

BIOLOGICAL EFFECTS

Neurotoxicity of fluoride: neurodegeneration in hippocampus of female mice

Light microscopic study of hippocampal sub-regions demonstrated significant numbers of degenerated nerve cell bodies in the CA3, CA4 and dentate gyrus areas of sodium fluoride administered adult female mice. Ultrastructural studies revealed neurodegenerative characteristics like involution of cell membranes, swelling of mitochondria, clumping of chromatin material, etc. can be observed in cell bodies of CA3, CA4, and dentate gyrus. Fluoride intoxicated animals also performed poorly in motor coordination tests and maze tests. Inability to perform well increased with higher fluoride concentration in drinking water.

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Keywords: Fluoride toxicity, Hippocampus, Mouse study, Neurodegeneration, Neurotoxicity.

Source: Indian J Exp Biol 2002;40(5):546-54.

Esthetically objectionable fluorosis attributable to water fluoridation

Objective: We compared estimates of fluorosis prevalence and risk attributable to fluoridation using an index applied to the entire dentition and to the maxillary anterior teeth. We also estimated the prevalence of perceived esthetic problems attributable to current fluoridation policy (Attributable Burden).

Methods: Fluorosis prevalence estimates were obtained from the National Survey of Oral Health in US School Children (1986-87) for the 1839 survey children aged 12-14 years who were scored for fluorosis, had never received fluoride drops or tablets, and had lived in only one home. For each child we calculated Dean's fluorosis index, and an anterior fluorosis index (value of the highest scored maxillary anterior tooth). We used each index to calculate risk of fluorosis attributable to fluoridation by subtracting at each level of severity the prevalence of fluorosis among those living in low fluoride areas ($F \leq 0.3$ ppm) from the prevalence among those living in optimally fluoridated areas ($0.7 \text{ ppm } F \leq 1.2 \text{ ppm}$). Findings from five published studies were used to calculate risk of perceived esthetic problem attributable to fluorosis by severity, i.e., the difference in the mean percentage of respondents who were satisfied with the appearance of their teeth with and without fluorosis. Finally, Attributable Burden was estimated by summing the products of risk of perceived esthetic problems attributable to fluorosis and risk of fluorosis attributable to fluoridation for each level of fluorosis severity.

Results: Prevalence of fluorosis, very mild or greater, was 26% with Dean's Index, which was significantly higher than the 18% figure calculated with the anterior index. Using the anterior index, fluoridation was a risk factor for very mild (attributable risk = 15%) and mild fluorosis (attributable risk = 3%). Risk of fluorosis (very mild or greater) attributable to fluoridation was significantly higher when calculated from prevalence estimates using Dean's Index than estimates calculated with the anterior index (24% versus 18%). The mean values of risk of perceived esthetic problems attributable to very mild and mild fluorosis were 9% and 33%, respectively.

Conclusion: We found that approximately 2% of US schoolchildren may experience perceived esthetic problems which could be attributed to the currently recommended levels of fluoride in drinking water. The findings further suggest that both estimates of fluorosis prevalence and risk of fluorosis attributable to fluoridation will be higher when calculated with an index applied to the entire dentition. Data were unavailable for fluoridated toothpaste and diluted formula consumption, thus the risk of fluorosis attributable to fluoridation may be overestimated if consumption was higher in fluoridated areas. The risk of perceived esthetic problems attributable to fluoridation must be weighed against its lifetime benefits and the associated costs of alternative solutions such as educating parents about appropriate toothpaste use and lowering the fluoride content of children's toothpaste.

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Keywords: Children, Dental fluorosis, Esthetically objectionable fluorosis, USA, Water fluoridation.

Source: Community Dent Oral Epidemiol 2002 Jun;30(3):199-209.

Dental fluorosis decline after changes to supplement and toothpaste regimens

In 1989/90, in 659 12-year-olds in Perth (F 0.8 mg/L) and the Bunbury region of Western Australia (WA) (F approximately 0.25 mg/L), dental fluorosis prevalences were 40.2% and 33.0%. Fluoride supplements (OR 4.63) and extended residence in a fluoridated area (OR 4.06) were significant risk factors; toothpaste ingestion variables had ORs greater than unity; in 1990, DMFT for this age group was 0.84. School Dental Service took steps to discourage supplement and toothpaste ingestion and to promote low fluoride toothpaste for children < 6 years of age.

Objectives: To evaluate the effect of this campaign on fluorosis and caries.

Methods: Between May-July 2000, 582 10-year-olds were examined for dental fluorosis (TF index) and dental caries (DMFT) in school dental clinics.

Results: Fluorosis prevalence was 22.2% in Perth and 10.8% in the Bunbury region. Overall prevalence was 18.0% and of this, 80.2% was TF 1, 17.9% was TF 2 and just 1.9% was TF 3. In 1989/90, 79 children had used supplements before the age of 4 year; in 2000 only 40 had done so ($P < 0.001$). Mean DMFT values in Perth and Bunbury were 0.32 and 0.28 ($P > 0.05$). Low F toothpaste, unavailable in 1989/90, had been used by 24.5%. The only significant risk factor was residence, OR 2.0. **CONCLUSIONS:** Fluorosis prevalence seems to have fallen in parallel with a reduction in discretionary intake from supplements and toothpaste. No increase in dental caries experience was recorded. Because the teeth examined in this study were at risk of fluorosis in 1992-95, very soon after policies changed, and because people are slow to change health habits, it seems reasonable to expect a further improvement when teeth mineralised in the late 1990s become visible.

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Keywords: Australia, Children, Dental fluorosis, Dental fluorosis prevention, Fluoride in toothpaste, Fluoride supplements, Perth.

Source: Community Dent Oral Epidemiol 2002 Jun;30(3):233-40..

Burns caused by dilute hydrofluoric acid in bleach

Two male cleaning workers aged 62 (patient 1) and 28 (patient 2) presented with red, swollen, aching hands and fingers. At the first interview, the fingers of the right hand of both patients were swollen from the proximal interphalangeal joint to the tip of the finger. The fingers were red and intensely painful. The bleach that both patients used contained 9.5% hydrofluoric acid, and therefore we diagnosed their symptoms as those of burns caused by this agent. We subcutaneously injected 8.5% calcium gluconate into the affected fingers and dressed them with gauze soaked in cooled 0.025% benzethonium chloride. The patients did not use gloves at work, neither of them knew that the cleaning fluid contained dilute hydrofluoric acid, and they were unaware of the danger of this agent. They had not received proper education about the care and handling of poisons and deleterious substances such as hydrofluoric acid. The doctors who had examined the patients in the emergency ward overlooked the possibility of hydrofluoric acid burns, although they suspected chemical burns and confirmed the trade name of the cleaning agent. In addition, although the patients presented with intense pain, no white areas of coagulation and blistering, or surrounding erythemas, which are characteristic symptoms of such burns, were evident. The component of the cleaning agent was described on the container label in very small print. Had the words "poison" and "hydrogen fluoride" been printed in large characters, the examining doctors in the emergency ward

would probably not have overlooked the presence of hydrofluoric acid, and the patients would perhaps have been more careful when using it. Hydrofluoric acid can be easily obtained by anyone through the Internet, although general consumers could not obtain industrial quantities. Therefore, the number of burn patients who are not familiar with hydrofluoric acid may increase in the future.

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Keywords: Burns, Chemical burns, Hydrofluoric acid, Hydrofluoric acid in bleach.

Source: J Nippon Med Sch 2002 Apr;69(2):180-4.

Fluoride ion toxicity in rabbit kidney thick ascending limb cells

Background and Objective: Some halogenated agents, especially methoxyflurane, because of a higher level of fluoride production, induce a renal concentrating defect that could be related to an ascending limb impairment. We investigated the mechanisms of fluoride toxicity on an immortalized cell line.

Methods: Cells were cultured for 2, 6, or 24 hr in the presence of fluoride. Toxicity evaluation was based on: cell numbers, protein content, leucine incorporation, lactate dehydrogenase (LDH) and N-acetyl-beta-glucosaminidase (NAG) releases, Na-K-ATPase and Na-K-2Cl activities, electron microscope studies. Infrared analysis and fluoride microdetermination allowed crystal components.

Results: At 5 mmol after 24 h, fluoride decreased cell numbers (-14%, *P < 0.05), protein content (-16%*), leucine incorporation (-54%*), Na-K-2Cl activity (-84%*), increased LDH (+145%*) and NAG release (+190%*). Na-K-ATPase was more sensitive and impaired from 1 mmol for 24h and after 2 hr at 5 mmol. Crystal formation in mitochondria occurred after 6 hr at 5 mmol. Infra-red analysis and fluoride microdetermination established that crystals contained sodium, phosphate, and fluoride.

Conclusions: The results suggest that the Na-K-ATPase pump is a major target for fluoride toxicity in Henle's loop.

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Keywords: Fluoride toxicity, Methoxyfluorane, Rabbit kidney, Renal concentrating defects, Sodium-potassium-ATPase pump.

Source: Eur J Anaesthesiol 2002 May;19(5):341-9.

DIETARY FLUORIDE

Fluoride content of alcoholic beverages

Background: In view of the conflicting reports of the extent and severity of dental caries in alcohol misusing subjects, a systematic survey of the fluoride content of alcoholic beverages was undertaken.

Results: The fluoride content of beverages varied widely particularly if non-UK European products were considered.

Conclusions: Beers brewed in locations with high fluoride water levels may contribute significantly to the daily fluoride intake, particularly in alcohol misusing subjects and this may contribute to alcohol-associated bone disease.

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Keywords: Fluoride in alcoholic beverages.

Source: Clin Chim Acta 2002 Jun;320(1-2):1-4.

BIOCHEMICAL EFFECTS

Bone cell mitogenic action of fluoroaluminate and aluminum fluoride but not that of sodium fluoride involves up-regulation of the insulin-like growth factor system

The fluoroaluminate ion (AlF_4^-) and sodium fluoride (NaF) have previously been shown to be bone cell mitogens. This study sought to determine whether the bone cell mitogenic action of AlF_4^- and/or NaF would involve the insulin-like growth factor (IGF) regulatory system. We evaluated the effect of mitogenic doses of AlF_4^- and NaF on the mRNA levels and the protein level (in conditioned media [CM]) of several components of the IGF system (i.e., IGF-2, IGF binding protein [IGFBP]-4, and IGFBP-5) in human TE85 osteosarcoma cells. Aluminum fluoride (AlF_3) was included for comparison. NaF, AlF_3 , and AlF_4^- , each at 50-100 $\mu\text{mol/L}$, increased [^3H]thymidine incorporation in TE85 cells. Mitogenic concentrations of AlF_3 and AlF_4^- : (1) increased the mRNA (up to twofold after 24 hr treatment) and protein (in CM) levels (up to 2.5-fold after 48 hr treatment) of IGF-2; (2) increased the mRNA level (twofold) and the protein level in CM (up to threefold) of stimulatory IGFBP-5; and (3) either reduced slightly or had no effect on the mRNA and protein (in CM) levels of the inhibitory IGFBP-4. Conversely, mitogenic concentrations of NaF had no significant effects on the protein (in CM) or mRNA level of IGF-2, IGFBP-4, or IGFBP-5. The addition of an inhibitory concentration of IGFBP-4 completely abolished the

bone cell mitogenic activity of AlF_3 and AlF_4^- but not that of NaF. The findings of this study provide strong evidence that the bone cell mitogenic activity of AlF_4^- and AlF_3 , but not that of NaF, is mediated by the upregulation of the IGF regulatory system.

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Keywords: Aluminum fluoride, Bone cell mitogenic action, Fluoroaluminate, Insulin-like growth factor system, Sodium fluoride.

Source: Bone 2002 May;30(5):705-11.

Lipid peroxidation and antioxidant defense systems in liver of rats in chronic fluoride toxicity

Lipid peroxidation and response of antioxidant defense systems were examined in the liver of rat litters exposed to 30 and 100 ppm fluoride through drinking water during early stages of life till puberty. Increased lipid peroxidation was seen as reflected by increased malondialdehyde levels. Total and reduced glutathione levels decreased and glutathione peroxidase activity increased. The ratio of reduced glutathione to total glutathione was decreased. Glutathione S-transferase was more elevated in the 30 than in the 100 ppm fluoride group. Ascorbic acid levels were decreased.

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Keywords: Antioxidant systems, Ascorbic acid, Fluoride toxicity, Glutathione, Glutathione peroxidase, Glutathione S-transferase, Lipid peroxidation, Rat study.

Source: Bull Environ Contam Toxicol 2002;68(4):612-6.

Editor's Note: For another account of this research by these authors, see pages 197-203 in this issue of *Fluoride*.

The influence of fluoride ingestion on urinary aluminum excretion in humans

Fluoride (F) and aluminum (Al) are both ingested daily in water, foods, and pharmaceuticals. Owing to the strong chemical affinity between F and Al, these elements can interact in biological systems. The purpose of the present study was to investigate the influence of F ingestion on Al excretion in humans. Six healthy volunteers ingested 100 mL of distilled water at 6:00 a.m. on day 1 (control period) and the same volume of sodium fluoride solution containing 5 mg of F at 6:00 a.m. on day 2 (test period). A schedule for meals and for blood and urine collection was followed for the two suc-

cessive days. The concentration of F was measured with an F-electrode, and Al was determined by ion-pair RP-HPLC with its complexation with 8-quinolinol. The mean concentration of serum F peaked within 30 minutes after ingestion of F and rapidly decreased thereafter, reaching baseline 24 hours later. In the control period, there was no increase of Al concentration in serum in 24 hours. In the test period, Al concentration in serum did not increase significantly compared with those in control serum. Although some variation was observed among subjects, cumulative amounts of F and Al excreted in urine during the test period were significantly higher than those during the control period. The results suggest that absorbed F enhanced urinary Al excretion and that the Al in urine may be, at least in part, derived from endogenous tissues.

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Keywords: Aluminum in urine, Fluoride in urine, Fluoride-aluminum interactions.

Source: Tohoku J Exp Med 2002 Mar;196(3):139-49.

Effects of sodium fluoride on total serum protein levels and transaminase activity in rats

Transaminase activity and serum total protein level were investigated in adult rats after oral treating with sodium fluoride at three doses, 10, 20, and 30 mg/kg daily for 90 days. After 90 days, the average total serum protein level of the rats in the treatment group decreased significantly compared with that in the control [1.9 ± 0.1 (mean \pm S.D., $n = 140$) vs. 3.1 ± 0.2] mg/dL, $P < 0.05$. Serum transaminase activity in the treatment group increased compared with that in the control [5.3 ± 0.4 (mean \pm S.D., $n = 140$) vs. 3.2 ± 0.3] $\mu\text{mol/min per ml}$, $P < 0.05$.

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Keywords: Rat study, Serum protein, Serum transaminase, Sodium fluoride.

Source: Biomed Pharmacother 2002 Jun;56(4):169-72.

Modulation of fluoride toxicity in rats by calcium carbonate and by withdrawal of fluoride exposure

In order to assess the effect of calcium on the toxic effects of fluoride, adult female Wistar rats were treated with sodium fluoride (NaF, 500 ppm in drinking water) alone or in combination with calcium carbonate (CaCO_3 , 50 mg/kg by oral intubation) daily for 60 days. Food, water and fluoride intake were measured daily for 60 days. Body weight gain, exploratory motor ac-

tivity, rota-rod motor coordination, dental structure, activities of acetylcholinesterase (AChE, brain and skeletal muscle) and $\text{Na}^+ \text{K}^+$ ATPase (erythrocyte membrane and skeletal muscle) and the concentrations of protein (serum and skeletal muscle), calcium (serum) and fluoride (serum) were determined in these animals 24 hr after the last treatment. The same parameters were tested in another group 60 days after withdrawal of NaF exposure (500 ppm in drinking water daily for 60 days). NaF treatment decreased food and water intake, reduced body-weight gain, and impaired exploratory motor activity and rota-rod performance. Dental lesions, inhibition of the activities of AChE and $\text{Na}^+ \text{K}^+$ ATPase, and a decrease in the concentration of protein, and serum calcium were also observed in these animals. These effects were accompanied by a marked elevation of fluoride concentration in the serum. CaCO_3 decreased the concentration of fluoride in the serum of NaF-treated animals. A decrease in serum fluoride concentration was found also after NaF withdrawal. A prevention of locomotor behavioural, biochemical, and dental toxicities of fluoride was observed in both these groups. It is concluded that the dose of CaCO_3 used in the present study has a potential to prevent the toxicity of fluoride by maintaining serum fluoride at a less toxic level. Further, the toxic effects of fluoride are reversible if its exposure is withdrawn for 2 months.

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Keywords: Calcium carbonate, Fluoride toxicity, Fluoride toxicity amelioration.

Source: *Pharmacol Toxicol* 2002 Feb;90(2):53-8.

Fluoride in groundwater in selected villages in Eritrea (North East Africa)

A study was undertaken to estimate fluoride content in the groundwater in certain parts of rural Eritrea. North-East Africa, along the River Anseba. Standard procedure was adopted for fluoride detection. Results indicate elevated concentration of fluoride in groundwater. The highest concentration was found to be 3.73 mg/L, well above the safety level for consumption. Geological basis for the high concentration of high fluoride has been established; it is presumed to be the pegmatite intrusion hosted by a granitic batholith. Extensive dental fluorosis has been observed in the population exposed to drinking water of high fluoride content.

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Keywords: Africa, Eritrea, Fluoride in groundwater, Fluoride in village water, Pegmatite.

Source: *Environ Monit Assess* 2002 Apr;75(2):169-77.