

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 CdCl₂, 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, cen-

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Key words: Cadmium; neurotoxicity; SH-SY5Y neuronal cells.

Acknowledgments: The authors acknowledge the MIUR funding (FFABR 2017 to CU). This abstract is an outcome of "Progetto Dipartimenti di Eccellenza", funded by MIUR. This work is dedicated to prof. Maurizio Bruschi.

Conference presentation: This paper was presented at the Third Centro 3R Annual Meeting - L'era delle 3R: modelli *in silico*, *in vitro* e *in vivo* per promuovere la ricerca traslazionale - 30 September - 1 October 2021, Evento online organizzato dal Politecnico di Torino.

Received for publication: 9 July 2021.

Accepted for publication: 7 September 2021.

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Biomedical Science and Engineering 2021; 4(s1):190
 doi:10.4081/bse.2021.190

tered at 1100 cm^{-1} . These results are correlated with the stretching and bending of CH₂ and CH₃ 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.

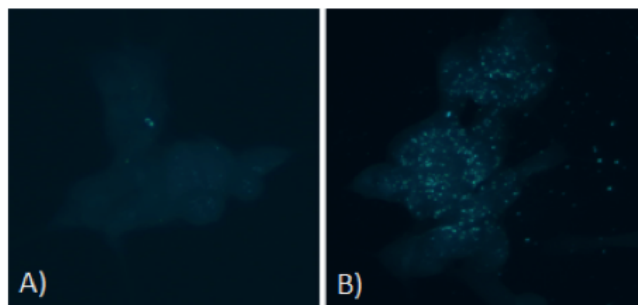


Figure 1. Intracellular visualization of labile Zn by Zinquin probe. A) Control cells show undetectable levels of free Zn; B) In Cd-treated cells an increased fluorescence signal due to free Zn is visible.

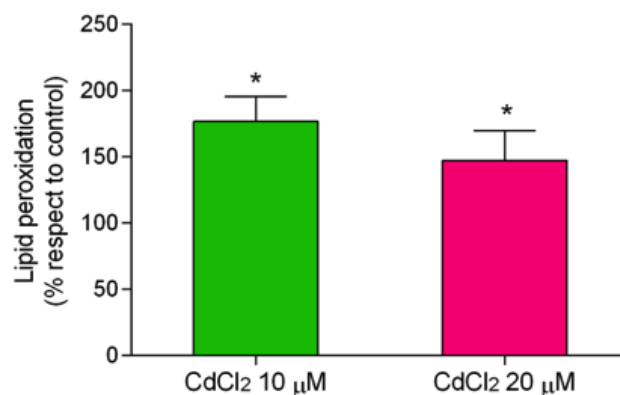


Figure 2. Lipid peroxidation level expressed as a percentage of the control. Statistically significant: * $p < 0.05$

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

1. Forcella M, Lau P, Oldani M, et al. Neuronal specific and non-specific responses to cadmium possibly involved in neurodegeneration: A toxicogenomics study in a human neuronal cell model. *Neurotoxicology* 2020; 76:162-73.
2. Urani C, Melchiorretto P, Brsichi M, et al. Impact of cadmium on intracellular zinc levels in HepG2 cells: quantitative

evaluations and molecular effects. *BioMed Res Int* 2015;949514.

3. Cheung YT, Lau WK, 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.
4. Rossi S, Serrano A, Gerbino V, et al. Nuclear accumulation of mRNAs underlies G4C2-repeat-induced translational repression in a cellular model of C9orf72 ALS. *J Cell Sci* 2015;128: 1787-99.