In vitro Multi-level Approaches to Study Cadmium Neurotoxicity

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Abstract

Epidemiological data have related cadmium exposure to neurotoxicity. The combination of multi-level and complementary approaches provides a comprehensive view of mechanistic processes in neurotoxicity.

Introduction

Epidemiology data link neurodegeneration to environmental factors, such as metals. Cadmium (Cd) is the 7th most dangerous chemical for human health. After exposure (e.g., inhalation, ingestion), it is bioaccumulated up to 30 years and can pass the blood-brain barrier with possible functional neuronal damage. A proposed mechanism of toxicity is the interference with essential metals. Thus, this work aims to evaluate the mechanisms of Cd toxicity, with particular regard to essential metal balance.

Materials and Methods

Human neuronal cell line (SH-SY5Y, ATCC® CRL-2266™) was used as an *in vitro* model. Microarray expression profiling: RNA was purified through the RNeasy Plus kit (Qiagen) and microarray experiments according to Forcella et al. (2020);[1] Cd and essential metal(loid)s (Fe, Zn, Ca) within the cells were quantified by plasma atomic emission spectrometry (ICP); Zn release after Cd exposure was visualized by fluorescence microscopy and the Zinquin probe.[2] The extent of lipid peroxidation was determined by the levels of malondialdehyde (MDA) measured using the thiobarbituric acid reactive substances assay and was expressed as nmol of MDA/mg proteins.

Finally, by using Raman spectroscopy analysis technique, morpho-functional markers and modifications in neuronal cells were investigated.

Results

Overall, the transcriptomics, enzymatic, chemical and fluorescence results all converge to the direction of a dyshomeostasis of essential metals (Fe, Zn, Ca) as a consequence of Cd accumulation.

ICP analyses reveal an increase of Cd accumulation in neuronal cells accompanied by a dysregulation of essential elements (Fe, Zn, Ca, Mg), which increase or decrease their basal concentration.

Raman spectra of cells treated with cadmium, in particular 20 µM $CdCl_2$, showed a lower intensity and shifts of vibrational modes in the high frequency region, centered at 2500 cm^{-1}, variable but mostly lower intensity and some shift of vibrational modes in the low frequency region, centered at 1100 cm^{-1}. These results are correlated with the stretching and bending of CH_2 and CH_3 groups of lipids, possibly due to lipid peroxidation. In Tab. 1 some Raman peaks are presented.

SH-SY5Y cell line, after 24 hours treatment to either 10 µM or 20 µM Cd, showed an increase in lipid peroxidation levels, slightly higher at 10 µM Cd than at 20 µM Cd. These results are in agreement with those of Raman spectroscopy.

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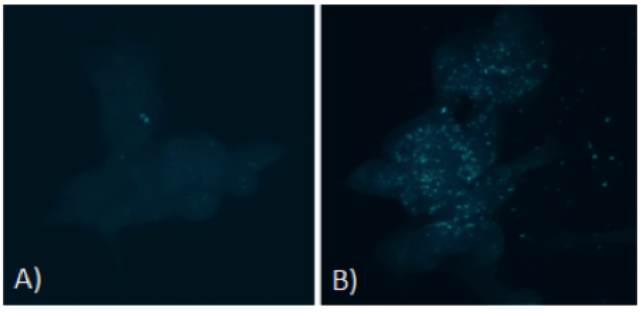

Figure 1. Intracellular visualization of labile Zn by Zinquin probe. A) Control cells show undetectable levels of free Zn; B) In Cd-treated cells an increased fluorescence signal due to free Zn is visible.

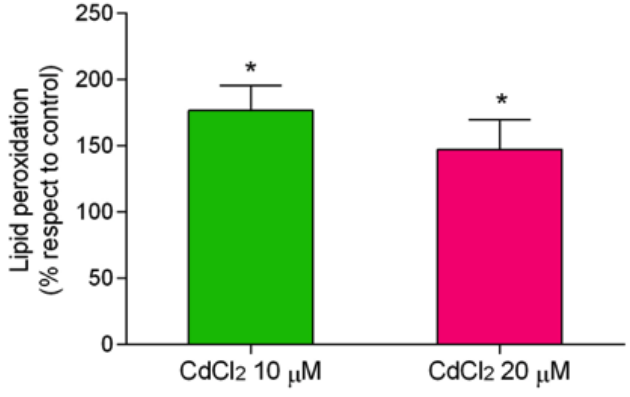

Figure 2. Lipid peroxidation level expressed as a percentage of the control. Statistically significant: *p<0.05

Table 1. Assignments of Raman peaks found in the spectra of Cd-treated cells compared with those found in control cells.

Peak position in control cells (cm^{-1})	Peak position in Cd-treated cells (cm^{-1})	Vibrational mode
1453	1455 (shift, +2)	CH_2/CH_3 bending-Lipids
2882	2882 (lower intensity)	CH_2 stretching-Lipids

Conclusions

Even though 2D cell cultures have known limitations, SH-SY5Y neuroblastoma cell line, is widely used as *in vitro* model for neurotoxicity studies and neurodegenerative diseases,[3,4] providing a tool for different methodological approaches.

In addition, according to the recommendations of the National Research Council of the National Academy of Sciences described in a recent report on toxicity testing in the 21st century, SHSY5Y are from human origin. It is worth noticing how the combined use of multiple methodologies provides an overall mechanistic view on target cells that could be compared to and complemented with epidemiology data. In addition, to the best of our knowledge, Raman spectroscopy was one of the first applications in cytotoxicology analyses to identify the effects of contaminants. The further development of Raman spectroscopy to *in vitro* and cytotoxicity studies in general will provide a means to identify in human cells specific biomarkers.

References

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