

Fluoride and Aluminum: Possible Risk Factors in Etiopathogenesis of Autism Spectrum Disorders

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Abstract: Fluoride and aluminum ions (Al^{3+}) are considered as new ecotoxicological factors. While aluminum has been involved among the possible culprits of autism spectrum disorders (ASD), fluoride is rarely considered. Al^{3+} is non-essential for all forms of life and serves no known biological role. Fluoride and Al^{3+} can elicit impairment of homeostasis, growth, development, cognition, and behavior. Several symptoms induced by Al^{3+} and/or fluoride overload can be seen in ASD. Several laboratory studies demonstrate that many effects primarily attributed to fluoride are caused by synergistic action of fluoride plus Al^{3+} . In water solutions, Al^{3+} forms in the presence of fluoride, water soluble aluminofluoride complexes (AlFx). AlFx has been widely used as an analogue of phosphate groups to study heterotrimeric G proteins involvement. AlFx affects numerous receptors and signaling systems. It is evident that the long-term intake of low amounts of fluoride and Al^{3+} can evoke receptor malfunctions. The synergistic interactions of fluoride plus Al^{3+} may thus evoke several histological, neurological, biochemical, and behavioral symptoms of ASD. This chapter brings evidence that AlFx represents a hidden potential danger for pathogenesis of ASD.

INTRODUCTION

The heterogeneity of pathophysiological, histological, neurological, biochemical, clinical, and behavioral symptoms provide us little reason to assume that there is one cause of ASD pathogenesis. Nevertheless, we suggested in our previous reviews [1,2] that multiple risk factors may cause the dysregulation of immune-glutamate pathways and evoke various multiple symptoms. Blaylock coined the term immunoexcitotoxicity as the possible central mechanism in ASD etiopathogenesis [3-6]. The interactions between excitotoxins, free radicals, lipid peroxidation products (LPP), inflammatory cytokines, and disruption of neuronal calcium homeostasis can result in brain changes suggestive of the pathological findings in cases of ASD. There is compelling evidence from a multitude of studies of various designs indicating that environmental and food borne excitotoxins can elevate blood and brain glutamate to levels known to cause neurodegeneration, brain inflammation, and alterations in the developing brain. We will focus in this chapter on the potential contributions of fluoride and Al^{3+} . While aluminum has been involved among the possible culprits of ASD, fluoride is rarely considered.

Despite the abundance of aluminum in nature, it has no biological function in humans. On the contrary, wide ranges of toxic effects of Al^{3+} to hundreds of cellular processes have been demonstrated [7]. There is compelling evidence that an accumulation of aluminum in the body appeared recently as the inevitable consequence of the activities of modern human civilization. There are a number of aluminum sources, such as the drinking water, nutrition, cosmetics, and the widespread use of aluminum in medicine. It means that humans are not able to avoid exposure to aluminum at present. Fluoride exposure is common in fetuses, newborns, and small children as a result of the artificial fluoridation of drinking water and a dramatic increase in the volume of man-made industrial fluoride compounds released into the environment. Many investigations of the long-term administration of fluoride to laboratory animals have demonstrated that fluoride and aluminum can elicit impairment of homeostasis, growth, development, cognition, and behavior.

Numerous epidemiological, ecological, and clinical studies have shown the effects of fluoride and aluminum burden on humans. Fluoride could complex with any pre-existing Al^{3+} within body fluids to produce the aluminofluoride

complexes (AlF_x), the most potent activators of G protein regulated systems known [8]. AlF_x increases the potential neurotoxic effect of Al^{3+} and fluoride alone particularly in children, whose brains are uniquely sensitive to environmental toxins. The synergistic interactions of fluoride plus Al^{3+} may evoke several histological, neurological, biochemical, and behavioral symptoms of ASD [2]. We suggest that the chronic burden with these new ecotoxicological factors could contribute to an alarming increase in the incidence of ASD.

ALUMINUM AND FLUORIDE BIOAVAILABILITY FROM ENVIRONMENT

Aluminum

Aluminum, the most abundant metal of the earth's lithosphere, is everywhere: in water sources, in nourishment, in different food additives and also in air in the form of dust particles. It has, until relatively recently, existed in forms not generally available to living organisms, and was therefore regarded as non-toxic. On the other hand, it remains one of the great paradoxes of life on the Earth that the most abundant metal in the lithosphere has no biological function.

With the use of aluminum in industry and presence in acid rain, there has been a dramatic increase in the amount of bioavailable Al^{3+} in the environment. Aluminum occurs in only one oxidative state (Al^{3+}). Al^{3+} can be formed in solution and this ion is known to exist in groundwater in concentrations ranging from 0.1 mg to 8.0 mg /L. The water supply industry uses aluminum sulphate to produce a less turbid drinking water. Aluminum sulfate used for water treatment was ranked 43rd among the top 50 additives during the years 1993 and 1994. Al hydroxide or sodium aluminate can be present in tap water as residuals from these processes. At present, there is no Environmental Protection Agency (EPA) health standard for aluminum in drinking water, only a recommended Secondary Maximum Contaminant Level (SMCL), which is 0.05 to 0.2 mg/L. This would represent the safe level of a contaminant in drinking water below which there is no known or expected risk to health. Although water is the most extensively studied aluminum source, it provides only about 1% of normal daily human intake [9].

The primary normal source of aluminum is food, which provides approximately 16-100 fold more Al^{3+} to systemic circulation than drinking water. For example, aluminum is found in a number of baked goods, such as pancakes and biscuits, processed cheeses, frozen foods, and beverages [10, 11]. Several papers report that all tea samples release aluminum (0.70-5.93 mg/L) during a standard infusion period. The results indicated that tea consumption must be

considered in any assessment of the total dietary intake of aluminum. Aluminum is added to frozen foods to improve their appearance. Aluminum salts are added to processed cheeses and to beer. The aluminum accumulation continues from cookware, cans, tetrapacks, and antacids. Krewski *et al.* [12] estimated that for the general population, intake of aluminum from food (7.2 mg/day for females and 8.6 mg/day for males) dominated that from drinking water (0.16 mg/day) and inhalation exposure (0.06 mg/day). Antacids and buffered aspirin can contribute on the order of thousands of mg/day to aluminum intake.

This evaluation places the gastrointestinal tract (GIT) as the focal point of understanding human exposure to aluminum. Al^{3+} may form complexes with other dietary acids, for example, malic, oxalic, tartaric, succinic, aspartic, and glutamic acids, which may dramatically increase its GIT absorption.

There are a number of ways in which humans are exposed to aluminum, such as the skin, the lung, the nose, and of course, the intramuscular vaccination. Human can be exposed to aluminum through aerosol and topical application of anti-perspirants, topical application of sunscreens, suntan lotion, skin moisturizers, and smoking [13]. Such items as deodorants, vaginal douches and baby wipe not only have high aluminum content, but are applied to areas where there is far greater tendency to absorption through the skin. However, because inhaled aluminum is approximately seven times more bioavailable than aluminum in drinking water, the contribution of inhaled aluminum exceeds the corresponding contribution from drinking water [12]. Aluminum absorbed across olfactory epithelia would bypass the defense of blood- brain barrier (BBB) and pass directly to the hippocampal region of the brain by way of the olfactory tract.

Other major contributors include aluminum used in medicines: antacids, dialysis solutions, parental, and intravenous nutrition solutions used in pediatrics [9,14,15]. Vaccines, allergy skin tests, human serum albumin, baby skin creams, baby diaper wipes, and antacids, which are frequently given to infants, are extremely high in aluminum.

Recently, the content of aluminum in vaccines has been discussed [16]. According to Yokel and McNamara [10] calculations based on 20 injections in the first 6 years of life and an average weight of 20 kg, children receive 0.07 - 0.4 $\mu\text{g/kg}$ of aluminum. This value is comparable with the amount of aluminum absorbed from food.

The natural barrier systems for Al^{3+} such as low absorption in the GIT, and various physiological ligands, such as citrate, phosphate, and silicic acid, were efficient buffers preventing the increased absorption of this metal in natural conditions. The population is now exposed to more bioavailable aluminum. The skin and mucus-lined epithelia of the GIT and respiratory systems are initially barriers to exposure to aluminum and they are also likely transitory sinks for biologically available Al^{3+} . No one has determined the level of Al^{3+} in the hair and nails.

There are few data addressing the excretion of aluminum from the body. It is assumed that a majority of ingested aluminum is excreted in feces, but the amount of aluminum in feces has not been measured. Several studies have demonstrated that the kidney is a route of excretion of systemic aluminum and that a proportion of aluminum in the blood is continually removed by the kidney [17]. An estimated $t_{1/2}$ in one human subject who received intravenous ^{26}Al was 7 years.

Exley [18] suggests that the lack of an Al-specific mechanism to enable its excretion from the body allows for the accumulation of aluminum and the persistence of any symptoms which might be the consequence of a body burden. We can assume that every compartment of human body including cells, organelles, cytoplasm, and extracellular fluids, contain a few atoms of potentially biologically available Al^{3+} . It seems that humans are not able to avoid exposure to aluminum at present. The question thus arises: What is the consequence of a burgeoning body burden of aluminum? Since Al^{3+} has no known function in life, then the first manifestations of the effect on human physiology in its presence will almost certainly be negative and most likely in the form of chronic disease.

Fluoride

Fluoride

It is noteworthy that fluorine, which is the most abundant halogen and the thirteenth most abundant element in the earth's crust, was not involved as a regular component of organic compounds of living organisms [19]. The concentration of available fluoride in the living environment, water, and food chain was very low before the Second World War. In 1942, H. Trendley Dean published his famous 21 City study in which he showed that at 1 ppm fluoride (1 milligram per litre) there was a marked decrease in tooth decay [20]. The artificial fluoridation of drinking water as a way of preventing dental caries has been a practice for many years in several countries. While a majority of European countries stop water fluoridation, the U.K. introduced it in 1999. Currently about 6 million people (10% of the U.K. population, mainly in the West Midlands and Newcastle) receive water artificially fluoridated to 1 ppm. Approximately 60-70% of the American people and more than 50% of the population of Australia, Columbia, Ireland, New Zealand, and Singapore are supplied with fluoridated drinking water. The U.S. EPA and the National Research Council (NRC) considered safe levels of fluoride in drinking water ≤ 4 ppm (4 mg/L). The WHO has a guideline for a recommended fluoride concentration of 1.5 ppm of drinking water. The problem of fluoride concentration higher than 2 ppm in ground water is one of the most important health-related environmental issues in many parts of India and China [1]. In central and northern Mexico millions of people are affected by high fluoride content in household-use groundwater.

The past 50 years have seen a dramatic increase in the volume of man-made industrial fluoride compounds released into the environment, so total fluoride intake has become an issue of particular concern [21]. People now get fluoride from many other sources, such as food and beverages, pesticide and fertilizers, industry, dental treatments, fluorinated drugs, and fluorine air pollution. Exposure to airborne fluorides from many diverse manufacturing processes such as phosphate fertilizer production, aluminum smelting, uranium enrichment facilities, coal-burning and nuclear power plants, petroleum refining and vehicle emissions, can be considerable. Several studies reported a high content of fluoride in most of tested teas. Tea is very high in fluoride because tea leaves accumulate more fluoride from pollution of soil and air than other plant species. Fluoride content in tea has risen dramatically over the last 20 years due to industrial contamination. Recent analyses have revealed that consuming tea infusions may lead to exposure to a high amount of fluoride [22-25].

Overexposure to fluoride among infants is a widespread problem in most major American cities. The investigation of the Environmental Working Group of the Fluoride Action Network found that up to 60% of formula-fed babies in

US cities were exceeding the upper tolerable limit for fluoride. Using fluoridated water, a bottle-fed baby will receive up to 250 times more fluoride than from the mother's milk (<http://www.fluoridealert.org>).

Under most conditions, fluoride is rapidly and extensively absorbed from the GIT. The rate of gastric absorption is inversely related to the pH of the gastric contents. High concentrations of Ca^{2+} and Al^{3+} can reduce the uptake of fluoride at this stage and the complexes or insoluble fluoride usually exit the body in the feces. Fluoride removal from plasma occurs by calcified tissue uptake and urinary excretion. A great part of the body burden of fluoride is associated with calcified tissues, and most of it is not exchangeable. In a healthy adult, about 50% of the fluoride, which enters plasma, is excreted by the kidney. Numerous studies document the increase of fluoride in blood, bones, and urine in children and adults occurring during the last two decades (for a review see [1]).

SYMPTOMS OF ALUMINUM AND FLUORIDE INTOXICATION

Aluminum

The studies showing the increased bioavailability of Al^{3+} due to acid rains reveal that aluminum may be toxic for many organisms. Toxic effects of aluminum concentration, which under legislation of both EPA and EU is allowed in drinking water (0.200 mg/L), have already been observed in some animal and plant species living in lakes with acidic water. Experiments with rats showed that the toxicity of 0.5 ppm aluminum in the drinking water was significantly greater than at 5 or even 50 ppm [26]. The reason for this paradoxical concentration effect is obscure.

The human body does not normally use aluminum, yet human physiology does not preclude its participation in wide and varied functions. Berend *et al.* [17] described the large content of aluminum in the lungs, liver, bone, myocardium, and parathyroid cells. The amount of aluminum that is not eliminated in the urine is retained in the body and removed very slowly.

Despite intensive research, we do not know exactly the conditions under which biologically reactive Al^{3+} has produced an aberrant physiological response in the affected cell or tissue. Exley [18,27] suggested that aluminum may be stored, thus being a silent “visitor” in tissues such as hair, skin, and bone. Its toxicity may be via a mechanism of free radical generation; in addition, aluminum may be recycled and used over and over again in promoting oxidative damage.

Much has already been written about human diseases attributed to exposure to Al^{3+} . These clinical observations demonstrate that severity and development of the symptoms increased long-lasting burden depend on a person's age, genetic background, nutrition status, kidney function, and many other factors. Aluminum exposure is linked to neurodegenerative diseases such as Alzheimer's disease, Parkinson disease, and multiple sclerosis [7,27,28]. A new distinct neurological disease, dialysis encephalopathy, which is associated with speech disturbances, personality changes, seizures and myoclonus, has been described in dialysis patients [29,30].

A new emerging syndrome, ***macrophagic myofasciitis***, was first described in France [31]. Victims of this syndrome suffer severe muscle and joint pains and severe weakness. Subsequent studies indicate widespread, severe brain injury as well—confirmed by MRI scanning. This brain syndrome has been described in American children as well. Recently, Exley *et al.* [16] reported the coincidence of macrophagic myofasciitis, chronic fatigue syndrome, and aluminum overload in an individual. This condition developed progressively following five vaccinations over a period of four weeks. Each of these vaccinations included an Al-based adjuvant and, three years later, the persistence of aluminum salt at an injection site was confirmed by muscle biopsy in the diagnosis of macrophagic myofasciitis. Aluminum overload was diagnosed four years post vaccination though the prevalence of this condition is unknown. This case has highlighted potential dangers associated with Al-containing adjuvants and authors have elucidated a possible mechanism whereby Al could trigger a cascade of immunological events, which are associated with autoimmune conditions [32]. Because vaccine adjuvants are designed to produce prolonged immune stimulation, they pose a particular hazard to the developing nervous system. Studies have shown that immune activation following vaccination can last up to two years. This means that the brain's microglial cells are also primed for the same length of time or possibly longer.

Another interesting question is connected with the potential of Al^{3+} to act as a potential antigen. It is because there is an immune response against Al^{3+} that it is used as a vaccine adjuvant. Aluminum is used as adjuvant in a wide range

of common vaccination. This is one of the reasons why vaccination has been assumed as the possible trigger for ASD. The Al - based adjuvants are known as long-lived depots of antigen but it is known that these Al-adjuvants activate innate immune signals, even in the absence of an adsorbed antigen. Research in rabbits show that labelled ^{26}Al , when injected into muscle as an adjuvant, was present after one hour in the blood sample. The distribution profile of ^{26}Al demonstrated its presence in various tissues; kidney > spleen > liver > heart > lymph node > brain [33]. Adjuvant aluminum is now implicated in a wide spectrum of human diseases, including adverse skin reactions, macrophagic myofasciitis, Alzheimer's disease, Parkinson's disease, ALS, cutaneous lymphoid hyperplasia, vaccine-related hypersensitivity to Al, immunotherapy-related hypersensitivity to Al, Gulf War illness, and Guillain-Barre syndrom [18].

The burgeoning use of Al-based adjuvants in vaccinations, which cover the full spectrum of human diseases and are administered throughout life, from new born babies through to the elderly, may already have created cohorts of individuals which are hypersensitive to aluminum exposure. Bergfors *et al.* [34] followed the group of 76, 000 vaccinees with Al- adsorbed diphtheria-tetanus/acellular pertussis vaccines and observed that 645 children (0, 8%) had persistent itching nodules at the vaccination site. The itching was intense and long-lasting. So far, 75% still have symptoms after a median duration of 4 years. Contact hypersensitivity to Al was demonstrated in 77% of the children with itching nodules and in 8% of the symptomless siblings who had received the same vaccines ($P < 0.001$). Identifiable diseases may then be the manifestation of immune-mediated responses to the burgeoning body burden of aluminum.

It has been known for a long time that local administration or application of aluminum to the brain can cause experimental animals to develop seizures [17].

Fluoride

The apparent side-effect of fluoride overload appears to be dental fluorosis. WHO recently estimated that dental fluorosis is endemic in at least 25 countries across the globe [1]. Millions of people live in endemic fluorosis area. WHO estimated that 2.7 million people have skeletal fluorosis in China, and over 6 millions suffer this crippling bone disease in India.

The toxic action of fluoride has been attributed to the fact that fluoride acts as enzymatic poison, inhibiting activities of many important enzymes such as enolase, lipase, phosphofructokinase, pyruvate kinase, glycogen synthase, succinate dehydrogenase, cytochrome oxidase, various phosphatases, ATP-ases, urease, and cholinesterases, to name a few. Strunecka *et al.* [1] review found that fluoride has been found to inhibit 22 various enzymes. On the other hand, 20 enzymes including adenylyl cyclase, lactate dehydrogenase, and glycogen phosphorylase, are stimulated by fluoride in milimolar concentrations. The use of fluoride in laboratory investigations helped in the discovery of glycolytic and Krebs-cycle pathways and provided key evidence of fluoride effects on the biochemical and physiological processes.

During the last decade numerous animal studies have been published, which have raised the level of concern about the impacts of increasing fluoride exposure on the brain [35-41]. These studies further highlight that it is not just the teeth, but the brain, that may be impacted by too much fluoride during infancy and childhood. Reduction of children's intelligence, various psychiatric symptoms in adults, such as memory impairment, and difficulties with concentration and thinking were reported [42,43].

Several studies appeared from China, which indicated a lowering of IQ in children associated with fluoride exposure [42,44,45]. Their conclusions have been criticized because of the possibility of unaccounted confounding variables. However, the later study by Xiang *et al.* [46] controlled for parental economic status and education, as well as exposure to iodide and lead. These authors found that IQ scores below 80 were significantly associated with higher serum fluoride level and estimated that children's IQ would be lowered at 1.8 ppm fluoride in their drinking water. Such a finding represents little margin of safety considering the potentially serious outcome for infants drinking fluoridated water. Rocha-Amador *et al.* [47] found that the increased content of fluoride in urine was associated with reduced performance, verbal, and full IQ scores. The effects on individuals indicated that for each mg increase of fluoride in urine, a decrease of 1.7 point in full IQ might be expected. Fluoride may belong to the class of developmental neurotoxicants such as arsenic, lead, and methylmercury.

Elevated fluoride content was found in embryonic brain tissues obtained from required abortions in areas where fluorosis was prevalent. These studies showed poor differentiation of brain nerve cells and delayed brain development [48]. The fetal BBB is immature and readily permeable to fluoride [35]. Mullenix *et al.* [49] demonstrated that fluoride accumulated in the brain of rats exposed to sodium fluoride in drinking water. The accumulations of fluoride were found in all the regions of the brain, with the highest levels in the hippocampus, one of the most sensitive areas of the brain to neurotoxicity. Mullenix and co-workers compared behavior, body weight, plasma, and brain fluoride levels after NaF exposures during late gestation, at weaning or in adults. Rats exposed prenatally had dispersed behaviors typical of hyperactivity, whereas rats exposed as adults displayed behavior-specific changes typical of cognitive deficits.

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 [50,51]. In their study using aluminum fluoride and sodium fluoride, Varner *et al.* [26] found damage in the superficial layers of the cortex, amygdala, and cerebellum—all areas endowed with abundant GluRs. Others have described a loss of Purkinje cells with chronic fluoride exposure, a cell type containing abundant AMPA GluRs. Also consistent with excitotoxicity is the finding of elevated levels of reactive oxygen species (ROS), reactive nitrogen species (RNS), and LPP in the brain, following fluoride exposure, both *in vitro* and *in vivo* [40,52]. Also of interest is the finding of elevation in nitric oxide (NO) via induced nitric oxide synthase (iNOS), again a critical component of excitotoxicity. 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 have shown that inflammatory cytokines and chemokines can markedly enhance excitotoxicity. This could represent fluoride alterations in cerebral glutamate levels, which are known to play a vital role in neuron migration and pruning of synaptic connections and dendrites [50,51].

The endocrine glands such as the thyroid and the pineal gland, are extremely sensitive to fluoride. It was shown that normal healthy individuals had thyroid function lowered when consuming water at 2.3 ppm [53]. The thyroid gland appears to be the most sensitive tissue in the body to fluoride burden, which is able to increase the concentration of **thyroid-stimulating hormone** (TSH) and decrease the concentration of T_3 and T_4 hormones, thereby producing hypothyroidism [54]. Up until the late 1950's, the doses of fluoride 2.3 - 4.5 mg/day were recommended in Europe to reduce the activity of the thyroid gland of those suffering from hyperthyroidism [55]. The search for a mechanism to explain how fluoride might lower thyroid activity has a very long and elusive history. A possible explanation has come from Tezelman *et al.* [56] who have suggested that overproduction of cAMP leads to a feedback mechanism resulting in a desensitization of the TSH receptor, thus ultimately leading to reduced activity of the gland.

Disturbance of thyroid hormone production has been found in correlation with lowered IQ in children in China [46]. A decreased level of T_3 was found in residents of Villa Ahumada, Mexico, where fluoride concentration in drinking water averages 5.3 ppm [57]. Susheela *et al.* [58] compared the production of thyroid hormones and TSH of 90 children living in fluoride endemic, non-iodine deficient areas of Delhi, India, along with 21 children from non-endemic areas. The data indicated an association of excess of fluoride intake and thyroid hormone disturbances leading to manifestation of iodine deficiency disorders (IDD). This study clearly documents that the primary cause of IDD may not always be iodine deficiency, but rather an excess of fluoride might induce it. Susheela *et al.* [58] suggests that iodine metabolism is being disturbed through fluoride's effect on deiodinases, the three enzymes, which regulate the conversion of T_4 to T_3 in target tissues. The role of excess of fluoride in development of IDD has been largely unnoticed at present, despite the fact that millions of children suffer with IDD. More targeted research is needed, considering the globally increasing problem of IDD.

The Synergistic Effects of Aluminum and Fluoride

Several laboratory studies have demonstrated that many effects primarily attributed to fluoride are caused by synergistic action of fluoride plus Al^{3+} . In water solution, Al^{3+} forms, in the presence of fluoride, water soluble aluminofluoride complexes (AlF_x) whose average stoichiometry depends on the excess concentration of fluoride ions and the pH of the solution (for a review see [1]). AlF_x has been widely used as an analogue of phosphate groups to study phosphoryl transfer reactions and heterotrimeric G proteins involvement [59]. Numerous laboratory studies demonstrated that AlF_x interacts with all known G protein-activated effector enzymes. Fluorides in the presence of Al^{3+} affect the levels of second messenger molecules, including cAMP, inositol phosphates, and Ca^{2+} . These studies

brought a great deal of understanding concerning the involvement of G proteins in cell signaling. Moreover, they bring evidence that AlFx influences various functions and biochemical reactions of many cells and tissues of animals or human organisms. Fluoride in the presence of trace amounts of Al^{3+} affects blood elements, endothelial cells and blood circulation, the function of lymphocytes and cells of the immune system, bone cells, fibroblasts and keratinocytes, ion transport, calcium influx and mobilization, processes of neurotransmission, metabolism of the liver, growth and differentiation, protein phosphorylation, and cytoskeletal proteins. These effects are not surprising in respect to the extensive role of G proteins in the cell. Physiological agonists of G protein-coupled receptors (GPCR) include neurotransmitters and hormones, such as dopamine, epinephrine, norepinephrine, serotonin, acetylcholine, glucagon, vasopressin, melatonin, TSH, neuropeptides, opioids, excitatory amino acids, prostanoids, purines, photons, and odorants.

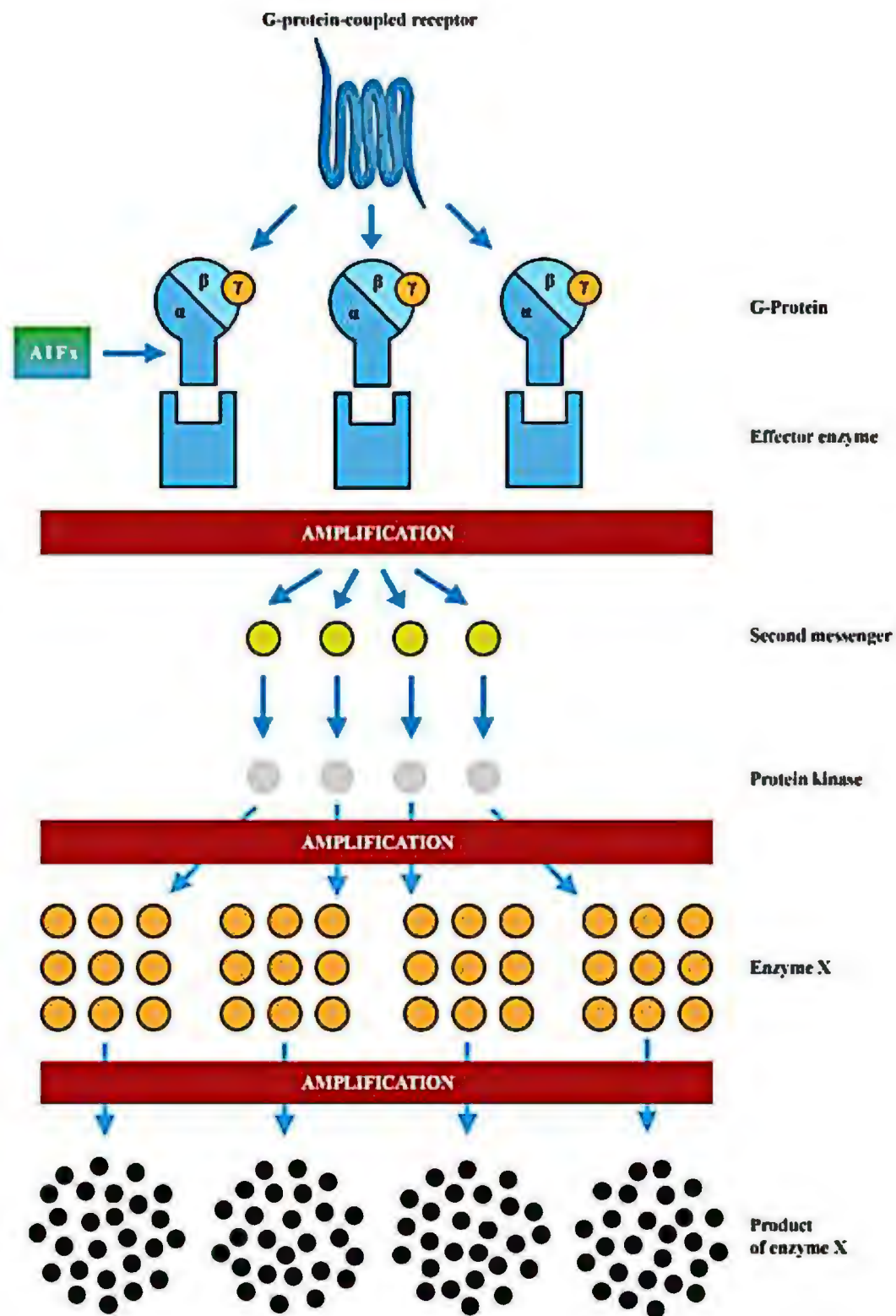


Figure 1: AIFx acts as the messenger of false information. Its message is greatly amplified during the conversion into the functional response of a cell. The second messenger molecule could be cAMP, Ins(1,4,5)P₃, and DAG.

The synergistic action of fluoride and Al^{3+} has the important implication for pathology. The effects of fluoride or Al^{3+} alone substantially differ from the effects of AlFx . While during the burgeoning accumulation of these elements, cell or tissue manage to cope with them until a threshold concentration is reached. Al^{3+} in micromolar concentrations avidly binds with fluoride to form AlFx . AlFx has a potency that allows it to activate hundreds of GPCRs. This means that the effects of AlFx result in pathophysiological consequences at several times lower concentrations than either Al^{3+} and fluoride acting alone. Moreover, the effects of AlFx are amplified by processes of signal transduction (Fig. 1). The principle of amplification of the initial signal during its conversion into a functional response has been a widely accepted tenet in cell physiology. It is evident that AlFx is a molecule giving a false message.

Toxicological potential of fluoride is thus markedly increased in the presence of trace amounts of Al^{3+} . It has been also observed that Al -induced neural degeneration in rats is greatly enhanced when the animals were fed low doses of fluoride. The presence of fluoride caused more Al^{3+} to cross the BBB and be deposited in the brain of rat [26].

THE POTENTIAL ROLE OF ALUMINUM AND FLUORIDE IN ETIOPATHOGENESIS OF ASD

Several symptoms induced by aluminum and/or fluoride overload can be seen in ASD. ASD is strongly correlated with neurodevelopmental alterations in prenatal, as well as the postnatal period. A considerable amount of evidence supports the conclusion that fluorides belongs to a group of other recognized causes of neurodevelopmental disorders and subclinical brain dysfunctions. Currently, fluoride exposure is common in fetuses, newborns, and small children mostly as a result of the artificial fluoridation of drinking water and a dramatic increase in the volume of man-made industrial fluoride compounds released into the environment. With over half of mothers using infant formula reconstituted using tap water, infant exposure to significant levels of AlFx has become a major problem. Chronic exposure of humans to Al and fluoride begins during the first trimester in the womb. While we have known for a long time that fluoride might cross the placenta and that the immature fetal BBB is readily permeable to both fluoride and Al^{3+} [12, 35], their impacts on the development of human fetal brain are not yet fully recognized. The association between embryonic errors and the development of autism has been reported in the literature. The implication is that the origin of ASD can be much earlier [60] in embryologic development than has been frequently reported.

A study by Du [48] revealed adverse effects of fluoride overload on the brains of 15 aborted fetuses between the 5-8th months of gestation from an endemic fluorosis area in China compared with those from a non-endemic area. Stereological study of the brains showed that the numerical density and the volume of the neurons were abnormal and the presence of undifferentiated neuroblasts as well as the increase in the nucleus-cytoplasm ratio of neurons indicated disordered neurodevelopment. The overall mean volume of the neurons was reduced. These results indicated that chronic fluoride overload in the course of intrauterine fetal life may produce certain harmful effects on the developing brain of the fetus. The recent study found more premature births in fluoridated than non-fluoridated upstate New York communities (<http://apha.confex.com/apha/137am/webprogram/Paper197468.html>). Gillberg and Gillberg [61] reported significantly more incidences of prematurity and postmaturity at birth of ASD patients. May-Benson *et al.* [62] investigated 467 children with ASD and found that the incidence of premature birth was 16%. Brimacombe *et al.* [63] studied a cohort of 164 families of autistic children referred to The Autism Center at New Jersey Medical School. Prevalence rates in this cohort for prematurity were higher than comparable rates reported nationally and in New Jersey.

Fluoridated drinking water contains up to 200 times more fluoride than breast milk (1000 ppb in fluoridated tap water vs 5-10 ppb in breast milk). As a result, babies consuming formula made with fluoridated tap water are exposed to much higher levels of fluoride than are breast-fed infants. Although there are some other reasons for encouraging breastfeeding, several authors are warning against ingestion of excessive fluoride from high quantities of intake of fluoridated water used to reconstitute concentrated infant formula early in infancy [64-66]. Newborn and premature infants also are particularly at risk for aluminum neurotoxicity. High permeability of the immature BBB to aluminum, the increased uptake via poorly developed GIT, and immature function of the kidney increases the risk of Al^{3+} intoxication. While aluminum content of breast milk is very small (about 20 $\mu g/L$), formulas based milk contain about ten times as much [17].

Tanoue and Oda [67] statistically compared weaning times of 145 children diagnosed as autistic with those of 224 normal children in the same catchment area: 24.8% of the patients and 7.5% of the controls were weaned by the end of first week, a significant difference. Early weaning because of the mother's rather than the child's condition occurred with 17.9% of the patients and 5.8% of the controls, also a significant difference. Historical studies on infantile autism revealed that the disorder developed more prevalently in the socioeconomic status where the incidence of breast-feeding was less frequent. These results suggest that early weaning may contribute to the etiology of infantile autism.

Some symptoms of ASD such as the sleep problems and the early onset of puberty suggest abnormalities in melatonin physiology and dysfunctions of the pineal gland. Luke [68,69] reported that fluoride accumulates in the pineal gland and that mongolian gerbils fed higher doses of fluoride excreted less melatonin metabolite in their urine and took a shorter time to reach puberty. When Luke had the pineal glands from 11 human corpses analyzed, the fluoride in the apatite crystals averaged about 9,000 ppm and in one case went as high as 21,000 ppm. Many studies indicate clearly that nocturnal production of melatonin is reduced in ASD [70]. Melatonin is responsible for regulating numerous life processes, including development and aging [71]. It is also known that production of melatonin by the pineal is controlled by mGluR and that excess aspartate or glutamate activity can inhibit melatonin release. Being a G protein type receptor, AIFx could also activate mGluR. Melatonin has been shown to have powerful neutralizing effects on ROS and LPP and to increase the levels of several of the antioxidant enzymes in the brain. A study of Tauman *et al.* [72] revealed that babies with the lowest melatonin production had the most neurobehavioral problems. In the light of these findings it is interesting to note that the Newburgh-Kingston fluoridation trial (1945-55) found that the girls in fluoridated Newburgh were menstruating on average 5 months earlier than girls in unfluoridated Kingston [73]. Considering the importance of melatonin in ASD, this issue warrants further study.

Mullenix *et al.* [49] demonstrated significant behavioral changes in rats exposed neonatally to fluoride in drinking water, with the effects dependent on the timing of the dose and sex of the animals. They found males were more affected with prenatal exposure than females if the exposure occurred after weaning or adulthood.

The contribution of aluminum to symptoms of ASD has been discussed for a long time; mainly the contribution of Al from vaccination in development of ASD pathology. Al-adjuvants potentiate the immune response, thereby ensuring the potency and efficacy of typically sparingly available antigen. While we do not understand fully how Al-based adjuvants work; it could be assumed that their efficacy is based upon similar principles in that their injection into tissue results in a threshold concentration of aluminum being reached instantaneously [32]. Thus, aluminum from vaccines is redistributed to numerous organs including the brain where it can accumulate. Each vaccine adds to this tissue level of aluminum. A total dose of 30.6 mg (and not the 0.85 mg considered safe by the FDA) is available when we calculate the total aluminum dose available from 36 vaccinations [4-6]. Of course, not all of this aluminum ends up in the tissues. However, aluminum can accumulate in substantial amounts from ingesting foods containing aluminum and from drinking water.

Recent research has also demonstrated sensitization to food allergens following their co-administration with Al salts such as antacid preparations, via childhood vaccinations, and an ongoing aluminum overload. While the body may cope robustly with a mild but persistent immune response to aluminum overload, the coping mechanism could possibly be suddenly and dramatically overwhelmed by a new exposure to Al-adjuvant. The latter, will not only enhance the antigenicity of itself, but it will raise the level of the immune response against all significant body stores of aluminum. Under these conditions, an individual's everyday exposure to aluminum will continue to fuel the response and myriad symptoms of associated autoimmunity will take over the life of the affected individual [32]. The individual will now respond adversely to aluminum exposures which previously were not sufficient to elicit a biological response and the only solution will be to treat the aluminum overload and to reduce everyday exposure to aluminum.

Moreover, we show that fluoride could complex with any pre-existing Al^{3+} within body fluids to produce the AlFx and this could lead to a combination of chronic activation of G protein regulated systems, dysregulation of calcium homeostasis, and sustained activation of receptor functions. A number of studies have shown that AlFx can evoke all symptoms of ASD. We have reviewed studies that indicate that fluoride and Al^{3+} can exacerbate the pathological

and clinical problems by worsening excitotoxicity, immune activation, and microglial priming. Moreover, AIFx accumulation in various compartments of the body may enhance the subclinical pathological alterations and/or the genetic susceptibility [1]. A number of ASD-related symptoms including intellectual disability, seizures, persistence of primitive reflexes, stereotypies, self-injurious behavior, among others, are known as potentially being emergent. We can assume that AIFx might play a role of trigger of these emergent phenomena [2].

AMELIORATION OF FLUORIDE AND ALUMINUM EFFECTS

Laboratory studies have revealed that withdrawal of fluoride resulted in some recovery of symptoms of fluoride toxicity. In 2006, a 12-person U.S. NRC committee reviewed the health risks associated with fluoride in the water and concluded:

"After reviewing the collective evidence, including studies conducted since the early 1990s, the committee concluded unanimously that the concentration of 4 mg/L for fluoride should be lowered. Such exposure clearly puts children at risk of developing severe enamel fluorosis, a condition that is associated with enamel loss and pitting." (<http://www.epa.gov/safewater/contaminants/index.html>).

Many scientists are calling for a stop to water fluoridation namely in the USA. On the other hand, authorities from the CDC claim the safety and effectiveness of fluoride at levels used in community water fluoridation. The Director of CDC J. L. Geberding wrote:

"Experts have weighed the findings and the quality of the available evidence and found that the weight of peer-reviewed scientific evidence does not support an association between water fluoridation and any adverse health effect or systemic disorder. Current evidence supports water fluoridation as being safe and effective for all population groups; nevertheless, CDC and other health organizations constantly review the scientific literature and safety evidence for information that might indicate a need for closer examination or additional research." (Personal letter to AS from April 29, 2008).

Most recently, proponents of water fluoridation have put their efforts into introducing mandatory fluoridation on a statewide level. They did this recently in the USA and are trying to do it presently in New Jersey. New Jersey has the highest overall prevalence of ASD among the US states - 9.9 autistic children per 1,000 children aged 8 years. New Jersey was the first state, where fluoride burden occurred during the Second World War [21] due to the production of enriched uranium for atomic bomb. In this area ASD has recently been diagnosed for one from every 67 boys (14.8 ASD cases per 1000 healthy boys). (<http://www.cdc.gov/mmwr/preview/mmwrhtml/ss5601a1.htm>).

Since tea is considered as the source of aluminum, it is interesting to know that adding milk to the tea can form insoluble Al-phosphate, thereby reducing absorption of aluminum in GIT. Yet, addition of lemon, which contains substantial amounts of the organic acid citrate, forms an Al-citrate complex, which has been shown to increase aluminum absorption from the gut 6-fold. [17]. Citrate is known to increase both GI absorption as well as tissue accumulation of aluminum. The bone aluminum content in rats treated with Al-hydroxide plus citrate was 41 times higher than in the bones of control rats treated with Al-hydroxide alone. However, in one study with human volunteers administered aluminum and citrate in the drinking water, no difference was observed between the plasma concentrations of Al^{3+} in subjects receiving citrate with the drinking water and those that did not. While increasing aluminum absorption, citrate may also enhance urinary excretion in humans, resulting in no significant increase in Al^{3+} plasma concentration in individuals with normal kidney function [17].

Ameliorative effects of vitamins C, E, and D alone, and in combination, were reported in laboratory animals [74]. Vitamins C and E act as antioxidants scavengers of free radicals and peroxides, which accumulate after fluoride exposure. Vitamin E channels the conversion of oxidized glutathione to reduced glutathione, which in turn helps conversion of mono- and dehydroascorbic acid to maintain ascorbic acid levels. Oral administration of vitamin C (50 mg/kg body weight/day) and vitamin E (2 mg/0.2 ml olive oil/animal/day) from day 6 to 19 of gestation along with NaF (40 mg/kg body weight) significantly ameliorates NaF-induced total percentage of skeletal and visceral abnormalities in rats. Vitamin E was comparatively less effective than vitamin C [75]. Vitamin D is known to promote GI absorption of Ca^{2+} and phosphate. Cotreatment with vitamins C, D, and E ameliorates NaF-induced

reduction in serum Ca^{2+} and phosphorus [76]. Ekambaram and Paul [77] reported that calcium carbonate prevents not only fluoride-induced hypocalcemia but also the locomotor behavioral and dental toxicities of fluoride by decreasing bioavailability of fluoride in rats. Toxic effects of fluoride were reversible if its exposure was withdrawn for two months. Recovery was also possible by feeding antioxidants (superoxide dismutase, glutathione, β -carotene, and some herbal extracts) [78]. Liu *et al.* [79] reported that synthetic catalytic scavengers of ROS proved beneficial in mouse brain for reversal of age related learning deficits and oxidative stress in mice.

Reversal of fluoride induced cell injury and fluorosis through the elimination of fluoride and consumption of a diet containing essential nutrients and antioxidants, have been shown in humans [80]. Increasing dietary proteins, calcium, and vitamins may help in its prevention especially in pregnant and nursing women and children [81]. Treatments of vitamins C, D, and Ca^{2+} showed significant improvement in skeletal, clinical, and biochemical parameters in children consuming water containing 4.5 ppm of fluoride.

There is a need for a non-invasive method to both reduce the absorption of aluminum in the GIT and facilitate the excretion of systemic aluminum in the urine. Exley and co-workers are currently working on solutions to reduce the human body burden of Al [82,83]. Based on the knowledge that silicon is the natural antagonist to aluminum, researchers have shown that silicon-rich mineral waters can be used to reduce the body burden of aluminum in individuals with Alzheimer's disease, macrophagic myofasciitis, and chronic fatigue syndrome. In one case report they demonstrated that regular drinking of a silicon-rich mineral water over a three month period dramatically reduced the body burden of aluminum from aluminum overload, to a normal range [16].

The iron chelating drug desferrioxamine (DFO) has been used extensively to treat individuals with suspected aluminum overload and, in spite of the significant side effects associated with its use, DFO remains, as yet, the only accepted course of treatment for the removal of systemic aluminum. Chelation with DFO may increase urinary aluminum excretion. A new oral agent, Feralex-G, in experimental tests, have shown less toxicity and greater removal of aluminum from tissues than more toxic intramuscular agents [84]. Several *in vitro* tests have also show a number of flavonoids to be excellent aluminum chelators, such as curcumin, quercetin and fisetin [85-87].

CONCLUSIONS

Fluoride and Al^{3+} exposure is common in fetuses, newborns, and small children as a result of the dramatic increase in the volume of man-made industrial fluoride and aluminum compounds released into the environment and food chain. The long-term fluoride burden has several health effects with a striking resemblance to ASD. These include hypocalcemia, hypomagnesemia, hypothyroidism, sleep-pattern disturbance, and IQ deficits. Conceivably, fluoride inhibits the release of pineal melatonin by elevating glutamate levels. Fluoride interferes with a number of glycolytic enzymes, resulting in a significant suppression of cellular energy production, which has been shown to dramatically increase excitotoxicity. Several studies demonstrate that autistic children have altered energy metabolism. Fluoride and Al^{3+} also reduce the antioxidant potential in the cells. This could be another significant source of priming of microglia as well as excitotoxicity. In addition, both fluoride and Al^{3+} have effects on cell signaling that can affect neurodevelopment and neuronal function.

The discovery of synergistic action of fluoride plus Al^{3+} expanded our understanding of mechanisms of their effects on living organism. The widespread use of AlFx as a general activator of heterotrimeric G proteins provided evidence that AlFx is a molecule giving false messages, which are amplified by processes of signal transduction. It is evident that AlFx might evoke receptor malfunction. Importantly, mGluRs operate by G-proteins. AlFx accumulation in various compartments of the body may enhance the subclinical pathological alterations and/or genetic susceptibility to various pathologies. This mechanism could explain the emergence phenomena in etiopathogenesis of ASD on a molecular and cellular level. Signaling disorders represent a major cause for the etiopathology of ASD. A number of studies have shown that AlFx can affect learning and behavior, and induce a loss of cerebrovascular integrity both in experimental animals and humans.

Scientific studies have already provided accumulating evidence demonstrating that environmental and dietary excitotoxins, as well as fluoride and aluminum, can exacerbate the pathological and clinical problems by worsening

excitotoxicity. The awareness of increasing load of fluoride and Al^{3+} as a new ecotoxicological phenomenon could contribute to the qualified assessment of their widespread use in water sources, food chains, and medicine.

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