 100 μl of Locke solution, 20 μl of [^3H] taurine (65 nM) and 20 μl of ZnSO_4 or DEDTC. Solutions of substrate were prepared for each experiment and counted previously to performance in order to achieve the desired final concentration. Concentrations of 40 or 100 μM of ZnSO_4 were used for the experiments of [^3H] taurine transport, according to results with the wide range by Marquez et al. [13]. DEDTC, intracellular chelator of zinc, at various concentrations, 1-500 μM and 40 μM was considered for next experiments in combination with ZnSO_4 . The samples are incubated 30 min with ZnSO_4 or DEDTC or ZnSO_4 +DEDTC.

Subsequently as previously described the cells are pre-incubated for 5 min and incubated for 25 s. The cells were washed with Locke buffer and the process was stopped by filtration as already described by Marquez et al. [13].

Treatment with the intracellular zinc chelator diethylenedithiocarbamate acid *in vivo*: The dose of intracellular zinc chelator DEDTC, 1000 mg/kg of weight [23] was administered (a single injection) and they studied different periods of time 1, 6 and 24 h according to zinc level reduction was selected the time for the following tests [23]. Two experimental groups of rats were used: 1) Intra-peritoneal administration (ip) of 1000 mg/kg of DEDTC diluted in saline solution (0.9% NaCl). 2) Control group was injected with saline solution (0.9% NaCl) [24]. To corroborate the results in retina, we evaluated zinc levels in another electrically excitable tissue such as heart (specifically a sample of the right ventricle).

Obtaining a heart sample

To control and treated animals: Heart was dissected by cardiac puncture with saline solution (0.9% NaCl) to wash and remove traces of blood and get a sample of the right ventricle. Subsequently the heart (tissue) was homogenized in deionized and distilled water to determine proteins, zinc concentration and taurine.

Levels of zinc in retina and heart

The retina tissue (pool the four retinas) was homogenized in 320 μl of deionized and distilled water or the heart tissue sample (right ventricle) was homogenized in 1020 μl of de-ionized and distilled water. 20 μl of the sample (retina or heart) according to the case, it was collected for protein determination by Bicinchoninic acid assay (BCA) [25] and the rest was used for determination of zinc after 1, 6 or 24 h of ip injection of DEDTC. The determination of zinc was determined according to the method used by Marquez et al. [15]. The spectral line for zinc was 213.856 nm [26,27]. It was chosen for the next experiments, 1000 mg/kg of DEDTC, 24 h after ip injection the tests were done. Saturation of taurine transport and effect of intracellular chelator of zinc, diethylenedithiocarbamate acid *in vivo* on taurine transport, in accordance Marquez et al. [13] with the transport parameters were standardized: Number of cells, temperature and time of pre-incubation. For saturation assays of taurine transport, the cells preparation (250,000 cells per tube) were incubated in the presence of various concentrations of [^3H] taurine from 5 to 105 nM (163 and 400 GBq) 99.613–1832,787 dpm per test tube. For each concentration, tubes were prepared by duplicate in a final volume of 200 μl (140 μl of Locke solution, 20 μl of [^3H] taurine and 40 μl of cells preparation). The preparation of cells was pre-incubated at 37°C during 5 min in Locke solution. The incubation of the cells was during 25 s and the transport was started by the addition of the substrate [^3H] taurine. After the incubation of cells during 25s, the process was stopped by rapid filtration through filters of fiberglass (Watman GF/C), followed by two washed with cold Locke solution 5 ml as previously described [13]. The capacity of transport (V^{max}) and affinity constant (K^{t}) were calculated for control and treated rats with DEDTC (1000 mg/kg at 24 h) by Lineweaver-Burk linearization.

Levels of taurine in retina and heart

According to Marquez et al. [15], taurine was determined in retina of rat: Isolated cells and in heart of rat; right ventricle, after 24 h of ip injection of DEDTC in isolated cells by high performance liquid

chromatography (HPLC). HPLC with detection of fluorescent was use by a modified method. The equipment consisted of a System of Separation Waters 2690 and a detector of fluorescent Shimadzu RF-551. Separation was done with a column Supelco LC-18 with measures 4.6*100 mm, 5 µm. The details of the methodology are described in Marquez et al. [15]. Taurine levels were quantified by the method of the external standard and expressed in nmol/mg of protein using the program Millenium (Waters, MA, USA) [17].

Data Analysis

All values were presented as mean ± standard error of the mean (SEM). The analysis of variance ANOVA with the program INSTAT followed by Tukey test [28] were used to compare the values and determined the statistical significance of the specific data. The program GraphPad Prisma 2 was used for analyzed the saturation curves and V_{max} and K^t of taurine transport were calculated either by Lineweaver-

Burk plots or curvilinear analysis. Values of $p < 0.05$ were considered for statistically significance.

Results

Taurine transport after *in vitro* treatment with intracellular zinc chelator diethylendithiocarbamate acid and zinc sulphate: Intermediate and high concentrations of DEDTC significantly decreased [3H]taurine transport (Figure 1A). Low concentrations did not produce significant effects on transport (Figure 1A), is observed the graph the data as a dosage curve (Figure 1B). [3H] Taurine transport significantly is lower with 40 µm DEDTC, incubation of the cells in the presence of DEDTC plus zinc, 40 µm and 100 µm, did not produce effects on the [3H] taurine transport modified by the chelator (Figure 2) and with $ZnSO_4$, 40 µm, in the medium, produced an increase in [3H] taurine transport (Figure 2). The incubation of the cells with zinc 100 µm decreased of transport (Figure 2).

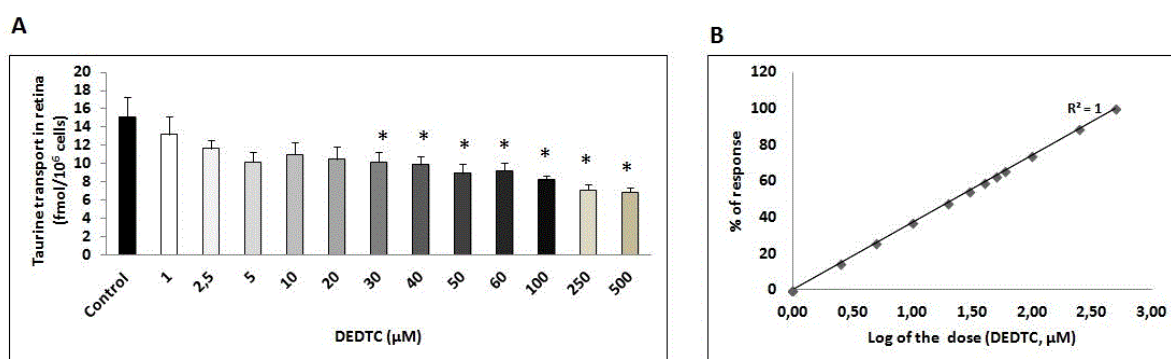


Figure 1: Intermediary and high concentrations of DEDTC significantly decreased [3H] taurine transport.

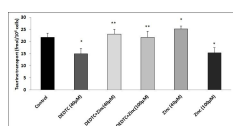


Figure 2: The incubation of the cells with zinc 100 µm decreased of transport.

Intra-peritoneal administration of diethylendithiocarbamate acid and levels of zinc in retina and heart: After 6 h of ip administration of saline solution (control group), the zinc levels was 66.03 ± 10.10 µg/mg of protein. The DEDTC 1000 mg/kg after 6 h produce decreased of concentration of zinc in retina in (29%) to 43.96 ± 2.52 µg/mg of protein in comparison with the controls (Figure 3). At 24 h 65.44 ± 10.24 µg/mg of protein of zinc for controls. The levels of zinc was lower in (27%) to 40.54 ± 5.64 µg/mg of protein at 24 h after the ip administration of DEDTC of in comparison with the controls (Figure 3). At 1 h, there were no effects on the levels of zinc in the retina after ip administration 1000 mg/kg of DEDTC in comparison with controls (Figure 3). The DEDTC did not modify the levels of other metals in the retina, such as: Calcium, magnesium, iron and sodium. For the next experiments a treatment time of 24 h was chosen because with 6 h we can cause more stress to the animals, besides that laboratory rats

should be sacrifice between 8:00 am and 10:00 am for their circadian rhythm. At 6 and 24 h the controls groups were 181.33 ± 29.57 and 193.00 ± 34.37 µg/mg of protein respectively. The ip administration of DEDTC produced that levels of zinc were lower in (17%) to 150.53 ± 25.15 and in (43%) to 110.45 ± 15.36 µg/mg of protein respectively, at 6 and 24 h in comparison with controls groups (graphs not shown). At 1 h there were no effects on levels of zinc in heart (graphs not shown).

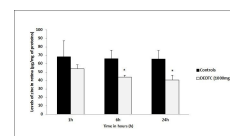


Figure 3: The levels of zinc was lower in (27%) to 40.54 ± 5.64 µg/mg of protein at 24 h after the ip administration of DEDTC in comparison with the controls.

Intra-peritoneal administration of diethylendithiocarbamate acid and transport of taurine in retina: The kinetic parameters of the controls were: V_{max} 19.60 ± 2.85 fmol/10⁶ cells and $K^t = 0.84 \pm 0.07$ µm (Table 1). 24 h after the ip administration of 1000 mg/kg of DEDTC, the transport of taurine was lower in comparison with controls (Figure 4 and Table 1). Transport capacity (V_{max}) decreased in 32% and the affinity constant for taurine increased in 42% (Table 1).

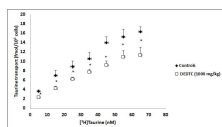


Figure 4: 24 h after the ip administration of 1000 mg/kg of DEDTC, the transport of taurine was lower in comparison with controls.

[3H]Taurine Transport		
Days	V_{max}	
(fmol/10 ⁶ cells)	K_t (μ m)	
Controls (Saline solution) 24 h	19.60 \pm 2.85	0.84 \pm 0.07
1000 mg/kg of DEDTC 24 h	13.37 \pm 1.98*	0.49 \pm 0.06*

Table 1: The effect of io administration 1000 mg/kg of diethylenedithiocarmate add (DEDTC) *in vivo* on [³H]taurine transport in isolated cells from rat retina after 24 h of ip injection, V_{max} and K_t were calculated by Lineaweaver-Burk linearization. Each Value is the mean \pm SEM, n=6. *P<0.05 with respect to controls.

Intra-peritoneal administration of diethylenedithiocarbamate acid and levels of taurine in retina and heart: The levels of taurine in retina from controls were 35.87 \pm 9.41 nmole/mg of protein and were lower in 62% (13.42 \pm 3.63 nmole/mg of protein) at 24 h after the ip administration of 1000 mg/kg of DEDTC (Figure 5A). The concentration of taurine in right ventricle of heart from control group was 28.18 \pm 4.31 nmole/mg of protein and was significantly reduced in 40% (16.81 \pm 2.63 nmole/mg of protein) at 24 h after the ip administration of DEDTC (Figure 5B).

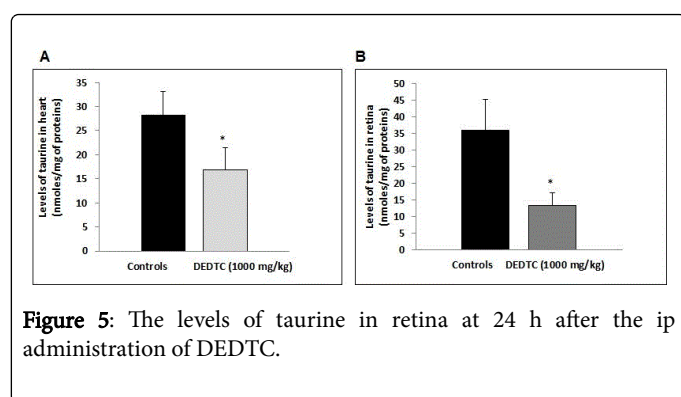


Figure 5: The levels of taurine in retina at 24 h after the ip administration of DEDTC.

Discussion

Divalent zinc is an essential mineral for the organism, highly concentrated in the brain and retina of mammals, where it is attached to metallo-enzymes and other proteins and it has catalytic and structural functions [1,29]. Among the clinical manifestations produced by their deficiency include: Anorexia, retarded growth, weight loss, impaired immune function, delayed sexual maturation, testicular atrophy, blindness and others [1].

Zinc plays an important role in the central nervous system (CNS), where there are high levels of chelated metal in regions of the brain

[1,30]. To study the functions of zinc, it is necessary to design and use of instruments that allow having the specific information of the location and concentrations of the zinc in defined extra and intracellular compartments, which would contribute with the understanding of the functions of the metal.

We have used zinc chelators as an alternative to zinc diets that will require much more time for the experimental design. We have designed assays with extracellular and intracellular zinc chelators administered *in vitro* or in a specific area *in vivo*, in this case intracellular to evaluate zinc deficiency on the retinal taurine system [13-16].

The zinc chelators specific for metal and most used so far are: TPEN and DEDTC, both membrane-permeable and DTPA a membrane-impermeable. The use of chelating agents allows the study of zinc levels in specific cell compartments, intra and extracellular by be able to do local injections [9]. The TPEN and DEDTC for being intracellular and membrane-permeable chelators, they generally have a greater effect on the decrease of zinc both within the cytoplasm and in other cell compartments and as well as separating the metal from specific proteins [31].

However, we have reported that the administration of TPEN *in vivo* has not affected other metal ions in the rat retina as calcium, magnesium, iron and sodium [15], similarly we observed it with ip treatment with DEDTC in the present study. The DEDTC-zinc complex has been reported to be more stable than those formed by other agents, since they are complexes without charge that do not release zinc into the cytoplasm and do not serve as ionophores [32].

We could observe that a wide range of concentrations *in vitro* of DEDTC chelator affect taurine transport by decreasing it and that the presence of zinc depending on the concentration increases or decreases the transport, confirming its biphasic effect previously reported by [13]. Additionally a wide range of concentrations of the TPEN chelator was studied: The dose of TPEN (between 10–60 μ m) significantly decreased [³H] taurine transport [13]. The combination of TPEN 20 μ m plus ZnSO₄ reverses the effect produced by the chelator. From the wide range of concentrations studied of DTPA high concentrations (100, 250 and 500 μ m) cause a decrease in taurine transport. DTPA 500 μ m plus ZnSO₄, they do not affect to transport [13]. Moreover, in the same study, [13], have shown that zinc has a biphasic effect, low concentrations of ZnSO₄ (30 and 40 μ m) augmented the transport; while high doses (100, 150 and 200 μ m) they decline it. Similar to what was observed in this study in *in vitro* transport assays.

There are many unknowns regarding the precise mechanisms of taurine-zinc interactions that affect to taurine system and that it can affect visual health, despite the evidence on the protective role of taurine and zinc. The TAUT could have a zinc binding site that affects the binding of taurine for transport, the zinc could moderate affection or specific target of one of TAUT, TAUT-1 or TAUT-2 or maybe it could affect transport by the formation of a taurine-zinc complex which would show that both molecules would act together on certain parameters related to the integrity and function of the retina.

Zinc acts as a non-competitive inhibitor of dopamine transporter since it binds to a specific site of the transporter protein and affects the binding of dopamine for transport [33]. In fact, histidine residues have been demonstrated in domains of the transporter molecule, that allow the recognition of zinc as for the serotonin transporter [34] or dopamine transporter [35], it remains to be clarified if the TAUT has histidine residues in any of its domains that allow the recognition of

the metal. Stockner et al. [35] by mutational analysis of the structure of the dopamine transporter, they ascertain the histidine residues which indicate the zinc binding site. An association of zinc and taurine has been suggested by observations showing that zinc deficiency results in taurine mobilization and loss through elevated concentrations in blood and urine. Conversely, in taurine deficient animals, a loss of zinc in ocular tissues has been detected, mentioned by Hyun and You [36]. Therefore, it may be speculated that zinc and taurine are related to each other. Although some studies have reported, still the relationship between zinc and taurine is not clear [6,37].

Conclusion

In this work we focus on using the DEDTC because in addition to being an effective chelator to produce zinc deficiency, is the only that have used in a systematic way. Systemic administration is closer to possible applicable treatments Yu and Li [38] reported that the application of DEDTC reduced intracellular zinc accumulation and increased the freshwater fish (zebra fish) survival. The latter was probably achieved by reducing oxidative stress that was triggered by zinc. Therefore, this study supports that the increased level of intracellular zinc is a critical factor in brain damage caused by hypoxic stress. Hypoxic-ischemic brain damage is often seen in patients who suffered a stroke or cardiac arrest. This allowed us to apply an intraperitoneal treatment to the experimental rats with which we observed that their effect was similar to the TPEN and DTPA intraocular on the retinal taurine system in retina. The amounts of zinc in retina and in heart human have been previously reported as in a range 22.5-31 µg/g in heart [39] and 100 µg/g in retina [40,41]. This treatment with DEDTC also affected the amounts of taurine and zinc in heart (right ventricle), which indicates that the systemic treatment not only affects this levels in retina but also other electrically excitable tissues.

The experimental conditions *in vitro* and *in vivo* are different, according to the approaches, the preliminary studies and the literature. *In vivo* tests are appropriate for observing direct effects and regulatory responses in the system. The DEDTC *in vivo* and *in vitro* affects the taurine system: Levels of taurine and transport.

There is a wide field of research with this intracellular chelator, its effect on retinal cells such as ganglion cell (GC) and glial cell on the taurine transporter and the different zinc transporters in retina: Isolated cells and tissue. These findings would allow complementing the reports about the functions of both molecules in this structure. Since it was previously we have shown that the intracellular chelator of zinc, TPEN, affects the location of the TAUT (decreases) in the different layers of the retina [15]. The extracellular chelator, DTPA, produced a decrease in GC of the total cells of retina. It also produced a significant decrease in TAUT and in the localization of ZnT-1 and ZnT-3 in the retina layers [16].

The presence of the taurine and zinc transporters is necessary to maintain the adequate levels of both molecules in the cells and thus facilitating cross talking and influencing their interactions. Our studies have shown that adequate concentrations of zinc are required for a functional taurine system in the retina, which involves the levels and taurine transport. These studies open the way to the design of new experimental protocols to elucidate the specific mechanisms of action of taurine and zinc in tissues of the CNS. In addition, the systemic administration is closer to the possible applicable treatments and

makes it possible to show that Taurine and Zinc Probably interact in relation to relevant functions, such as cellular protection.

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Conflict of Interest

None of the authors has conflict of interest with this submission.

References

1. Vallee BL, Falchuk KH (1993) The biochemical basis of zinc physiology. *Physiol Rev* 73: 79-118.
2. Takeda A, Tamano H, Tochigi M, Oku N (2005) Zinc homeostasis in the hippocampus of zinc-deficient young adult rats. *Neurochem Int* 46: 221-225.
3. Hurley LS, Swenerton H (1966) Congenital malformations resulting from zinc deficiency in rats. *Proc Soc Exp Biol Med* 123: 692-696.
4. Warkany J, Petering, HG (1972) Congenital malformations of the central nervous system in rats produced by maternal zinc deficiency. *Teratology* 5: 319-334.
5. Rogers JM, Hurley LS (1987) Effects of zinc deficiency on morphogenesis of the fetal rat eye. *Development* 99: 231-238.
6. Jacobson SG, Meadows NJ, Keeling PW, Mitchell WD, Thompson RP (1986) Rod mediated retinal dysfunction in cats with zinc depletion: Comparison with taurine depletion. *Clin Sci (Lond)* 71: 559-564.
7. Wen GY, Sturman JA, Wisniewski HM, MacDonald A, Niemann WH (1982) Chemical and ultrastructural changes in tapetum of beagles with a hereditary abnormality. *Invest Ophthalmol Vis Sci* 23: 733-42.
8. Srivastava VK, Varshney N, Pandey DC (1992) Role of trace elements in senile cataract. *Acta Ophthalmol (Copenh)* 70: 839-841.
9. Frederickson CJ, Suh SW, Koh JY, Cha YK, Thompson RB, et al. (2002) Depletion of intracellular zinc from neurons by use of an extracellular chelator *in vivo* and *in vitro*. *J Histochem Cytochem* 50: 1659-1662.
10. Hyun HJ, Sohn JH, Ha DW, Ahn YH, Koh JY, et al. (2001) Depletion of intracellular zinc and copper with TPEN results in apoptosis of cultured human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 42: 460-465.
11. Kanekiyo M, Itoh N, Mano M, Kawasaki A, Tanaka J, et al. (2000) Cellular zinc status regulates cytomegalovirus major immediate-early promoter. *Antiviral Res* 47: 207-214.
12. Libin C, Schoene NW, Zhu L, Fanzo JC, Alshatwi A, et al. (2002) Zinc depletion reduced Egr-1 and HNF-3β expression and apolipoprotein A-I promoter activity in Hep G2 cells. *Am J Physiol Cell Physiol* 283: C623-630.
13. Marquez A, Urbina M, Lima L (2014) Zinc and zinc chelators modify taurine transport in rat retinal cells. *Neurochem Res* 39: 2234-2239.
14. Marquez A, Salazar V, Lima L (2015) Localization of taurine transporter and zinc transporters in rat retinal cells and tissue: Effect of intracellular zinc chelation. *J Mol Pathophysiol* 4: 42-50.
15. Marquez A, Urbina M, Quintal M, Obregón F, Lima L (2016) Effects of intracellular zinc chelator *in vivo* on taurine transport, taurine concentrations and taurine transporter in rat retina cells. *J Memb Sci Technol* 6: 149.
16. Márquez A, Salazar V, Lima L (2017) Efecto de un quelante de zinc extracelular sobre el número de células ganglionares en retina de rata, y transportadores de taurina y de zinc en estas células. *Acta biol.Colomb* 22: 137-148.
17. Nusetti S, Obregón F, Quintal M, Benzo Z, Lima L (2005) Taurine and zinc modulate outgrowth from goldfish retinal explants. *Neurochem Res* 30: 1483-1492.

18. Lourenço R, Camilo ME (2002) Taurine: A conditionally essential amino acid in humans? An overview in health and disease. *Nutr Hosp* 17: 262-270.
19. Schuller-Levis G, Park E (2006) Is taurine a biomarker? *Adv Clin Chem* 41: 1-21.
20. Militante J, Lombardini JB (2004) Age-related retinal degeneration in animal models of aging: Possible involvement of taurine deficiency and oxidative stress. *Neurochem Res* 29: 151-160.
21. Told R, Schmidl D, Palkovits S, Boltz A, Gouya G et al. (2014) Antioxidative capacity of a dietary supplement on retinal hemodynamic function in a human lipopolysaccharide (LPS) model. *Invest Ophthalmol Vis Sci* 56: 403-11.
22. Jayo M, Cisneros J (2015) Guía para el cuidado y uso de los animales de laboratorio. Efecto de un quelante de zinc extracelular sobre el número de células ganglionares en retina de rata, y transportadores de taurina y de zinc en estas células *Acta Biol Colomb* 22: 137-148.
23. Jin L, Murakami TH, Janjua NA, Hori Y (1994) The effects of zinc oxide and diethyldithiocarbamate on the mitotic index of epidermal basal cells of mouse skin. *Acta Med Okayama* 48: 231-236.
24. Utrera J, Romero R, Verdaguier E, Junyent F, Auladell C (2011) Recovery of axonal myelination sheath and axonal caliber in the mouse corpus callosum following damage induced by N,N-diethyldithiocarbamate. *Eur J Neurosci* 34.
25. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, et al. (1985) Measurement of protein using bicinchoninic acid. *Anal Biochem* 150: 76-85.
26. Lima L, Obregón F, Matus P (1998) Taurine, glutamate and GABA modulate the outgrowth from goldfish retinal explants and its concentrations are affected by the crush of the optic nerve. *Amino Acids* 15: 195-209.
27. Nusetti S, Urbina M, Obregon F, Quintal M, Benzo Z, et al. (2010) Effects of zinc *ex vivo* and intracellular zinc chelator *in vivo* on taurine uptake in goldfish retina. *Amino Acids* 38: 1429-1437.
28. Tapia F (1994) GraphPad InStat [programa de ordenador. Versión 2.05a]. San Diego, CA, EUA: Software GraphPad
29. Kambe T, Tsuji T, Hashimoto A, Itsumura N (2015) The Physiological, biochemical and molecular roles of zinc transporters in zinc homeostasis and metabolism. *Physiol Rev* 95: 749-84.
30. Radford RJ, Lippard SJ (2013) Chelators for investigating zinc metalloneurochemistry. *Curr Opin Chem Biol* 17: 129-36.
31. Cho YE, Lomeda RA, Ryu SH, Lee JH, Beattie JH, et al. (2007) Cellular Zn depletion by metal ion chelators (TPEN, DTPA and chelex resin) and its application to osteoblastic MC3T3-E1 cells. *Nutr Res Pract* 1: 29-35.
32. Kay AR, Toth K (2008) Is zinc a neuromodulator? *Sci Signal* 1: re3.
33. Norgaard-Nielsen K, Norregaard L, Hastrup H, Javitch JA, Gether U (2002) Zn²⁺ site engineering at the oligomeric interface of the dopamine transporter. *FEBS Lett* 524: 87-91
34. White K, Kiser P, Nichols D, Barker EL (2006) Engineered zinc-binding sites confirm proximity and orientation of transmembrane helices I and III in the human serotonin transporter. *Protein Sci* 15: 2411-2422.
35. Stockner T, Montgomery T, Kudlacek O, Weissensteiner R, Ecker GF, et al. (2013) Mutational analysis of the high-affinity zinc binding site validates a refined human dopamine transporter homology model. *PLoS Comput Biol* 9: 1002909.
36. Hyun JK, You YK (2000) Synergism between zinc and taurine in the visual sensitivity of the bullfrog's eye. *J Photosci* 7: 115-121
37. Pasantes-Morales H, Cruz C (1984) Protective effect of taurine and zinc on peroxidation-induced damage in photoreceptor outer segments. *J Neurosci Res* 11: 303-311.
38. Yu X, Li YV (2013) Neuroprotective effect of zinc chelator DEDTC in a zebrafish (Danio rerio). Model of Hypoxic Brain Injury. *Zebrafish* 10: 30-5.
39. Lech T, Sadlik JK (2011) Zinc in postmortem body tissues and fluids. *Biol Trace Elem Res* 142: 11-17.
40. Grahn BH, Paterson PG, Gottschall-Pass KT, Zhang Z (2001) Zinc and the eye. *J Am Coll Nutr* 20: 106-118.
41. Ugarte M, Osborne NN (2001) Zinc in the retina. *Prog Neurobiol* 64: 219-249.