Superoxide dismutase 1 (SOD1) and cadmium: A three models approach to the comprehension of its neurotoxic effects

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Abstract

In three different biological models, the recombinant protein expressed in *E. coli*, the neuronal cells SH-SY5Y and the nematode *Caenorhabditis elegans*, cadmium inhibits SOD1 activity without affecting its expression level.

Introduction

The widespread toxic pollutant cadmium is released into the environment mainly by anthropogenic activities. Human exposure can occur through different sources and once absorbed it has a biological half-life of 20-30 years. Brain is a target of cadmium and inside the nervous system it can interfering with essential metal ions homeostasis, as well as substituting zinc or copper affecting protein structures and functions, or depleting cell's antioxidant defence systems.[1] Moreover, cadmium exposure has been related to amyotrophic lateral sclerosis (ALS) neurodegenerative diseases, in which the 15-20% of familial cases is attributed to mutations in superoxide dismutase 1 (SOD1) gene. SOD1 is a homodimeric metalloenzyme of 32 kDa mainly located in the cytoplasm, in which each monomer binding one copper and zinc ions within a disulfide bonded conformer. Zinc is involved in structure stability, while the copper is responsible for the catalytic activity.

Cadmium neurotoxicity on SOD1 has been analysed in a comparative study, using three model organisms with different levels of complexity: the *in vitro* human neuroblastoma SH-SY5Y cell line, a well-known model for *in vitro* studies on neurotoxicity and neurodegenerative diseases;[2] the *in vivo* organism *Caenorhabditis elegans*, largely used as a model in different research fields, like metal toxicology and neurotoxicology[3,4] and the simple microorganism model *E. coli,* in which the overexpression of the recombinant human SOD1 (hSOD1) was useful to study not only cadmium toxicity but also to investigate its ability to substitute zinc or copper in SOD1.

Materials and Methods

At first, we have investigated the cadmium effect on neuronal cells and *C. elegans* viability through MTT assay and acute toxicity test respectively. Subsequently, we have evaluated the effect of sublethal cadmium doses on both SOD1 enzymatic activity, according to Vance *et al.*,[5] and SOD1 expression through Western blot analysis. These analyses were also performed on *E. coli* in presence of cadmium alone or mixed with copper and/or zinc.

Cadmium administration was performed by addition to culture medium or as a food source for *C. elegans*. Statistical

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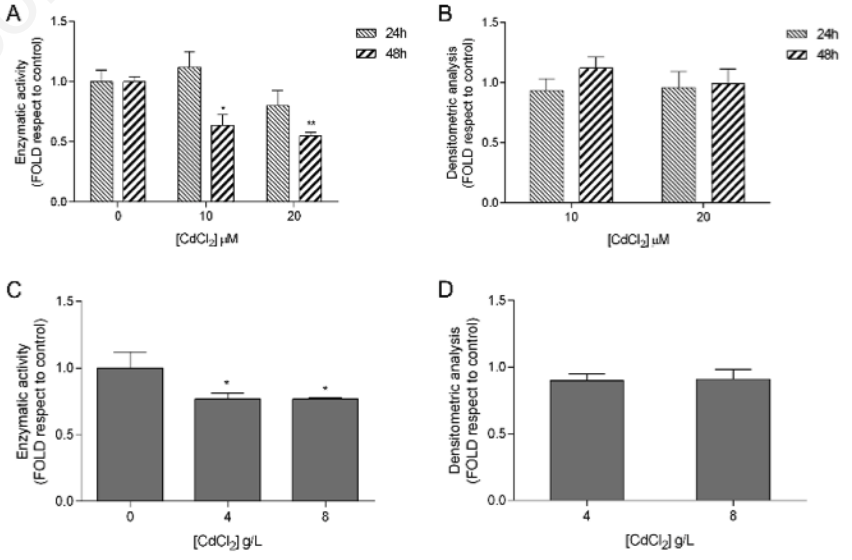

Figure 1. A-C) SOD1 activity in SH-SY5Y exposed to either 10 or 20 µM $CdCl_2$ for 24 and 48 hours (A) and in *C. elegans* after a 16 hours treatment with 0-4-8 g/L $CdCl_2$ (C). Data were normalized to the control and represent mean ± E.S. of three independent experiments. B-D) Densitometric analysis of SOD1 expression in SH-SY5Y (B) and *C. elegans* (D) after cadmium treatment, presented as means ± standard error (SE) of three independent experiments. *p<0.05, **p<0.01.

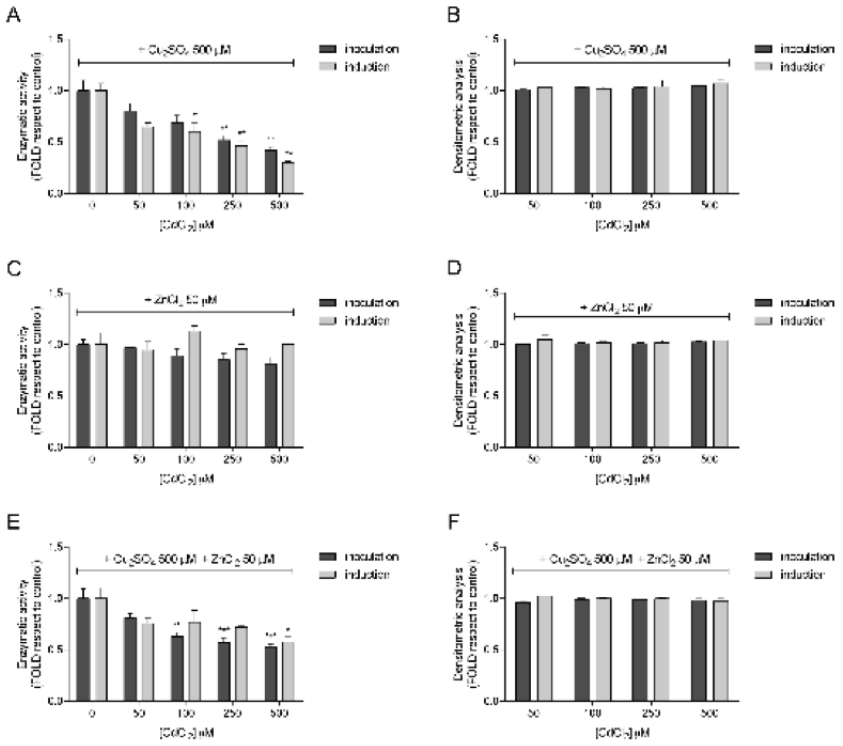

Figure 2. Enzymatic activity of hSOD1 after *E. coli* exposure to different $CdCl_2$ concentrations (0-500 µM), in the presence of either 500 µM Cu_2SO_4 (A) or 50 µM $ZnCl_2$ (C) or both 500 µM Cu_2SO_4 and 50 µM $ZnCl_2$ (E). Densitometric analysis of recombinant hSOD1 after exposure to different $CdCl_2$ concentrations (0-500 µM), in the presence of either 500 µM Cu_2SO_4 (B) or 50 µM $ZnCl_2$ (D) or both 500 µM Cu_2SO_4 and 50 µM $ZnCl_2$ (F). Data were normalized to the control and represent mean ± E.S. of three independent experiments. *p<0.05, **p<0.01, ***p<0.001.

analysis was done by Dunnet multiple comparison procedure.

Results

The viability assays allowed us to determine the optimal sublethal doses for SOD1 investigation on both neuronal cells and *C. elegans*, in detail 10 and 20 µM $CdCl_2$ for 24 and 48 hours and 4 and 8 g/L $CdCl_2$ for 24 hours respectively (data not shown). In both model cadmium exposure reduced SOD1 activity, without interfering with its expression level (Figure 1).

In the *E. coli* system copper and zinc have been introduced to avoid limitation in SOD1 production, due to protein overexpression. In these conditions we observed a reduction in SOD1 activity only when copper is given to the cells, while in the presence of zinc we observed a protective effect (Figure 2). Like the other models, we did not seen changes in SOD1 expression level.

Discussion and Conclusions

Our results clearly demonstrate that cadmium leads to a loss of SOD1 enzymatic activity, without altering its expression in all the three different model systems tested; moreover zinc seems to exert a protective effect against cadmium neurotoxicity on SOD1, as seen in the *E. coli* overexpressing system. Our data show that cadmium can be harmful at low doses, mimicking environmental contamination. Thus, although insights into the contribution of both environmental and genetic (or genetic-like) factors are essential to the comprehension of ALS pathogenesis, understanding the environmental contribution is a critical aspect being the only component of the risk which can be modified. Our approach underlying the advantage to use three different models to overcome this biological problem without needing more complex animals' models (*e.g.* mouse), fulfilling the 3R principles.

References

1. Wang B, Du Y. Cadmium and its neurotoxic effects. Oxid Med Cell Longev 2013;2013:898034.
2. Cheung YT, Lau WKW, Yu MS, et al. Effects of all-trans-retinoic acid on human SH-SY5Y neuroblastoma as in vitro model in neurotoxicity research. Neurotoxicology 2009;30:127–35.
3. Moyson S, Vissenberg K, Fransen E, et al. Mixture effects of copper, cadmium, and zinc on mortality and behavior of Caenorhabditis elegans. Environ. Toxicol Chem 2018;37:145–59.
4. Chia SJ, Tan EK, Chao YX. Historical Perspective: Models of Parkinson's Disease. Int J Mol Sci 2020;21:2464.
5. Vance PG, Keele BB Jr, Rajagopalan KV. Superoxide dismutase from Streptococcus mutans. Isolation and characterization of two forms of the enzyme. J Biol Chem. 1972:247:4782-6