FLUORIDE NEUROTOXICITY AND EXCITOTOXICITY/MICROGLIAL ACTIVATION: CRITICAL NEED FOR MORE RESEARCH

Summary: The exact mechanism of the neurotoxic effect of fluoride and aluminofluoride complexes on the brain has not been fully elucidated, although there is compelling evidence that it is closely related to that of heavy metal neurotoxicities as well as a host of neuropathological conditions. These involve an interaction between excitotoxic amino acids and proinflammatory cytokines, both of which are released in high concentrations with microglial activation. It is important that research be undertaken to explore and assess fluoride activation of microglia, the resident immune cells in the brain.

Keywords: Excitotoxic amino acids; Excitotoxicity; Fluoride neurotoxicity; Microglial activation; Proinflammatory cytokines; Research topics.

Despite an overwhelming number of studies demonstrating neurotoxicity of fluoride compounds, I have been unable to find a single study that specifically examined fluoride activation of the excitotoxic cascade or of brain microglia, the resident immune cells in the brain.1-5 The excitotoxic cascade is triggered when an excess of extraneuronal glutamate and/or cellular energy suppression occurs in the central nervous system.

Glutamate is the most abundant neurotransmitter in the brain and operates through a series of specific receptors, either ionotropic such as NMDA (N-methyl-D-aspartate), AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid), and kainate or G-protein-operated metabotropic receptors. When these receptors are overstimulated, a series of intracellular signaling events occurs that can lead to cell death, a process called excitotoxicity.

Microglia, when activated, not only secrete high levels of immune cytokines and other immune factors, but also release excitotoxic levels of glutamate and aspartate.

The interaction between chronic microglial activation and activation of the excitotoxic cascade has been demonstrated in a growing number of neurological disorders, including neurodegenerative diseases such as Alzheimer’s dementia, Parkinson’s disease, Lou Gehrig’s disease (amyotrophic lateral sclerosis, ALS), Huntington’s disease, and olivopontocerebral degeneration. These activations are also found in strokes, ischemia/hypoxia, hypoglycemic brain injury, glaucoma, brain trauma, meningitis, viral encephalitis, multiple sclerosis, and autism.

This same immune/excitotoxic reaction has been found to play a dominant role in a number of metal-related neurotoxic reactions, including those involving mercury, lead, cadmium, tin, and aluminum. In the case of mercury, microglial activation and glutamate-triggered excitotoxicity occur at concentrations well below neuronal cytotoxic concentrations. Likewise, blocking glutamate receptors ameliorates mercury toxicity.

Although I have proposed such an excitotoxic mechanism, I am concerned that no one as yet has actually looked at it in relation to fluoride neurotoxicity, despite the strong evidence that fluoride can accumulate in certain areas of the brain in
relatively high concentrations. For example, Mullenix demonstrated that fluoride accumulated in the hippocampus of rats exposed to sodium fluoride in drinking water. Similarly, fluoride has been shown to accumulate selectively in the pineal gland of adult humans, primarily in hydroxyapatite calcifications.

Histopathological studies of fluoride-exposed animals have demonstrated damage to CA1 and CA4 areas of the hippocampus and to the dentate gyrus, which is also consistent with excitotoxicity. In their study using aluminum fluoride and sodium fluoride, Varner et al. found damage in the superficial layers of the cortex, amygdala, and cerebellum—all areas endowed with abundant glutamate receptors. Others have described a loss of Purkinje cells with chronic fluoride exposure, a cell type containing abundant AMPA glutamate receptors. It is also of interest that gliosis (activation of microglia and astrocytes) is only mentioned in passing in these studies and never quantified.

Also consistent with excitotoxicity is the finding of elevated levels of reactive oxygen species (ROS), reactive nitrogen species (RNS), and lipid peroxidation products (LPO) in a number of organs, including brain, following fluoride exposure, both in vitro and in vivo. A major portion of the excitotoxic cascade involves ROS/RNS/LPO production. Also of interest is the finding of elevation in nitric oxide (NO) via induced nitric oxide synthase (iNOS), again a critical component of excitotoxicity.

Not only do excitatory amino acids (glutamate, aspartate, cysteine, cysteic acid, homocysteine, etc) generate abundant free radicals, but, in addition, free radicals and LPO products also markedly enhance excitotoxicity. Microglia and astrocytes release abundant levels of glutamate and aspartate when activated.

A number of studies have shown activation of protein kinase C (PKC) by fluoride and aluminofluoride complexes. Activation of PKC is essential to excitotoxicity, and blocking this enzyme blocks excitotoxicity. Likewise, PKC plays a major role in microglial activation. Fluoride activation of PKC moves it from the cytosol to the membrane, and in turn activates MAPK (mitogen activated protein kinase), which then activates IP3 (inositol 1,4,5-triphosphate) and DAG (diacylglycerol), a process essential to macrophage/microglial activation.

Despite this clear activation of the cell signaling pathways for microglial activation, no one appears to have conducted definitive studies to demonstrate brain microglia activation by fluoride, as has been done for other neurotoxins. But there is strong circumstantial evidence. For example, a shift of a Th1 (T-helper cell-1) to a Th2 (T-helper cell-2) proinflammatory cytokine profile from in vitro fluoride exposure has been demonstrated. In addition, there is evidence of macrophage activation from pulmonary exposure to fluoride with dramatic increases in IL-1β (interleukin-1β), TNF-α (tumor necrosis factor-alpha) and various chemokines. Microglia are derived from macrophages and utilize the same activation mechanisms. It is also of interest that silica combined with fluoride produces a greater pulmonary pathology than calcium fluoride alone.
Another mechanism connected to excitotoxicity is the microglial release of inflammatory eicosanoids. Several studies, both in vitro and in vivo, have shown elevation in eicosanoids with fluoride exposure. Inflammatory prostaglandin E2 (PGE2) eicosanoids play a significant role in excitotoxicity. Blocking PGE2 production markedly attenuates excitotoxicity.

Finally, at least two studies have shown that fluoride compounds can activate immune pathways that can lead to, or enhance, autoimmunity. A growing number of studies has shown that inflammatory cytokines and chemokines can markedly enhance excitotoxicity, perhaps by cross-talk between glutamate receptors and cytokine receptors.

To date I have been able to find only one investigation that even looked at glutamate levels with fluoride exposure. The authors found elevated glutamate in the region of the exposure. It is clear, therefore, that well-designed studies need to be undertaken to answer the following two questions:

(1) Does fluoride in the brain chronically activate microglia?

(2) Are glutamate receptors activated by fluoride or the aluminofluoride complex?

We know that metabotropic glutamate receptors (mGluR) operate by a GTPase-type (G-protein) receptor and that stimulation of group I mGluR (metabotropic glutamate receptor) enhances excitotoxicity. With the demonstration by Strunecká et al. of G-protein activation by aluminofluoride complexes, it would be surprising if excitotoxicity was not involved in fluoride neurotoxicity.

Appropriate research is needed to answer these critically important questions.

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REFERENCES
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