TSQ FLUOROCHROME INCUBATION INCREASE AUTOMETALLOGRAPHIC ZINC DETECTION IN PRIMARY CELL CULTURES OF ASTROCYTES

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INTRODUCTION

Zinc plays a critical role for a wide range of cellular functions. However, free zinc ions outside a narrow concentration range are toxic to a variety of cell types. In CNS, zinc ions are involved in synaptic transmission and the fine regulation of its concentration in the neuronal milieu is therefore critical for neuronal survival and brain function. In this regard, altered free zinc amounts have been reported to be associated with many illnesses and syndromes affecting most body organs, including the brain. Cells have developed a variety of mechanisms to control free zinc concentrations, including zincosome formation. Among the glial cells in CNS, astrocytes play a central role in regulating neuronal milieu composition and thus brain homeostasis. As astrocytes envelop synaptic structures in the brain, these cells play an active role in the withdrawal of the zinc ions released during the transmission of the nerve impulse. Zinc ions are labile and the monitoring of these ions after their capture by the cells has become an experimental challenge.

Objective: The present study compares side by side direct (TSQ) and indirect (AMG) zinc detection methods with the aim to increase the sensitivity in monitoring of zinc uptake in rat astrocytes in culture.

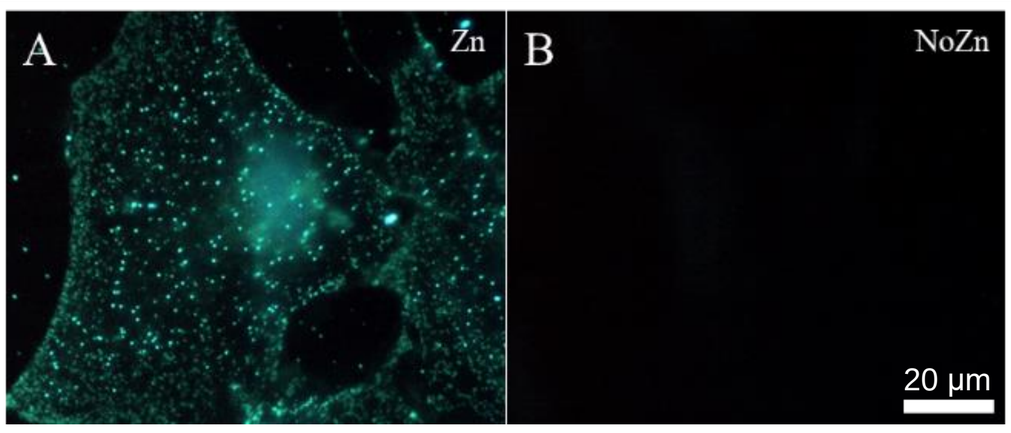

Figure 1. TSQ labels zincosomes in cultures of astrocytes incubated with (A) or without (B) an exogenous zinc pulse. This fluorescence is very labile.

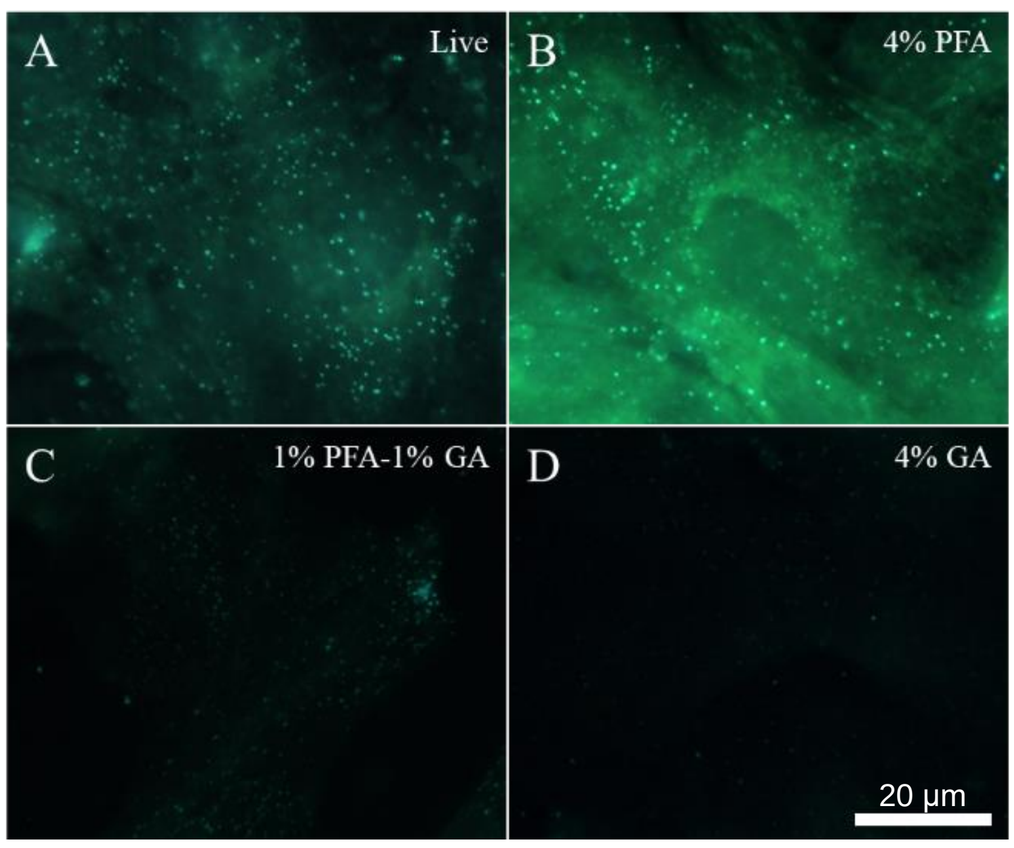

Figure 2. TSQ labels zincosomes in living cells (A), after fixation labelling is more stable but was greatly reduced (B-D).

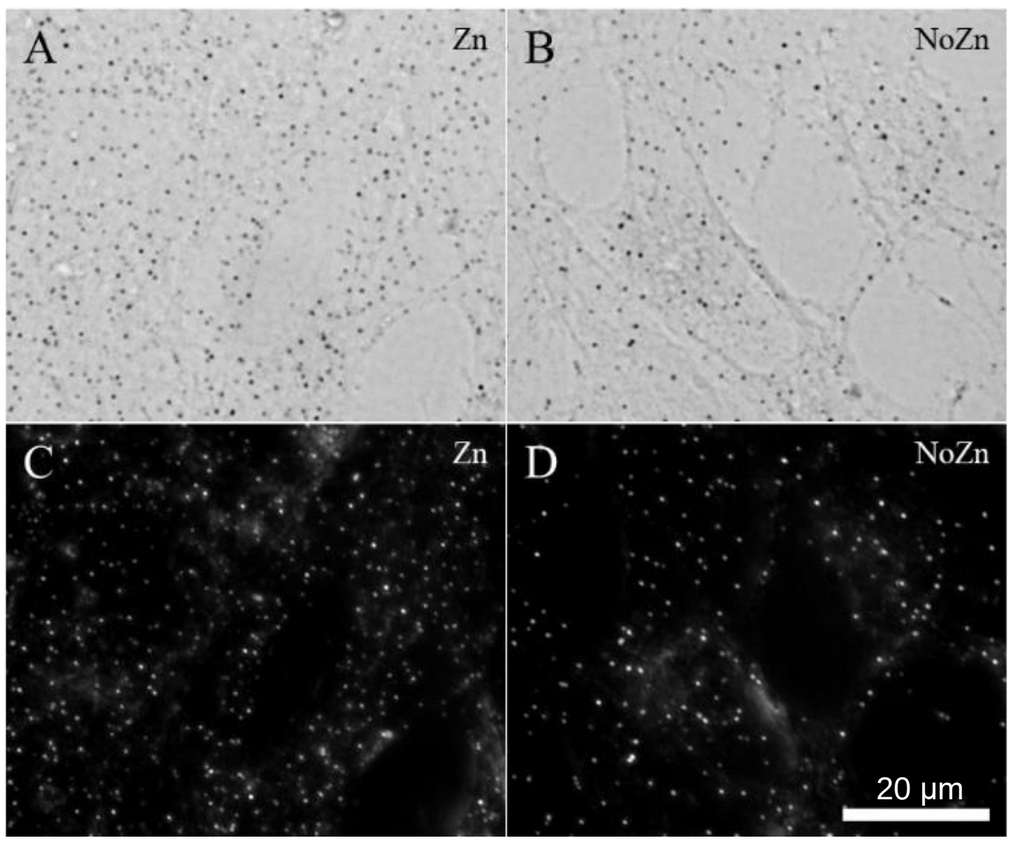

Figure 3. Precipitation of zinc with sodium selenite and further development with the autometallographic method of Neo-Timm resulted in artefactual labelling of both cell cultures, incubated in the presence (A-C) or absence (B-D) of exogenous zinc.

CONCLUSION

We found that for the accurate study the zinc ion content of zincosomes in cell monolayers is necessary a combination of different parameters. We conclude that the combination of TSQ fluorochrome incubation, fixation with 4% PFA, zinc precipitation with 0.1 g/100 ml sodium sulphide followed by a second incubation with TSQ preserves the zinc ion content during all the experimental procedures, leading to an accurate detection of zinc by AMG that appropriately reflects the intracellular zinc contents in cells. These improvements to the existing zinc monitoring techniques will facilitate the examination and better understanding of the role that zinc plays in health and disease.

Acknowledgments: This work was supported by grants from FIS (Red de Trastornos Adictivos (RTA), RD06/0001/0009) and MICINN, (SAF2008-00305).

MATERIAL AND METHODS

Astrocyte Primary Culture: cell cultures were prepared from P0 rat fetuses. Cells were cultures in DMEM medium with 5% FCS and were maintained during 7 days at 37ºC with 5% CO_2 in a humidified atmosphere.

Cell treatments: cells were incubated in the presence or absence of 50 µM $ZnSO_4$ after incubation with TPEN, a zinc chelating agent, to increase both zinc requirement and extracellular zinc uptake.

Fluorescence Microscopy: intracellular zinc was detected by fluorescence microscope after incubation with 50 µg/ml of zinc specific TSQ fluorochrome for 10 minutes.

Zinc Precipitation: two methods were used: 1) Fixed cells, 0.1% sodium sulfide in PB (phosphate buffer pH 7.2) during 30 minutes. 2) Living cells, 5 mM sodium selenite during 10 minutes.

Fixation: cells were fixed with different fixatives: 4% PFA, 4% GA and the mixture 1% PFA-1% GA.

Zinc Autometallography (AMG): cell monolayers were developed during 30 minutes at 37ºC in order to obtain specific silver particles over the zinc deposits.

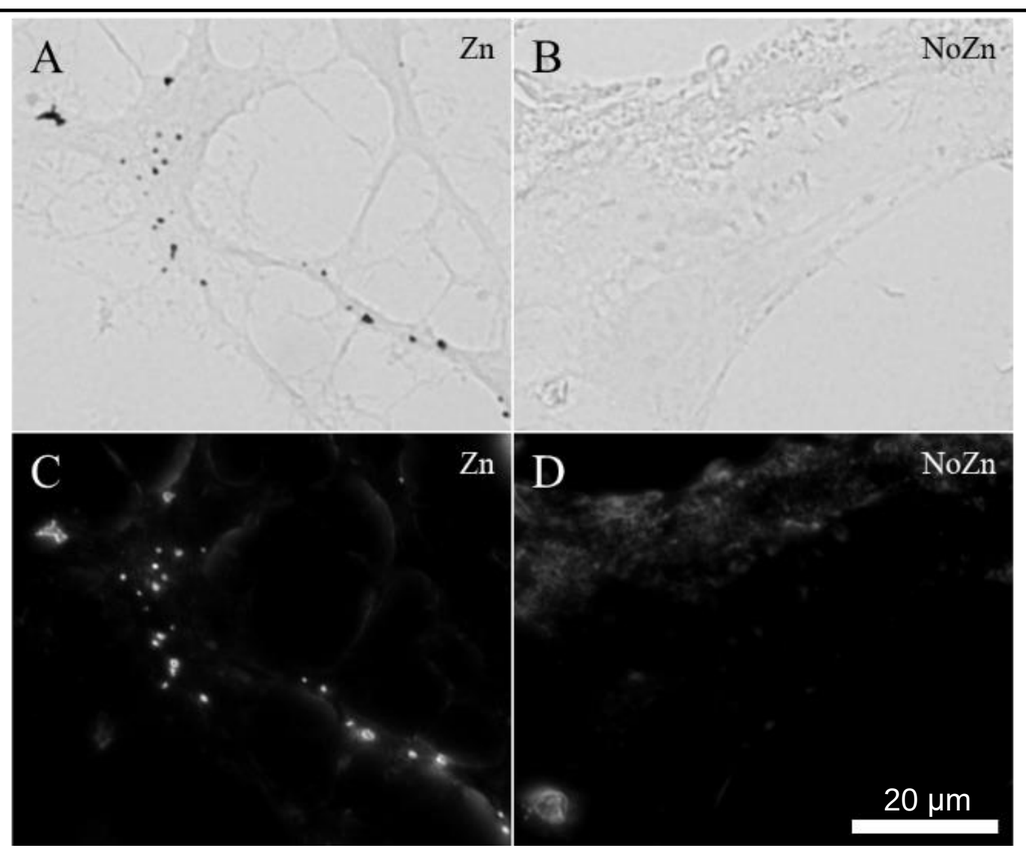

Figure 4. Precipitation of zinc with sodium sulphide and further development with the autometallographic method of Neo-Timm resulted in specific labelling, only in cell cultures incubated with exogenous zinc (A-C).

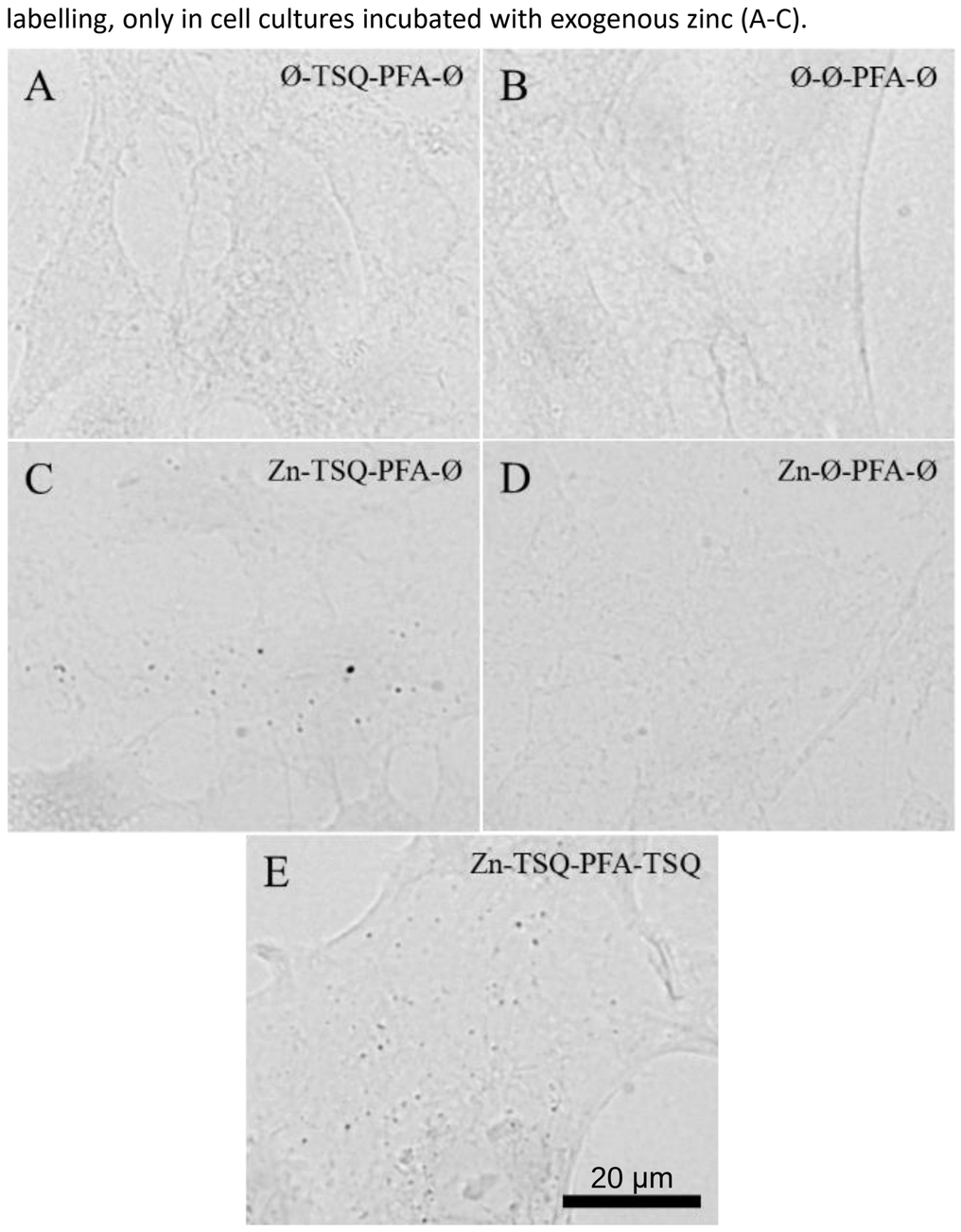

Figure 5. Incubation of cells with TSQ increased the detection of zincosomes. All samples were developed with the autometallographic method of Neo-Timm following the treatments shown in the figures.

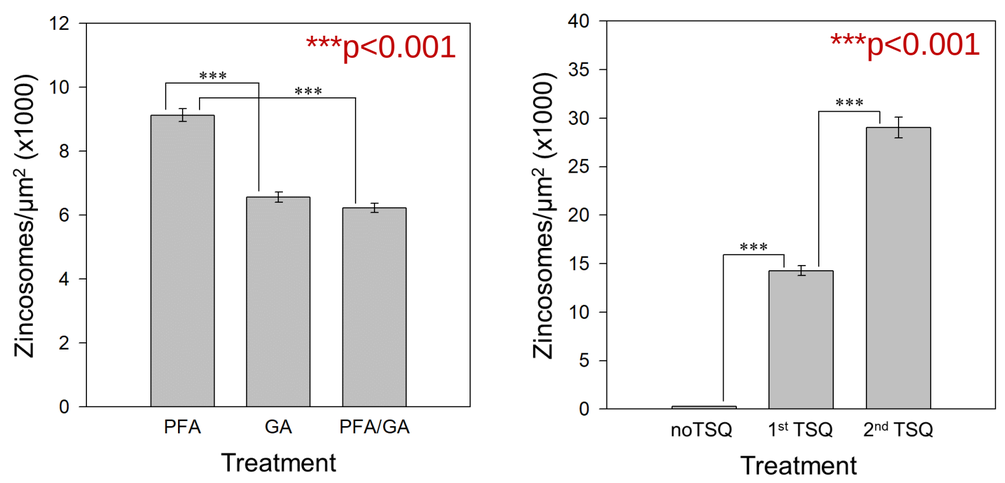

Figure 6. Quantification of zincosomes after development with Neo-Timm. Comparison between fixatives (left) and between different TSQ incubations (right). PFA and 2nd TSQ incubations are better options.