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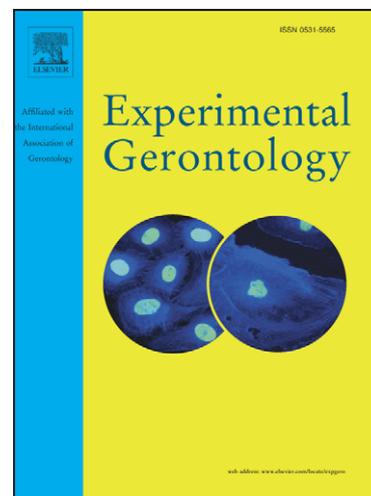
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Zinc supplementation in the elderly subjects: effect on oxidized protein degradation and repair systems in peripheral blood lymphocytes

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Running title: Zinc supplementation and oxidized protein status.

Keywords: Methionine sulfoxide reductases; Proteasome; Zinc supplementation; Oxidized proteins; Human aging.

Abstract

Aging has been associated with zinc deficiency, leading to chronic inflammation and subsequent oxidative stress, especially in the immune system. The increased oxidative stress provokes the accumulation of oxidized proteins, raising the problem of the efficacy of intracellular protein maintenance systems responsible for the elimination of oxidatively modified proteins. Our objective was to analyse the effect of zinc supplementation in the elderly on protein maintenance in peripheral blood lymphocytes. The status of the proteasome, which is in charge of oxidized protein degradation and the repair enzymes peptide methionine sulfoxide reductases, which can reverse methionine oxidation in proteins, were analysed on peripheral blood lymphocytes collected from 20 elderly subjects (age-range between 59-85 years old) before and after zinc supplementation (10 mg of zinc per day for 48 +/- 2 days). A decrease of oxidized protein content in zinc supplemented subjects was observed and was associated with an increase of expression levels and/or activities of proteasome and methionine sulfoxide reductases. Our results indicate that zinc treatment could enhance the anti-oxidative defences of peripheral blood lymphocytes by increasing the activities of protein maintenance systems responsible for the elimination of oxidatively modified proteins.

Introduction

Accumulation of oxidatively modified proteins is a hallmark of cellular aging (Beckman and Ames 1998; Berlett and Stadtman 1997) that raises the problem of the efficacy of intracellular protein maintenance systems responsible for the elimination of oxidized proteins. Protein degradation and repair are critical for eliminating oxidized proteins from the cell (Petropoulos and Friguet 2005). Oxidized protein degradation is mainly achieved by the proteasomal system (Grune et al., 2003) and it is now well established that proteasomal function is impaired in human aging (Chondrogianni and Gonos 2005; Farout and Friguet 2006). In the immune system, a decline of proteasome proteolytic activity has been reported in peripheral blood lymphocytes (PBL) with age of human donors (Carrard et al., 2003; Ponnappan et al., 1999) along with increased oxidative modification of 20S proteasome subunits (Carrard et al., 2003). More recently, in human T lymphocytes, age-related decline of proteasome has been attributed to both a lower expression of catalytic and structural proteasome subunits and increased oxidative modification of certain subunits (Ponnappan et al., 2007). Oxidized protein repair is limited to few modifications, such as methionine oxidation, that can be reversed within proteins by the methionine sulfoxide reductase A and B enzymes (MsrA and MsrB) (Hansel et al., 2005; Weissbach et al., 2002). All MsrB are zinc-containing enzymes (Kumar et al., 2002). Importantly, the Msr system has been implicated in increased longevity and resistance to oxidative stress in different cell types and model organisms such as mouse and drosophila (Moskovitz et al., 2001; Ruan et al., 2002). Moreover, we have reported an age-related decline of Msr activity in different rat organs such as brain, kidney and liver (Petropoulos et al., 2001) and replicative senescence of WI 38 fibroblasts has been associated with a decreased Msr activity and expression of both MsrA and MsrB2 together with an increase in protein carbonyl content (Picot et al., 2004). No focused research has been performed thus far to clarify the impact of nutritional zinc on protein oxidation status and

oxidized protein degradation and repair systems. In this study, we have analysed the status of both the proteasome and the Msr system in PBL cellular extracts, before and after zinc supplementation of elderly subjects. Our results indicate that zinc treatment could enhance the anti-oxidant defences of PBL by increasing the activities of protein maintenance systems responsible for the elimination of oxidized proteins.

Material and methods

Zinc supplementation

Zinc supplementation was performed in healthy elderly subjects aged between 59-85 years and according to some inclusion and exclusion criteria (Kanoni et al., 2006) in Italy with 10 mg/day of zinc-aspartate (Unizink 50, KÖHLER PHARMA Corp., Alsbach-Hähnlein, Germany) and in Poland with an identical form of zinc-aspartate (Zincas, Zakład Chemiczno-Farmaceutyczny FARMAPOL, Poznań, Poland) approved by the respective local Health Authorities. The supplementation period was 48 +/- 2 days. PBL were collected before and after supplementation following the protocols described below.

Peripheral blood lymphocytes recovery

Individual blood samples were drawn in a plastic syringe containing heparin. The whole blood was collected and centrifuged at 450 x g for 10 min at 4°C to separate plasma. The remaining blood was diluted 1:3 with phosphate buffered saline (PBS), pH 7.4, without Mg⁺⁺ and Ca⁺⁺. Granulocytes were purified from red blood cells through sedimentation with 8-10% HAES (final concentration) and the remaining cells were carefully stratified with a pipette on the top of a Ficoll-Hypaque solution (d = 1,077 g/mL). The solution was centrifuged at 450 x g for 30 min at 20°C and the PBL were recovered and washed two times with PBS.

Plasma zinc level determination

Plasma samples were taken from each subject before and after zinc supplementation. Plasma zinc determination was performed with a Thermo XII series Induction Coupled Plasma-Mass Spectrometry (ICP-MS) against zinc reference standards following the application note (AN_EO604) of the manufacturer (Thermo Electron Corporation, Waltham, MA, USA). A cut-off of $\leq 11\mu\text{M}$ zinc was taken as an index of marginal deficiency (Maret and Sandstead 2006).

Gel electrophoresis and immunoblotting

Thirty microgram of total protein extracts were separated on 12% SDS-polyacrylamide gel electrophoresis and then electrotransferred for 75 min at room temperature. Western blotting experiments were performed with either anti-MsrA polyclonal antibodies at a dilution of 1/5000, or anti-actin monoclonal antibodies (Santa-Cruz, Biotechnology, Inc., California, USA) at a dilution of 1/1000. Detection of carbonyl groups was performed with the OxyBlot oxidized protein detection kit (AbCys S.A., Paris, France), according to the manufacturer. Proteins carbonyls were revealed by anti-dinitrophenol antibodies. Blots were developed with chemiluminescence using the SuperSignal West Femto chemiluminescent substrate (Perbio Sciences, Brebières, France). Films were scanned and the amount of signal was quantified by densitometric analysis using Image Master 1D software (Amersham-Biosciences, Orsay, France).

Determination of Msr activity and proteasome peptidase activities

Total Msr enzymatic activity was measured in cellular homogenates using *N*-acetyl-[^3H] Met *R,S* (O) as substrate as previously described (Brot et al., 1982). 30 μg of crude protein cellular extracts was incubated at 37°C for 45 min. The chymotrypsin-like activity of the proteasome was assayed using the fluorogenic peptide, succinyl-Leu-Leu-Val-Tyr-amidomethylcoumarin (LLVY-AMC) as described previously (Bulteau et al., 2002) with minor modifications. The

mixture, containing 20 μg of crude protein cellular extracts in 25 mM Tris-HCl, pH 7.5, was incubated at 37° C with the peptide substrate LLVY-AMC at 25 μM in a final volume of 200 μL . Enzymatic kinetics were conducted in a temperature-controlled microplate fluorimetric reader (Fluostar Galaxy, BMG, Stuttgart, Germany). Excitation/emission wavelengths were 350/440 nm for amidomethylcoumarin.

Statistical analysis

All results are expressed as the mean \pm SEM. Comparisons were analysed with the Student *t*-test and were assumed to be statistically significant if $P \leq 0.05$.

Results

The concentration of plasma zinc was measured in plasma of the 20 elderly subjects before and after zinc supplementation. The 11 subjects from Italy presented a slight deficiency in plasmatic zinc concentration before the supplementation (mean of 9.33 $\mu\text{M} \pm 0.74$) and showed a statistical significant increase in zinc concentration after supplementation (mean of 11.73 $\mu\text{M} \pm 1$). The situation was different in Poland given that the 9 subjects did not exhibit a plasmatic zinc deficiency before the supplementation (mean of 11 $\mu\text{M} \pm 0.76$) and did not show a statistically significant increase after zinc treatment (mean of 11.95 $\mu\text{M} \pm 0.835$) (Figure 1A). To analyse whether zinc supplementation protects PBL against oxidative damages, we monitored carbonyl groups on intracellular proteins, which is a good index of global protein oxidation, using OxyBlot experiments. Quantification of carbonyl levels showed that protein oxidation is lower of at least 10% when we compared the subjects before and after supplementation in Italy and Poland (15% and 8% decrease compared to the non-supplemented subjects, respectively, Figure 1B). The prevention of oxidized protein accumulation can be due, at least in part, to an increase activity of the enzymatic systems in charge of the elimination of oxidized proteins in the cell, i.e. proteasome and Msr. To test this

hypothesis, the chymotrypsin-like peptidase activity of the proteasome was measured using a fluorogenic peptide as substrate and was shown to be significantly higher in zinc supplemented subjects from Italy and Poland (132% and 123% of the activity of the non-supplemented subjects, respectively, Figure 1C). Msr activity was measured with *N*-acetyl-³H] Met *R,S* (O), a radioactive substrate, allowing the determination of the MsrA and MsrB activities. Figure 1D show that Msr activity is increased in zinc supplemented subjects in Italy and Poland (126 % and 182 % of the activity the non-supplemented subjects, respectively). In order to determine whether the increase in Msr activities could be due to a higher expression of MsrA, western blotting experiments were conducted in PBL protein extracts using a polyclonal antibody raised against the MsrA protein. The amount of MsrA was evaluated relative to that detected in samples from non-supplemented subjects. As shown in Figures 2A and 2B, the amount of MsrA is 18% higher in subjects after supplementation.

Discussion

In this study, we showed that zinc supplementation during 7 weeks in the elderly (age range between 59 and 85 years old) can help them to maintain plasma zinc homeostasis and to protect proteins against oxidative damages by increasing the activities of the cellular protein maintenance systems. It is well known that zinc deficiency may affect the immune system, caused, at least in part, by the increasing of oxidative stress (Finamore et al., 2005; Mocchegiani et al., 2006a). A zinc deficiency, due to a lower intake and a decreased absorptive efficiency, was reported in elderly (Mocchegiani et al., 2006b). In this study, the Polish elderly subjects did not show zinc deficiency contrary to the Italian elderly subjects. Zinc supplementation leads to a slight increase in plasma concentrations from Polish elderly and to significant increase in Italian elderly (Figures 1A). This result indicates that a 7 week-zinc supplementation is sufficient to restore zinc homeostasis and most likely to reduce oxidative stress. In fact, zinc supplementation was shown to reduce oxidative stress and

incidence of infections in the elderly (Prasad et al., 2007). The observed increase of plasma zinc in zinc-supplemented subjects is associated with a higher protection against protein oxidative damages since the oxidized protein content is lower in PBL proteins in zinc supplemented-elderly, as shown by the detection of carbonyl groups (Figures 1B). In this context, it was also important to study the status of the proteasome, the major proteolytic system involved in the degradation of oxidized proteins (Grune et al., 2003) and, in fact, chymotrypsin-like proteasome activity is slightly increased in PBL of zinc supplemented donors (Figures 1C). In a previous study on the effect of aging on proteasome structure and function in human PBL, a decrease in proteasome proteolytic activity was associated with the modification of certain catalytic and regulatory subunits by glycation or by conjugation with a lipid peroxidation product (Carrard et al., 2003). Thus, zinc supplementation, by reducing oxidative stress, could protect the proteasome against oxidative modifications of its subunits and preserve the proteasome activity in the elderly. Since not only the proteasome but also the Msr system have been shown to be implicated in oxidized protein elimination (Petropoulos and Friguet 2006), we investigated the Msr system in PBL before and after zinc supplementation. Interestingly, Msr activity is strongly increased after zinc supplementation in PBL (Figures 1D). Despite the fact that zinc-dependent MsrB enzymes (Kumar et al., 2002) were not detected by western blot in the PBL samples before or after zinc supplementation, it is likely that a significant part of the Msr activity increase upon zinc supplementation is due to MsrB activity. Indeed, MsrA expression is increased only by about 20% while total Msr activity is increased up to 82% in the Polish subjects. Further studies are needed to address the fate of MsrB proteins upon zinc supplementation. The zinc supplementation mediated increase in Msr activity could explain, at least in part, the protection against protein oxidative damages.

In conclusion, our results indicate that zinc supplementation, by stimulating the enzymatic systems involved in the elimination of oxidized proteins, could serve as a potential anti-aging strategy.

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References

- Beckman, K. B., Ames, B. N., 1998. The free radical theory of aging matures. *Physiol Rev.* 78, 547-581.
- Berlett, B. S., Stadtman, E. R., 1997. Protein oxidation in aging, disease, and oxidative stress. *J Biol Chem.* 272, 20313-20316.
- Brot, N., Werth, J., Koster, D., Weissbach, H., 1982. Reduction of N-acetyl methionine sulfoxide: a simple assay for peptide methionine sulfoxide reductase. *Anal Biochem.* 122, 291-294.
- Bulteau, A. L., Moreau, M., Nizard, C., Friguier, B., 2002. Impairment of proteasome function upon UVA- and UVB-irradiation of human keratinocytes. *Free Radic Biol Med.* 32, 1157-1170.
- Carrard, G., Dieu, M., Raes, M., Toussaint, O., Friguier, B., 2003. Impact of ageing on proteasome structure and function in human lymphocytes. *Int J Biochem Cell Biol.* 35, 728-739.
- Chondrogianni, N., Gonos, E. S., 2005. Proteasome dysfunction in mammalian aging: steps and factors involved. *Exp Gerontol.* 40, 931-938.
- Farout, L., Friguier, B., 2006. Proteasome function in aging and oxidative stress: implications in protein maintenance failure. *Antioxid Redox Signal.* 8, 205-216.
- Finamore, A., Devirgiliis, C., Panno, D., D'Aquino, M., Polito, A., Venneria, E., Raguzzini, A., Coudray, C., Mengheri, E., 2005. Immune response in relation to zinc status, sex and antioxidant defence in Italian elderly population: the ZENITH study. *Eur J Clin Nutr.* 59 Suppl 2, S68-S72.
- Grune, T., Merker, K., Sandig, G., Davies, K. J., 2003. Selective degradation of oxidatively modified protein substrates by the proteasome. *Biochem Biophys Res Commun.* 305, 709-718.
- Hansel, A., Heinemann, S. H., Hoshi, T., 2005. Heterogeneity and function of mammalian MSRs: enzymes for repair, protection and regulation. *Biochim Biophys Acta.* 1703, 239-247.
- Kanoni, S., Dedoussis, G., Manios, Y., Malavolta, M., Mocchegiani, E., 2006. Health status, blood and anthropometrical indices from Greek old and nonagenarian subjects. *Biogerontology.* 7, 329-337.
- Kumar, R. A., Koc, A., Cerny, R. L., Gladyshev, V. N., 2002. Reaction mechanism, evolutionary analysis, and role of zinc in *Drosophila* methionine-R-sulfoxide reductase. *J Biol Chem.* 277, 37527-37535.
- Maret, W., Sandstead, H. H., 2006. Zinc requirements and the risks and benefits of zinc supplementation. *J Trace Elem Med Biol.* 20, 3-18.
- Mocchegiani, E., Malavolta, M., Marcellini, F., Pawelec, G., 2006a. Zinc, oxidative stress, genetic background and immunosenescence: implications for healthy ageing. *Immun Ageing.* 3, 6.
- Mocchegiani, E., Costarelli, L., Giacconi, R., Cipriano, C., Muti, E., Tesei, S., Malavolta, M., 2006b. Nutrient-gene interaction in ageing and successful ageing. A single nutrient (zinc) and some target genes related to inflammatory/immune response. *Mech Ageing Dev.* 127, 517-525.
- Moskovitz, J., Bar-Noy, S., Williams, W. M., Requena, J., Berlett, B. S., Stadtman, E. R., 2001. Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proc Natl Acad Sci U S A.* 98, 12920-12925.
- Petropoulos, I., Friguier, B., 2005. Protein maintenance in aging and replicative senescence: a role for the peptide methionine sulfoxide reductases. *Biochim Biophys Acta.* 1703, 261-266.

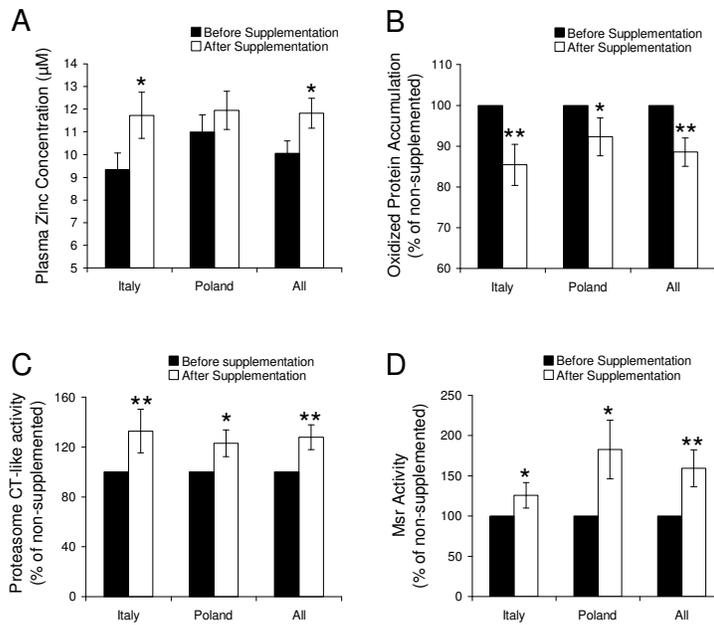
- Petropoulos, I., Friguet, B., 2006. Maintenance of proteins and aging: the role of oxidized protein repair. *Free Radic Res.* 40, 1269-1276.
- Petropoulos, I., Mary, J., Perichon, M., Friguet, B., 2001. Rat peptide methionine sulphoxide reductase: cloning of the cDNA, and down-regulation of gene expression and enzyme activity during aging. *Biochem J.* 355, 819-825.
- Picot, C. R., Perichon, M., Cintrat, J. C., Friguet, B., Petropoulos, I., 2004. The peptide methionine sulfoxide reductases, MsrA and MsrB (hCBS-1), are downregulated during replicative senescence of human WI-38 fibroblasts. *FEBS Lett.* 558, 74-78.
- Ponnappan, S., Ovaa, H., Ponnappan, U., 2007. Lower expression of catalytic and structural subunits of the proteasome contributes to decreased proteolysis in peripheral blood T lymphocytes during aging. *Int J Biochem Cell Biol.* 39, 799-809.
- Ponnappan, U., Zhong, M., Trebilcock, G. U., 1999. Decreased proteasome-mediated degradation in T cells from the elderly: A role in immune senescence. *Cell Immunol.* 192, 167-174.
- Prasad, A. S., Beck, F. W., Bao, B., Fitzgerald, J. T., Snell, D. C., Steinberg, J. D., Cardozo, L. J., 2007. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. *Am J Clin Nutr.* 85, 837-844.
- Ruan, H., Tang, X. D., Chen, M. L., Joiner, M. L., Sun, G., Brot, N., Weissbach, H., Heinemann, S. H., Iverson, L., Wu, C. F., Hoshi, T., Joiner, M. A., 2002. High-quality life extension by the enzyme peptide methionine sulfoxide reductase. *Proc Natl Acad Sci U S A.* 99, 2748-2753.
- Weissbach, H., Etienne, F., Hoshi, T., Heinemann, S. H., Lowther, W. T., Matthews, B., St John, G., Nathan, C., Brot, N., 2002. Peptide methionine sulfoxide reductase: structure, mechanism of action, and biological function. *Arch Biochem Biophys.* 397, 172-178.

Legends of figures

Figure 1 : Effects of zinc supplementation on oxidized protein content, proteasome and methionine sulfoxide reductase activity per country. **A**- Elderly subjects aged between 59-85 years were recruited and admitted for a zinc supplementation trial during 48 +/- 2 days. Zinc supplementation was performed with 10 mg/day of zinc-aspartate. Plasma zinc concentrations were determined as described in material and methods by induction plasma coupled mass spectrometry (ICP-MS). **B**- Relative quantification of carbonyl groups in proteins as a measure of oxidized protein accumulation. Results are shown as the mean percentage of non-supplemented samples from Italy, Poland and both. **C**- Chymotrypsin (CT)-like activity of the proteasome was assayed using the fluorogenic peptide LLVY-AMC. Results are shown as the mean percentage of non-supplemented samples. **D**- Msr activity was measured using the *N*-acetyl-[³H] Met *R,S* (O) as substrate. Results are shown as the mean percentage of non-supplemented samples. All results are expressed as the mean ± SEM. Values with statistical significance were obtained by the Student's t-test and considered for *P* under 0.05. (* for *P* < 0.05; ** for *P* < 0.01).

Figure 2 : Effect of zinc supplementation on MsrA expression levels. **A**- Western blotting experiments of total protein extracts from subjects before and after supplementation were performed using antibodies against the methionine sulfoxide reductase A. Actin was used as loading control. **B**- Relative quantification of MsrA expression levels. MsrA levels were quantified by densitometric analysis using the Image Master 1D software and were normalized with the value obtained for actin. Results are shown as the mean percentage of non-supplemented samples. All results are expressed as the mean ± SEM. Values with statistical significance were obtained by the Student's t-test and considered for *P* under 0.05. (***) for *P* < 0.001).

Figure 1

Figure 1
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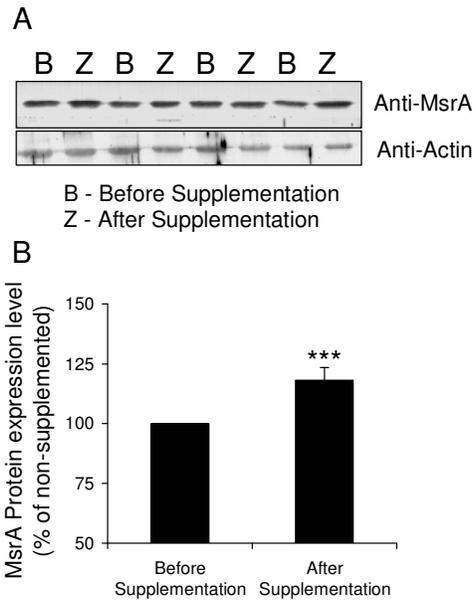


Figure 2
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