HEALTH/BIOLOGICAL EFFECTS

BONE SURFACE AND WHOLE BONE AS BIOMARKERS FOR ACUTE FLUORIDE EXPOSURE

This study compares fluoride concentrations [F] in surface and whole bone in rats for up to 27 days following an acute oral dose of F. Four groups of rats received a single oral F dose (50 mg/kg body weight), and the control group received deionized water (n = 10/group). Groups were euthanized at 1, 3, 9, or 27 days after F administration. Plasma and femurs were collected. F on the femur surface was removed from a circular area (4.52 mm²) by immersion in 0.5 M HCl for 15 s. The solution was buffered with total ionic strength adjustment buffer and analyzed with a F ion selective electrode. The subjacent bone was sectioned and ashed at 600°C. Ash and plasma were analyzed for F with the electrode following hexamethyldisiloxane-facilitated diffusion. Data were analyzed by Kruskall-Wallis and Dunn's test and by linear regression (p<0.05). Peak plasma and bone surface [F] occurred on day 1 (0.26 ± 0.14 µg/mL and 1801 ± 888 µg/g, respectively). Bone surface (F) at 3, 9, and 27 days were not statistically different from control. A significant increase in whole bone [F] was observed 3 days after F administration and the [F] remained relatively constant thereafter. The mean (± SD) surface/whole bone [F] ratios for the control and F groups were 2.45 ± 0.98, 3.92 ± 1.32, 1.61 ± 0.82, 1.73 ± 0.39, and 1.09 ± 0.28, respectively. Plasma and bone surface [F]s were positively correlated (r = 0.74). Thus, bone surface was found to be a suitable biomarker for acute, sublethal F exposure in rats 1 day after F administration. Whole bone [F]s were significantly increased at 3, 9, and 27 days after F administration.

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Keywords: Acute fluoride exposure; Bone biomarkers; Bone surface; Hexamethyldisiloxane diffusion; Plasma fluoride; Rat femur; Whole bone.


IN VIVO EFFECT OF FLUORIDE-SUBSTITUTE APATITE ON RAT BONE

Different types of calcium phosphate compounds are commercially available for medical and dental applications as bone substitute materials. Biological apatites contain several kinds of minor components such as carbonate (CO₃)²⁻, magnesium (Mg²⁺), and fluoride (F⁻) in enamel, dentin, and bone. It has been reported that F ion and F-substituted apatite promote osteoblast proliferation and inhibit osteoclast cell activity. The purpose of this study was to investigate the in vivo activity of F-substituted apatite (FAp) on rat tibia. Apatites of unsintered calcium deficient apatite (CDA) and FAp with low, medium, and high F concentrations were implanted in rat tibia for 1 and 2 weeks. Implanted tissues were embedded in paraffin blocks, stained with hematoxylin-eosin, and observed histomorphometrically. Results indicated that, in rats, low F apatites induced better and faster new bone formation in vivo compared to CDA. Therefore F as a minor element in bone was found to have a positive effect on bone formation in vivo.

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Keywords: Apatites; Bone histomorphology; Calcium deficient apatite; Fluoride apatites; Rat bone; Rat tibia.


EFFECT OF SODIUM FLUORIDE ON GASTRIC EMPTYING AND INTESTINAL TRANSIT IN MICE

Fluoride, well recognized as potentially harmful, is easily absorbed by the gastrointestinal mucosa. It is therefore conceivable that any alteration of the gastrointestinal motility can affect
the rate of absorption of fluoride and lead to aggravation of its toxic effects. The effects of fluoride on gastric emptying and intestinal transit were studied in the mouse using a carboxymethyl cellulose (CMC) solution as a non-nutrient meal. The participation of the cholinergic and nitrergic systems in these effects was also evaluated. Oral gavage with 5 mM NaF had no significant effect on gastric emptying and intestinal transit of the CMC meal, whereas a decrease of gastric emptying (−33%, p<0.05) and an increase in intestinal transit (+20.7%, p<0.05) were observed with 20 mM NaF. Atropine injection also induced a significant decrease of gastric emptying. Combined treatment of atropine with 20 mM NaF brought about a further, but not significant decrease in gastric emptying. N-G-nitro-L-arginine methyl ester (L-NAME) treatment with or without oral administration of NaF decreased gastric emptying. Atropine treatment significantly depressed intestinal transit from 56.5% to 37.7% in the absence of NaF and from 70.1% to 42.8% in its presence. In contrast, L-NAME administration either alone or with fluoride increased intestinal transit (p<0.05). The present results suggest that fluoride alters gastrointestinal motility, an effect that may partly involve the cholinergic pathway.

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Keywords: Carboxymethyl cellulose; Cholinergic and nitrergic systems; Gastric emptying; Gastrointestinal motility; Mice and fluoride; N-G-nitro-L-arginine methyl ester; Oral gavage in mice.

DENTAL EFFECTS

HOW DOES FLUORIDE AFFECT DENTIN MICROHARDNESS AND MINERALIZATION?

Fluoride (F) has been a useful instrument in caries prevention. However, only limited data exist on the effect of its long-term use on dentin mineralization patterns and microhardness. The objective of this study was to evaluate the influence of tooth F concentration ([F]) and dental fluorosis (DF) severity on dentin microhardness and mineralization. We collected 137 teeth in Montreal and Toronto, Canada, and Fortaleza, Brazil, where the mean levels of water F were 0.2 ppm, 1 ppm, and 0.7 ppm, respectively. Teeth were analyzed for DF severity, dentin [F], enamel [F], dentin microhardness, and dentin mineralization. Dentin [F] correlated with DF severity; enamel [F] correlated with dentin microhardness and dentin mineralization; DF severity correlated with dentin microhardness. Genetic factors (e.g., DF severity) and environmental factors (e.g., tooth [F]) influenced the mechanical properties (microhardness) of the teeth, while only the environmental factors influenced their material properties (e.g., mineralization). Fortaleza teeth were harder and less mineralized and presented higher dentin [F] values. Montreal teeth presented lower levels of DF when compared with both Toronto and Fortaleza teeth.

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Keywords: Dental fluorosis; Dentin microhardness; Enamel fluoride; Environmental factors; Genetic factors; Dentin mineralization; Fortaleza, Brazil; Montreal and Toronto, Canada.

FLUORIDE’S EFFECT ON HUMAN DENTIN ULTRASOUND VELOCITY (ELASTIC MODULUS) AND TUBULE SIZE

Despite fluoride (F) use in caries prevention, not much is known about its effects on tooth quality. This study evaluated the effect of tooth F concentration ([F]) on selected dentin structural and mechanical properties. Third molars (n = 136) from Toronto, which has 1 part per million (ppm.) water [F], Montreal (0.2 ppm. water [F]), and Fortaleza (Brazil) (0.7 ppm. water [F]), were analyzed for [F], dental fluorosis (DF) severity, ultrasound velocity, and dentin tubule size and density. The enamel [F] was found to vary between 32 and 940 ppm., the
dentin [F] was found to vary between 110 and 860 ppm., while the DF severity varied between TF0 and TF4. The enamel [F] showed no correlation with dentin [F], DF severity, ultrasound velocity, dentin tubule size or density. The dentin [F] correlated with DF severity, dentin tubule size, and ultrasound velocity. DF severity showed a correlation with dentin [F] and ultrasound velocity. It was concluded that dentin [F] is an indicator of dentin structural properties (dentin tubule size and ultrasound velocity), while DF severity is an indicator of dentin mechanical properties (ultrasound velocity).

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Keywords: dentin; elastic modulus; fluoride; tubule size; ultrasound.

**FLUORIDE ANALYSIS**

**PROTEOMIC ANALYSIS OF KIDNEY IN FLUORIDE-TREATED RATS**

The recent development of proteomic techniques has enabled investigators to directly examine the population of proteins present in biological systems. We first report here the proteomic changes of renal protein induced by fluoride. To investigate molecular mechanisms of renal injury induced by fluoride, proteins were isolated from rat kidney and profiled by two-dimensional gel electrophoresis (2DE). With the analysis of Image-Master 2D Elite software, 141 up-regulated and eight down-regulated protein spots in 2DE gels of the fluoride-treated group were gained by comparison to the control group, 13 of which were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). The identified proteins are mainly related to cell proliferation, metabolism, and oxidative stress, and provide a valuable clue to explore the mechanism of renal fluorosis. This study also shows that proteomic techniques are a powerful tool in fluoride toxicology research.

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Keywords: Cell proliferation; Fluoride metabolism; Gel electrophoresis; Proteomic analysis; Rat kidney; Renal fluorosis; Renal protein.

**HEALTH/TOXIC EFFECTS IN HUMANS**

**EFFECTS ON PROTEIN AND mRNA EXPRESSION LEVELS OF p53 INDUCED BY FLUORIDE IN HUMAN EMBRYONIC HEPATOCYTES**

In this research we investigated the effects of protein and mRNA expression levels on p53 induced by fluoride in human embryo hepatocyte L-02 cells. The protein and mRNA levels of p53 in L-02 cells were measured after in vitro cultured L-02 cells were exposed to sodium fluoride at different concentrations (40, 80, and 160 µg/mL) for 24 hr. The cell survival rate of L-02 cells in the high fluoride group was significantly lower than that of the control group. The protein expression levels of p53 in the middle and high level fluoride groups were significantly higher than in the control group and increased with increasing fluoride concentration. The mRNA expression levels of p53 in the fluoride groups were markedly higher than in the control group. The mRNA expression level of p53 in the high level fluoride group was, however, lower compared to the middle fluoride group, but similar to the low fluoride group. These finding suggest that fluoride can decrease the L-02 cells survival rate and induce protein and mRNA expressions of p53; however, there is no consistency between the protein expression level of p53 and the mRNA expression level.

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INFLAMMATORY MARKERS IN BRONCHOALVEOLAR LAVAGE FLUID FROM HUMAN VOLUNTEERS 2 HOURS AFTER HYDROGEN FLUORIDE EXPOSURE

Fluoride has been in focus as a possible causal agent for respiratory symptoms amongst aluminium potroom workers for several decades. Previously, using bronchoalveolar lavage (BAL), we demonstrated airway inflammation in healthy volunteers 24 hr after exposure to hydrogen fluoride (HF). The objective of the present study was to examine early lung responses to HF exposure. Bronchoscopy with BAL was performed 2 hr after the end of 1-hr exposure to HF. Significant reductions in the total cell number and the number of neutrophils and lymphocytes were observed in the bronchoalveolar portion (BAP), whereas there were no significant changes in the bronchial portion (BP). Significantly decreased concentrations of beta2-MG, IL-6, and total protein were found in both BAP and BP. Additionally, IL-8 was significantly reduced in BP, and ICAM-1 and albumin were present in lower concentrations in BAP. Lung function measurements were not affected by HF exposure. These reported effects are presumably transitory, as many were not present in the airways 24 hr after a similar HF exposure.

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HEALTH/TOXIC EFFECTS IN ANIMALS

EFFECTS OF CHRONIC INGESTION OF SODIUM FLUORIDE ON MYOCARDIUM IN A SECOND GENERATION OF RATS

Possible effects of long term exposure (6 months) to sodium fluoride (NaF) through drinking water on the morphology and biochemistry of myocardial tissue in second generation adult male rats were investigated. Wistar strain female and male rats were reared until the second generation of rats was obtained, during which they were given 1, 10, 50, and 100 mg/L NaF in drinking water. Of the second generation, 28 male rats were divided into four groups and had the same treatment. All the second generation rats were sacrificed and autopsied at the end of the 6 months. In the samples of myocardial tissues, the levels of fluoride and the activities of principal antioxidant enzymes were determined, and a histopathological examination was conducted. Significant histopathological changes were found in the myocardial tissue of rats treated with 50 and 100 mg/L NaF. These were myocardial cell necrosis, extensive cytoplasmic vacuole formation, nucleus dissolution in myosits, swollen and clumped myocardial fibers, fibrillolysis, interstitial oedema, small hemorrhagic areas, and hyperaemic vessels. Additionally, the increased activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and thiobarbituric acid-reactive substance (TBARS) levels were observed in the myocardial tissues of rats treated with 10 and 50 mg/L NaF. On the other hand, the activities of SOD, GSH-Px, and CAT decreased, but the TBARS levels increased in the myocardial tissues of rats treated with 100 mg/L. The present results revealed that prolonged ingestion of fluoride through drinking water, particularly at high concentrations, induced significant histopathological and biochemical changes leading to myocardial tissue damage.

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EXPOSURE TO HIGH FLUORIDE CONCENTRATION IN DRINKING WATER WILL AFFECT SPERMATOGENESIS AND STEROIDOGENESIS IN MALE ALBINO RATS

Sodium fluoride (NaF) administered orally to adult male Albino rats at a level of 4.5 ppm and 9.0 ppm for 75 days in the drinking water caused a significant decrease in the body weight, brain index, and testicular index. A significant decrease in sperm count, sperm motility, sperm viability, and sperm function (HOS positive) with increased sperm abnormalities was also observed. The activity levels of testicular steroidogenic marker enzymes 3-beta-hydroxysteroid dehydrogenase (3-beta-HSD) and 17-beta-hydroxysteroid dehydrogenase (17-beta-HSD) were significantly decreased in the NaF-treated rats indicating decreased steroidogenesis and in turn spermatogenesis in rats exposed to NaF.

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Keywords: Fluoride and male rats; Spermatogenesis; Steroidogenesis; Testicular steroidogenic marker enzymes.

PRENATAL EXPOSURE TO THE ACETYLCHOLINESTERASE INHIBITOR METHANESULFONYL FLUORIDE ALTERS FOREBRAIN MORPHOLOGY AND GENE EXPRESSION

Methanesulfonyl fluoride (MSF) is a CNS-selective acetylcholinesterase (AChE) inhibitor, currently being developed and tested for the treatment of symptoms of Alzheimer's disease. We have previously confirmed that a single in utero exposure to MSF at clinically appropriate doses inhibits AChE activity in fetal rat brain by 20%, and when administered throughout gestation, MSF achieves a 40% level of inhibition. Here, we show that rats chronically exposed in utero to MSF display marked sex-specific differences in morphological development of the cerebral cortical layers compared with controls at 7 days of age. Forebrain size and cortical thickness were increased in females and decreased in males. An analysis of gene expression in neonate brain on the day of birth revealed sex-specific differential expression of over 25 genes, including choline acetyltransferase (ChAT), which were affected by prenatal MSF exposure. Many of these genes are associated with sexual differentiation and brain development, while others are involved in more generalized cellular and metabolic processes. The changes observed in cortical morphology and gene expression suggest a critical developmental role for AChE in the fetal nervous system, most likely through its effect on cholinergic neurotransmission.

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Keywords: Alzheimer's disease; Brain development; Cellular processes; Cerebral cortex; Choline acetyltransferase; Cholinergic neurotransmission; Fetal rat brain; Forebrain; Genes; Metabolic processes; Methanesulfonyl fluoride; Sexual differentiation.
collected 7 days post-exposure and analyzed for radioactivity. During and after unlabeled SO$_2$F$_2$ exposures, blood, brain, and kidney were collected and analyzed for fluoride ion. SO$_2$F$_2$ was rapidly absorbed, reaching maximum concentrations of radioactivity in both plasma and red blood cells (RBC) near the end of the 4-hr exposure period. Radioactivity was rapidly excreted, mostly via the urine. Seven days post-exposure, small amounts of radioactivity were distributed among several tissues, with the highest concentration detected in respiratory tissues. Radioactivity associated with the RBC remained elevated 7 days post-exposure, and highly perfused tissues had higher levels of radioactivity than other non-respiratory tissues. Radioactivity cleared from plasma and RBC with initial half-lives of 2.5 hr after 30 ppm and 1–2.5 h after 300 ppm exposures. The terminal half-life of radioactivity was 2.5-fold longer in RBC than plasma. Based on the radiochemical profiles, there was no evidence of parent $^{35}$SO$_2$F$_2$ in blood. Identification of fluorosulfate and sulfate in blood and urine suggests that SO$_2$F$_2$ is hydrolyzed to fluorosulfate, with release of fluoride, followed by further hydrolysis to sulfate and release of the remaining fluoride.

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Keywords: Blood; Brain; Feces; Fumigant gas; Kidney; Metabolism; Pharmacokinetics; Radioactive labeling; Rats and sulfuryl fluoride; Sulfuryl fluoride; Urine; Wood insects.

METHANESULFONYL FLUORIDE, AN ACETYLCHOLINESTERASE INHIBITOR, ATTENUATES SIMPLE LEARNING AND MEMORY DEFICITS IN ISCHEMIC RATS

Methanesulfonyl fluoride (MSF), a highly selective CNS inhibitor of acetylcholinesterase, has recently been demonstrated to promote improvement in cognitive performance in patients with senile dementia of Alzheimer type. Because a similar cognitive impairment may accompany stroke, we investigated in the present study whether treatment with MSF could produce beneficial effects in adult rats subjected to an experimental stroke model. Sprague-Dawley rats received transient 60 min intraluminal occlusion of the right middle cerebral artery (MCAo) and were given i.p. injections of either MSF (1 mg/kg at 24 and 48 h post-MCAo and 0.3 mg/kg thereafter every other day) or the vehicle, peanut oil, for 4 weeks. Behavioral tests and biochemical assays were performed at 28 days post-surgery. MSF treatment produced about 90% inhibition of acetylcholinesterase in the brain. Ischemic animals that received the vehicle displayed significantly elevated body swing biased activity (84.8 ± 10%) and significantly prolonged acquisition (398 ± 62 s) and shortened retention (79 ± 26 s) of the passive avoidance task. Interestingly, while the ischemic animals that received the MSF exhibited elevated body swing biased activity (87.7 ± 8%), they performed significantly better in the passive avoidance task (255 ± 36 s and 145 ± 18 s in acquisition and retention) than the vehicle-treated animals. Moreover, whereas brains from both groups of animals revealed similar extent and degree of cerebral infarction, the MSF-treated ischemic animals showed more intense immunoreactivity, as well as a significantly higher number (10-15% increase) of septal choline acetyltransferase-positive cells than the vehicle-treated ischemic animals. These results show that MSF, possibly by preserving a functional cholinergic system, attenuated stroke-induced deficits in a simple learning and memory task.

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Keywords: Acetylcholinesterase; Cerebral infarction; Cholinergic system; Ischemic rats; Methanesulfonyl fluoride; Passive avoidance tasks; Rat brain; Stroke-induced deficits.