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Fluoride exposure regulates the elongation phase of protein synthesis in cultured Bergmann glia cells

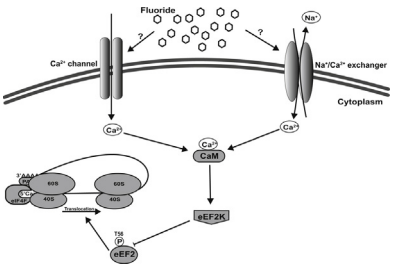
Marco Flores-Méndez, Diana Ramírez, Nely Alamillo, Luisa C. Hernández-Kelly, Luz María Del Razo, Arturo Ortega *

Departamento de Toxicología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado Postal 14-740, México D.F. 07300, Mexico

HIGHLIGHTS

- Glia cells are target of fluoride toxicity.
- Fluoride decreases protein synthesis.
- Glial glutamate transport is increased by fluoride.

GRAPHICAL ABSTRACT



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ABSTRACT

Fluoride is an environmental pollutant present in dental products, food, pesticides and water. The latter, is the greatest source of exposure to this contaminant. Structural and functional damages to the central nervous system are present in exposed population. An established consequence of the neuronal is the release of a substantial amount of glutamate to the extracellular space, leading to an excitotoxic insult. Glutamate exerts its actions through the activation of specific plasma membrane receptors and transporters present in neurons and in glia cells and it is the over-activation of glutamate receptors and transporters, the biochemical hallmark of neuronal and oligodendrocyte cell death. In this context, taking into consideration that fluoride leads to degeneration of cerebellar cells, we took the advantage of the well-established model of cerebellar Bergmann glia cultures to gain insight into the molecular mechanisms inherent to fluoride neurotoxicity that might be triggered in glia cells. We could establish that fluoride decreases [^{35}S]-methionine incorporation into newly synthesized polypeptides, in a time-dependent manner, and that this halt in protein synthesis is the result of a decrease in the elongation phase of translation, mediated by an augmentation of eukaryotic elongation factor 2 phosphorylation. These results favor the notion of glial cells as targets of fluoride toxicity and strengthen the idea of a critical involvement of glia cells in the function and dysfunction of the brain.

1. Introduction

Fluoride (F^-) is an environmental pollutant and exists only in combination with other elements as F^- compounds. Humans are exposed to F^- via dental products, food, pesticides and water. It has been estimated that F^- concentrations from 16 to 64 mg/kg are lethal to human adults while lower concentrations (3–16 mg/kg) are deleterious for infants. It has been established

* Corresponding author. Tel.: +52 55 5747 5410.
E-mail addresses: maflores@cinvestav.mx (M. Flores-Méndez), aalmilloi@cinvestav.mx (N. Alamillo), lcr@cinvestav.mx (L.C. Hernández-Kelly), ldelrazo@cinvestav.mx (L.M. Del Razo), arortega@cinvestav.mx, arortega@mac.com (A. Ortega).

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that drinking F^--contaminated water from subsoil constitutes the most common via of F^- exposure; it is relevant to mention that the minimal risk level for daily oral F^- uptake is 0.05 mg/Kg/d. In fact, it is known that concentrations higher than 0.15 mg/kg/d result in fluorosis, a disease caused by deposition of F^- in the body. This disorder affects primarily skeletal tissue and teeth, although soft tissues, such as liver, kidney, pancreas and importantly, brain are also affected (Council, 2006). Through the use of animal models, now it is known that F^- exposure results in structural and functional damages to the central nervous system (CNS). Further studies associated chronic F^- exposure to diminished childrens' intellectual ability, suggesting a strong negative correlation between F^- exposure and IQ performance (Chunxiang Wu et al., 2006; Shivarajashankara et al., 2002; Mullenix et al., 1995; Ding et al., 2011). A severe motor coordination impairment has also been demonstrated as a consequence of F^- exposure (Paul et al., 1998). Interestingly, F^- treatment leads to degeneration of cerebellar Purkinje neurons and glia cells (Chouhan et al., 2010).

Astrocytes outnumber neurons in the CNS and despite of this fact, their contribution to brain physiology has been a matter of debate for a number of years. In the last decades, their ability to produce and release neuroactive substances and their proven expression of most of the identified neurotransmitter receptors and transporters have paved the way to the re-evaluation of their role in brain physiology (Yang et al., 2008; Teichberg, 1991).

An excellent model in which the role of glia cells as partners of neurons can be documented is the primary culture of chick cerebellar Bergmann glia cells (BGC). These cells are the most abundant non-neuronal population of the cerebellum, span the molecular layer covering completely excitatory and inhibitory synapses (Somogyi et al., 1990). Their particular location is related to their involvement in neurotransmitter uptake and turnover, K^+ homeostasis, lactate supply and pH regulation (Lopez-Bayghen et al., 2007). In terms of glutamatergic transmission, BGC are in a very short proximity to the parallel fiber-Purkinje cell synapses, and participate in the glutamate (Glu)/glutamine (Gln) shuttle that assures the proper neurotransmitter supply to presynaptic terminals (Martinez-Lozada et al., 2013). In this sense, BGC respond to glutamatergic stimulation, as we have been able to characterize over the years (Barrera et al., 2010). Moreover, impairment of the glial Glu uptake activity results in an excitotoxic insult (Kim et al., 2011)

It has long been established that Glu-dependent transcription is essential for long-term memory (Hu et al., 2006). Activity-dependent gene expression regulation stabilizes the synaptic changes that underlie the late phase of long-term potentiation (Pittenger and Kandel, 1998). In this regard, it should be noted that most molecular studies are focused in Glu-dependent gene expression regulation at the transcriptional level and its disruption by xenobiotics. Nevertheless, the rate of protein synthesis has a crucial role in memory and synaptic plasticity (Cammalleri et al., 2003). Translational control offers the possibility of a rapid response to external stimulus without mRNA synthesis and transport. Therefore, immediacy is one of the advantages of translational over transcriptional control (Gebauer and Hentze, 2004). Most translational controls are mediated through the phosphorylation of several components of the translational machinery. In eukaryotic cells, peptide-chain elongation requires two elongation factors (eEFs): eEF1 and eEF2. In the elongation process, eEF1A binds GTP and recruits amino-acyl-tRNAs to the A-site of the ribosome to match the codon located in that site, whereas eEF1B (a heterotrimer) acts as the guanine nucleotide exchange factor (GEF) for eEF1A, while eIF2B is a GEF for eukaryotic initiation factor 2 (eIF2) (Mateyak and Kinzy, 2010).

The translocation step of elongation, meaning, the movement of the ribosome by one codon is carried out by eEF2, a monomer that binds GTP. This factor is phosphorylated in a single site (Thr56) impairing its interaction with the ribosome, preventing the translocation process, and by these means stopping protein synthesis. This phosphorylation is catalyzed by a highly specific enzyme, the eEF2 kinase (eEF2K), previously known as Ca^{2+}/calmodulin dependent protein kinase III (CaMKIII). Re-initiation of the elongation process depends on the dephosphorylation of eEF2, a process that involves the inactivation of eEF2K via its phosphorylation by the 70 kDa ribosomal S6 protein kinase ($p70^{S6k}$) and the 90 kDa ribosomal S6 protein kinase ($p90^{RSK}$) (Kaul et al., 2011).

In order to gain insight into the molecular mechanisms of F^- toxicity and taking into consideration that the cerebellum is one of the CNS targets for this halogen, we decided to explore a plausible impairment of protein synthesis upon exposure to this contaminant. To this end, we used the established model of chick cerebellar BGC primary cultures due to their involvement in glutamatergic transmission, through the Glu/Gln shuttle (Martinez-Lozada et al., 2013). We were able to find a transient decrease in peptide elongation upon exposure to low F^- concentrations. Furthermore, we could establish an F^-dependent Ca^{2+}influx and an eEF2 phosphorylation. These results provide a novel perspective of the molecular mechanisms affected by F^- toxicity.

2. Methods

2.1. Materials

Tissue culture reagents were obtained from GE Healthcare (Carlsbad, CA, USA). Sodium fluoride and the neutral red assay were purchased from Sigma–Aldrich (St. Louis, MO, USA). Glu and A23187 [6S-[6α (2S*,3S*), 8β(R*), 9β,11α]]-5-(Methylamino)-2-[[3,9,11-trimethyl-8-[1-methyl-2-oxo-2-(1Hpyrrol-2-yl) ethyl] 1,7dioxaspiro[5.5]undec-2-yl)methyl]-1,3-benzoxazole-4-carboxylic acid] were obtained from Tocris Cookson (St. Louis, MO, USA). [$^{45}Ca^{2+}$] was from PerkinElmer (Boston, MA, USA). Anti-eEF2 polyclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phospho eEF2 (Thr 56), anti-phospho EF2K (Ser366) and anti-EF2K polyclonal antibodies were purchased from Cell Signaling (Boston, MA, USA). Horseradish peroxidase-linked anti-rabbit antibody and the enhanced chemiluminescence reagent (ECL) were obtained from Amersham Biosciences (Buckinghamshire, UK). All other chemicals were from Sigma (St. Louis, MO, USA).

2.2. Cell culture and stimulation protocol

Primary cultures of cerebellar BGC were prepared from 14-day-old chick embryos as previously described (Ortega et al., 1991). Cells were plated in 6 or 24-well plastic culture dishes in DMEM containing 10% foetal bovine serum, 2 mM glutamine, and gentamicin (50 mg/ml) at 37 °C in 5% CO_2 and used on the 4th–7th day after culture. Before any treatment, confluent monolayers were switched to non-serum DMEM media containing 0.5% bovine serum albumin (BSA) for 2 h and then treated as indicated. Confluent monolayers were exposed to the indicated concentrations of F^- for different time periods, and samples were processed as detailed below.

2.3. Cell viability

F^- cytotoxicity was determined by the neutral red (NR) assay. This assay evaluates the number of viable cells after exposure to toxicants. It is based on the uptake and subsequent lysosomal