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PARADOXES OF FLUORIDE TOXICITY

ABSTRACT: Numerous literature sources reveal evidence that fluoride affects the activities of numerous enzymes *in vitro* as well as *in vivo*. Millions of people live in endemic fluoride areas with a severe public health problem. A plethora of data suggest that fluoride should be recognized as a developmental neurotoxicant for humans. The use of water fluoridation for the prevention of dental caries has increased the concern about adverse fluoride effects. The fluoride concentration at which a reduction in dental caries is expected is close to the level which might cause chronic pathological effects. We comment on how some of the conclusions of the recent review by Guth et al., published in *Archives of Toxicology*, are the emerging paradoxes in fluoride research. We show that fluoride has pluripotent effects, which might contribute to unexpected epidemics in the future.

Keywords: Aluminofluoride complexes; Autism spectrum disorders; Enzymes; Fluoride; G proteins; Neurotoxicity.

Fluoride has been used in laboratory investigations as a tool affecting the activities of isolated enzymes or tissue slices since the beginning of the 20th century. These studies contributed to the discovery of fundamental biochemical processes such as glycolysis, the citric acid cycle, lipolysis, and ion transportation across membranes.[1] Simultaneously, they opened the understanding of the mechanisms of the toxic effects of fluoride. The expanding research provided evidence that fluoride affects life processes from fertilization to aging, and from gene transcription to mental activities with a powerful efficacy. Such findings contradict the practice of community water fluoridation (CWF), which is used as a way of preventing dental caries in developed countries, such as the United States of America (USA), Canada, Australia, New Zealand, the United Kingdom (UK), and the Republic of Ireland. The obvious argument of the proponents of CWF for the safety of the practice has been that the concentration of fluoride in the blood plasma from the use of fluoridated water cannot reach concentrations comparable with those used in the *in vitro* studies. Such an argument has recently appeared in the comprehensive review of Guth et al.[2] published on May 8, 2020, in *Archives of Toxicology*. Guth et al. declared: "Specific molecular targets for most of the effects of fluoride remain to be established and many of the findings from *in vitro* studies were only observed in the millimolar range. … The *in vivo* relevance of such concentrations in humans is questionable, since fluoride plasma concentrations in healthy adults generally range between 0.4 and 3.0 µM."[2] However, rather than discussing in detail here the finding that fluoride plasma concentrations in persons in endemic areas may reach levels of 7.37–39.5 µM,[3,4] we would like to comment on how some of the other conclusions of the above-mentioned review are the emerging paradoxes in fluoride research.

PARADOX NUMBER ONE:

ENZYMES AND FLUORIDE HORMESIS

A review of laboratory studies of fluoride effects on enzymes reveals that competing reactions might produce paradoxical dose-response effects.[5] For example, Zakrzewska et al.[6] found that the activity of lactate dehydrogenase in ram semen displayed a nine-fold decrease with 20 µM fluoride, but at the much higher concentration of 100 mM fluoride, its activity is nearly 40% above that of the control. Burgstahler[5] describes several examples of the existence of such hormesis effects of

fluoride, in both *in vitro* and *in vivo*. There are a considerable number of situations in which the response to low-dose fluoride concentration is potentially adverse.[7]

Since the early 20th century, it has been evident that fluoride interferes with Mg^{2+} activation and with the interactions of enzymes with phosphate groups.[1] Such interactions can fundamentally alter the physicochemical properties of several enzymes in very low fluoride concentrations.

Competing reactions can thus elicit unexpected responses of enzymes to fluoride in micromolar concentrations in both *in vitro* and *in vivo*.[1,5]

Paradox number two:

Fluoride in complex with aluminum acts in nanomolar concentrations

Fluoride played an important role as a tool in the discovery of guanine nucleotide-binding proteins (G proteins).[8] The breakthrough for the explanation of the fluoride effects came with the observation that free aluminum ions (Al^{3+}) are a requirement for the activation of the regulatory component of adenylyl cyclase by fluoride.[9,10] The contribution of fluoride to the discovery of G proteins has been evident since Rodbell mentioned fluoride 15 times in his Nobel Prize lecture.[11] Bigay et al.[10] demonstrated that aluminofluoride complexes (AlFx) activate stoichiometric amounts of G protein in the micromolar range. The nuclear magnetic resonance analysis with ^{19}F of a GDP-AlFx complex confirmed that they could mimic bound GTP.

GTP binds with nanomolar affinity to the α subunits of heterotrimeric G proteins. AlFx might act as a general activator of G proteins giving false messages. Moreover, such a false message is amplified by the processes of signal transduction.[12] These discoveries heralded a new field of research into the structure and mechanism of AlFx. An unexpected paradox is that fluoride, in synergy with Al^{3+}, might affect G proteins at concentrations several times lower than those for fluoride acting alone.[13]

Paradox number three:

Pluripotent danger of fluoride toxicity

The phosphate analog model of AlFx shows that fluoride in concentrations of 10^{-1} –10^{-6} M, in the presence of trace amounts of Al^{3+}, may evoke several signaling disorders, exacerbate alterations in neurotransmission, and act as an endocrine disruptor. AlFx mimics the phosphate monoesters in studies of ATPases, GTPases, kinases, mutases, phosphohydrolases, and phosphatases.[12,13]

Phosphate groups have a key role in the regulation of metabolism and the most fundamental biological processes. The famous pronouncement of Sir Alexander Todd[14] —"Where there's life, there's phosphorus"—expressed the centrality of phosphates for life on the Earth. An awareness of the increasing load of fluoride and Al^{3+} as a phosphate analog could contribute to a critical reassessment of their widespread use.

Albert Gilman said in his Nobel prize lecture: "The ultimate dream is to design drugs that will prevent aberrant G protein action."[15] The number of known G protein-coupled receptors (GPCRs) exceeds 800 types, which makes them the largest family

of membrane proteins encoded in the human genome. Recently, it was found that between 30% and 60% of the drugs marketed to improve health target G proteins.[16] To the contrary, AlFx can affect G proteins, impair health, and lead to symptoms of pathology by causing alterations to various physiological processes. For example, numerous endocrinopathies are frequently reported from areas of endemic fluorosis, such as the deficient thyroid function with abnormal levels of T3, T4, and TSH found in India and Pakistan.[1,3,4] The pluripotent targets of fluoride in the whole organism might contribute to a paradox of unexpected response and adverse fluoride effects.

PARADOX NUMBER FOUR:

FLUORIDE NEUROTOXICITY

Three hundred and fifteen laboratory, clinical, epidemiological, and ecological studies, from over the whole world have documented fluoride neurotoxicity.[17,18] There is now overwhelming evidence that prolonged exposure to fluoride in the prenatal and early postnatal stages might have toxic effects on the development and metabolism of the human brain.[19]

A long-term burden of fluoride may result in numerous health effects which have a striking resemblance to the pathophysiology of autism spectrum disorder (ASD), such as oxidative stress and inflammation. Fluoride may impair cognition and produce IQ deficits, sleep-pattern disturbance, a reduced ability to learn, and behavioral problems in some individuals.[17,20]

The effect of chronic fluoride exposure on children's intelligence, measured as the intelligence quotient (IQ), has been traditionally investigated as an indication of the neurotoxic effect of fluoride in various geographical areas.[17-20]

Guth et al.[2] evaluated 23 epidemiological studies reporting an association between high fluoride exposure and reduced intelligence as a marker of fluoride-induced neurotoxicity. Most of these reports were also analyzed in the above-mentioned papers as well as in several editorials by Spittle in *Fluoride*.[21-23] An updated review by Philippe Grandjean, published in *Environmental Health* on December 19, 2019, of developmental fluoride neurotoxicity concluded that recent epidemiological results support the notion that elevated fluoride intake during early development can result in IQ deficits.[24] On the other hand, Guth et al.[2] found in their evaluation that, so far, almost all studies investigating the effect of fluoride on intelligence are of low quality with some limitations leading to confounding effects related to a constellation of factors, including, in comparison to the "reference populations," the "exposed populations" being in relatively poor rural communities with less developed healthcare systems, lower educational and socioeconomic status, lower overall nutritional status and intake of essential nutrients, and higher exposure to environmental contaminants, such as lead, cadmium, mercury, and manganese. The authors thus concluded that only two prospective cohort studies[25,26] considered possible confounding factors and that these two studies reported conflicting results. They called for high-quality epidemiological studies to be carried out since their assessment of the presently available studies does not support the presumption that fluoride is a human developmental neurotoxicant. In the present situation of increasing dental and skeletal fluorosis, systemic fluorosis affecting millions of

people, and a rising ASD epidemy, the challenge of Guth et al.[2] sounds like a great paradox.

Paradox number five:

Fluoride is not a developmental neurotoxicant in the European Union

Guth et al.[2] do not support the presumption that fluoride should be assessed as a human developmental neurotoxicant at the current exposure levels in Europe. This is true due to the fact that most of the European states rejected water fluoridation shortly after its introduction in the 1970s–1990s. It was not easy, and the readers of *Fluoride* will know the names of Dr Hans Moolenburgh and Professor Arvid Carlsson, Swedish Nobel laureate, who fought against water fluoridation in Europe. However, there is still the danger of an excessive intake of fluoride occurring in parts of Europe where the drinking water is fluoridated, such as in the Republic of Ireland and in some areas in Great Britain, While some European countries without water fluoridation, such as Germany, France, and the Czech Republic, have a very low incidence of children with ASD,[17] the prevalence of ASD in the Republic of Ireland and in the UK is comparable with the USA, Canada, and The People's Republic of China.

The Panel of the European Food Safety Authority (EFSA) considered that fluoride is not an essential nutrient and no average requirement for the performance of its essential physiological functions can be defined.[27] The Panel considered that data on the dose-response relationship between caries incidence and consumption of drinking water with different fluoride concentrations are adequate to set an Adequate Intake (AI) of 0.05 mg F/kg body weight per day for children, pregnant, and lactating women. In the European Union (EU), the AI covers fluoride intake from all sources, including toothpaste, and other dental hygiene products. The available data on fluoride intake of the European population is variable but generally at or below 0.05 mg/kg/day.[27] For younger children (1–6 years of age) the Upper Tolerable Intake Levels is exceeded when consuming more than one liter of water at 0.8 mg F/L and fluoride from other sources.

It is a remarkable paradox, that Guth et al.[2] concluded that the consumption of drinking water with extremely high fluoride concentrations (>8 mg F/L), which may result in plasma concentrations of approximately 10 μM fluoride, was not dangerous as a developmental neurotoxicant because it was still 100-fold below the critical *in vitro* cytotoxic concentration of 1 mM fluoride.[2]

A fluoride concentration >8 mg F/L occurs in endemic areas such as in Pakistan and Sri Lanka, where serious health impacts such as dental and skeletal fluorosis, ASD, and thyroid disturbances have been reported.[4,17,18,20]

Hirzy et al.[18] assessed the findings of a recent IQ study on water fluoridation and estimated a daily dose of fluoride that might protect children from lowered IQ. Benchmark dose analysis (BMD) showed that the possible safe dose to protect against a five-point IQ loss is about 0.045 mg F/day. The safe dose estimated with the LOAEL/NOAEL method is about 0.047 mg F/day. Based on their calculations, a protective daily dose should be no higher than 0.05 mg/day, or 0.0010 mg/kg-day for children.[18]

The greatest paradox of the Guth et al. review[2] may be their rejection of the broad range of evidence that is widely accepted over the whole world that fluoride may act as a developmental neurotoxicant.

CONCLUSIONS

Recently, there has been a renewed public concern about whether or not fluoride supplementation via the drinking water has harmful effects. The dose at which dental caries reduction is expected is not far away from the one which may cause chronic pathological effects. Many authors suggest that preventative efforts should focus on the prevention of developmental fluoride neurotoxicity by reducing of fluoride intake of pregnant women and small children.[17-24,26] We should change the focus of the research on fluoride toxicity from a reductionistic approach to investigating the underlying integrative networks. The pluripotent toxic effects of fluoride may result in unexpected epidemics in the future.

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