NBMI RELATED PUBLICATIONS

EmeraMed is a biotechnology company whose main goal is targeting the cause of diseases related to toxic heavy metal exposures. EmeraMed is primarily dedicated to the prevention and treatment of illnesses related to oxidative stress induction in the Central Nervous System.

This article was prepared by EmeraMed's founder, Dr. Boyd Haley, PhD and retired professor of Chemistry and Biochemistry and Professor Emeritus University of Kentucky. The initial development of NBMI was done when Dr. Haley was Chair of Chemistry at the University of Kentucky.

Emeramide® - NBMI is a heavy metal antidote. It binds the cations of mercury, iron, lead and cadmium among others, into thermodynamically irreversible NBMI-metal complexes totally eliminating their chemical reactivity and toxicity even while the bound metals remain in the body. The resultant non-reactive NBMI-metal complex is primarily oxidized and modified by the P450 Detox system, transported to the liver where it enters the biliary transport system and eventually is excreted into the feces. NBMI is also a powerful anti-oxidant which directly scavenges hydroxyl free radicals in a manner similar to reduced glutathione. This supports its positive health effects for treatment of oxidative stress induced illnesses caused by other non-metal factors, such as pesticides and herbicides. It is my hypothesis that organic compounds that induce oxidative stress do so by binding to specific iron-proteins causing the release of Fe^{2+} which is a redox metal that catalytically, through Fenton type reactions, induces the catalytic production of hydroxyl free radicals inducing oxidative stress.

Oxidative stress released free radicals is a major part of many illnesses. NBMI scavenging of free radicals, which are unstable molecules that can damage the cells in your body prevents this damage to cellular macromolecules preventing cell death and injury. Additionally, NBMI, in addition to scavenging hydroxyl free radicals chelates the cations of the heavy metals mercury and unbound iron, which most likely start the creation excess free radicals and thereby helps restore normal function to the mitochondria and overall health.

EmeraMed is looking for assistance to apply for new drug approval outside of the United States, in countries in Latin America and Asia. EmeraMed has completed most of the FDA required safety studies to apply for consideration for marketing authority in the USA, the regulatory body indicates it will consider this when a human Phase II/III trial on mercury toxic humans is presented. This type of trial is now underway.

PROGRESS, RESEARCH AND CLINICAL TRIALS COMPLETED TO DATE

Mercury poisoning: Orphan Drug Designation has been granted for the prevention and treatment of mercury toxicity in the EU and USA. Phase 1 and multiples Phase 2a clinical studies have been performed with no adverse drug effects noted.

Mercury exposure from Small Scale Gold Mining: Careful examination of the mercury-intoxicated gold miners Phase 2 trial in Ecuador shows that pain was one of the main symptoms of mercury poisoning that dropped dramatically with the 300 mg/day dose of NBMI.

COPD TREATMENT: A Phase 2a clinical trial has been completed in summer of 2018 and found Emeramide® to be safe in COPD subjects with no adverse effects. Anecdotal observations indicate NBMI has a very positive effect with some COPD patients.

Kidney Damage: The kidney is one of the main targets for mercury cation (Hg2+). Our studies to chronically expose rats with enough Hg2+ to induce measurable kidney damage, and after the animals were injured, they were given Emeramide®. Not surprisingly, they quickly recovered.

Environmental Uses: NBMI is strongly attracted to arsenic (As). Previous research found NBMI containing columns could lower arsenic in water passed through them to non-detectable (ND) levels. Videos of treatment of mercury/lead toxic predator birds near death saving their lives and returning them to nature. Go to https://www.youtube.com/playlist?app=desktop&list=PLVXDa7_NtVcs59VHTG8HygeZ9UPBRS1Uz .

SAFETY AND USE OF NBMI TO TREAT "UNBOUND IRON" RELATED ILLNESSES: NBMI has been shown to bind free iron in laboratory studies as well as subjects and test animals and has shown a lack of drug-related adverse effects in all 5 clinical studies performed to date and could be a much safer treatment for these diseases. Because it is a capsule and appears to be completely non-toxic, NBMI has great potential to replace Deferasirox as the leading iron chelator.

SIGNIFICANT PUBLICATIONS:

Reversal of genetic brain iron accumulation by N,N′-bis(2-mercaptoethyl)isophthalamide, a lipophilic metal chelator, in mice. Ruiying Cheng1 · Rajitha Gadde2 · Yingfang Fan3 · Neha Kulkarni2 · Nachiket Shevale3 · Kai Bao4 · Hak Soo Choi4 ·Swati Betharia2 · Jonghan Kim1,3
Received: 4 January 2022 / Accepted: 21 March 2022

Abstract: N,N'-bis(2-mercaptoethyl)isophthalamide (NBMI) is a novel lipophilic metal chelator and antioxidant used in mercury poisoning. Recent studies have suggested that NBMI may also bind to other metals such as lead and iron. Since NBMI can enter the brain, we evaluated if NBMI removes excess iron from the iron-loaded brain and ameliorates iron-induced oxidative stress. First, NBMI exhibited preferential binding to ferrous (Fe2+) iron with negligible binding affinity to ferric (Fe3+) iron, indicating a selective chelation of labile iron. Second, NBMI protected SH-SY5Y human neuroblastoma cells from the cytotoxic effects of high iron. NBMI also decreased cellular labile iron and lessened the production of iron-induced reactive oxygen species in these cells. Deferiprone (DFP), a commonly used oral iron chelator, failed to prevent iron-induced cytotoxicity

or labile iron accumulation. Next, we validated the efficacy of NBMI in Hfe H67D mutant mice, a mouse model of brain iron accumulation (BIA). Oral gavage of NBMI for 6 weeks decreased iron accumulation in the brain as well as liver, whereas DFP showed iron chelation only in the liver, but not in the brain. Notably, depletion of brain copper and anemia were observed in BIA mice treated with DFP, but not with NBMI, suggesting a superior safety profile of NBMI over DFP for long-term use. Collectively, our study demonstrates that NBMI provides a neuroprotective effect against BIA and has therapeutic potential for neurodegenerative diseases associated with BIA.

Parkinsonian diseases (elevated brain-iron) – Phase 2a study: A series of in vivo studies showed that Emeramide® reduces brain-iron levels and normalizes brain-oxidative stress. We have seen substantial effects of NBMI on brain-iron chelation in the brain-iron accumulating H67D knock-in mice also involving Deferiprone, DFP. The figure shows (see study https://emeramed.com/information/) that while NBMI in six weeks decreases brain-iron level with high statistical significance (** = $p<0.01$), Deferiprone did not – it is so inefficient that it needs months to show results.

Emeramide® currently has a pilot Phase 2a ongoing for Atypical Parkinson's Diseases Progressive Supranuclear Palsy (PSP) and Multiple System Atrophy (MSA), severe and lethal Parkinsonian orphan diseases that today lack safe and effective treatments.

LEAD TOXICITY:

Neuroprotective Effects of N,N′bis-(2-mercaptoethyl) Isophthalamide (NBMI) Against Lead Induced Toxicity in U-87 MG Cells

Rajitha Gadde and Swati Betharia First published: 17 April 2020

Abstract
Background and Objective
Lead is one of the most dangerous and ubiquitous environmental toxins with no levels safe for human exposure. Lead causes neurotoxicity primarily by inducing oxidative stress. After crossing the blood brain barrier, lead is taken up by glial cells which serve as protective lead-binding proteins. While chelation is the mainstay of therapy for lead poisoning, currently FDA approved chelators such as dimercaptosuccinic acid (DMSA) are associated with many adverse effects and safety concerns. N,N′bis-(2-mercaptoethyl) isophthalamide (NBMI) is a newer lipophilic water insoluble compound with potential metal chelating and antioxidant properties. The purpose of this study was to determine whether NBMI displayed neuroprotective effects in U-87 malignant glioblastoma (U-87 MG) cells exposed to lead acetate (PbAc).
Materials and Methods
U-87 MG cells were treated with either vehicle, NBMI (0–100 μM), or DMSA (0–100 μM) for 24 hours followed by PbAc (0–300 μM) for 48 hours. Cell viability (CellTiter-Glo® Assay), cell morphology (trypan blue staining), degree of oxidative stress (GSH-GloTM Glutathione Assay),

and impact on apoptosis (Bax and Bcl-2 protein levels using Western blotting) were determined. Comparisons between multiple groups were performed using one-way or two-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test. Results with a p value <0.05 were considered statistically significant. **Results:** Exposure of U-87 MG cells to PbAc decreased cell viability in both a concentration and time-dependent manner. Trypan blue staining revealed that PbAc negatively altered the cell morphology. In addition, PbAc exposure led to an increase in total glutathione levels, as well as the Bax/Bcl-2 ratio, indicating increased oxidative stress and initiation of the cell apoptotic pathway. Pretreatment with NBMI diminished PbAc-induced cell death, morphology changes, increase in total GSH, and increase in the Bax/Bcl-2 ratio. Interestingly, the effects of NBMI were more prominent compared to DMSA in attenuating PbAc-induced cell death.

Conclusion

Lead exposure induces cell death and oxidative stress which are reversed by pretreatment with NBMI implying that NBMI is a new and promising chelator for treating PbAc-induced neurotoxicity.

N,N′bis-(2-mercaptoethyl) isophthalamide (NBMI) exerts neuroprotection against lead-induced toxicity in U-87 MG cells

Rajitha Gadde1 · Swati Betharia1

Received: 29 March 2021 / Accepted: 15 June 2021

Abstract

N,N'-bis(2-mercaptoethyl)isophthalamide (NBMI) is a novel lipophilic heavy metal chelator and thiol redox antioxidant. This study was designed to investigate the neuroprotective activity of NBMI in U-87 MG cells exposed to lead acetate (PbAc). Cells were pretreated with NBMI for 24 h prior to a 48 h exposure to PbAc. Cell death (55%, $p < 0.0001$) and reduction of intracellular GSH levels (0.70-fold, $p < 0.005$) induced by 250 μM Pb were successfully attenuated by NBMI pretreatment at concentrations as low as 10 μM. A similar pretreatment with the FDA-approved Pb chelator dimercaptosuccinic acid (DMSA) proved ineffective, indicating a superior PKPD profile for NBMI. Pretreatment with NBMI successfully counteracted Pb-induced neuroinflammation by reducing IL-1β (0.59-fold, $p < 0.05$) and GFAP expression levels. NBMI alone was also found to significantly increase ferroportin expression (1.97-fold, $p < 0.05$) thereby enhancing cellular ability to efflux heavy metals. While no response was observed on the apoptotic pathway, this study demonstrated for the first time that necrotic cell death induced by Pb in U-87 MG cells is successfully attenuated by NBMI. Collectively these data demonstrate NBMI to be a promising neuroprotective compound in the realm of Pb poisoning.

N,N'-Bis(2-mercaptoethyl)isophthalamide Binds Electrophilic Paracetamol Metabolites and Prevents Paracetamol-Induced Liver Toxicity

Johan L Å Nilsson [1], Anders Blomgren [1], Ulf J Nilsson [2], Edward D Högestätt [1], Lars Grundemar [1]

Abstract

Paracetamol overdosing may cause liver injury including fulminant liver failure due to generation of the toxic metabolites, N-acetyl-p-benzoquinone imine (NAPQI) and p-benzoquinone (p-BQ). Herein, the chelating agent, N,N'-Bis(2-mercaptoethyl)isophthalamide (NBMI), was examined for its potential ability to entrap NAPQI and p-BQ and to prevent paracetamol-induced liver injury. Both NBMI and the conventional paracetamol antidote N-acetylcysteine (NAC) were investigated with regard to their abilities to scavenge the NAPQI and p-BQ in a Transient Receptor Potential Ankyrin 1-dependent screening assay. Stoichiometric evaluations indicated that NBMI was able to entrap these metabolites more efficiently than NAC. Furthermore, oral administration of either NBMI (680 mg/kg) or NAC (680 mg/kg) prevented the development of the characteristic liver necrosis and elevation of serum alanine aminotransferase in a mouse model for paracetamol-induced liver injury. In summary, these results show that NBMI is able to entrap the toxic metabolites NAPQI and p-BQ and to prevent paracetamol-induced liver injury in mice.

STUDIES ON NBMI EFFECTS ON MERCURY TOXICITY

Efficacy of N,N'bis-(2-mercaptoethyl) isophthalamide on mercury intoxication: a randomized controlled trial

Paul Schutzmeier [1], Augusto Focil Baquerizo [2], Wilson Castillo-Tandazo [2], Nicholas Focil [2], Stephan Bose-O'Reilly [3 4]

Abstract

Background: Chronic mercury intoxication is a severe health issue and occurs especially in gold mining communities. Common chelators used for improving mercury elimination are not everywhere available and challenged by poor cell wall penetration. This study is part of a feasibility trial and the aim was to gather first information about the efficacy of the newly developed chelator N,N'bis-(2-mercaptoethyl) isophthalamide (NBMI) on chronic mercury intoxication.

Methods: In this three-armed, placebo-controlled randomized trial, 36 miners with mercury urine levels exceeding 15 µg/l were administered 100 mg NBMI, 300 mg NBMI or placebo for 14 days. Levels of mercury in urine [µg/l and µg/g creatinine] and plasma l were analyzed. Therapeutic effect was assessed using the medical intoxication score (MIS) and its single health outcomes (e.g. excessive salivation, sleeping problems), fatigue scores, a neuromotoric test battery (CATSYS) and a neurological outcome (Finger to nose test).

Results: Physical fatigue was significantly decreased in the 300 mg NBMI group compared to the control. Mercury concentration in urine following 300 mg NBMI treatment was significantly lowered compared to control, however, this effect was less distinct with adjustment for **creatinine.**

Conclusion: NBMI showed an effect on physical fatigue and there were indications to positive effects on other symptoms as well. More comprehensive studies are mandatory to verify the effects of NBMI as a novel tool for treating mercury intoxications.

Trial registration: ClinicalTrials.govIdentifier: NCT02486289 . Date of registration: June 24, 2015.

Keywords: Chelation therapy; Chronic mercury intoxication; Gold mining; Mercury; NBMI.

Reductions in plasma and urine mercury concentrations following N,N′bis-(2-mercaptoethyl) isophthalamide (NBMI) therapy: a post hoc analysis of data from a randomized human clinical trial.

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_Email :
Research Department Institute of Chronic Illnesses, Silver SpringMD20905USA, , , , , ,
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Abstract
Environmental mercury exposure possesses a significant risk to many human populations. At present there are no effective treatments for acute mercury toxicity. A new compound, N,N′bis-(2-mercaptoethyl) isophthalamide (NBMI), a lipophilic chelating agent was created to tightly/irreversibly bind mercury. A post hoc dose-dependent analysis of NBMI therapy was undertaken on data from a randomized controlled NBMI human treatment trial on 36 Ecuadorian gold miners with elevated urinary mercury concentrations. Study subjects were randomly assigned to receive 100 milligram (mg) NBMI/day, 300 mg NBMI/day, or placebo for 14 days. For each study subject daily milligram NBMI dose/Kilogram (Kg) bodyweight were determined and plasma and urine mercury concentrations(micrograms (µg)/Liter (L)) on study day 1 (pre-NBMI treatment), 15 (after 14 days of NBMI treatment) and 45 (30 days after NBMI treatment) were correlated with NBMI dosing using the linear regression statistic in SAS. Regression revealed significant inverse correlations between increasing per mg NBMI/Kg bodyweight/day and reduced concentrations of urinary and plasma mercury on study day 15 (reduced by in urine = 18–20 µg/L and plasma = 2 µg/L) and study day 30 (reduced by in urine = 15–20 µg/L and plasma = 4 µg/L) and significant correlations between reductions in mercury concentrations in urine and plasma. Significant 30% reductions in urinary mercury concentrations per mg NBMI/Kg bodyweight/day administered for 14 days were observed. This study supports the dose-dependent ability of NBMI therapy to significantly reduce mercury concentrations, particularly in the urine, in an acutely mercury exposed human population. NBMI therapy should be evaluated in other mercury exposed populations.

Thiol-redox antioxidants protect against lung vascular endothelial cytoskeletal alterations caused by pulmonary fibrosis inducer, bleomycin: comparison between classical thiol-protectant, N-acetyl-L-cysteine, and novel thiol antioxidant, N,N'-bis-2-mercaptoethyl isophthalamide

Rishi B Patel [1], Sainath R Kotha, Lynn A Sauers, Smitha Malireddy, Travis O Gurney, Niladri N Gupta, Terry S Elton, Ulysses J Magalang, Clay B Marsh, Boyd E Haley, Narasimham L Parinandi

Abstract:
Lung vascular alterations and pulmonary hypertension associated with oxidative stress have been reported to be involved in idiopathic lung fibrosis (ILF). Therefore, here, we hypothesize that the widely used lung fibrosis inducer, bleomycin, would cause cytoskeletal rearrangement through

thiol-redox alterations in the cultured lung vascular endothelial cell (EC) monolayers. We exposed the monolayers of primary bovine pulmonary artery ECs to bleomycin (10 µg) and studied the cytotoxicity, cytoskeletal rearrangements, and the macromolecule (fluorescein isothiocyanate-dextran, 70,000 mol. wt.) paracellular transport in the absence and presence of two thiol-redox protectants, the classic water-soluble N-acetyl-L-cysteine (NAC) and the novel hydrophobic N,N'-bis-2-mercaptoethyl isophthalamide (NBMI). Our results revealed that bleomycin induced cytotoxicity (lactate dehydrogenase leak), morphological alterations (rounding of cells and filipodia formation), and cytoskeletal rearrangement (actin stress fiber formation and alterations of tight junction proteins, ZO-1 and occludin) in a dose-dependent fashion. Furthermore, our study demonstrated the formation of reactive oxygen species, loss of thiols (glutathione, GSH), EC barrier dysfunction (decrease of transendothelial electrical resistance), and enhanced paracellular transport (leak) of macromolecules. The observed bleomycin-induced EC alterations were attenuated by both NAC and NBMI, revealing that the novel hydrophobic thiol-protectant, NBMI, was more effective at µM concentrations as compared to the water-soluble NAC that was effective at mM concentrations in offering protection against the bleomycin-induced EC alterations. Overall, the results of the current study suggested the central role of thiol-redox in vascular EC dysfunction associated with ILF.

Amelioration of Acute Mercury Toxicity by a Novel, Non-Toxic Lipid Soluble Chelator N,N'bis-(2-mercaptoethyl)isophthalamide: Effect on Animal Survival, Health, Mercury Excretion and Organ Accumulation

David Clarke[1], Roger Buchanan, Niladri Gupta, Boyd Haley

Abstract :

The toxic effects of mercury are known to be complex with specific enzyme inhibitions and subsequent oxidative stress adding to the damaging effects. There are likely other factors involved, such as the development of impaired metal ion homeostasis and depletion of thiol and selenium based metabolites such as cysteine and selenium. Much of the toxicity of mercury occurs at the intracellular level via binding of Hg2+ to thiol groups in specific proteins. Therefore, amelioration of mercury toxicity by the use of chelation would likely be enhanced by the use of a chelator that could cross the cell membrane and the blood brain barrier. It would be most favorable if this compound was of low toxicity, had appropriate pharmacokinetics, bound and rendered mercury cation non-toxic and had antioxidant properties. Herein we report on such a chelator, N,N'-bis(2-mercaptoethyl)isophthalamide (NBMI), and, using an animal model, show that it prevented the toxic effects associated with acute exposure induced by injected mercury chloride.

Novel lipid-soluble thiol-redox antioxidant and heavy metal chelator, N,N'-bis(2-mercaptoethyl)isophthalamide (NBMI) and phospholipase D-specific inhibitor, 5-fluoro-2-indolyl des-chlorohalopemide (FIPI) attenuate mercury-induced lipid signaling leading to protection against cytotoxicity in aortic endothelial cells

Jordan D Secor[1], Sainath R Kotha, Travis O Gurney, Rishi B Patel, Nicholas R Kefauver, Niladri Gupta, Andrew J Morris, Boyd E Haley, Narasimham L Parinandi

Abstract

Here, we investigated thiol-redox-mediated phospholipase D (PLD) signaling as a mechanism of mercury cytotoxicity in mouse aortic endothelial cell (MAEC) in vitro model utilizing the novel

lipid-soluble thiol-redox antioxidant and heavy metal chelator, N,N'-bis(2-mercaptoethyl)isophthalamide (NBMI) and the novel PLD-specific inhibitor, 5-fluoro-2-indolyl des-chlorohalopemide (FIPI). Our results demonstrated (i) mercury in the form of mercury(II) chloride, methylmercury, and thimerosal induced PLD activation in a dose- and time-dependent manner; (ii) NBMI and FIPI completely attenuated mercury- and oxidant-induced PLD activation; (iii) mercury induced upstream phosphorylation of extracellular-regulated kinase 1/2 (ERK1/2) leading to downstream threonine phosphorylation of PLD(1) which was attenuated by NBMI; (iv) mercury caused loss of intracellular glutathione which was restored by NBMI; and (v) NBMI and FIPI attenuated mercury- and oxidant-induced cytotoxicity in MAECs. For the first time, this study demonstrated that redox-dependent and PLD-mediated bioactive lipid signaling was involved in mercury-induced vascular EC cytotoxicity which was protected by NBMI and FIPI.

Heme Iron Causes Cytotoxicity to Lung Microvascular Endothelial Cells through Oxidant Lipid Signaling: Protection by Novel Lipophilic Thiol Chelator and Antioxidant Drug, N,N'-bis-2-Mercaptoethyl Isophthalamide (NBMI) and Implications in Treatment of Sickle Cell Disease ;Honors Research Thesis, Yizhou Wu. The Ohio State University April 2014 Project Advisor Narasimham L. Parinandi, Ph.D.
Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, Dorothy M. Davis Heart & Lung Research Institute, College of Medicine & Wexner Medical Center,
The Ohio State University

ABSTRACT

Hereditary hemoglobinopathies, mainly sickle cell disease (SCD) and β-thalassemia, 3+ contribute to iron overload through hemolysis and hemoglobin (Hb) degradation. Hemin (Fe - heme), released from the extracellular Hb, is identified as a critical player in hemoglobinopathies. Heme and iron released from hemoglobin during hemoglobinopathies lead to reactive oxygen species formation (ROS) and oxidant injury to cells and tissues, including the vascular endothelium. Pulmonary hypertension has been recognized as a serious complication in SCD and lung microvasculature appears to be a target to iron-induced lung microvascular endothelial dysfunction contributing to pulmonary hypertension in SCD. Currently available iron chelation therapies for the protection against iron-induced stress in SCD patients are limited and not efficacious. Therefore, here we hypothesized that iron overload would cause reactive oxygen species (ROS) generation and oxidative stress in SCD crisis triggering the redox-regulated activation of the membrane phospholipid-hydrolyzing and cellular lipid signaling enzyme, phospholipase D (PLD) in lung microvascular endothelium, which could be the underlying mechanism(s) of pulmonary hypertension in SCD patients. In our current investigation, we established the oxidant and thiol-redox mediation of iron-induced (hemin) cytotoxicity through PLD-mediated lipid signaling in the bovine lung microvascular endothelial cell (BLMVEC) model in vitro. Additionally, our study revealed that hemin caused cytotoxicity through ROS generation, oxidative stress, depletion of glutathione (GSH) redox status, membrane lipid peroxidation, and phospholipase D (PLD) activation, formation of PLD-generated bioactive lipid mediator, phosphatidic acid (PA), and lipid signaling in BLMVECs. The PLD-specific pharmacological inhibitor, 5-fluoro-2-indolyl des-chlorohalopemide hydrochloride hydrate (FIPI), not only attenuated hemin-induced PLD activation but also protected against cytotoxicity of hemin in BLMVECs suggesting the regulatory role of PLD and the PLD-generated bioactive lipid, PA in cytotoxicity of hemin in endothelial cells (ECs). Furthermore, our current study

demonstrated that N,N'-bis-2-mercaptoethyl isophthalamide (NBMI), a novel synthetic, non-toxic, and lipophilic thiol-redox heavy metal chelator and antioxidant drug, offered a greater efficacy in attenuating the hemin-induced oxidative stress and PLD activation and protection against cytotoxicity of hemin in lung microvascular ECs as compared to the currently used iron and trace metal chelating drugs and thiol-protectants, thus offering promise for the treatment of iron overload and associated vascular EC injury during hemoglobinopathies including SCD.

Aqueous mercury precipitation with the synthetic dithiolate, $BDTH_2$
Author links open overlay panel **Lisa Y.Blue, ParthaJana, D.A.Atwood**
Abstract
$BDTH_2$, 1,3-benzenediamidoethanethiol (common name) and closely related derivatives were specifically designed to become insoluble after the formation of linear, covalent bonds to aqueous mercury(II). $BDTH_2$ (IUPAC nomenclature, N,N′-bis(2-mercaptoethyl)isophthalamide) emerged as the preeminent reagent for the complete precipitation of mercury from water after several years of studies with a wide range of compounds having one, two, three, and four thiol groups. $BDTH_2$ does not become inactive through oxidation to disulfide and can be applied to mercury-containing water as acidic, basic, and ethanolic solutions. The BDT–Hg precipitate is extremely stable and leaches low-ppm levels of mercury only under extremely acidic and basic conditions. $BDTH_2$ is also effective in the aqueous precipitation of other soft, divalent metals, such as copper, cadmium, lead, and the main group elements, arsenic and selenium. The insolubility of the BDT–M compounds can be attributed to the presence of strong, non-polar, covalent M–S bonding within a water-insoluble organic framework. $BDTH_2$ has no known biological toxicity and is being sold as a nutritional supplement under the trade name OSR-1. This review describes the chemistry, precipitation, and leaching studies of $BDTH_2$ with mercury.

Mercury induces tight junction alterations and para-cellular transport in colon epithelial cells through oxidative stress and thiol-redox dysregulation—protection by novel lipid-solublethiolantioxidant,NBMI Aarti_Vala_Honors_Thesis_May2012_final.p(1.992Mb) Advisor: Parinandi, Narasimham

The Ohio State University. Department of Microbiology Honors Theses; 2012

Abstract:
Intestinal permeability, characterized as leaky-gut syndrome, is a debilitating gastrointestinal disorder that leads to inflammation and altered immune response. Tight junctions are crucial for cell-to-cell adhesion and regulation of paracellular transport of molecules across the intestinal epithelium. However, the exact mechanism of the leaky-gut state encountered in autistic spectrum disorders is not known. Mercury, both as inorganic and organic forms, has been identified as a serious environmental pollutant, occupational hazard, and pharmaceutical toxicant. Mercury, in the form of thimerosal in vaccines, has been implicated as one of the causative species of autism. Therefore, here, we hypothesized that mercury would cause intestinal epithelial cell tight junction alterations and paracellular hyperpermeability (leak) through oxidative stress and thiol-redox dysregulation which could lead to the leaky-gut condition. Hence, we investigated the mechanism of tight junction alteration and paracellular leak of macromolecules in the well-established in vitro intestinal (colon) epithelial Caco-2 cell model. We also identified efficacy of the thiol-redox stabilization drugs to protect against the

mercury-induced damage in the Caco-2 cells in vitro. Our studies revealed that the two forms of mercury, methylmercury and thimerosal caused (i) dose- and time-dependent cytotoxicity (lactate dehydrogenase release, decreased mitochondrial integrity, and cell morphological alterations), (ii) loss of intracellular glutathione (GSH), (iii) increase in the formation of reactive oxygen species, (iv) loss of barrier dysfunction; (v) loss of cell proliferation, (vi) actin cytoskeletal rearrangement (actin stress fiber formation), (vii) tight-junction (ZO-1 protein and occludins) alterations, and (viii) increase in paracellular leak of macromolecules in Caco-2 cells in vitro. Mercury-induced cytotoxicity, tight junction alterations, and increase in paracellular leak were significantly attenuated by the novel lipophilic thiol-redox-stabilizing antioxidant and heavy metal chelator, N,N'-bis-(2-mercaptoethyl)isophthalamide (NBMI). For the first time, the results of the current study demonstrated that mercury (methylmercury and thimerosal) caused intestinal epithelial cell damage and macromolecule leak through thiol-redox dysregulation and oxidative stress which was effectively protected by the novel lipophilic thiol-redox stabilizer and heavy metal chelator, NBMI. Our findings emphasize the significance of altered cellular functioning by the intestinal epithelium upon exposure to mercuric agents, and pharmacological attenuation by the novel drug, NBMI.

The effect of a new metal chelator emeramide on secondary iron overload in mice

A thesis presented By Hanting Phoebe Tsai to The Department of Pharmaceutical Sciences
in partial fulfillment of the requirements for the degree of Master of Science
In the field of Pharmaceutical Sciences
NORTHEASTERN UNIVERSITY BOSTON, MASSACHUSETTS
August 2019
GRADUATE APPROVAL RECORD NORTHEASTERN UNIVERSITY
Thesis Title: The effect of a new metal chelator emeramide on secondary iron overload in mice
Author: Hanting Phoebe Tsai Department: Pharmaceutical Sciences
Approved for Thesis Requirements of the Master of Science Degree
Graduate Office Notified of Acceptance Signature:

ABSTRACT

Iron (Fe) is an essential metal for facilitating several cellular functions, e.g. DNA synthesis, enzyme activities, and erythropoiesis, in our bodies. However, when the iron homeostasis is not tightly regulated, our body functions could not be carried out properly and would even cause diseases. Excessive iron deposition due to genetic disorders (primary iron overload) or chronic hemolytic anemia (secondary iron overload) could be harmful by inducing oxidative stress and further causing organ injuries and dysfunctions, e.g. hepatic cirrhosis and cardiac hypertrophy. Patients with sickle cells disease (SCD) may have secondary iron overload via hemolysis and ineffective erythropoiesis. Iron absorption is upregulated to complement ineffective erythropoiesis. In addition, chronic blood transfusions are necessary for those who have severe symptoms of SCD or anemia and will further intensify the iron burden and oxidative stress. Thus, iron chelators usually accompany blood transfusion in order to decrease excess iron disposition. However, currently FDA-approved iron chelators show significant side effects, which results in low patients' compliance. N,N' bis-(2-mercaptoethyl)isophthalamide) (NBMI; emeramide), a novel metal chelator, has shown efficacy in decreasing iron burden in a mouse model of primary iron overload. Previous in vivo test on rats also indicated its low toxicity. Therefore, NBMI was proposed to test its efficacy on iron chelation along with safety on a

secondary iron overload mouse model of SCD by comparing to the most widely used iron chelator, deferasirox (DFX). I found that NBMI had limited effect on decreasing iron level in sickling mice (HbSS). Drug toxicity was not observed. Interestingly, NBMI treatment resulted in decreased oxidative stress in the liver and kidneys of HbSS mice. Although the iron level was not considerably decreased by NBMI compared to DFX, it is possible that NBMI becomes hydrophilic after binding to iron and stays in the tissues. Since non-heme iron levels were measured, the assay does not differentiate NBMI- bound iron and free iron (unbound form). Since free, labile iron causes oxidative stress, NBMI-bound iron could be no longer harmful and may not induce oxidative damage. These results indicate that NBMI could potentially be used as an antioxidant supplement to protect the body from oxidative stress in SCD patients.