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PARTICULATE AIR POLLUTION AND ITS EFFECT ON HEART RATE VARIABILITY

Air pollution is a major public health problem, which has been associated with an increase in cardiopulmonary morbidity and mortality in many cities around the world after short-term exposures. Acute as well as chronic exposure to suspended particulate matter (PM) has been linked to a rise in hospital admissions and emergency room visits due to respiratory and cardiovascular causes, especially in children under 5 years of age with asthma and elderly people with known cardiac or pulmonary disease. Moreover, cohort epidemiological studies have linked long-term exposure to particulate air pollution to a reduction of life expectancy due to cardiovascular mortality. Although the underlying biological mechanism of these associations remains limited, several hypotheses have been postulated, from inflammation, accelerated atherosclerosis and altered cardiac autonomic function.

Since the 1980s, heart rate variability (HRV) has been widely used in clinical fields to stratify the risk of arrhythmic death of the patients with ischemic heart disease in whom a low HRV could be a negative predictor. Alterations of the autonomic nervous system accompanying the early stages of essential hypertension have also studied using HRV and it was found that sympathetic activity increases while parasympathetic decreases. Patients with congestive heart failure had clinical signs of enhanced sympathetic activity and progressive decrease in RR variance. A low HRV has been associated to an increased mortality rate in people with heart disease.

Recent studies that included elderly individuals with heart diseases in Baltimore, Boston and Mexico City suggest that $PM_{2.5}$ air pollution measured with ambient monitors was associated with a reduced HRV. The aim of the present study was to test whether this association could also be observed in young healthy adults, using personal exposure monitors, at ambient levels currently observed in the Mexico City Metropolitan Area.

Forty young healthy residents of the Mexico City Metropolitan Area underwent 13 h Holter electrocardiographic and $PM_{2.5}$ personal monitoring. HRV was evaluated in time domain: the standard deviation of normal RR intervals (SDNN) and the percentage of differences between adjacent normal RR intervals larger than 50 ms (pNN50). In multivariate analysis with mixed effects models, a significant negative association of pNN50 with $PM_{2.5}$ accumulative exposure was found. An increase in 30 □g/m^3 of the average $PM_{2.5}$ personal exposure in the previous 2 h decreased the pNN50 in 0.08% (P=0.01). This observation revealed an acute effect related to environmental exposure to $PM_{2.5}$ with regard to HRV in normal youngsters.

The public health consequences of the effects found in a young healthy population in this study could be different from those informed in previous studies. A decrease of HRV is a common finding in the elderly and in patients with heart disease, which has been demonstrated to be a predictor of an increased mortality in both populations. However, in the long term cardiac autonomic imbalance may play a role in the development of cardiovascular diseases. A reduction in the pNN50 suggesting a decreased parasympathetic cardiac autonomic control

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with sympathetic nervous system overactivity has been associated with the development of hypertension among previously normotensive men.

However, the significance of a decreased parasympathetic activity in the young healthy adults over a lifetime exposure in urban areas as Mexico City needs to be clarified. Further studies are needed to assess if these cardiac autonomic changes could be related to the association between $PM_{2.5}$ air pollution and the increase in cardiovascular morbidity and mortality.

Individuals included in this study are representative of a population with exposure patterns mainly in in-door microenvironments, characterized by a sedentary lifestyle. Therefore, conclusions are limited to these types of subjects. Studies with a larger sample size that include individuals with different exposure patterns should be carried out in order to corroborate the results of this study. Additionally, no other copollutants such as O_3, SO_2 or PM_{10} were included because corresponding measurements were not always available in the city environmental monitor net.

Source: Journal of Exposure Science and Environmental Epidemiology, Vol. 16, No. 2, March 2006.

Aluminum Exposure: Behavioral Effects in Rats

Both aluminum (Al) and aging have been associated with neurobehavioral changes in mammals. Among environmental metals, Al has a remarkable toxic potential for humans and it is well established that the element is neurotoxic in mammals.

It is known that gestational and lactational exposure to Al doses that do not produce maternal toxicity can result in persistent neurobehavioral deficits in the offspring of some mammals. Moreover, some experimental conditions such as maternal diet or gestational restraint stress can interact with Al adverse effects. While it is known that aging is associated with neurobehavioral deficits, data from studies on Al effects on aging are confounding. In this sense, several studies have demonstrated learning deficits in adult animals after Al exposure. However, other investigations found neither effects nor improvement in some tasks. These conflicting findings on learning may be a consequence of different Al formulations, doses and/or routes of exposure.

To date, only very few studies have raised the question of how life-long Al exposure could affect later performance in different learning tasks. Although learning deficits following prenatal exposure to Al were observed in different animal species, results are controversial. On the other hand, studies in rodents have demonstrated that, during pregnancy, maternal stress may be associated with adverse effects on embryonic/fetal development, while long-lasting effects on emotionality and learning have been reported. Interestingly, it has been found that interactions between maternal stress and some metals can enhance the potential developmental toxicity of these elements.

Now a new study has been carried out to evaluate in rats the effects of a long-term oral exposure to Al and prenatal stress on the neurobehavioral performance of the offspring at 1 year (adult) and two years (old) of age.

Pregnant females were orally exposed to 0, 50, and 100 mg Al/kg/day. Each Al-exposed group was divided into two subgroups. One of these was subjected to restraint stress (2 h/day on gestation days 6-20). The offspring of the treated females were maintained with the same Al treatment until sacrifice at 1 or 2 years of age. Activity in an open-field and learning in a water maze were evaluated. Although no significant differences were observed in motor activity, a biphasic effect of Al on learning could be observed. Thus, exposure to 100 mg Al/kg decreased performance of the task in both adult and old rats when compared to animals exposed to 50 mg Al/kg. An age-related effect on water maze performance, as well as an accumulation of Al in brain of rats exposed to 100 mg Al/kg at 2 years of age was found. Interestingly, while prenatal restraint stress did not modify behavioral parameters, Al accumulation was prevented by prenatal restraint.

The results of the present study indicate that a prolonged oral Al exposure produces behavioral effects in rats. At moderated Al levels, these effects consist of facilitating acquisition and retention in a water maze task, while subtle impairment in this task is associated with greater doses. Aluminum accumulation in brain has only been observed at high levels of Al exposure. An association between Al accumulation in brain and impaired water maze performance in both acquisition and retention tasks was noted. A general effect of age in the water maze task was also observed, while neither an Al X age interaction nor a prenatal mild stress X Al interaction could be noted. Since behavioral data obtained did not substantially differ between 1- and 2-year old rats, Al effects seem to be associated rather with the exposure doses than with the duration of the exposure. However, in further investigations it would be of interest to use rodent models with increased vulnerability to neurodegenerative processes. It would allow to better detect subtle neurobehavioral effects of oral Al exposure, as well as to elucidate its potential role in neurodegeneration.

Source: Toxicology, Vol. 218, February 2006.

Salivary Monitoring for Lead Poisoning: A Study in the Residents of Klity Village in Thailand

Lead poisoning usually results from cumulative absorption of small amounts of lead until toxic concentrations are reached in the body. The severity of lead toxicity depends on the duration, frequency, and amount of exposure. When lead enters the blood it can cause serious health problems in adults. In children, however, the effects can be devastating. Children can sustain lead in their bodies for up to 17-20 years, thus destroying the central nervous system and causing chronic toxicity in the entire body. Developed countries have long realized the widespread detrimental effects of lead poisoning and have conducted extensive research and drawn up new and strong measures to control lead levels in air, water, and food. In Thailand, the problem of lead contaminated water in Klity Creek, Kanchanaburi Province was reported in April 1998. The sources of lead are both natural and a result of lead mining. Lead has been mined for more than 30 years in this area, and the untreated wastewater from the ore-dressing was discharged into Klity Creek. The Department of Pollution Control reported the estimate of the lead in the sediment of the creek was about 15,000 tons at a distance of 13 kilometers down stream from the creek. Since 1994, many village children in this area have been diagnosed with Down's syndrome and have had physical deformities, while the adults have suffered from an unidentified illness which caused the body to swell and ache. The first blood tests of the villagers in 1999 revealed that the lead concentration was 4-5 times higher than 4-9 □g/dl, the average for Thai adults.

Salivary monitoring has been used to monitor environmental pollutants, since circulating chemicals can be present in the salivary glands, which is reflected in the saliva. The concentration of these substances in the saliva depends on the nature of the material and its transport process. There are many reports indicating the distribution of lead in the salivary glands and its diffusion into the saliva in high concentrations. Using salivary sampling may be an alternative way to study lead exposure due to its non-invasive technique compared to venipuncture of blood. Therefore, the objective of the present study was to evaluate the concentration of lead in saliva compared to the level in the blood.

Lead concentrations in whole blood and saliva were examined in 16 females and 13 males living in Klity village. The geometric mean for the lead content in the blood was 24.03 □g/dl (range 11.80-46.60 □g/dl) while the lead content in the saliva was 5.69 □g/dl (range 1.82-25.28 □g/dl). No significant differences were found between the concentrations of lead in blood and saliva in relation to the age of the subject. Males were found to have higher blood lead levels than females. The coefficient of correlation between salivary and blood lead levels was -0.025. This data suggests that saliva is not suitable material for biological monitoring with respect to lead exposure.

Source: Southeast Asia Journal of Tropical Medicine and Public Health, Vol. 36, No. 6, November 2005.

HEALTH BENEFITS OF FRUIT JUICE CONSUMPTION

There is considerable evidence revealing an association between diets rich in fresh fruit and vegetables and a decreasing incidence of cardiovascular and neurodegenerative diseases and cancer. These protective effects have been attributed mainly to compounds naturally present in juices such as phenolic compounds, carotenoids and vitamin C. World consumption of natural juices is increasing as a consequence of the human search for a healthier life. The juice production industry, especially for orange juice, is expanding in many countries. Despite scientific data reporting beneficial properties derived from juice consumption, some components of juices have been identified as mutagenic or carcinogenic. Carcinogenic or genotoxic effects may be mediated by the interaction of juice components with transition metals or by sub-products of juice auto-oxidation. In a recent study, the mutagenic potential of orange juice and two metallic agents used in dietary supplementation, $FeSO_4$ and $CuSO_4$, were investigated using the comet assay in mouse blood cells *(in vivo)*. Both metal compounds were genotoxic for eukaryotic cells after 24 h treatment at the doses used. Significant damage repair was observed after 48 h of treatment with the same compounds. Orange juice had a modulating effect on the action of metallic sulfates. In the case of iron treatment, the presence of the orange juice had a preventive, but not restorative, effect. On the other hand, in the case of copper treatment, the effects were both preventive and restorative. PIXE (particle induced X-ray emission) analysis indicated a positive correlation between DNA damage and the hepatic levels of iron and a negative correlation between whole blood copper and DNA damage. A negative correlation between hepatic iron and whole blood copper content was also seen in the treatment with both ferrous and cupric sulfates.

However, it should be mentioned that this interaction of metals and orange juice must be examined carefully, since treatments were not administered at the same time and there is a lack of knowledge about the kinetics of copper. Moreover, more extensive tracer studies would be necessary to shed light upon the kinetic of iron and copper.

Source: Food and Chemical Toxicology, Vol. 44, March 2006.

Effects of Nonthermal Microwaves from Mobile Telephones

The data on biologic effects of nonthermal microwaves (MWs) from mobile phones are diverse, and these effects are presently ignored by safety standards of the International Commission for Non-Ionizing Radiation Protection.

Growing public concern about possible effects of MW exposure from mobile telephones has been expressed by groups in many countries because of the increasing use of wireless communications systems.

Experimental data suggest that MW effects occur only under specific parameters of exposure, depending on several physical parameters and biologic variables.

Dependence of the MW effects on several physical parameters, including frequency, polarization, and modulation, and also several biologic variables could explain various outcomes of studies with nonthermal MWs.

MWs under specific conditions of exposure induce DNA strand breaks in rat brain cells as measured by single-cell electrophoresis. The mechanisms of this effect are not understood, but they could be related to induced changes in the interaction of DNA with proteins, rather than DNA damage.

Several proteins, such as the tumor suppressor p53-binding protein 1 (53BP1) and phosphorylated H2AX (γ-H2AX), have been shown to produce discrete intranuclear foci, which are believed to colocalize with DNA double-strand breaks (DSBs) providing a scaffold structure for DSB repair.

So-called hypersensitivity to electromagnetic fields (EMFs) is a fairly new phenomenon, and etiology of the hypersensitivity to EMFs is not yet known. There are several symptoms that people experience in proximity to different sources of EMFs, such as video display terminals of personal computers, electrical appliances, or mobile telephone. The symptoms are not specific for this illness, and there is no known pathophysiologic marker or diagnostic test.

There is a substantial lack of knowledge in the biophysical modeling of MW-induced nonthermal biologic effects. Resonance-like interactions of MWs with such targets as cellular membranes, chromosomal DNA, and ions in protein cavities have been proposed.

A new study has now analyzed the effects of MWs at different frequencies on chromatin conformation and 53BP1 and γ-H2AX foci in lymphocytes from healthy and hypersensitive subjects.

For the first time, the data obtained in this present study clearly show that MWs from GSM mobile telephones affect simultaneously the formation of 53BP1 and γ-H2AX foci in human lymphocytes as a function of carrier frequency. This result obtained in lymphocytes from both healthy and hypersensitive persons is of great importance. Such frequency dependence suggests a mechanism that does not deal with thermal heating. Investigation of this mechanism and the molecular targets of the frequency-dependent effects of MWs in the frequency range of mobile communication is a fundamental problem.

Another aspect of this finding is that criteria other than "thermal," based on Specific Absorption Rate (SAR) and power density in acute exposures, may be needed for accurate safety standards. In particular, these safety standards certainly cannot be based on data obtained at one specific frequency.

Source: Environmental Health Perspectives, Vol. 113, No. 9, September 2005.

MOBILE PHONE-INDUCED MYOCARDIAL OXIDATIVE STRESS

The use of mobile phones is one of the fastest growing technological developments. Electromagnetic radiation (EMR) or radiofrequency fields of cellular mobile phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation, and by changing the antioxidant defense systems of human tissues, thus leading to oxidative stress. Mobile phones are used in close proximity to the heart, therefore 900 MHz EMR emitting mobile phones may be absorbed by the heart. Caffeic acid phenethyl ester (CAPE), one of the major components of honeybee propolis, was recently found to be a potent free radical scavenger and antioxidant, and is used in folk medicine. The aim of this recent study was to examine 900 MHz mobile phone-induced oxidative stress that promotes production of reactive oxygen species (ROS) and the role of CAPE on myocardial tissue against possible oxidative damage in rats.

Thirty rats were used in the study. Animals were randomly grouped as follows: sham-operated control group *(N: 10)* and experimental groups: (a) group II: 900 MHz EMR exposed group *(N: 10)*; and (b) group III: 900 MHz EMR exposed + CAPE-treated group *(N: 10)*. A 900 MHz EMR radiation was applied to groups II and III 30 min/day, for 10 days using an experimental exposure device. Malondialdehyde (MDA, an index of lipid peroxidation), and nitric oxide (NO, a marker of oxidative stress) were used as markers of oxidative stress-induced heart impairment. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status. In the EMR exposed group, while tissue MDA and NO levels increased, SOD, CAT and GSH-Px activities were reduced. CAPE treatment in group III reversed

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HEALTH EFFECTS OF PULMONARY ZINC EXPOSURE

Exposure to particulate matter (PM) in ambient air has long been associated with adverse cardiopulmonary health effects. Cardiovascular damage induced by pulmonary exposure to environmental chemicals can result from direct action or, secondarily, from pulmonary injury. Researchers have developed a rat model of pulmonary exposure to zinc to demonstrate cardiac, coagulative. and fibrinolytic alterations. Male Wistar Kyoto rats were instilled intratracheally with saline or zinc sulfate, 131 □g/kg (2 □mol/kg); the alterations were determined at 1, 4, 24, and 48 h postexposure. High-dose zinc presented changes in circulating levels of zinc above normal and induced significant pulmonary inflammation/injury such that cardiac impairments were likely. At 1-24 h postexposure, plasma levels of zinc increased to nearly 20% above the base line. Significant pulmonary inflammation and injury were determined by analysis of bronchoalveolar lavage fluid and histopathology in zinc-exposed rats at all time points. Starting at 4 h postexposure, pulmonary damage was accompanied by persistently increased gene expressions of tissue factor (TF) and plasminogen activator-inhibitor-1 (PAI-1), but not thrombomodulin (TM). Cardiac tissues demonstrated similar temporal increases in expressions of TF, PAI-1, and TM mRNA following pulmonary instillation of zinc. In contrast to extensive pulmonary edema and inflammation, only mild, and focal acute, myocardial lesions developed in a few zinc-exposed rats; no histological evidence showed increased deposition of fibrin or disappearance of troponin. At 24 and 48 h postexposure to zinc, increases occurred in levels of systemic fibrinogen and the activated partial thromboplastin time.

These findings suggest that a modest but detectable increase in myocardial degeneration may have occurred in some zinc-exposed animals. This small effect of zinc on cardiac pathology despite a marked stimulation of coagulation and fibrinolytic pathways may suggest that a longer period of time is needed for discernible histopathological damage to occur following exposure, or blood coagulation changes may not necessarily result in visual pathological lesions in the heart in acute scenarios. One pertinent observation is that this acute exposure resulted in massive pulmonary-cell damage and neutrophilic inflammation, but not infiltration or accumulation of macrophages. The roles that pulmonary neutrophil versus macrophage infiltration may play in inducing procoagulative and degenerative changes in the heart are not clear; however, studies of these functions may provide insight into mechanisms of cardiovascular impairment for a variety of pulmonary ailments.

Despite procoagulative gene expression induced by zinc exposure, no indication of enhanced fibrin deposition in the heart could be found. The staining technique employed, while giving a good indication of large changes in fibrin deposits, was not useful for detecting increases in the deposition of fibrin in capillaries; further analysis is required for this specific detection. Furthermore, although no widespread myocardial injury occurred at the time points studied, it has been previously demonstrated that myocardial lesions do occur following chronic extended exposure to zinc-containing particles, and, thus, a chronic exposure to zinc or a predisposed disease susceptibility may be necessary to detect altered fibrin deposition and myocardial degeneration.

Evidence for a procoagulative status was strengthened in this study by the increased levels of plasma fibrinogen. Plasma fibrinogen in animals and humans has been shown to increase in response to PM. The increase in plasma fibrinogen following intratracheal instillation of zinc was not as remarkable as that seen with intratracheal exposure to metal-rich combustion particles, despite the greater severity of the pulmonary injury; these findings suggest that the mechanism of fibrinogen increase and its role in overall cardiac impairment may differ with different types of pulmonary insults. Although the increase in plasma fibrinogen is indicative of a procoagulative status, the actual clotting time of the blood increased after 24 and 48 h in the zinc-exposed animals. This increase is consistent with an increase in the mRNA expression of the clotting inhibitor TM in the heart. This may suggest an adaptive response in the heart and explain the lack of histologically discernible deposition of fibrin. The lack of TM expression in the lung may be reflective of overt pulmonary injury and the lack of compensatory ability.

The study discovered that pulmonary exposure to zinc resulted in a rapid increase in circulating zinc, which persisted for up to several hours. Acute zinc-induced pulmonary injury and inflammation were associated with marked procoagulative effects on the heart with suggestions of mild acute cardiac lesions. The cardiac procoagulative effects could be due either to pulmonary injury and neutrophilic inflammation and/or a direct effect exerted by zinc itself. While pulmonary injury/inflammation were marked and progressive, circulating zinc remained high for several hours following exposure, and the heart would potentially encounter high concentrations of zinc following its first passage through the pulmonary artery and, subsequently, the left ventricle.

Source: Toxicology and Applied Pharmacology, Vol. 211, February 2006.

MOBILE PHONE-INDUCED MYOCARDIAL OXIDATIVE STRESS

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these effects. In this study, the increased levels of MDA and NO and the decreased levels of myocardial SOD, CAT and GSH-Px activities demonstrate the role of oxidative mechanisms in 900 MHz mobile phone-induced heart tissue damage, and CAPE, via its free radical scavenging and antioxidant properties, ameliorates oxidative heart injury. These results show that CAPE exhibits a protective effect on mobile phone-induced and free radical mediated oxidative heart impairment in rats.

Source: Toxicology and Industrial Health, Vol. 21, No. 9, October 2005.

TBT-Induced Modulations of Neurotransmitters in the Brain of Adult Mice

Humans are exposed to tributyltin (TBT) compounds via the ingestion of TBT-contaminated fish and shellfish.

The compounds have been widely used as biocides and anti-fouling agents. Recently, restrictions on the application of TBT compounds as vessel antifouling agents have been introduced; however, TBT pollution in fish and shellfish, marine water and sediment are still being reported worldwide. The possible health hazard caused by exposure to TBT through ingesting contaminated fish and shellfish is a growing concern. According to a comparison of the tentative acceptable daily intake (ADI) of TBT for an adult male – 1.6 mg/kg b.w./day as TBT oxide – with the intake of TBT due to environmental exposure, the intake is not likely to be at a critical level. However, the toxicity of TBT has been studied mainly on the basis of acute exposure, and the tentative ADI was decided using such data. Subacute exposure is more accurate for the evaluation of the risk caused by environmental pollution.

Neurotoxicity is one of the major toxic effects of TBT. The alterations in concentrations of neurotransmitters (catecholamine and indoleamine) and their metabolites have been used as indexes of the toxic effects of TBT in the central nervous system. The ratios of neurotransmitters and their metabolites have been used as indexes of neural activity. In addition, TBT inhibits the synthesis of dopamine (DA) in PC-12 cells *in vitro*.

The toxic effects on the next generation, exposed to TBT via the placenta and their dam's milk, may be stronger than the toxic effects on adults. Reseachers have examined the effects of TBT on the behavior of F1 rats administered TBT chloride in their diet. TBT chloride was administered to dams during pregnancy and lactation, and to the F1 offspring until the end of the experiment. TBT at 5 or 125 ppm abolished the sex differences in behavior among the offspring. The toxic effects on behavior, which are related to the effects on the central nervous system, were induced in the offspring exposed to TBT. It is of interest to determine whether a low-dose exposure to TBT via the placenta and the dam's milk induces alterations in the metabolism of neurotransmitters. In a new study, pregnant mice were administered TBT chloride in their diet.

Pregnant ICR mice were exposed to TBT chloride at concentrations of 0, 15 or 50 ppm in water or 125 ppm in food. Male offspring were sacrificed at one, two and three weeks after birth. The concentrations of norepinephrine, dopamine (DA), dihydoxyphenylacetic acid, homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were determined in different brain regions by HPLC. All offspring from the 125 ppm group died immediately after birth. A significant decrease in the body weight of the TBT-treated F1 groups compared to the control group was observed in the first week. Significant increases compared to the controls were observed for the DA concentration in the striatum of the 50 ppm F1 group, and for the HVA concentration in the cerebrum and the 5-HT concentration in the medulla oblongata of the 15 and 50 ppm F1 groups in the third week. At three weeks of age, the neurotransmitters and their metabolites may be useful indexes for developmental neurotoxicity. For the dams, a significant decrease in the 5-HT concentration was observed in the cerebellum, medulla, midbrain and striatum of the 125 ppm group compared to the control group. A significant decrease in the 5-HIAA concentration was also observed in the cerebellum, midbrain and striatum of the dams in the 125 ppm group compared to the control. TBT may induce a decrease in the synthesis of 5-HT in the dams. The discrepancy between dams and offspring may be due to several factors such as age, dose, route, sex and pregnancy.

For developmental neurotoxicity, a direct assessment of neurotoxicity may be needed in addition to the analysis of neurotransmitters and their metabolites.

The limited number of mice in each group in the current experiment may have affected the power of detecting significant differences. The dissection of the brain must be finished within 3-4 h to avoid the diurnal changes of neurotransmitters; therefore, it was necessary to limit the number of mice. A comparison between two groups only (the control and high-dose group) may allow an increase in the number in each group. For a direct assessment of neurotoxicity, a cell death detection kit or TUNEL assay kit may be useful to apply the brain of mice from newborn to post-natal day 21. Further studies are required.

Source: Toxicology and Industrial Health, Vol. 22, No. 1, February 2006.

THE RELATIONSHIP BETWEEN LEAD EXPOSURE, MOTOR FUNCTION AND BEHAVIOR IN INUIT PRESCHOOL CHILDREN

Although blood lead concentrations have reduced dramatically in the general population, recent studies have suggested that prenatal and early postnatal exposure to very low doses of lead can adversely affect certain aspects of a child's development, including fine motor function, memory, learning and behavior. While the vulnerability of the developing brain to lead has been well documented, the neurotoxin's mechanism of action remains unclear. To date, epidemiological studies have attempted to define populations at greatest risk as well as the array of deficits found in exposed individuals. However, researchers have still not been able to decode 'lead's signature'. Many scientists believe that the divergent observations concerning lead's impact may be partly due to the complex nature of lead's mechanism of action. At lower levels of exposure, a number of variables including dose, time of exposure, individual variability and other moderating variables seem to interact in a complex fashion. This makes it difficult to define the direct effects of lead, the potential mediators and the threshold at which such effects can be seen. One possible explanation for this lack of understanding may be that most epidemiological research in this field has focused on specific domains of development, testing only a certain number of tasks per cohort without studying patterns of coexisting deficits in children exposed to background levels of lead.

In a recent study carried out in Nunavik, Inuit children were tested at birth and later on at 5 years of age for lead (Pb), mercury (Hg) and organochlorine compounds (OCs) in blood. The preschool aged children were then assessed using various neuromotor tasks. Data analysis revealed that lead exposure at testing time has an adverse effect on fine motor function including reaction time, poor alternating movements, increased mirror movements, irregularity and increased action tremor during pointing, but had no effects on postural tremor.

Now a new study has been conducted to determine whether behavior acted as a mediator, a moderator or acted independently in the relationship between lead exposure and fine motor function.

The sample consisted of 110 preschoolers, of age 5, from Nunavik. Lead concentration was measured at birth and at testing time. Average lead levels were of 4.9 □g/dL (0.24 □mol/L) and 5.3 □g/dL (0.26 □mol/L) for cord and child blood, respectively. Children's balance and fine motor capacities were tested. A modified version of the Infant Behavior Rating Scale (IBR) was used to assess behavior. Postnatal blood lead concentrations correlated positively with both impulsivity and activity. Neither pre- nor postnatal blood lead concentration correlated with attention level. The children's scores on impulsivity (I) and activity (A) were summed to create the independent variable IA, which was tested as a potential mediator between lead exposure and two dependent variables: the coefficient of covariation in alternating hand movements and transversal sway in tandem position. Mediation was significant only for the latter variable. IA and attention were then tested as potential moderators in the relation between postnatal lead exposure and motor function. No significant interaction between independent variables could be observed. These results do not support the hypothesis that, at low levels of postnatal exposure, lead acts indirectly on motor function via behavior. However, there may also be an indirect effect of lead acting via behavior on transversal sway. By studying the relationship between the various outcomes measured to date, researchers can disentangle the direct versus indirect effects of lead exposure. Similar methods could be useful to determine the mechanism of action of lead or other neurotoxins on a broader spectrum of developmental domains.

Source: Neurotoxicology and Teratology, Vol. 28, January-February 2006.

Acute Neurotoxic Effects of the Fungal Metabolite Ochratoxin-A

Ochratoxin-A (OTA) is a fungal metabolite with potential toxic effects on the central nervous system. Its complex mechanisms of action include evocation of oxidative stress, bioenergetic compromise, inhibition of protein synthesis, production of DNA single-strand breaks and formation of OTA-DNA adducts.

Investigation of the effects of acute and chronic exposure to OTA on the nervous system has been scarce, even though development of nervous tissue appears to be very susceptible to the deleterious effects of OTA. OTA has been reported to induce teratogenic effects in neonates (rats and mice) exposed in utero, characterized by microcephaly and modification of the brain levels of free amino acids. OTA was also reported to be neurotoxic to adult male rats fed OTA in the diet. Neurotoxicity, indicated by concentration of lactic dehydrogenase released from the dissected brain tissue, was more pronounced in the ventral mesencephalon, hippocampus, and striatum than in the cerebellum. The bioconcentration of OTA in these brain regions did not correlate with toxicity.

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Acute Neurotoxic Effects of the Fungal Metabolite Ochratoxin-A

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The mechanism responsible for toxicity to neural tissues is not clear, but studies in peripheral organs and tissues reveal a spectrum of actions. The mechanisms of toxicity implicated include inhibition of protein synthesis, mitochondrial impairment, oxidative stress and DNA damage.

OTA-induce damage to DNA, evidenced by formation of single-strand breaks, has been reported to occur both *in vitro* and *in vivo*. The DNA damage was shown to be reversible with time suggesting that variation in capacity to repair DNA may account in part for differences in vulnerability to OTA between tissues. OTA was also reported to induce single-strand breaks in a concentration-dependent manner in canine kidney cells and this effect could be potentiated by inhibition of DNA repair. Other studies have demonstrated OTA-DNA adducts in mouse and monkey kidney after OTA treatment. In kidney, liver and spleen, several modified nucleotides were clearly detected in DNA, 24 h after administration of OTA, but their levels varied significantly in a tissue and time-dependent manner over a 16-day period. The OTA-DNA adducts were not quantitatively and qualitatively the same in the three organs examined due to differences of metabolism in these organs and differences in the efficiency of DNA repair processes.

OTA treatment can increase oxidative stress in peripheral organs. Administration of OTA (1 mg/kg) to rats resulted in a 22% decrease in alpha-tocopherol plasma levels and a five-fold increase in the expression of the oxidative stress responsive protein heme oxygenase-1, specifically in the kidney. More direct evidence of oxidative stress was derived from studies, which utilized electron paramagnetic resonance spectroscopy to measure the generation of hydroxyl radicals, in rat hepatocyte mitochondria and microsomes incubated with OTA and metabolites.

OTA toxicity is associated with inhibition of both protein and RNA synthesis. OTA is known to interfere with the charging of transfer ribonucleic acids (tRNA) with amino acids. In particular, OTA has been shown to inhibit bacterial, yeast and liver phenylalanyl-tRNA synthetases. The inhibition is competitive to phenylalanine and is reversed by an excess of this amino acid. OTA has also been shown to inhibit enzymes that use phenylalanine as a substrate such as phenylalanine hydroxylase.

Mitochondrial dysfunction has been shown to be involved in the development of OTA-induced toxicity in proximal renal tubule cells. Respiration was reduced in the absence and presence of a phosphate acceptor using site I (glutamate/malate) and site II (succinate) respiratory substrates 15 and 30 min after exposure to 10^{-3} M OTA, implicating an action of OTA at both electron transport sites. However, in isolated rat liver mitochondria, inhibition kinetic studies revealed that OTA is an uncompetitive inhibitor of both succinate-cytochrome c reductase and succinate dehydrogenase while sparing cytochrome oxidase and NADH dehydrogenase activity (Complex I) at concentrations less than 10^{-5} M.

Now researchers have carried out a new study to evaluate the extent of OTA neurotoxicity across six brain regions in the mouse brain in the context of oxidative stress, oxidative DNA damage and DNA repair.

Oxidative DNA damage, as measured with the "comet assay", was significantly increased in the six brain regions at all time points up to 72 h, with peak effects noted at 24 h in midbrain (MS), CP (caudate/putamen) and HP (hippocampus). Oxidative DNA repair activity (oxyguanosine glycosylase or OGG1) was inhibited in all regions at 6 h, but recovered to control levels in cerebellum (CS) by 72 h, and showed a trend to recovery in other regions of brain. Other indices of oxidative stress were also elevated. Lipid peroxidation and superoxide dismutase (SOD) increased over time throughout the brain. In light of the known vulnerability of the nigro-striatal dopaminergic neurons to oxidative stress, levels of striatal dopamine (DA) and its metabolites were also measured. Administration of OTA (0-6 mg/kg i.p.) to mice resulted in a dose-dependent decrease in striatal DA content and turnover with an ED50 of 3.2 mg/kg. A single dose of 3.5 mg/kg decreased the intensity of tyrosine hydroxylase immunoreactivity (TH+) in fibers of striatum, TH+ cells in substantia nigra (SN) and TH+ cells of the locus ceruleus. TUNEL staining did not reveal apoptotic profiles in MS, CP or in other brain regions and did not alter dopamine and cAMP-regulated phosphoprotein (DARPP32) immunoreactivity in striatum. In conclusion, OTA caused acute depletion of striatal DA on a background of globally increased oxidative stress and transient inhibition of oxidative DNA repair.

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Correspondence should be addressed to:

ICEIT NEWSLETTER
Chulabhorn Research Institute
Office of Academic Affairs
Vibhavadee-Rangsit Highway
Bangkok 10210, Thailand
Tel: (66-2) 574-0615
Fax: (66-2) 574-0616
CRI Homepage: <http://www.cri.or.th>

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