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MOLECULAR INTERACTIONS OF TOXIC METALS

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Project Description

This project examines the associations between selenium and mercury and between nickel subsulfide and deoxyribonucleic acid (DNA) to explore the common aspect of all toxic metal pathologies: disruptive chemical interactions that functionally impair important biomolecules. Selenium bioavailability is inversely related to methylmercury bioaccumulation in fish and is also inversely related to methylmercury toxicity. Although the mechanisms responsible are not well characterized, both effects may be the result of mercury selenide formation occurring as a by-product of a side reaction that occurs during normal selenium metabolism within animal cells. In the case of the nickel subsulfide–DNA interaction, it appears a unique and unexpected molecular mechanism of DNA adduct formation may be responsible for the damaging effects associated with respiratory exposure. This project examines the molecular reactions responsible for the physiological and ecological effects of these toxic metals.

Goals

The overall goal of this project is to increase the understanding of toxic metals and the direct, indirect, and consequent biochemical mechanisms by which they disrupt cell physiology and by which they exert their pathological effects. The goal of the first two studies is to examine the biogeochemistry of Hg–Se interactions and assess the effects of endogenous selenium on Hg bioaccumulation and retention. The third study will examine the nature of nickel subsulfide-dependent DNA damage in an effort to qualitatively and quantitatively characterize its effects relative to other particulate-associated toxic metals.

Rationale

The chemical mechanisms involved in toxic metal interactions with biologically important molecules are instrumental in the pathophysiology accompanying the signs and symptoms of toxicity. Although directly induced chemical mechanisms are commonly expected in metal toxicity syndromes, it is clear that indirect as well as consequent mechanisms are also important. For example, toxic metals may cause direct damage through competitive binding interactions which inhibit enzyme activity or indirect damage through free radical formation, resulting in altered signal transduction pathways or cell damage

and apoptosis. Still other mechanisms of toxic metal pathology extend beyond direct and indirect effects, and occur through dose-dependent consequent effects.

One type of consequent mechanism of metal toxicity involves exclusive displacement or sequestration of vital trace metals. For example, it is well recognized that the major mechanism of zinc intoxication arises from the zinc ion's preferential absorption across the luminal membrane of gut epithelial cells.

In the presence of excessive amounts of dietary zinc, intestinal absorption of other elements such as copper will be compromised because they utilize the same binding sites on the transporter molecules. Thus the chief physiological effect of chronic zinc intoxication is depletion of intracellular copper stores. Loss of copper leads to loss of copper-dependent enzymes. Since these enzymes perform numerous essential functions, the pathological syndrome associated with zinc toxicity arises because of the consequent loss of copper-dependent enzyme activities.

This proposal addresses the possible consequent mechanism of metal toxicity involving mercury and selenium. Mercury has the ability to sequester selenium in tissues as biologically unavailable mercury selenide. Loss of bioavailable selenium can lead to the loss of selenium-dependent enzyme formation. Thus, similar to zinc toxicity, the pathological syndrome associated with mercury toxicity may arise because of a decrease in selenium-dependent enzyme activities. The consequent selenium deficit may be overlooked because of the high accumulation of selenium and mercury in tissues as unavailable HgSe. The paradoxical nature of this interaction becomes even more pronounced when considering aquatic ecosystems. Increased availability of selenium diminishes the accumulation of mercury in fish. Selenium affects mercury distribution in the ecosystem while mercury simultaneously affects selenium's distribution, bioavailability, and physiology. The interactions between mercury and selenium occur as a result of the extraordinarily high binding coefficients between oxidized mercury and reduced selenium. These forms are both available intercellularly in creatures exposed to mercury in the aquatic environment.

The ability of selenium compounds to decrease the toxic action of mercury has been established in all investigated species of mammals, birds, and fish (1, 2). Selenium and Hg influence each other's bioavailability (3), toxicology (4), and remediation (5, 6). Several studies suggest an important role of Se in the bioaccumulation of Hg in fish (7–9). Fish tissue Hg is inversely related to the abundance of Se present in the ecosystem (6, 10). Selenium supplementation of lake waters in Sweden resulted in a 75%–85% reduction in Hg levels of fish over a 3-year period (5). Elimination of Se-rich discharges of fly ash to an artificial lake caused a steady increase in Hg concentrations (11, 12). Turner and Rudd (13) studied the uptake of radiolabeled Se and Hg into several fish species, zoobenthos, and plankton in experimental enclosures in a mercury-contaminated lake in northwestern Ontario. Their studies showed aquatic biota bioaccumulate selenium rapidly with a concomitant reduction in accumulation of mercury. The major sink for mercury and selenium was found to be the sediment. Studies such as these confirm the importance of Se-dependent Hg retirement from aquatic ecosystems.

Selenium is heterogeneously distributed across geographic regions of the globe such that certain regions and continents have far more soil Se than others. Although regional Se follows broad trends, soil Se distributions can vary dramatically across short distances. The plains of North America have robust soil Se concentrations, while the coasts and Canadian Shield are much lower. In general, regions of northern Europe such as Finland and Sweden are quite low in soil Se. Some of the world's lowest soil Se concentrations are found in New Zealand, certain parts of China, and many parts of Africa.

Recent data suggest mercury concentrations in fish from northern Canada are not declining as fast as fish from similar ecosystem settings in southern Canada. This observation may be a direct effect of the lower selenium content generally present in soils of northern Canada. The primary means of depuration is

expected to occur through formation of insoluble mercury selenides. Since the selenide binding partner can only form and endure in a reducing environment, mercury binding will be expedited in anaerobic environments and in the reducing intracellular environments of living tissues. Since Se suspended in the water column will be in an oxidized state that is unable to bind Hg, the mechanism of HgSe formation is apt to be biologically mediated.

In this regard, important contributions to mercury selenide formation appear likely to occur in plants, bacteria, protozoa, crustaceans, arthropods, and mollusks. Additionally, unlike higher organisms, these creatures do not have an acidic gut environment. It is, therefore, assumed the HgSe will pass through its digestive tract and be deposited in the silt. Therefore, at each level of the aquatic food chain, the mercury selenide present in the body mass of the prey would be expected to be far less bioavailable for absorption by the predator. This reduction in bioavailability is expected to result in less mercury bioaccumulation in the top-end predator fish consumed by humans.

Likewise, interactions between toxic doses of inorganic Hg and toxic doses of selenite in rodents have been thoroughly examined and described as “the mutual alleviation of toxicity through formation of inert Hg–Se complexes.” It has also been shown that marine mammals, having the highest mercury burdens in the marine biota, are able to detoxify methylmercury by formation and storage of HgSe in the liver. Wageman et al. (14) found that as much as 53% of the total mercury in the liver of ringed seals was in the form of HgSe. Whether or not HgSe protects not only the animal in question against mercury poisoning but also consumers of livers which contain this compound is unknown. Although, based on the very low solubility of HgSe, it is not expected to be absorbed by the mammalian alimentary tract. This specific area of research is yet to be investigated.

Numerous studies do, however, indicate that the Se naturally present in foods, such as fish and seafood, provides a physiological protection against Hg toxicity. This protected effect is assumed to be due to selenium sequestering mercury through the formation of HgSe thereby rendering mercury unavailable to exert its toxic effects. Studies have also shown mercury exposure reduces the activity of selenium-dependent enzymes. In this regard, mercury sequesters selenium and reduces selenium’s bioavailability for formation of essential selenium-dependent enzymes. The protective effect of additional Se may simply support continued selenoenzyme synthesis by overcoming the loss of selenium through formation of HgSe. Therefore, measuring the amount of mercury present in the environment or food sources may provide an inadequate reflection of the potential for health risks if the protective effects of selenium are not also considered.

Another area of concern regarding exposure to toxic metals that will be investigated in this study involves the risk associated with exposure to inhaled nickel subsulfide-containing particulates. Nickel and other reactive oxygen species (ROS)-forming metal ions are present in varying distributions in combustion-derived particulates such as residual oil fly ash (15, 16). Therefore, these elements primarily operate through indirect molecular mechanisms of toxicity since the ROS formed by these elements causes the molecular damage associated with their toxic effects. Respiratory and cardiovascular effects of exposure to these airborne particulates are associated with formation of ROS occurring at the particle interface or from water-soluble transition metal ions arising from the particulates. However, there may also be direct mechanisms of molecular interactions that may accompany exposure to certain of these elements.

An earlier study performed at the EERC applied an assay that provides a sensitive and rapid assessment of ROS generating capacity inherent in the materials analyzed (17). The results of this study indicate that potential health hazards associated with exposure to respirable particulate materials may be qualitatively and quantitatively assessed. However, the molecular forms of the chemical species present appear to be just as important as the mass quantities of the potentially toxic elements. Distinctions in

solubility and stability of various forms of nickel influence cellular uptake rates as well as kinetics of ROS formation (18). As a result, more severe consequences are associated with pulmonary exposure to chemical species such as nickel subsulfide than with nickel oxide (15, 16). This correlates well with analytical observations that the damage induced by nickel subsulfide are not only quantitatively greater, but also qualitatively different than that induced by other nickel species.

This project will examine molecular mechanisms responsible for the toxic effects of metals known to be biologically hazardous. Our first task is designed to test the hypothesis that selenium contributes to diminishment of the biological availability of methylmercury in aquatic food webs through formation of insoluble and biologically unavailable mercury selenides, resulting in long-term retirement of mercury from actively cycling pools in the environment. Likewise, our second task will test the hypothesis that the formation of HgSe in the cell contributes to the toxic effects of methylmercury by sequestering necessary selenium required for selenoenzyme synthesis. Our third task will test the hypothesis that nickel subsulfide-dependent DNA damage occurs through a molecular mechanism that is distinct from other free radical-generating metal species.

Approach

S.A.M.P.L.E. (Selenium Analysis in the METAALICUS Project Lake Ecosystem)

The objectives of this project are to investigate the potential influence of selenium on mercury dynamics in an extremely well-defined aquatic ecosystem. We are enhancing the METAALICUS (Mercury Experiment to Assess Atmospheric Loading In Canada and the United States) study by determining the selenium concentrations of representative aquatic biota samples in the food web and will correlate our results with total mercury and methylmercury analyses already being obtained as part of that study. METAALICUS is a whole-ecosystem experiment in which mercury loading to a headwater lake and its watershed is being experimentally manipulated. Mercury is being added in distinct forms of stable, nonradioactive isotopes of inorganic mercury. Different mercury stable isotopes are being added to the upland, wetland, and lake surface to determine the relative contributions of these sources to fish mercury levels. The mercury concentrations are being tracked in all major compartments in the lake, watershed, and atmosphere. Our effort in this project is to examine the selenium content in selected fish and invertebrate samples and investigate correlations between endogenous selenium concentrations with the mercury concentrations present in these same samples.

Electron Microscopy-Based Identification of Mercury Selenide in Tissues

Utilizing tissue samples from experimental animals that have been chronically exposed to large quantities of dietary mercury and tissue samples obtained from whales and squid, we are applying electron microscopy methods to identify and quantify HgSe in mercury-rich tissues. Direct measurement of HgSe in tissues will support the hypothesis we have proposed and will definitively show mercury's role in inhibiting selenoprotein synthesis. Further studies will examine the possible accumulation of HgSe in mercury-rich sediments. Direct measurement of HgSe accumulated in sediments will provide an indication of selenium's involvement in mercury retirement from the ecosystem.

Mechanisms of Nickel Subsulfide Mediated DNA Damage

Nickel subsulfide-dependent DNA damage appears to cause DNA damage that is distinct from that caused by other free radical-generating metal species. To evaluate ROS-dependent damage from insoluble forms of metalloparticulates, supercoiled circular DNA from bacteriophage Φ X174 DNA will be used as a test substrate in a highly sensitive monitoring assay. Samples of National Institute of Standards and

Technology (NIST) standard particulate materials, nickel subsulfide, and nickel oxide will be extracted using 1 M NaOAc-0.5 M HOAc at pH 5, 25°–28°C followed by exhaustive rinsing with deionized H₂O. DNA substrate suspensions in aqueous solutions will be exposed to equivalent mass quantities of these insoluble residues in multiple parallel samples that will be exposed and processed identically. Because of its compact hydrodynamic diameter, undamaged DNA (DNA Form 1) moves more rapidly through a 0.6% agarose gel during electrophoresis. The relative quantities of DNA migrating as either form will be visualized using ethidium bromide following separation. Free radical damage creates “nicks” in the DNA molecule, resulting in relaxed, partially unwound forms (DNA Form 2) that migrate through the gel at a slower rate than DNA Form 1. ROS-dependent damage changes DNA Form 1 into DNA Form 2 in a time- and dose-dependent manner. Exposure to nickel subsulfide rapidly eliminates DNA Form 1 and results in quantitative conversion into DNA Form 2 and a third uncharacterized form that has a migration rate during electrophoresis that is intermediate between DNA Forms 1 and 2. Working in collaboration with the Mayo Foundation Molecular Core Facility, the nickel subsulfide-dependent DNA damage will be characterized and differentiated from the damage caused by other forms of nickel present in inhaled airborne particulates. Distinctions in form and mechanisms of nickel subsulfide-dependent molecular damage to DNA will be assessed using comparison of defined effects of other DNA treatments using differential chromatography studies.

Progress

Samples of fish and invertebrates from the METAALICUS lakes are being identified and will be sent to the EERC for selenium analysis. Analysis of numerous geochemical databases has been performed to identify regions with heterogeneous selenium distributions where studies of mercury bioaccumulation in fish will be appropriate.

Arrangements to obtain tissues from animals (whale, squid, and experimental animals) and humans that have had high levels of mercury exposure are being made. Preliminary work with scanning electron microscopy has been performed and methodologies for mercury selenide recognition and quantification are being developed.

Initial studies of analysis of time- and dose-dependent DNA damage from exposure to nickel subsulfide are being performed.

Quality Objectives

The quality objective of Task 1 is to produce analytical results of selenium analysis of the selected samples from various organisms collected from the aquatic ecosystem. The analysis will be performed by the EERC Analytical Research Laboratory (ARL) which has extensive experience in selenium analysis. The results may then be evaluated to test the hypothesis and determine the influence of selenium on mercury accumulation.

The quality objective of Task 2 is to produce analytical results of HgSe particulate analysis by electron microscopy methods in order to identify and quantitate the formation of these HgSe species in mercury-rich tissues obtained from experimental animals and marine mammals. The identification of the HgSe species will be based on prior reports of HgSe characterization and available standards.

The quality objective of Task 3 is to produce repeatable results of a DNA damage technique which comparatively assesses the inherent ROS formation rates of various compounds.

Measurement/Data Acquisition

The methods used in this project are appropriate for analysis according to established protocols in place at the EERC. All sampling procedures, instrument calibrations, and quality control (QC) considerations are included in the protocols. Selenium analysis (Task 1) will be performed by graphite furnace atomic absorption spectrometry according to established analytical protocols of the ARL. Mercury selenide species (Task 2) will be identified by using various electron microscopy methods according to established analytical protocols of the Natural Materials Analytical Research Laboratory. The DNA damage technique (Task 3) will be performed in the Mayo Foundations' Molecular Core Facility, following established and appropriate analytical protocols.

Assessment and Validation

Based upon the expected reproducibility of the sample analyses, the numbers of samples used for each task of the work plan will provide statistically meaningful results, using standard statistical analysis. In each of the three tasks, we will only utilize data that are complete, can be verified by similar studies and pertinent literature, and produces repetitive results.

The project is designed with a time line and milestones that will facilitate in determining whether or not the objectives and the goal of the project are achieved. During the course of the project, the schedule and milestones will be reviewed in order to assess progress of the project.

Status

In order to examine selenium effects on mercury bioaccumulation in the experimentally supplemented and control lakes of the METAALICUS project, samples of invertebrate biomass are being examined alongside fish samples. Appropriate samples have been identified among the samples obtained from these lakes.

Methodological development has progressed, and preliminary analysis of whale and squid tissues has been initiated. Analysis of mercury selenide formation is planned in both in situ and ex vivo isolates from these tissues. Digestion procedures that degrade materials other than HgSe have been studied and are being developed for this project.

Studies of nickel subsulfide-mediated DNA damage are under way, and standardization of sample-handling protocols for the EERC and Mayo Clinic's molecular biology core facility has been developed.

Potential Applications and Benefits

Increased information regarding the effects of background selenium availability on methylmercury bioaccumulation will help regulatory agencies interpret differences in methylmercury concentrations in fish collected from lakes. Because selenium's geologic distribution can vary dramatically over short distances, lake selenium contents have the potential to cause considerable differences in mercury bioaccumulation rates between even neighboring lakes that may have no other perceptible differences in atmospheric deposition or other innate influences. Selenium-dependent mercury retirement will contribute to making previously deposited mercury unavailable, leaving only the most recently deposited mercury in a bioavailable condition. If selenium-dependent mercury retirement is occurring, it will also change

current understanding of the global mercury cycle, particularly regarding input–output mass balance results.

The study of the molecular mechanism of nickel subsulfide-dependent DNA damage may provide important information for regulatory agencies to consider. If the mechanism occurs through direct adduct formation rather than through indirect damage from reactive oxygen species, it will guide regulatory requirements for limiting human exposure.

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