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Original Research Communication

Effect of Zinc Ions on the Cytotoxicity Induced by the Amyloid β-Peptide

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ABSTRACT

Insoluble aggregates of the amyloid β-peptide (Aβ) are a major constituent of senile plaques found in brains of Alzheimer's disease patients. The β-amyloid fragment $A\beta_{1-40}$ is toxic to rat pheochromocytoma PC12 cells, leading to a concentration-dependent decrease in the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The detrimental effects of $A\beta_{1-40}$ are enhanced in the presence of 1 mM zinc, whereas 50 μM zinc exerts a protective effect against $A\beta_{1-40}$-induced toxicity. Exposure of PC12 cells to low zinc concentrations (50 μM) affords a decrease (1.4-fold) in the extent of lipid peroxidation, a decrement in protein oxidation (1.1-fold), and an increase in ATP levels (1.2-fold), although the differences were not statistically significant. However, treatment of cells with high concentrations of zinc (1 mM) led to significant increases in lipid peroxidation (3.7-fold) and protein oxidation (1.5-fold) and to depletion of the ATP pool (21-fold). These data suggest that zinc has a concentration-dependent dual effect, protective and toxic, thus playing an important role in the pathogenesis of Alzheimer's disease. Antiox. Redox Signal. 2, 317–325.

INTRODUCTION

ALZHEIMER'S DISEASE (AD), the most common type of dementia in the elderly, is characterized by the presence in the brain of numerous extracellular proteinaceous amyloid deposits and intracellular neurofibrillary tangles and also by the selective loss of neurons (Iversen *et al.*, 1995). The major protein component of these amyloid deposits are fibers composed of β-amyloid (Aβ), a 39- to 43-residue peptide (Glenner and Wong, 1984) derived by proteolysis from a large transmembrane glycoprotein, denoted as β-amyloid precursor protein (β-APP) (Goldgaber *et al.*, 1987; Bendotti *et al.*, 1988).

Various lines of genetic evidence support a central role of Aβ in the pathogenesis of AD, and an increasing number of studies show that Aβ may be one molecular link between oxidative stress and AD-associated neuronal cell death (Yankner, 1996). High levels of oxidative stress (Smith *et al.*, 1995; Yankner, 1996; Mark *et al.*, 1996) and increased levels of protein oxidation (Smith *et al.*, 1991) and lipid peroxidation (Lovell *et al.*, 1995) occur in vulnerable regions of AD brain. It has been proposed that oxidative stress could initiate a cascade of events leading to the neuronal cell death that occurs in AD (Yankner, 1996).

Many factors have been proposed as risk factors for AD, namely the exposure to certain

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metals, such as zinc (Cuajungco and Lees, 1997). Although enough information is not yet available with regard to the toxic mechanisms of zinc, it has recently been shown that zinc neurotoxicity occurs by oxidative stress (Kim *et al.*, 1999). In most systems, zinc is described as an antioxidant (Bray and Bettger, 1990). Previous results have shown that zinc deprivation leads to increased lipid peroxidation in animals (Coudray *et al.*, 1991; Faure *et al.*, 1991) and impairs copper zinc-superoxide dismutase activity (Coudray *et al.*, 1992), whereas zinc supply, in a cellular system, is able to provide protection against oxidative stress by inhibiting lipid peroxidation, reactive oxygen species (ROS) generation, DNA fragmentation, glutathione depletion, and lactate dehydrogenase (LDH) leakage (Bagchi *et al.*, 1997, 1998). However, in some studies, zinc has been shown to exert a pro-oxidant activity, for example, increasing the cytotoxicity of hydrogen peroxide applied on cultured fibroblasts (Richard *et al.*, 1993). Furthermore, it has been shown that zinc binds to APP controlling its conformation and stability (Bush *et al.*, 1993). In an *in vitro* study, Bush *et al.* (1994) observed that high concentrations of zinc ions caused the $A\beta_{1-40}$ protein to form clumps resembling the amyloid plaques found in the brains of Alzheimer's patients.

In previous studies, using rat pheochromocytoma PC12 cells, we have shown that the amyloid β-peptide $A\beta_{1-40}$ leads to the inhibition of cell survival by a mechanism involving oxidative stress and mitochondrial dysfunction (Pereira *et al.*, 1998, 1999). In the present study, the effect of zinc metal ions (50 μM and 1 mM) on the amyloid β-peptide $A\beta_{1-40}$-induced toxicity was evaluated by determining the reduction of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Furthermore, to clarify the mechanisms by which zinc interferes with $A\beta_{1-40}$-induced inhibition of cell survival, the effect of zinc on lipid and protein oxidation and also on the metabolic function of PC12 cells was analyzed. Data from this study clearly demonstrate that zinc has a concentration-dependent dual effect, probably related with the disruption of the oxidant/pro-oxidant status of PC12 cells and to the compromise of the cellular energy metabolism.

MATERIALS AND METHODS

Chemicals

Amyloid beta peptide fragment ($A\beta_{1-40}$) was obtained from Bachem AG (Bubendorf, Germany). Zinc chloride ($ZnCl_2$) was obtained from Fluka (Portugal). MTT and RPMI-1640 medium were purchased from Sigma Chemical Co. (St. Louis, MO). Fetal calf serum (FCS) was obtained from Biokrom KG (Berlin, Germany), and horse serum from GIBCO (Paisley, UK). All other reagents were of analytical grade.

Cell culture and toxicity studies

Stock cultures of PC12 cells (established line) (Greene and Tischler, 1976), purchased from ATCC (American Type Culture Collection), were grown routinely in 75-cm^2 tissue culture flasks in RPMI-1640 medium supplemented with 10% heat-inactivated horse serum and 5% heat-inactivated FCS, to which 50 U/ml penicillin and 50 μg/ml streptomycin (Sigma Chemical Co., St. Louis, MO) were added and maintained at 37°C in a humidified incubator containing 95% air and 5% CO_2. Twenty-four hours after seeding, the medium was replaced by fresh medium containing the desired concentration of $A\beta_{1-40}$ and $ZnCl_2$.

Measurement of cytotoxicity

Cell viability was measured using the MTT reduction ability of PC12 cells. In brief, after incubation of the cells with the compounds to be tested, the medium was aspirated and 0.5 mg/ml MTT dissolved in serum-free RPMI-1640 medium, was added. After an additional 2 hr incubation at 37°C, isopropanol/HCl was added and the absorbance at 570 nm of solubilized MTT formazan products was measured. Results were expressed as the percentage (%) of MTT reduction, assuming the absorbance of control cells as 100%.

Quantification of lipid peroxidation

The extent of lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS), which include mal-

ondialdeyde (MDA), using the Thiobarbituric Acid Test (TBA) according to a modified procedure described by Ernster and Nordenbrand (1967). The amount of TBARS formed was calculated using a molar extinction coefficient of 1.56×10^5 mol^{-1} cm^{-1} and expressed as nmol TBARS/mg protein.

Analysis of ATP

After the incubation period, the medium was removed, and PC12 cells were extracted, in ice, with 0.3 *M* perchloric acid. The cells were scraped from the wells and centrifuged at 15,800 × *g* for 5 min. The supernatants were neutralized with 10 *M* KOH in 5 *M* Tris and centrifuged at 15,800 × *g* for 5 min. The resulting supernatants were assayed for ATP by separation in a reverse-phase high performance liquid chromatography (HPLC). The chromatography apparatus was a Beckman-System Gold, consisting of a 126 Binary Pump Model and 166 Variable UV detector, controlled by a computer. The detection wave length was 254 nm, and the column was a Lichrospher 100 RP-18 (5 μm) from Merck. An isocratic elution with 100 m*M* phosphate buffer (KH_2PO_4), pH 6.5, and 1.0% methanol was performed with a flow rate of 1 ml/min. The required time for each analysis was 6 min.

Carbonyl content

The carbonyl content was determined by reaction of the carbonyl groups with 2,4-dinitrophenylhydrazine according to a procedure described by Levine *et al.* (1990). All of the samples had a blank prepared by treatment with HCl instead of 2,4-dinitrophenylhydrazine in HCl. The carbonyl content was calculated from the maximum absorbance (360 nm) using a molar absorption coefficient of 22,000 M^{-1} cm^{-1}.

Data analysis

Data were expressed as means ± SEM of the indicated number of determinations, from at least three independent experiments. Statistical significance analysis was determined by using the unpaired two-tailed Student's *t*-test or by the one-way analysis of variance for multiple comparison (MANOVA), followed by the Tukey-Kramer posthoc test (a value of $p < 0.05$ was considered significant).

RESULTS

Effect of zinc on the cellular viability

The effect of zinc on the cellular viability of PC12 cells was evaluated by determining the percentage (%) of MTT reduction upon incubation of cells for 24 hr in the absence or in the presence of two zinc concentrations (50 μ*M* and 1 m*M*). As shown in Fig. 1, treatment of PC12 cells with 50 μ*M* zinc did not induce any significant alteration on the cellular viability. However, when PC12 cells were exposed during the same period of time to 1 mM zinc, a significant inhibition of MTT reduction occurred, as compared to control conditions (62.30 ± 3.50% and 99.23 ± 2.59%, respectively).

Dose-dependent Aβ-induced toxicity in the presence/absence of zinc

The dose-dependent amyloid β-peptide toxicity was evaluated by determining the percentage (%) of MTT reduction upon incubation of PC12 cells for 24 hr with increasing concen-

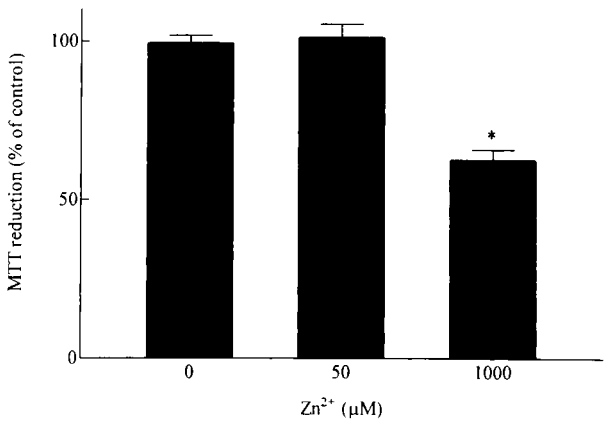

FIG. 1. Effect of zinc (50 μ*M* and 1 m*M*) on the cellular viability of PC12 cells. After the desired incubation period, control and zinc-treated cells were analyzed for their ability to reduce the tetrazolium salt MTT. Data are the arithmetic mean ± SEM of triplicate determinations of 3–15 different experiments. *$p < 0.05$ (*t*-test) when compared with control conditions.

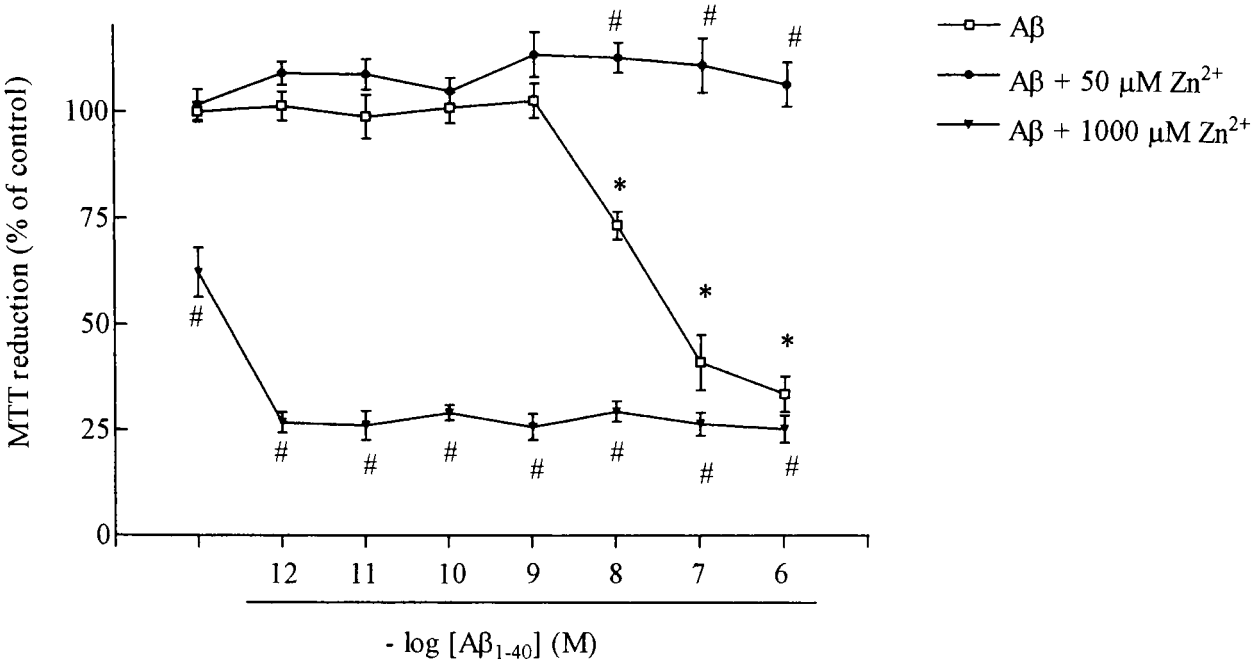

FIG. 2. Dose–response curve for the $A\beta_{1-40}$ peptide-mediated toxicity on PC12 cells and the effect of zinc (50 mM and 1 mM). After the desired incubation period, control and drug-treated cells were analyzed for their ability to reduce the tetrazolium salt MTT. Data are expressed by the arithmetic mean ± SEM of triplicate determinations of 3–15 experiments. $^*p < 0.05$ (MANOVA test; Tukey-Kramer test) when compared with control conditions; $\#p < 0.05$ (MANOVA test; Tukey-Kramer test) when compared with the values determined in $A\beta_{1-40}$-treated cells, in the absence of zinc.

trations of $A\beta_{1-40}$ peptide in the presence/absence of 50 μM and 1 mM zinc (Fig. 2).

The $A\beta_{1-40}$ peptide induced a decrease in cellular viability in a dose-dependent manner, with a significant decrease of MTT reduction ability measured after treatment of the cells with 10 nM of the amyloid peptide. The toxic effect of $A\beta_{1-40}$ peptide was enhanced by 1 mM zinc when the cells were exposed for 24 hr to $A\beta_{1-40}$ peptide in the presence of 1 mM zinc, $A\beta_{1-40}$ concentrations that per se did not affect cell viability (1 pM–1 nM) became toxic, and the toxicity induced by $A\beta_{1-40}$ peptide alone (10 nM–1 μM) was potentiated. On the other hand, 50 μM zinc protected the cells against the amyloid peptide toxicity. The percentage of MTT reduction determined in cells treated with toxic concentrations of $A\beta_{1-40}$ peptide (10 nM–1 μM) in the presence of 50 μM zinc is similar to that determined under control conditions, in the absence of $A\beta_{1-40}$ and zinc.

Influence of zinc on lipid peroxidation

As shown in Fig. 3, the extent of lipid peroxidation in PC12 cells incubated with 50 μM zinc was not different from that determined under control condition although it was slightly lower (1.74 ± 0.06 and 1.24 ± 0.04 nmol TBARS/mg protein, respectively). However, 1 mM zinc induced a significant enhancement in the extent of lipid peroxidation as compared with controls (4.62 ± 0.37 nmol MDA/mg protein and 1.74 ± 0.06 nmol TBARS/mg protein, respectively).

Effect of zinc on protein oxidation

Data from Fig. 4 show that the cellular oxidation of proteins, evaluated by determining the content in carbonyl groups, was significantly increased in PC12 cells treated for 24 hr with 1 mM zinc (19.76 ± 0.75 nmol/mg protein). It was also observed that the incubation of cells with 50 μM zinc did not affect significantly the protein oxidation of PC12 cells, although it leads to a small decrease in carbonyls content (11.43 ± 1.27 nmol/mg protein).

Effect of zinc on ATP levels

The intracellular levels of ATP were determined in PC12 cells upon incubation for 24 hr

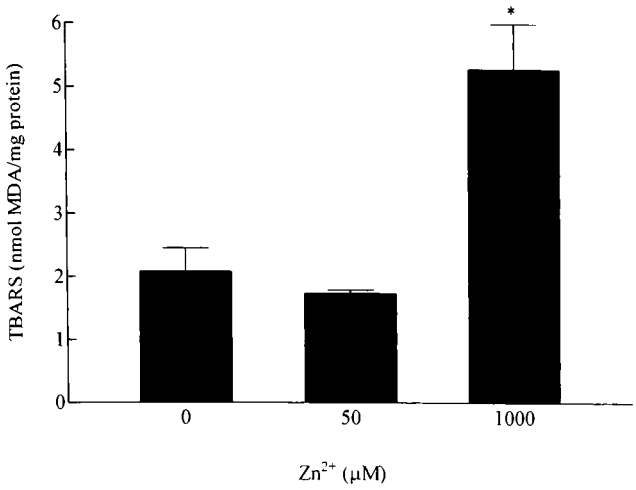

FIG. 3. Effect of zinc on the extent of lipid peroxidation in PC12 cells. The peroxidation of lipid membrane lipids was evaluated by the quantification of TBARS. Data are expressed by the arithmetic mean ± SEM of four independent preparations. $^{*}p < 0.05$ (*t*-test) when compared with the control condition.

with or without 50 μ*M* or 1 m*M* zinc (Fig. 5). In the presence of 50 μ*M* zinc, the ATP levels were slightly increased as compared with those determined upon incubation of PC12 cells in the absence of zinc (4.33 ± 0.15 and 3.62 ± 0.54 nmol/mg protein, respectively). However, this increase in ATP content, induced by 50 μ*M* zinc, was not statistically significant. On the other hand, when PC12 cells were incubated with 1 m*M* zinc, a significant depletion on ATP levels occurred (0.18 ± 0.02 nmol/mg protein) in comparison with control conditions, in the absence of metal ions (3.62 ± 0.54 nmol/mg protein).

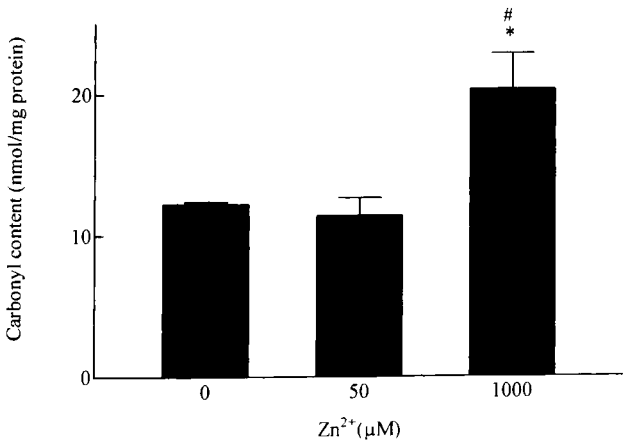

FIG. 4. Effect of zinc (50 μ*M* and 1 mM) on protein oxidation. The oxidation of cellular proteins, determined as an increase in the content of carbonyl groups, was analyzed in control and zinc-treated cells. Data are expressed by the arithmetic mean ± SEM of four independent preparations. $^{*}p < 0.05$ (*t*-test) compared with control condition; $^{\#}p < 0.05$ (*t*-test) when compared with the 50 μ*M* condition.

DISCUSSION

In the present study it was observed that zinc (50 μ*M* and 1 m*M*) has a dual effect on PC12 cells, affording protection against $A\beta_{1-40}$ at low concentrations and enhancing toxicity at high concentrations (Fig. 2). To elucidate the mechanisms involved in zinc-induced protection or potentiation of Aβ toxicity, the following parameters were analyzed in PC12 cells treated in the absence or in the presence of zinc (50 μ*M* and 1 m*M*): cellular viability (Figs 1 and 2), lipid peroxidation (Fig. 3), protein oxidation (Fig. 4), and energy levels (Fig. 5).

The results presented in this paper, obtained using PC12 cells incubated with increasing concentrations of $A\beta_{1-40}$ (10 n*M*–1 μ*M*) in the presence of 50 μ*M* zinc or 1 m*M* zinc, are in agreement with a recent study reporting that zinc produces significant protection against Aβ-induced toxicity (at Aβ/Zn molar ratios of 1:0.1 and 1:0.01) in cultured primary hippocampal neurons, while it offers no protection or enhances Aβ toxicity at higher concentrations (1:1 molar ratio) (Lovell *et al.*, 1999).

It has been proposed (Lovell *et al.*, 1999) that the protective effect of zinc against Aβ toxicity, in hippocampal neurons, is due, in part, to the enhancement of Na^+/K^+ ATPase activity, which prevents the disruption of calcium homeostasis on cell death associated with Aβ toxicity (Mattson *et al.*, 1992). Zinc ions may also affect the perturbation of calcium homeostasis induced by the Aβ through its interactions with calcium-permeable, zinc-sensitive channels formed by the Aβ peptides (Kawahara *et al.*, 1997; Rhee *et al.*, 1998). Furthermore, in previous studies it was shown that zinc exposure at a nonphysiological dose (≥600 μ*M*), for 15 min, resulted in neuronal death in mixed cultures of cortical and glial cells, while exposure for a longer period (18–24 hr) at lower concentrations (≤300 μ*M*) induces glial cell death (Choi *et al.*, 1988; Yokoyama *et al.*, 1986).

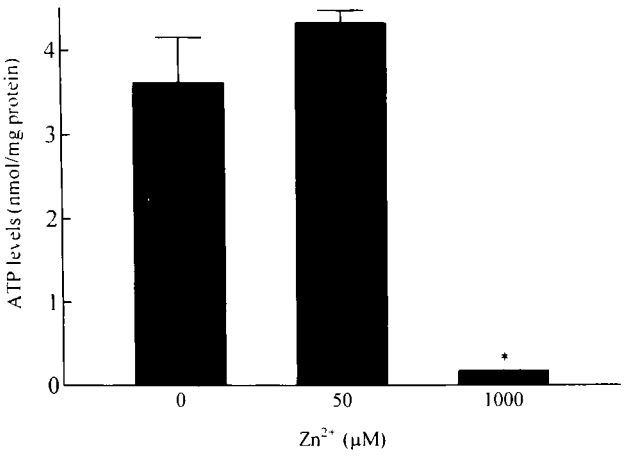

FIG. 5. Effect of zinc exposure on intracellular ATP levels. Cells were exposed, or not, to zinc (50 mM and 1 m*M*) for 24 hr, and then the ATP concentration of control and zinc-treated cells was determined by reverse-phase HPLC with UV detection. Data are expressed by the arithmetic mean ± SEM of 3–4 independent preparations. $^*p < 0.05$ (*t*-test) compared with control conditions.

Several studies have shown that zinc protects fatty acids from peroxidation by inhibiting the production of ROS catalyzed by transition metals, and zinc deprivation in animal models can cause increased lipid peroxidation (Coudray *et al.*, 1991; Faure *et al.*, 1991). Furthermore, zinc salts have been shown to provide protection against oxidative stress, leading to inhibition of lipid peroxidation, ROS generation, and reduced glutathione (GSH) depletion (Bagchi *et al.*, 1997, 1998).

Our results suggest that at high concentrations (1 m*M*) zinc leads to oxidative stress promoting lipid peroxidation (Fig. 3) and protein oxidation (Fig. 4). Kim *et al.* (1999) have shown that zinc-induced cortical neuronal death is mediated by free radicals, which increased membrane lipid peroxidation. On the other hand, data obtained with 50 μM zinc suggest that, at low concentrations, zinc has antioxidant potency decreasing the extent of lipid peroxidation and protein oxidation.

When PC12 cells were treated with 1 m*M* zinc, the ATP levels decreased when compared to control conditions, which suggests that perhaps this is an extremely toxic concentration that affects the oxidative phosphorylation. It has been proposed that ATP depletion within the cell, as a result of an alteration of mitochondrial function, is one of the major reasons for the cell death following exposure to oxidant agents (Carini *et al.*, 1992; Imberti *et al.*, 1993). Oxidative stress, induced by endogenous agents or during the aging process, is known to inhibit the mitochondrial respiratory chain (Bowling *et al.*, 1993; Bates *et al.*, 1995; Schapira and Cooper, 1992; Glinka and Youdim, 1994; Cleeter *et al.*, 1992). Zinc can inhibit the cellular respiratory chain ($K_i = 10^{-7}$ *M*) by blocking the initial step of respiration at the level of electron transfer between ubiquinone (coenzyme Q) and cytochrome *b* and *bc* 1 complex (complex III) (Link and von Jagow, 1995). At higher concentrations (10^{-3} *M*), zinc may further inhibit the mitochondrial electron transport at the levels of flavoprotein 1 or 2 (complex I or II) and cytochrome *c* oxidase (complex IV) activities (Skulachev *et al.*, 1967).

The stimulation of Aβ-induced toxicity by high concentrations of zinc (Fig. 2) could be explained by the promotion of Aβ aggregation, because the formation of insoluble aggregates of the peptide is related with its neurotoxicity (Pike *et al.*, 1991). Huang and colleagues have suggested that zinc leads to $A\beta_{1-40}$ aggregation due to the promotion of conformational changes, such as the modification of sulfhydryl groups of the Aβ peptide (Huang *et al.*, 1997). Previous work (Mantyh *et al.*, 1993; Esler *et al.*, 1996) indicated that salts of zinc at high, possibly nonphysiological, concentrations $\geq 10^{-4}$ *M* significantly enhance the rate of Aβ aggregation *in vitro*. These results stand in quantitative disagreement with one study that reported significant aggregation of Aβ by zinc at physiological concentrations (Bush *et al.*, 1994). However, zinc-mediated $A\beta_{1-40}$ precipitation levels (≤ 5 μM) per se is not sufficient for neurodegeneration, as co-addition of zinc to rat hippocampal primary cultures, specifically and in a dose-dependent manner, reduced $A\beta_{1-40}$ neurotoxicity (Fuson *et al.*, 1996).

Zinc can act as an antioxidant at low concentration (50 μM), protecting the PC12 cells against ROS. Probably zinc competes with certain oxygen radicals-inducing agents, such as the $A\beta_{1-40}$ peptide (Pereira *et al.*, 1999), relative to its binding site on the cellular membrane. Transition metals attach themselves to active

binding sites and induce enhanced production of reactive species, leading to tissue-damaging effects. It has been described that zinc inhibits the toxicity of paraquat, a widely used herbicide, through a mechanism that involves the displacement of copper from its binding sites, thus preventing free radical production (Chevion *et al.*, 1990). It has also been shown that zinc, at low concentrations (micromolar), has an antagonistic effect on the oxidative properties of copper, decreasing ·OH formation (Powell *et al.*, 1994). Zinc competes with iron and copper for their binding sites, providing protection against metal-mediated DNA single- and double-strand breaks (Har-El and Chevion, 1991). Therefore, zinc may function as a site-specific rather than a general antioxidant. By competing with other metal ions for binding to cell membranes and proteins, zinc may displace redox-active metals, namely iron and copper, and make them more available for binding to ferritin and metallothionein, respectively (Bettger, 1993). Furthermore, zinc may affect Aβ toxicity through its action on copper induced amyloid β-chain aggregation (Atwood *et al.*, 1998). Zinc may also alter the amyloid-induced hydrogen peroxide (H_2O_2) formation that results from copper binding and reduction by Aβ (Huang *et al.*, 1999), and it protects from oxidation by binding to zinc sulfhydryl groups in proteins (Bagchi *et al.*, 1998).

In conclusion, we can say that zinc might have a concentration-dependent dual action, protective and toxic, thus playing an important role in the pathogenesis of Alzheimer's disease. However, further experiments are needed to clarify the mechanisms underlying those effects.

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ABBREVIATIONS

AD, Alzheimer's disease; Aβ, amyloid β-peptide; $A\beta_{1-40}$, 1–40 fragment from amyloid β-peptide; β-APP, β-amyloid precursor protein; FCS, fetal calf serum; GSH, reduced glutathione; H_2O_2, hydrogen peroxide; HPLC, high-performance liquid chromatography; LDH, lactate dehydrogenase; MANOVA, one-way analysis of variance for multiple comparison; MDA, malondialdeyde; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ·OH, hydroxyl radical; PC12, pheochromocytoma cell line; ROS, reactive oxygen species; TBA, Thiobarbituric Acid Test; TBARS, thiobarbituric acid reactive substances; Zn, zinc.

REFERENCES

ATWOOD, C.S., MOIR, R.D., HUANG, X., SCARPA, R.C., BACARRA, N.M., ROMANO, D.M., HARTSHORN, M.A., TANZI, R.E., and BUSH, A.I. (1998). Dramatic aggregation of Alzheimer A beta by Cu (II) is induced by conditions representing physiological acidosis. J. Biol. Chem. **273(21),** 12817–12826.

BAGCHI, D., BAGCHI, M., and STOHS, S.J. (1997). Comparative in vitro oxygen radical scavenging ability of zinc methionine and selected zinc salts and antioxidants. Gen. Pharmacol. **2,** 85–91.

BAGCHI, D., VUCHETICH, P.J., BAGCHI, M., TRAN, M.X., KROHN, R.L., RAY, D.S.D., and STOHS, S.J. (1998). Protective effects of zinc salts on TPA-induced hepatic and brain lipid peroxidation, glutathione depletion, DNA damage and peritoneal macrophage activation in mice. Gen. Pharmacol. **30,** 43–50.

BATES, T.E., HEALES, S.J.R., DAVIES, S.E.C., BOAKYE, P., and CLARCK, J.B. (1995). Effects of 1-methyl-4-phenylpyridinium on isolated rat brain mitochondria: evidence for a primary involvement of energy depletion. J. Neurochem. **63,** 640–648.

BENDOTTI, C., FORLONI, G., MORGAN, R., O'HARA, B., OSTER-GRANITE, M., REEVES, R., GEARHART, J., and COYLE, J. (1988). Neuroanatomical localization and quantification of amyloid precursor protein mRNA by the situ hybridization in the brains of normal, aneuploid, and lesioned mice. Proc. Natl. Acad. Sci. USA **85,** 3628–3632.

BETTGER, W.J. (1993). Zinc and selenium, site-specific versus general antioxidation. Cn. J. Physiol. Pharmacol. **71,** 721–724.

BOWLING, A.C., MUTISYA, E.M., WALTER, L.C., PRICE, D.L., CORK, L.C., and BEAL, M.F. (1993). Age-dependent impairment of mitochondria function in primate brain. J. Neurochem. **60,** 1964–1967.

BUSH, A., MULTHAUP, G., MOIR, R.D., WILLIAMSON, T.G., SMALL, D.H., POLLWEIN, B., BEYREUTHER, K., and MASTERS, C.L. (1993). A novel zinc (II) binding modulates the function of βA4 amyloid protein precursor of Alzheimer's disease. J. Biol. Chem. **268,** 16109–16112.

BUSH, A., PETTINGELL, W., MULTHAUP, G., PARADIS, M., VON-SANTTEL, J., GUSELLA, J., BEYRUTHER, K., MASTERS, C., and TANZI, R. (1994). Rapid induction of Alzheimer β-amyloid formation by zinc. Science **265,** 1464–1467.

CARINI, R., PAROLA, M., DIANZANI, M.U., and ALBANO, E. (1992). Mitochondria damage and its role in causing hepatocyte injury during stimulation of lipid peroxidation by iron nitriloacetate. Arch. Biochem. Biophys. **297,** 110–118.

CLEETER, M.J., COOPER, J.M., and SCHAPIRA, A.H.V. (1992). Irreversible inhibition of mitochondria complex I by 1-methyl-4-phenylpyridinium: evidence for free radical involvement. J. Neurochem. **58,** 786–789.

CHEVION, M., KORBASHI, P., KATZHANDLER, J., and SALMAN, P. (1990). Zinc–A redox-inactive metal provides a novel approach for protection against metal mediated free radical induced injury: study of paraquat toxicity in E. coli. Adt. Exp. Med. Biol. **264,** 217–222.

CHOI, D.W., YOKOYAMA, M., and KOH, J. (1988). Zinc neurotoxicity in cortical cell culture. Neuroscience **24, (1),** 67–69.

COUDRAY, C., BOUCHER, F., RICHARD, M.J., ARNAUD, J., DE LEIRIS, J., and FAVIER, A. (1991). Zinc deficiency, ethanol, and myocardial ischemia affect lipoperoxidation in rats. Biol. Trace Element Res. **30,** 103–118.

COUDRAY, C., RICHARD, M.J., LAPORTE, F., FAURE, P., ROUSSEL, A.M., and FAVIER, A. (1992). Superoxide dismutase activity and zinc status: a study in animals and man. J. Nutr. Med. **3,** 13–26.

CUAJUNGCO, M.P., and LEES, G.J. (1997). Zinc metabolism in the brain: relevance to human neurodegenerative disorders. Neurobiol. Dis. **4,** 137–169.

ERNSTER, L., and NORDENBRAND, K. (1967). Microsomal lipid peroxidation. Methods Enzymol. **10,** 574–580.

ESLER, W.P., STIMSON, E.R., JENNINGS, J.M., GHILARDI, J.R., MANTYH, P.W., and MAGGIO, J.E. (1996). Zinc-induced aggregation of human and rat β-amyloid peptides in vitro. J. Neurochem. **66,** 723–732.

FAURE, P., ROUSSEL, A.M., RICHARD, M.J., FOULON, T., GROSLAMBERT, P., HADJIAN, A., and FAVIER, A. (1991). Effect of an acute zinc depletion on rat lipoprotein distribution and peroxidation. Biol. Trace Element Res. **28(2),** 135–146.

FUSON, K.S., BOGGS, L.N., and MAY, P.C. (1996). Zinc promotes Aβ aggregation but attenuates Aβ neurotoxicity. Neurobiol. Aging **17** (Suppl.), S108 (431).

GLENNER, G.G., and WONG, C.W. (1984). Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun. **120,** 885–890.

GLINKA, Y.Y., and YOUDIN, M.B.H. (1994). Inhibition of mitochondria complexes I and IV by 6-hydroxydopamine. Eur. J. Pharmacol. Environ. **292,** 329–332.

GOLDGABER, D., LERMAN, M.I., MCBRIDGE, O.W., SFFIOTTI, U., and GAJDUSEK, D.C. (1987). Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science **235,** 877–890.

HUANG, X., ATWOOD, C.S., MOIR, R.D., HARTSHORN, M.A., VONSATTEL, J.P., TANZI, R.E., and BUSH, A.I. (1997). Zinc-induced Alzheimer's $A\beta_{1-40}$ aggregation is mediated by conformational factors. J. Biol. Chem. **272,** 26464–26470.

HUANG, X., ATWOOD, C.S., HARTSHORN, M.A., MULTHAUP, G., GOLDSTEIN, L.E., SCARPA, R.C., CUAJUNGCO, M.P., GRAY, D.N., LIM, J., MOIR, R.D., TANZI, R.E., and BUSH, A.I. (1999). The Aβ peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. Biochemistry **38,** 7609–7616.

HUNTER, F.E.J., and FORD, L. (1955). Inactivation of oxidative and phosphorylative systems in mitochondria by preincubation with phosphate and other ions. J. Biol. Chem. **216,** 138–144.

IMBERTI, R., NIEMINEN, A., HERMAN, B., and LMASTERS, J. (1993). Mitochondria and glycolytic dysfunction in lethel injury to hepatocytes by t-butylhydroperoxide: protection by frutose, cyclosporin A, and trifluoperazine. J. Pharmacol. Exp. Ther. **265,** 393–400.

IVERSEN, L.L., MORTISHIRE-SMITH, R.J., POLLACK, S.J., and SHEARMAN, M.S. (1995). The toxicity in vitro of β-amyloid protein. Biochem. J. **311,** 1–16.

KAWAHARA, M., ARISPE, N., KURODA, Y., and ROJAS, E. (1997). Alzheimer's disease amyloid beta-protein forms Zn^{2+}-sensitive, cation-selective channels across excised membrane patches from hypothalamic neurons. Biophys. J. **73,** 67–75.

KIM, E.Y., KOH, J.Y., KIM, Y.H., SOHN, S., JOE, E., and GWAG, B.J. (1999). Zn^{2+} entry produces oxidative neuronal necrosis in cortical cell cultures. Eur. J. Neurosci. **11,** 327–334.

LEVINE, R.L., GARLAND, D., OLIVER, C.N., AMICI, A., CLIMENT, I., LENZ, A.J., AHN, B-W., SHALTIEL, S., and STADTMAN, E.R. (1990). Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. **186,** 464–478.

LINK, T.A., and VON JAGOW, G. (1995). Zinc ions inhibit the Qp center of bovine heart mitochondrial bcl complex by blocking a protonable group. J. Biol. Chem. **270,** 25001–25006.

LOVELL, M.A., EHMANN, W.D., BUTLER, S.M., and MARKESBERY, W.R. (1995). Elevated thiobarbituric acid-reactive substances and antioxidante enzyme activity in the brain in Alzheimer's disease. Neurology **45,** 1594–1601.

LOVELL, M.A., XIE, C.S., and MARKESBERY, W.R. (1999). Protection against amyloid beta peptide toxicity by zinc. Brain Res. **823,** 88–95.

MANTYH, P.W., GHILARDI, J.R., ROGERS, S., DEMASTER, E., ALLEN, C.J., STIMSON, E.R., and MAGGIO, J.E. (1993). Aluminium, iron, and zinc ions promote aggregation of physiological concentrations of β-amyloid peptide. J. Neurochem. **61,** 1171–1174.

MARK, R.J., BLANC, E.M., and MATTSON, M.P. (1996). Amyloid β-peptide and oxidative injury in Alzheimer's disease. Mol. Neurobiol. **12,** 211–224.

MATTSON, M.P., CHENG, B., DAVIES, D., BRYANT, K., LIEBERBURG, I., and RYDEL, R.E. (1992). β-amyloid peptides destabilize calcium homeostasis and render

human cortical neurons vulnerable to excitotoxicity. J. Neurosci. **12,** 379–389.

PEREIRA, C., SANTOS, M.S., and OLIVEIRA, C. (1998). Mitochondrial function impairment induced by amyloid β-peptide on PC12 cells. NeuroReport **9,** 1759–1755.

PEREIRA, C., SANTOS, M.S., and OLIVEIRA, C. (1999). Involvement of oxidative stress on the impairment of energy metabolism induced by Aβ-peptides on PC12 cells. Protection by antioxidants. Neurobiol. Dis. **6,** 209–219.

PIKE, C.J., WALENCEWICZ, J., GLABE, C.G., and COTMAN, C.W. (1991). Aggregation-related toxicity of synthetic β-amyloid protein in hippocampal cultures. Eur. J. Pharmacol. **207,** 367–368.

POWELL, S.R., HALL, D., AIUTO, L., WAPNIR, R.A., TELCHBERG, S., and TORTOLANI, A.J. (1994). Zinc improves postischemic recovery of the isolated rat heart through inhibition of oxidative stress. Am. J. Physiol. **266,** H2497–H2507.

RHEE, S.K., QUIST, A.P., and LAL, R. (1998). Amyloid beta-protein-(1-42) forms calcium-permeable, Zn^{2+}-sensitive channel. J. Biol. Chem. **273,** 13379–13382.

RICHARD, M.J., GUIRAUD, P., LECCIA, M.T., BEANI, J.C., and FAVIER, A. (1993). Effect of zinc supplementation on resistance of cultured human skin fibroblasts towards oxidant stress. Biol. Trace Element Res. **37,** 187–199.

SCHAPIRA, A.H.V., and COOPER, J.M. (1992). Mitochondrial function in neurodegeneration and ageing. Mutation Res. **275,** 133–143.

SKULACHEV, V.P., CHISTYAKOV, V.V., JASAITIS, A.A., and SMIRNOVA, E.G. (1967). Inhibition of the respiratory chain by zinc ions. Biochem. Biophys. Res. Commun. **26,** 1–6.

SMITH, M.A., CARNEY, J.M., STARKE-REED, P.E., OLIVER, C.N., STADTMAN, E.R., FLOYD, R.A., and MARKESBERY, W.R. (1991). Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer's disease. Proc. Natl. Acad. Sci. USA **88,** 10540–10543.

SMITH, M.A., SAYRE, L.M., MONNIER, V.M., and PERRY, G. (1995). Radical ageing in Alzheimer's disease. Trends Neurosci. **18,** 172–176.

YANKNER, B.A. (1996). Mechanisms of neuronal degeneration in Alzheimer's disease. Nature **382,** 685–691.

YOKOYAMA, M., KOH, J., and CHOI, D.W. (1986). Brief exposure to zinc is toxic to cortical neurons. Neurosci. Lett. **71,** 351–355.

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1. Lisa M Martorano, Christian J Stork, Yang V Li. 2010. UV irradiation-induced zinc dissociation from commercial zinc oxide sunscreen and its action in human epidermal keratinocytes. *Journal of Cosmetic Dermatology* **9**:4, 276-286. [CrossRef]
2. Sanja Pavlica, Rolf Gebhardt. 2010. Comparison of uptake and neuroprotective potential of seven zinc-salts. *Neurochemistry International* **56**:1, 84-93. [CrossRef]
3. Sanja Pavlica, Frank Gaunitz, Rolf Gebhardt. 2009. Comparative in vitro toxicity of seven zinc-salts towards neuronal PC12 cells. *Toxicology in Vitro* **23**:4, 653-659. [CrossRef]
4. M. Grazina, J. Pratas, F. Silva, S. Oliveira, I. Santana, C. Oliveira. 2006. Genetic basis of Alzheimer's dementia: role of mtDNA mutations. *Genes, Brain and Behavior* **5**, 92-107. [CrossRef]
5. Bruce L. Martin, Abigail M. Tokheim, Patrick T. McCarthy, Brendan S. Doms, Andrew A. Davis, Ian M. Armitage. 2006. Metallothionein-3 and neuronal nitric oxide synthase levels in brains from the Tg2576 mouse model of Alzheimer's disease. *Molecular and Cellular Biochemistry* **283**:1-2, 129-137. [CrossRef]
6. Keiko Konoha, Yutaka Sadakane, Masahiro Kawahara. 2006. Zinc Neurotoxicity and its Role in Neurodegenerative Diseases. *JOURNAL OF HEALTH SCIENCE* **52**:1, 1-8. [CrossRef]
7. S. M. Cardoso, A. C. Rego, C. Pereira, C. R. Oliveira. 2005. Protective effect of zinc on amyloid-ß 25–35 and 1–40 mediated toxicity. *Neurotoxicity Research* **7**:4, 273-281. [CrossRef]
8. Paul E Beaumont, Hyong K Kang. 2005. Correspondence. Balancing the risks and benefits in AMD. *Clinical and Experimental Ophthalmology* **33**:1, 108-110. [CrossRef]
9. Glenda M. Bishop, Stephen R. Robinson. 2004. The Amyloid Paradox: Amyloid-#-Metal Complexes can be Neurotoxic and Neuroprotective. *Brain Pathology* **14**:4, 448-452. [CrossRef]
10. Math P. Cuajungco, Kyle Y. Fagét. 2003. Zinc takes the center stage: its paradoxical role in Alzheimer’s disease. *Brain Research Reviews* **41**:1, 44-56. [CrossRef]
11. Michael Grundman, Patrick Delaney. 2002. Antioxidant strategies for Alzheimer's disease. *Proceedings of the Nutrition Society* **61**:02, 191-202. [CrossRef]
12. C Resende Oliveira. 2001. Research on aging in Portugal. *Experimental Gerontology* **36**:10, 1599-1607. [CrossRef]