BIOCHEMICAL EFFECTS

Selective decreases of nicotinic acetylcholine receptors in PC12 cells exposed to fluoride

In an attempt to elucidate the mechanism by which excessive fluoride damages the central nervous system, the effects of exposure of PC12 cells to different concentrations of fluoride for 48 hr on nicotinic acetylcholine receptors (nAChRs) were characterized here. Significant reductions in the number of binding sites for both [3H]Epibatidine and [125I]α-bungarotoxin, as well as a significant decrease in the Bmax value for the high-affinity of Epibatidine binding site, were observed in PC12 cells subjected to high levels of fluoride. On the protein level, the α3 and α7 subunits of nAChRs were also significantly decreased in the cells exposed to high concentrations. of fluoride. In contrast, such exposure had no significant effect on the level of the β2 subunit. These findings suggest that selective decreases in the number of nAChRs may play an important role in the mechanism(s) by which fluoride causes dysfunction of the central nervous system.

Authors: Chen J, Shan K-R, Long Y-G, Wang Y-N, Nordberg A, Guan Z-Z.
Correspondence: Department of Pathology, Guiyang Medical College, Guizhou, PR China.
Keywords: [125I]α-Bungarotoxin; [3H]Epibatidine; Fluoride-induced CNS dysfunction; Nicotinic acetylcholine receptors; PC12 cells.
Source: Toxicology 2003;183(1-3):235-42.

Amiodarone attenuates fluoride-induced hyperkalemia in vitro

Poisoning by hydrofluoric acid or fluoride salts results in hypocalcemia, hypomagnesemia, and hyperkalemia with subsequent cardiac arrhythmias. In previous studies, quinidine attenuated fluoride-induced hyperkalemia in vitro, and enhanced survival in animals. Like quinidine, amiodarone is a potassium channel blocker, although amiodarone is more familiar to clinicians due to its recent inclusion in advanced cardiac life support (ACLS) protocols.

Objectives: This in-vitro study of human erythrocytes was designed to determine whether amiodarone could attenuate fluoride-induced hyperkalemia.

Methods: Six healthy volunteers each donated 60 mL of blood on three occasions. Each specimen was divided into 12 tubes, incubated at 37 degrees C, and oxygenated with room air. An aqueous sodium fluoride (F^-) solution was added to tubes 1-9. Incremental amounts of quinidine were added to tubes 1-4 (Q(1)-Q(4)) to attain calculated concentrations of 0.73 μg/mL, 1.45 μg/mL, 2.9 μg/mL, and 5.8 μg/mL, respectively. Incremental amounts of amiodarone were added to tubes 5-8 (A(1)-A(4)) to attain calculated concentrations of 0.38 μg/mL, 0.75 μg/mL, 1.5 μg/mL, and 3.0 μg/mL, respectively. Tubes 9-12 were controls for each of F^-, amiodarone, quinidine alone, and no additive, respec-

tively. Extracellular potassium concentration of K^+ was followed, and an objective endpoint was defined as the rise in potassium concentration at 6 hours.

Results: Fluoride produced a significant change in K^+ by 6 hours in all samples. Quinidine produced a J-shaped curve in its ability to attenuate the rise in K^+, with only one concentration, Q(3), demonstrating significance versus tube 9 (control). Amiodarone also demonstrated a J-shaped dose-response effect, with statistical significance at A(1), A(2), and A(3) versus tube 9 (control). There was no significant difference among the effective concentrations (Q(3), A(1), A(2), and A(3)) of both drugs.

Conclusions: In this in-vitro model using human blood, amiodarone and quinidine both attenuated F^--induced hyperkalemia. Further study is indicated to determine whether amiodarone enhances survival in F^--poisoned animals.

Authors: Su M, Chu J, Howland MA, Nelson LS, Hoffman RS.
Correspondence: Department of Emergency Medicine, New York University/Bellevue Hospital Center, New York, NY 11203, USA. marksu@pol.net
Keywords: Amiodarone; Cardiac arrhythmias; Fluoride-induced hyperkalemia; Fluoride toxicity; Potassium channel blockers; Quinidine.
Source: Academic Emergency Med 2003 Feb;10(2):105-9.

NaF induces early differentiation of murine bone marrow cells along the granulocytic pathway, but not the monocytic or pre-osteoclastic pathway, *in vitro*

The stimulatory effects of sodium fluoride (NaF) on bone formation have been explained solely by its activation of osteoblasts. However, the action of F on the osteoclast lineage is poorly understood. We previously found that NaF differentiates HL-60 cells to granulocytic cells. To further test this action, we have employed here primary cultures of progenitor cells derived from murine bone marrow. NaF at subtoxic concentrations (< 0.5 mM) significantly up-regulated several intracellular enzymes [lactate dehydrogenase (LDH), beta-glucuronidase (beta-GL), acid phosphatase (ACP)], cellular reduction of nitroblue tetrazolium (NBT), and nitric oxide (NO) production, which are all accepted as general differentiation markers. NaF (< 0.5 mM) also up- regulated granulocyte-specific markers [chloroacetate esterase (ChAE), cell surface antigens (Mac-1, Gr-1)], but none of any of the monocyte-specific markers [non-specific esterase (NSE), cell surface antigens (F4/80, MOMA-2)]. Although other general differentiation markers (phagocytosis, adhesion, appearance, nuclear/cytoplasmic ratio) were not appreciably influenced by NaF, essentially in support of our previous data from HL-60 cells, the present findings suggest that NaF induces early differentiation of bone marrow hemopoietic progenitor cells along the granulocytic pathway, but not the monocytic pathway that is linked to osteoclast formation. Therefore, in addition to its potent stimulatory effects on

osteoblastic bone formation, NaF applied to patients with osteoporosis could be expected to indirectly reduce osteoclastic bone resorption.

Authors: Oguro A, Kawase T, Orikasa M.
Correspondence: Meirin College, Niigata, Japan.
Keywords: Murine bone marrow cells; Granulocytic pathway; HL-60 cells; Lactate dehydrogenase; Osteoblastic bone formation; Osteoclastic bone resorption; Sodium fluoride and bone formation.
Source: In Vitro Cell Dev Biol Anim 2003 Jul 24.

EFFECTS ON TEETH

Dental caries and fluorosis in low- and high-fluoride areas in Turkey

Objective: The aim of this study was to investigate the caries prevalence of children living in either low- or high-fluoride areas and to relate caries experience to the severity of dental fluorosis.

Method and materials: A total of 278 12- to 14-year-old schoolchildren, 149 in a low-fluoride area (LFA) and 129 in a high-fluoride area (HFA), were included in the study. The naturally occurring fluoride concentrations in the drinking water were 0.30 to 0.40 ppm in the LFA, 1.42 to 1.54 ppm in the HFA1, and 1.55 to 1.66 ppm in the HFA2. Dental caries was recorded with the World Health Organization criteria, and dental fluorosis was measured using the Tooth Surface Index of Fluorosis.

Results: The percentages of children who had an average TSIF > or = 1 were 0%, 29%, and 77% in the LFA, HFA1, and HFA2, respectively. The mean decayed, missing, and filled permanent teeth (DMFT) and decayed, missing, and filled permanent surfaces (DMFS) were 0.84 ± 0.98 and 1.58 ± 2.24 in LFA, 1.30 ± 1.46 and 1.78 ± 2.52 in HFA1, and 1.26 ± 1.42 and 1.97 ± 2.60 in HFA2, respectively. There was no significant difference in caries prevalence among children living in low- and high-fluoride areas when evaluated with an analysis of covariance model, including the frequency of toothbrushing. Toothbrushing frequency had a significant effect on the decayed teeth, decayed surfaces, DMFT, and DMFS. In high-fluoride areas, there was no relationship between caries prevalence and severity of fluorosis.

Conclusion: Increasing water fluoride levels were associated with higher prevalence and severity of dental fluorosis and had no influence on caries experience in children with poor oral hygiene.

Authors: Ermis RB, Koray F, Akdeniz BG.
Correspondence: Department of Restorative Dentistry and Endodontics, School of Dentistry, Suleyman Demirel University, Isparta, Turkey. banu_ermis@yahoo.com
Keywords: Caries in children, Dental fluorosis; Toothbrushing; Water fluoridation; WHO criteria.
Source: Quintessence Int 2003 May;34(5):354-60.

COMMENT

This study compared dental fluorosis and caries in regions of Turkey where fluoride levels in the drinking water were quite similar to those found in the US and other countries that do not have endemic fluorosis. Despite many epidemiological studies examining the relationship between dental fluorosis and caries, the 'end point' where the dental benefits from fluoride exposure reaches a maximum and, thereafter, starts to decline, has never really been firmly established. The unexpected finding of this study was that dental fluorosis prevalence and severity was high in the areas where the drinking water levels were only 1.42 and 1.66 ppm. This is well below the maximum contaminant level (MCL) of 4.0 ppm, currently considered by the US Environmental Protection Agency to be the level at which fluoride in drinking water is still 'safe'. One might argue that having more than 13% of the children with TSIF (tooth surface index of fluorosis) scores of 3, as measured in this study, would suggest that a 'safe' level of fluoride has been exceeded.

The authors also confirmed the finding of Heller *et al*,[1] which was based on historical data, that increased levels of fluoride in drinking water above 0.7 ppm were not significantly associated with any further decline in dental decay. While the numbers of subjects studied were relatively small (only 63–66 children in the high-fluoride groups), the authors took care to calibrate the examiners and to include only children who did not have any nutritional deficiency. Despite the limitations of the study, there were significant differences between the groups in terms of dental fluorosis but *not* in terms of caries. The authors attempted to rationalize their findings by noting that the children in the low-fluoride areas brushed their teeth more often. However, they conceded that, based on their study, "alternative sources of drinking water with lower fluoride concentration in Isparta should be identified with regard to anticaries effect of fluoride and disadvantages of fluorosis."

Hardy Limeback BSc PhD DDS
Head, Preventive Dentistry
University of Toronto

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REFERENCE

1 1 Heller KE, Eklund SA, Burt BA. Dental caries and dental fluorosis at varying water fluoride concentrations. J Public Health Dent 1997;57(3):136-43.

The prevalence of developmental enamel defects in permanent molars in a group of English school children

Aim: An epidemiological study was designed to determine the prevalence of enamel defects in first permanent molars in English children of ethnic backgrounds.

Materials And Methods: A population of school children aged seven years, living in the low water fluoride City of Leeds (UK) were examined for the presence of developmental enamel defects in first permanent molars. The examination criteria were based on the DDE index for screening surveys. The ethnic background to the children examined was determined by school records, name and visual assessment.

Results: The results for 307 children (154 females) showed an overall prevalence of defective enamel in first permanent molars of 14.5% and tooth prevalence of 7.2%. There were effects of gender or tooth site. There was no significant difference in prevalence between White-Caucasian (17%) or Asian-Caucasian (10%) children. The demarcated opacity was the most frequent type of defect seen, followed by diffuse opacities and hypoplasia. The occlusal and buccal surfaces were the most commonly affected.

Conclusion: As there were no significant differences in prevalence between children of different ethnic groups it was concluded that the aetiology of enamel defects in permanent molars was most likely affecting all children.

Authors: Zagdwon AM, Toumba KJ, Curzon ME
Correspondence: Department of Paediatric Dentistry Leeds Dental Institute, University of Leeds, UK.
Keywords: Enamel defects; English school children; Hypoplasia; Permanent molars.
Source: Eur J Paediatr Dent 2002 Jun;3(2):91-6.

The 2001 Kentucky Childrens Oral Health Survey: findings for children ages 24 to 59 months and their caregivers

Purpose: This study was performed to provide a 2001 benchmark of oral health status of children in Kentucky with a comparison to the most recent state (1987) and national surveys.

Methods: Using Basic Screening Survey protocols for visual screenings, a sample of 572 children ages 24 to 59 months was screened in health department clinics and physicians' and pediatric dentists' offices across Kentucky after caregivers completed a questionnaire. Screeners were provided modified Association of State and Territorial Dental Directors training materials. Analyses on the sample and population estimates were done with SAS and SUDAAN software. This weighted population estimate analysis is based on the assumption that sampled children at participating sites are representative of other children at that site, as well as children at refusing sites.

Results: Sample data and adjusted population estimates closely approximated each other. Population estimates indicated that 43% had untreated caries, 47% had caries experience (early childhood caries), and 31% had severe early childhood caries. Thirty-seven percent of the children needed early care, 9% needed urgent care, 39% had never been to the dentist, 44% had a history of "bad bottle

behaviors," and 35% of the parents had not been to the dentist within the last year.

Conclusions: Dental caries is a major health and early childhood development problem in high-risk preschool children in Kentucky.

Authors: Hardison JD, Cecil JC, White JA, Manz M, Mullins MR, Ferretti GA.
Correspondence: Division of Dental Public Health, College of Dentistry, University of Kentucky, Lexington, KY, USA.
Keywords: Baby bottle caries; Dental caries in children; Kentucky USA; Visual screening.
Source: Pediatr Dent 2003 Jul-Aug;25(4):365-72.

Note: In view of these overall adverse dental findings, the anti-caries effectiveness of statewide mandatory water fluoridation in Kentucky is questionable. (See related reports and comment in *Fluoride* 2003;36:207, 210-2).

Association of salivary *Streptococcus mutans* with caries in young children: effect of dental health education on salivary levels

Aim: This study aimed to determine the effect of a long-term dental health education (DHE) for mothers with young children on the level of salivary *Streptococci mutans* (SM) and their association with caries in young children.

Methods: A randomly selected cohort of 228 children born between 1 January and 30 September 1995, in a low socioeconomic high caries suburb of Leeds (UK), was divided into the following groups: A) DHE focused on diet; B) DHE focused on oral hygiene instruction (OHI) using fluoride toothpaste; C) DHE by a combined diet and OHI message. DHE was given using an interview and counseling for at least 15 minutes in each child's home, every three months for the first two years and twice a year in the third year of the study. A fourth group D was given diet and OHI, at home, but once a year only. The children in a fifth group E (control), received no DHE and were never visited, but examined at three years of age only. All children and mothers were examined for caries using the BASCD criteria. The levels of salivary SM were determined by sampling of bacteria from the oral cavity with a 1.8 cm wide wooden spatula, after giving the mother a paraffin pellet to chew for a minute and in children using unstimulated saliva. Bacteria were plated out and counted using image analysis for counting colonies.

Results: At three years of age the difference in the level of salivary SM between groups was not statistically significant. However, in group E there was a statistically significant relationship ($p<0.05$) between salivary SM and caries in children.

Conclusion: The difference in the level of salivary SM between groups given various programs of dental health education was not statistically significant.

There was a statistically significant ($p<0.05$) relationship between salivary SM and caries in children.

Authors: Kowash MB, Curzon ME, Hart P.
Correspondence: Department of Paediatric Dentistry, Leeds Dental Institute, University of Leeds, UK.
Keywords: Caries in young children; Fluoride toothpaste; Salivary *streptococcus mutans.*
Source: Eur J Paediatr Dent 2002 Dec; 3(4): 199-204.

FLUORIDE CONTROL

Monitoring report on status in coal-burning-induced fluoride poisoning area in Luoyang, 1991-2000

Objective: To understand the effects of stove-improvement measures in coal-burning-induced fluoride poisoning area in Luoyang. *Methods:* Dynamic monitoring on the fluoride concentration. in indoor air, grain (corn and wheat), vegetable (chili, radish and Chinese cabbage), and the urine fluoride concentration and prevalence of dental fluorosis among 8–12 yr-old children was conducted. This followed stove-improvement measures in a coal-burning-induced fluoride poisoning area, Jiangzhuang village, Xin'an county, Luoyang, during 1991-2000. The obtained data were compared with those in 1990. *Results:* After the performance of stove-improvement measures, all of the fluoride contents in indoor air, grain and vegetable, the urine fluoride concentration and the prevalence of dental fluorosis of 8-12 aged children revealed a decreasing trend. This compared favorably with the corresponding standards for non-fluoride poisoning area, despite the fluctuation of data in different years. *Conclusion:* The stove-improvement measures could effectively prevent the indoor air, grain and vegetable from fluoride pollution, and control the prevalence of fluoride poisoning in coal-burning-induced fluoride poisoning area.

Authors: Geng S-S, Yang F-L, Liu SM, Geng A-Q, Han J-Q, Ma J, Zhu Q-P, Yin G-J, Xu B, Zhang L.
Correspondence: Luoyang Institute for Prevention and Treatment of Endemic Diseases, Luoyang, Henan, PR China.
Keywords: Chinese grains and vegetables; Improvements in coal-burning stoves; Fluoride toxicity reduction.
Source: Huanjing Yu Jiankang Zazhi 2003; 20(4): 233-4. [Article in Chinese].

Fluoride and aluminium concentrations of tea plants and tea products from Sichuan Province, PR China

Some Tibetans in Sichuan Province in southwestern China have been suffering from fluorosis, due to drinking and eating tea with high fluoride (F) and aluminium (Al) contents. Tea plants, soils of tea plantations and tea products from Yaan, Gaoxian and Yibin Cities in Sichuan Province were investigated to

evaluate the factors affecting F and Al contents in tea products. The F and Al concentrations of four commercial brands of brick teas were significantly higher than those of 11 brands of green teas. Chemical analysis indicated that total and available F and Al concentrations in tea plantation soils in Yaan and Gaoxian were within the normal range compared with acid soils in South China and tea soils in Fujian Province. Edaphic conditions did not contribute to the high F and Al concentrations in brick tea. Analysis of raw materials of brick tea indicated that old leaves were the major contributors to the high F and Al contents contained in brick tea. There were also great variations among different tea varieties in accumulating F and Al, and concentrations of F and Al in tea variety Qianmei 303 were about 2-3-fold higher than the other three varieties. Selection of appropriate varieties would be important to lower F and Al contents in tea products.

Authors: Shu WS, Zhang ZQ, Lan CY, Wong MH.
Correspondence: State Key Laboratory for Bio-control, School of Life Sciences, Zhongshan University, 510275, Guangzhou, PR China.
Keywords: Aluminum; Brick tea; Fluoride; Fluorosis in China.
Source: Chemosphere 2003 Sep;52(9):1475-82.

An improved method for defluoridation

Fluoride is a naturally occurring toxic mineral present in drinking water and is the root cause of many diseases and disorders. The present international drinking water standards set by World Health Organization (WHO) for fluoride is 1.5 ppm. In order to find the wide spread concentration of fluoride in drinking water of a fluoride contaminated aquifer, 30 bore well water samples have been collected from different villages of Natrampalli Union which comes under Tiruvannamalai Circle, Tamil Nadu, India. In the present work, an attempt to remove fluoride by the use of coagulant, Poly Aluminum Chloride (PAC) was made and is compared with the most common existing technique "Nalgonda Technique" where there was a reversible reaction. The coagulant used in Nalgonda technique is Alum [$(Al_2SO_4)_3$]. Results of the present work show that Poly Aluminum Chloride (PAC) can be an effective coagulant for the removal of fluoride from water with a higher removal efficiency of about 75–85% in less processing time. It was also observed that the fluoride removal was dependent on the initial fluoride concentration and dose of coagulants.

Authors: Muthu GI, Vinodhini V, Padmapriya G, Sathiyanarayanan K, Sabumon PC.
Dept of Civil Engineering, Vellore Institute of Technology, (Deemed University), Vellore, India.
Keywords: Defluoridation in India; Nalgondo technique; Poly aluminum chloride coagulant.
Source: Indian J Environ Health 2003;45(1):65-72.

HEALTH/BIOLOGICAL EFFECTS

Antioxidant defense system and lipid peroxidation in patients with skeletal fluorosis and in fluoride-intoxicated rabbits

Fluorosis is a serious public health problem in many parts of the world where drinking water contains more than 1 ppm of fluoride. The main manifestations of skeletal fluorosis are crippling bone deformities, spinal compressions, and restricted movements of joints. Although fluorosis is irreversible, it could be prevented by appropriate and timely intervention through understanding the process at biochemical and molecular levels. As in the case of many chronic degenerative diseases, increased production of reactive oxygen species (ROS) and lipid peroxidation has been considered to play an important role, even in the pathogenesis of chronic fluoride toxicity. However, there is inconclusive proof for an altered oxidative stress and antioxidant balance in fluorosis, and the existing data are not only conflicting but also contradictory. In the present communication we have evaluated the antioxidant defense system (both enzymatic and nonenzymatic) and lipid peroxidation in both humans from an endemic fluorosis area (5 ppm fluoride in the drinking water) and in rabbits receiving water with 150 ppm of fluoride for six months. There was no significant difference in lipid peroxidation, glutathione, and vitamin C in the blood of human fluorotic patients and fluoride-intoxicated rabbits as compared to controls. Neither were there any changes in the activities of catalase, superoxide dismutase, glutathione peroxidase, or glutathione S-transferase in the blood due to fluoride intoxication (of rabbits) or fluorosis in humans. The results together do not subscribe to oxidative stress theory in fluorosis. Thus, in the absence of clear proof of oxidative damage and to counter toxic effects of fluoride through supplementation of antioxidants, extensive investigations are needed to conclusively prove the role of oxidative stress in skeletal fluorosis.

Authors: Reddy GB, Khandare AL, Reddy PY, Rao GS, Balakrishna N, Srivalli I.
Correspondence: National Institute of Nutrition (ICMR), Hyderabad, India.
Keywords: Catalase; Glutathione S-transferase; Humans; Lipid peroxidation; Rabbits; Reactive oxygen species (ROS); Skeletal fluorosis; Vitamin C.
Source: Toxicol Sci 2003;72(2):363-8.

Fluoride-induced disruption of reproductive hormones in men

Fluoride-induced reproductive effects have been reported in experimental models and in humans. However, these effects were found in heavily exposed scenarios. Therefore, in this work our objective was to study reproductive parameters in a population exposed to fluoride at doses of 3-27 mg/day (high-fluoride-exposed group-HFEG). Urinary fluoride levels, semen parameters, and reproductive hormones in serum (LH, FSH, estradiol, prolactin, inhibin-B, free and total testosterone) were measured. Results were compared with a group of

individuals exposed to fluoride at lower doses: 2-13 mg/day (low-fluoride-exposed group-LFEG). A significant increase in FSH ($P<0.05$) and a reduction of inhibin-B, free testosterone, and prolactin in serum ($P<0.05$) were noticed in the HFEG. When HFEG was compared to LFEG, a decreased sensitivity was found in the FSH response to inhibin-B ($P<0.05$). A significant negative partial correlation was observed between urinary fluoride and serum levels of inhibin-B ($r = –0.333$, $P = 0.028$) in LFEG. Furthermore, a significant partial correlation was observed between a chronic exposure index for fluoride and the serum concentrations of inhibin-B ($r = –0.163$, $P = 0.037$) in HFEG. No abnormalities were found in the semen parameters studied in the present work, neither in the HFEG, nor in the LFEG. The results obtained indicate that a fluoride exposure of 3-27 mg/day induces a subclinical reproductive effect that can be explained by a fluoride-induced toxic effect in both Sertoli cells and gonadotrophs.

Authors: Ortiz-Perez D, Rodriguez-Martinez M, Martinez F, Borja-Aburto VH, Castelo J, Grimaldo J, de la Cruz E, Carrizales L, Diaz-Barriga F.
Correspondence: Laboratorio de Toxicologia Ambiental, Facultad de Medicina, Universidad Autonoma de San Luis Potosi, Av. Venustiano Carranza 2405, Col. Lomas Filtros, CP 78210, San Luis Potosi, Mexico.
Keywords: Fluoride effects on reproductive hormones; Gonadotrophs; Inhibin -B; Semen; Sertoli cells; Urinary fluoride levels.
Source: Environ Res 2003 Sep;93(1):20-30.

HEALTH/TOXIC EFFECTS IN ANIMALS

Lipid peroxidation and antioxidant systems in the blood of young rats subjected to chronic fluoride toxicity

Wistar albino rats were exposed to 30 or 100 ppm fluoride in drinking water during their fetal, weanling, and post-weaning stages of life up to puberty. Extent of lipid peroxidation and response of the antioxidant systems in red blood cells and plasma to prolonged fluoride exposure were assessed in these rats in comparison to the control rats fed with permissible level (0.5 ppm) of fluoride. Rats treated with 100 ppm fluoride showed enhanced lipid peroxidation. as evidenced by elevated malondialdehyde (MDA) levels in red blood cells, but 30 ppm fluoride did not cause any appreciable change in RBC MDA level. 30 ppm fluoride-intake resulted in increased levels of total and reduced glutathione in red blood cells and ascorbic acid in plasma while 100 ppm fluoride resulted in decreases in these levels. The activity of RBC glutathione peroxidase was elevated in both the fluoride-treated groups, which was more pronounced with 100 ppm. Reduced to total glutathione ratio in RBC and uric acid levels in plasma decreased in both the groups. RBC superoxide dismutase activity decreased significantly on high-fluoride treatment. These results suggest that long-term high-fluoride intake at the early developing stages of life enhances oxidative stress in the blood, thereby disturbing the antioxidant defense of rats. Increased oxidative

stress could be one of the mediating factors in the pathogenesis of toxic manifestations of fluoride.

Authors: Shivarajashankara YM, Shivashankara AR, Bhat PG, Rao SH.
Correspondence: Department of Biochemistry, M.R. Medical College, Gulbarga, India.
Keywords: Anti-oxidant systems; Lipid peroxidation; Malondialdehyde; RBC glutathione dismutase; RBC superoxide dismutase; Rats.
Source: Indian J Exp Biol 2003;41(8):857-60.

Fluoride toxicity to aquatic organisms: a review

Published data on the toxicity of fluoride (F^-) to algae, aquatic plants, invertebrates and fishes are reviewed. Aquatic organisms living in soft waters may be more adversely affected by fluoride pollution than those living in hard or sea water because the bioavailability of fluoride ions is reduced with increasing water hardness. Fluoride can either inhibit or enhance the population growth of algae, depending upon fluoride concentration, exposure time and algal species. Aquatic plants seem to be effective in removing fluoride from contaminated water under laboratory and field conditions. In aquatic animals, fluoride tends to be accumulated in the exoskeleton of invertebrates and in the bone tissue of fishes. The toxic action of fluoride resides in the fact that fluoride ions act as enzymatic poisons, inhibiting enzyme activity and, ultimately, interrupting metabolic processes such as glycolysis and synthesis of proteins. Fluoride toxicity to aquatic invertebrates and fishes increases with increasing fluoride concentration, exposure time, and water temperature, and decreases with increasing intraspecific body size and water content of calcium and chloride. Freshwater invertebrates and fishes, especially net-spinning caddis fly larvae and upstream-migrating adult salmons, appear to be more sensitive to fluoride toxicity than estuarine and marine animals. Because, in soft waters with low ionic content, a fluoride concentration as low as 0.5 mg F^-/L can adversely affect invertebrates and fishes, safe levels below this fluoride/L concentration are recommended in order to protect freshwater animals from fluoride pollution.

Author: Camargo JA.
Correspondence: Departamento Interuniversitario de Ecologia, Edificio de Ciencias, Universidad de Alcala, Alcala de Henares, Madrid E-28871, Spain. E-mail: Julio.camargo@uah.es
Keywords: Enzymatic poisons; Fluoride toxicity in aquatic organisms; Fluoride effects in soft water.
Source: Chemosphere 2003 Jan;50(3):251-64.

Loss of T-type calcium current in sensory neurons of rats with neuropathic pain

Background: Pathophysiology in the primary sensory neuron may contribute to chronic neuropathic pain. Ca channels play a central role in neuronal processes,

and sensory neurons are rich in low-voltage-activated calcium channels (LVACCs). However, the physiological function of these channels is unknown. Their possible role in rebound burst firing makes them a candidate for increased excitability after neuropathic injury.

Methods: This study uses pharmacological methods to isolate LVACC in cells from the dorsal root ganglia of neuropathic and sham-operated rats, including the blockade of high-voltage-activated Ca channels with fluoride and selective toxins. LVACCs were examined with conventional whole cell patch clamp electrophysiology techniques.

Results: After chronic constriction injury of the peripheral axon, LVACC was significantly reduced compared to sham rats as shown by a 60% reduction in peak current density and an 80% reduction in total calcium influx. A depolarizing shift in the voltage dependence of activation and an increase in the rate of deactivation and inactivation appear to cause this reduction of LVACC. Either Ni^{2+} or mibefradil, blockers of LVACC, applied in the bath to normal dorsal root ganglion cells during current clamp significantly and reversibly increased excitability.

Conclusions: These results suggest that loss of LVACC may contribute to decreased spike frequency adaptation and increased excitability after injury to sensory neurons. Through decreased Ca^{2+} influx, the cell becomes less stable and more likely to initiate or transmit bursts of action potentials. Consequently, modulation of Ca^{2+} currents at the dorsal root ganglion may be a potential method of therapeutic intervention.

Authors: McCallum JB, Kwok W-M, Mynlieff M, Bosnjak ZJ, Hogan QH.
Correspondence: Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226, USA. mccallum@mcw.edu
Keywords: Calcium channels; Calcium currents; Dorsal root ganglion; Electrophysiology; Mibefradil; Neuropathic pain; Nickel ions; Sensory neurons.
Source: Anesthesiology 2003 Jan;98(1):209-16.

Toxicity of aqueous and sediment-associated fluoride to freshwater organisms

Inorganic fluorides were declared toxic under the Canadian Environmental Protection Act in 1993 based on their potential to cause long-term harmful effects in aquatic and terrestrial ecosystems, but information on the toxicity of sediment-associated fluoride to freshwater benthic organisms was considered incomplete. The purpose of this study was to determine the toxicity of aqeous and sediment-associated fluoride to several species of freshwater organisms and to determine if toxic effects could be expected under environmentally realistic exposures. Toxicity of fluoride (as NaF) in short-term (48-96 hr) lethality tests was greatest for the amphipod *Hyalella azteca* (median lethal concentration. [LC50] = 14.6 mg F^{-}/L), followed by the mayfly *Hexagenia limbata* (32.3), the

midge *Chironomus tentans* (124.1), the fathead minnow *Pimephales promelas* (262.4), and the cladoceran *Daphnia magna* (282.8). Relative toxicity in long-term (10-28 day) growth and survival tests in spiked sediment was similar. *Hyalella azteca* was the most sensitive species for growth (25% inhibitory concentration [IC25] = 290.2 μg F^-/g), followed by *C. tentans* (661.4), *H. limbata* (1,221.3), and *P. promelas* (>5,600); *H. azteca* was also the most sensitive species for survival (LC50 = 1,114.6 μg F^-/g), followed by *H. limbata* (1,652.2) and *P. promelas* and *C. tentans* (>5,600 for both). Concentrations of fluoride measured in sediments near some industrial point sources exceed some of these toxicity thresholds. Fluoride is highly mobile in aquatic systems and could potentially reach toxic levels in the water column during dredging to remove fluoride-contaminated sediment.

Authors: Metcalfe-Smith JL, Holtze KE, Sirota GR, Reid JJ, De Solla SR.
Correspondence: Aquatic Ecosystem Impacts Research Branch, National Water Research Institute, Burlington, ON, Canada.
Keywords: Fluoride-contaminated sediment; Fluoride toxicity in freshwater organisms.
Source: Environ Toxicol Chem 2003;22(1):161-6.

Effects of fluoride on *Xenopus* embryo development

Fluoride was first associated with fetal malformation shortly after water fluoridation was initiated in the 1940s. Since many chemicals can interact directly with the embryo to cause malformation, the effects of fluoride on embryonic and fetal development were investigated. The effects of sodium fluoride on the development of frog embryos were studied under conditions described by the Frog Embryo Teratogenesis Essay-*Xenopus* (FETAX), a screening assay for teratogens. The most prominent malformations caused by sodium fluoride are reduction in the head-tail lengths and dysfunction of the neuromuscular system of the tadpoles. The values for LC(50), EC(50), and minimal concentration to inhibit growth (MCIG) of sodium fluoride met the limits established for a teratogen in frog embryos, showing that sodium fluoride is a direct-acting teratogen on developing embryos. Since FETAX has a high degree of success in identifying mammalian teratogens, the observed teratogenic action of sodium fluoride on frog embryos would indicate a strong possibility that sodium fluoride may also act directly on developing mammalian fetuses to cause malformation.

Authors: Goh EH, Neff AW.
Correspondence: Department of Pharmacology and Toxicology, Indiana University School of Medicine, Medical Sciences Program, Jordan Hall 009A, 47405, Bloomington, IN, USA.
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HEALTH/TOXIC EFFECTS IN PLANTS

Possible pathways of the negative influence of acid gases on plants

A review. The degree of negative influence of acid gases on plants is considered in dependence of their solubility in water. The linkage of water in the processes of hydration of gases forming acids can decrease the chemical potential of water in leaf apoplast. It causes the decrease in water inflow into leaf symplast. The higher the solubility. of acid gases in water and the higher their concentration in the air, the lower the water inflow from apoplast to symplast. At high concentration of toxicant water chemical potential in leaf apoplast is lower than in symplast, and water flows out into apoplast, i.e. plasmolysis takes place. Plasmolysis leads to the increase in toxicant concentration in leaf symplast and finally to the necrosis of cells. When air with acid gases dissolves in apoplast water, "concentration" of acid gases takes place because the acid components are more soluble in water than the main components of the air (nitrogen and oxygen). The lower acid dissociation in apoplast water, the higher speed of receipt from apoplast to symplast and even to inner cell compartments through cell membranes. It can explain why sulfur dioxide and hydrogen fluoride are more toxic than nitrogen dioxide. Exogenous acids, producing hydrogen ions, negatively influence different metabolic processes of plants.

Author: Vasfilov, SP.
Correspondence: Botanical Garden, Ural Branch, Russian Academy of Science, Yekaterinburg, Russia.
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