HHS Public Access

Author manuscript

Ann N Y Acad Sci. Author manuscript; available in PMC 2021 November 01.

Published in final edited form as:

Ann N Y Acad Sci. 2020 November ; 1479(1): 196–209. doi:10.1111/nyas.14347.

Methylene blue and monosodium glutamate improve neurologic signs after fluoroacetate poisoning

Vanessa E. DeLey Cox[1], **Matthew A. Hartog**[1], **Erin Pueblo**[1], **Michelle Racine**[1], **Laura Jennings**[1], **Justin Tressler**[1], **Wing Y. Tuet**[1], **Samuel Stone**[1], **Samuel A. Pierce**[1], **Lily Thompson**[1], **Aliyah Dukes**[1], **Heidi Hoard-Fruchey**[1], **Benjamin Wong**[1], **Bryan J. McCranor**[1]

[1]Pharmaceutical Sciences Department, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD

Abstract

Fluoroacetate (FA) is a tasteless, odorless, water-soluble metabolic poison with severe toxicological effects. Characterized in the mid-1900s, it has been used as a rodenticide but is comparably lethal to all mammals. Many countries have restricted its use, and modern-day accidental human exposures are rare, but recently, concerns have been raised about its application as a chemical weapon with no known antidote. A combined treatment of methylene blue (MB), an antioxidant, and monosodium glutamate (MSG), a precursor of the citric acid cycle substrate alpha-ketoglutarate, has been recommended as an effective countermeasure; however, no peer-reviewed articles documenting the efficacy of this therapy have been published. Using a rodent model, we assessed the effects of MB and MSG on the neurologic, cardiac, and pulmonary systems. Transcriptomic analysis was used to elucidate inflammatory pathway activation and guide bioassays, which revealed the advantages and disadvantages of these candidate countermeasures. Results show MB and MSG can reduce neurologic signs observed in rats exposed to sodium FA and improve some effects of intoxication. However, while this strategy resolved some signs of intoxication, ultimately it was unable to significantly reduce lethality.

Graphical Abstract

Using a rodent model, we assessed the effects of methylene blue (MB) and monosodium glutamate (MSG) on the neurologic, cardiac, and pulmonary systems. Results show MB and MSG can reduce neurologic signs observed in rats exposed to sodium fluoroacetate and improve some effects of intoxication. However, while this strategy resolved some signs of intoxication, ultimately it was unable to significantly reduce lethality.

Address for correspondence: Bryan J. McCranor, Pharmaceutical Sciences Department, U.S. Army Medical Research Institute of Chemical Defense, 8350 Ricketts Point Rd., Aberdeen Proving Ground, MD 21010. bryan.j.mccranor.civ@mail.mil.

Author contributions

V.E.D.C. wrote the manuscript, designed experiments, and analyzed data. L.J., E.P., and M.R. performed and analyzed the biochemical assays. M.A.H. wrote the transcriptomics sections. M.A.H. and L.T. performed and analyzed the transcriptomics analysis. E.P., M.R., S.S., A.D., S.A.P., J.T., W.Y.T., and L.J. performed and analyzed the animal experiments. H.H.F., B.W., and B.J.M. conceptualized this work, edited the manuscript, and performed analysis. B.J.M. accepts responsibility for the integrity of data presented.

Disclosure

The views expressed are solely those of the authors and do not necessarily represent the official views of the CCRP, NIAID, NIH, HHS, USAMRICD, or DoD.

Competing interests

The authors declare no competing interests.

Keywords

metabolic toxin; 1080; fluorocitrate; rat models; antioxidant; TCA cycle

Introduction

Fluoroacetate (FA) was first identified as a botanically derived metabolic poison.[1] In the 1940s, a synthetic version, sodium FA (1080), was characterized and mass-produced as a rodenticide.[2, 3] Exposure to 1080 can be fatal to any species, but mammals are especially sensitive. In humans, the median lethal dose (LD_{50}) of 1080 is estimated to be 2–10 mg/kg.[4] Since no antidote or therapeutic treatment currently exists, many countries, including the United States, heavily restrict its use; however, some countries, including Mexico, South Korea, Australia, and New Zealand, have more liberal regulations on the use of 1080, leading to debates about both public safety and the ethics of using 1080 to control invasive and nuisance species.[5–10]

Signs and symptoms of 1080 intoxication are nonspecific. Across species, neurologic effects are the most common with secondary symptoms including respiratory distress and cardiac dysfunction.[11] In humans, symptoms include nausea, muscle weakness, nerve pain, tachypnea, cyanosis, and decreased body temperature.[12–14] The most common clinical symptom of 1080 poisoning, metabolic acidosis, is observed in many disease states including cancer and alcohol intoxication, which confounds proper clinical identification. Additionally, the onset of symptoms is often delayed postexposure in warm-blooded animals. The lack of specific signs and symptoms combined with the delayed onset complicates efforts to diagnose poisoning. In the past 10 years, the identification of 1080 intoxication in livestock and domesticated animals has continued to be a focus.[15–18] These studies have been complemented by novel strategies to identify 1080 contamination in food and water sources.[19, 20] However, despite ongoing research, there is still no reliable treatment for intoxication.

Owing to the severity of intoxication following exposure, 1080 has recently become recognized as a potential chemical weapon that may be used by assassins or terrorists.[21–23] In 2007, the CIA released a statement identifying it as a weapon of interest for the Iraqi Security Service under Saddam Hussain.[24] In 2011, a dozen people were arrested for smuggling 1080 into New York City along with other poisons.[25] The route of administration does not influence lethality; while ingestion is the most common route, 1080 can be inhaled, injected, or absorbed through dermal abrasions.

Despite limited progress identifying medical countermeasures, the mechanism of toxicity has been understood for decades. FA itself is nontoxic. As a small molecule, it readily passes through the cellular membrane, and because it mimics acetic acid, FA is converted into F-acetyl-SCoA and can enter the citric acid cycle. Citrate synthase then catalyzes the reaction between F-acetyl-SCoA and oxaloacetate to form fluorocitrate (FC), a highly toxic molecule that inhibits aconitase, thereby blocking cellular respiration.[26–28] The conversion of 2-carbon FA to 6-carbon FC is known as lethal synthesis.[29]

FC is a competitive inhibitor of aconitase; however, it binds very strongly, mimicking a suicide substrate.[30] Only 3% of FA is estimated to convert to FC.[31, 32] The remaining FA is defluorinated via a glutathione-dependent mechanism and excreted in the urine.[33] The binding affinity of FC for aconitase is significantly greater than that of the native substrate, citrate, so that even a small amount of FC can be fatal. A number of structurally similar molecules are also converted *in vivo* to FC with comparable cellular effects and physiological signs and symptoms.[34] Countermeasures against 1080 intoxication would apply to any chemicals in this group.

Currently, treatment of 1080 poisoning is limited to supportive care. Animal studies have evaluated the efficacy of various acetate donors (i.e., ethanol, glycerol, and acetate), but the therapeutic potential is limited, and these countermeasures must be administered close to the time of exposure.[34–36] Investigations into naturally occurring 1080 tolerance in mammals and microorganism have yet to establish clear routes to developing an antidote, though amino acid metabolism has been suggested to play a role in 1080-tolerant bacteria.[37–39] Expectations of providing prompt treatment are unrealistic as symptoms present several hours postexposure. Some *in vitro* studies have evaluated the therapeutic potential of antioxidants, but further research in animal models would be required.[40]

In 2010, a U.S. patent application was published outlining a therapeutic treatment to counter 1080 intoxication.[41] The patent named methylene blue (MB) and monosodium glutamate (MSG) as a combined treatment to decrease the lethality of 1080 in rats. Data were presented in the patent, and preliminary data have been included in textbook chapters; however, no comprehensive peer-reviewed article has been published.[14, 42]

As an antioxidant, MB can mitigate damage from reactive oxygen species originating from mitochondrial dysfunction.[43] As a medical therapeutic, MB is FDA approved for the treatment of methemoglobinemia and has also shown efficacy in treating hypoxia, hypotension, and cyanide intoxication.[44–47] MSG is metabolized to alpha-ketoglutarate, a substrate of the citric acid cycle that would bypass the aconitase blockade following 1080 poisoning. By supporting glutamine metabolism, MSG may circumvent the bottleneck in the citric acid cycle that prevents pyruvate oxidation. Administration of MSG may allow cells to produce ATP despite the damaging cellular effects of 1080.

Through analysis of biosamples and evaluation of respiratory parameters, the therapeutic benefit of MB and MSG as countermeasures against 1080 was assessed in an ingestion rat model. MB and MSG were administered in combination at 0.5 and 2 h after exposure. Though we found MB and MSG had some therapeutic benefits, in our hands, these compounds did not reduce lethality in any of the therapeutic models evaluated. These data suggest important directions for future research.

Materials and methods

Animals

Male Sprague–Dawley rats (300–500 g) were housed individually under standard conditions with a 12 h light/dark cycle, and LabDiet® Rodent Feed 5001 (Lab Supply, Fort Worth, TX)

and water available ad libitum. All research complied with relevant federal statutes and regulations regarding animal care and research including the Animal Welfare Act of 1966 (and amendments) and the National Research Council's Guide for the Care and Use of Laboratory Animals (National Academy Press, 2011). The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee, the United States Army Medical Research Institute of Chemical Defense (USAMRICD), Aberdeen Proving Ground, MD.

In vivo *exposure*—Before exposure, animals were placed in individual whole-body plethysmography (WBP) chambers for 10 min to acclimate. Baseline measurements of respiratory parameters were then recorded for 10 minutes. After baseline, animals were removed and administered either 1080 or water via a non-invasive assisted-drinking method.[48, 49] To mimic real-world conditions, animals were not fasted prior to experiments. Control animals not exposed to 1080 were used to establish a baseline for healthy animals. Exposure concentrations of 1080 were calculated based on an LD_{50} of 2.08 mg/kg.[48] Doses of 1080 and therapeutics were scaled to preexposure animal weight, and liquid volumes administered were normalized. Animals were then returned to WBP chambers where respiratory function was tracked for 24 hours. Physiological and behavioral signs were observed continuously for at least 2 h postexposure. Therapeutics were administered after exposure via either a subcutaneous (SC) or an intraperitoneal (IP) injection. A sham treatment (injectable sterile water) was used as vehicle control. Treatment cohorts for 1080-exposed and control animals were as follows: (1) exposure to 1 × LD_{50} and administration at 0.5 h postexposure of a single injection of both MB (5 mg/kg, SC) and MSG (250 mg/kg, IP) or sham injections (water, SC and IP) ($n = 39$); (2) exposure to 1 × LD_{50} and two administrations at 0.5 and 2 h postexposure of MB (5 mg/kg SC) and MSG (250 mg/kg IP) or sham injections (SC and IP) ($n = 40$); (3) exposure to 1 × LD_{50} and no treatments, and animals euthanized for genomic analysis at 1 h postexposure ($n = 8$), 2 h postexposure ($n = 8$), and 3 h postexposure ($n = 8$); and (4) exposure to 0.5 × LD_{50} and administration at 0.5 h postexposure of a single injection of both MB (5 mg/kg, SC) and MSG (250 mg/kg, IP) or sham injections (water, SC and IP) ($n = 24$). The total number of animals used was 127.

Prior to euthanasia, animals were deeply anesthetized via an intramuscular injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). Animals were then euthanized via exsanguination. Blood was collected from the descending aorta and placed in an untreated collection tube. The left lung was tied off, and bronchoalveolar lavage was performed on the right lung using 3 mL of phosphate-buffered saline. Tissues were collected, processed, and flash-frozen in liquid nitrogen. For biochemical analysis, animals were euthanized, and tissue and biosamples were collected 24 h postexposure.

Comprehensive metabolic panel and complete blood count—For the comprehensive metabolic panel, blood was collected from the descending aorta and placed in an untreated collection tube. Samples were run on an automated hematology analyzer to determine concentrations of blood urea nitrogen (BUN), aspartate transaminase (AST), and alanine aminotransferase (ALT). For the complete blood count, blood was collected from the

descending aorta and placed in a blood collection tube treated with EDTA, and samples were run on an automated hematology analyzer.

BALF analysis—Bronchoalveolar lavage fluid (BALF) was centrifuged at 2254 × *g* for 10 min at 10 °C. The supernatant was collected, aliquoted, and stored at −8 0 °C. Total protein content was assessed via the Pierce 660 protein assay (Cat. No. 22662, Thermo-Fisher Scientific) using the company's recommended protocol with the following modifications: 10 μL of BALF and 150 μL of Pierce® 660 nm protein assay reagent were added directly to a 96-well plate. The plate was shaken for 1 min, then incubated for 4 min at room temperature. Results were read on a SpectraMax M5 microplate reader. Samples were prepared in triplicate. Prediluted protein assay standards (bovine serum albumin, Cat. No. 23208, Thermo Scientific, Rockford, IL) were used to generate a protein concentration curve. Hemoglobin (Hb) concentrations were based on the absorption of BALF at 540 nanometers. BALF samples were aliquoted into a 96-well plate and read on a SpectraMax M5 microplate reader. Samples were prepared in triplicate.

Inflammatory cytokine analysis—Cytokine-specific assays were run to quantify serum concentrations of interleukin (IL)-10. A serum separator tube was used to fractionate blood by centrifugation. Isolated serum samples were aliquoted and frozen at −80 °C. Thawed samples were run on a Bio-Plex® 200 system (Bio-Rad Laboratories, Hercules, CA) using the ProcartaPlex Rat IL-10 Simplex Kit (Cat. No. EPX10A-36049–901, Thermo-Fisher Scientific) following the manufacturer's instructions with a 21-h incubation at 4 °C. Undiluted samples were assayed in triplicate and analyzed using the Bio-Plex manager software (v6.1, Bio-Rad Laboratories). The analyte concentration range used to generate the standard curve for IL-10 was 9.89–40500 pg/mL.

Data and statistical analysis—Unless otherwise noted, data are presented as the mean ± one standard deviation. When appropriate, two groups were compared using a Student's *t*-test or >2 groups were analyzed using an ANOVA followed by Dunnett's test. Significant differences were defined as $P < 0.05$. Specialized software was used to collect respiratory dynamics (FinePointe Software v2.3.1.16, DSI). All raw data were exported and analyzed using custom-designed programs (Microsoft Visual Basic for Applications v.7.0.1639, Microsoft Corporation, Redmond, WA), spreadsheet software (Microsoft Excel v.14.1.7166.5000 [32-bit], Microsoft Corporation, Redmond, WA), and statistical and graphing software (GraphPad Prism v5.04, v.7.04, GraphPad Software, Inc., La Jolla, CA).

Genomics—At 1, 2, or 3 h postexposure the heart, lungs, liver, kidneys, and brain were collected from 1080-exposed and vehicle control animals. The tissues were immediately snap-frozen in liquid nitrogen and stored at −80 °C. Total RNA was isolated using RNeasy® Plus Mini Kits (QIAGEN, Germantown, MD). RNA concentration, quality, and integrity were determined using a NanoDrop® ND-8000 UV–Vis spectrophotometer (Thermo-Fisher Scientific; Waltham, MA) and an Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, CA). RNA was processed for hybridization to GeneChip® HT RG 230 PM array plates using the GeneChip 3' IVT PLUS Reagent Kit (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. The array plates were processed and scanned

using an Affymetrix GeneTitan system. The raw signal intensities were imported into Partek® Genomics Suite® version 7.0 (Partek Inc., St. Louis, MO) and normalized using robust multi-array averaging (RMA). Principal component analysis (PCA) was used to identify patterns and major sources of variability in the data. An analysis of variance (ANOVA) was used to identify significant changes in gene expression induced by 1080 exposure (≤−1.5 fold change ≥1.5; false discovery rate adjusted $P \leq 0.05$). The probeset ID, fold change, and P value of genes with significant changes in expression level were imported into the Ingenuity® Pathway Analysis (IPA; Qiagen Inc., Valencia, CA) and mapped to biological and toxicological pathways.

Chemicals—Sterile water for injection (SWFI; Cat. No. A1287301, Thermo-Fisher Scientific, Waltham, MA) was used as a vehicle. Control groups received only SWFI without dissolved chemicals added. Sodium FA (Cat. No. N-13216–250MG, Chem Service Inc., West Chester, PA), MB (Cat. No. M9140, Sigma Aldrich, St. Louis, MO), and L-glutamic acid monosodium salt (Cat. No. RES5063G-A701X, Sigma Aldrich, St. Louis, MO) were used for animal studies. All chemicals were dissolved in SWFI.

Results

Based on the patent application from Goncharov *et al.*, we pursued similar treatment strategies using a combination of MB and MSG as countermeasures to 1080 intoxication.[41] Additionally, upstream regulator analysis of the transcriptomic data predicted that MSG may be a regulator of response to 1080 exposure in cardiac tissue at 2 h postexposure (Z-score: 2.31) and in hepatic tissue at 3 h postexposure (Z-score: 3.06) (Fig. S1, online only). Previously we identified convulsions as a common neurologic sign in rats following 1080 exposure. One cohort of rats was treated with a single administration of 5 mg/kg MB and 250 mg/kg MSG at 0.5 h, which significantly reduced the number of rats experiencing convulsions within 2 h following exposure. In the exposed group treated 0.5 h postexposure, 10% of animals experienced convulsions compared with 70% of sham-treated animals. A second cohort was treated with the same concentrations of MB and MSG, which were administered at 0.5 h and again at 2 hours. Of these animals, 50% of treated animals experienced convulsions as compared with 90% of sham-treated animals, supporting the argument that MB and MSG reduce neurologic signs in rats exposed to 1080. However, despite these promising data, treatment with MB and MSG did not improve survival as compared with sham-treated animals. In fact, more sham animals survived 24 h postexposure than treated animals (treatment at 0.5 h led to 70% sham and 50% treated survival; and treatment at 0.5 and 2 h resulted in 90% sham and 70% treated survival.) In subsequent studies, the effects of MB and MSG on rats exposed to 1080 were evaluated for metabolic changes, respiratory function, and inflammatory response.

Prior research has established multiorgan failure as a characteristic of 1080 exposure.[11] Metabolic panels run on blood collected 24 h postexposure have been consistent with hepatic, renal, and cardiac damage.[48] In this study, treatment with a combination of 5 mg/kg MB and 250 mg/kg MSG, at 0.5 and 2 h postexposure seemed to reduce markers of organ damage. Concentrations of enzymes indicative of multiorgan damage were similar in treated animals and control animals but elevated in sham-treated animals (Fig. 1A and B). Elevated

ALT and AST levels are indicators of liver damage. Levels of both enzymes were elevated in serum collected from sham-treated animals, but concentrations were normal in animals treated at 0.5 and 2 h with MB and MSG. Results from the cohort treated at 0.5 h showed a similar trend in ALT, but owing to low numbers of blood samples collected from treated survivors ($n = 4$), definitive statements cannot be made from this data set (Table S1, online only). Since hepatocytes are highly metabolically active and can be especially susceptible to mitochondrial dysfunction, increases in serum markers of liver damage in 1080-exposed animals are not surprising.[50] The decrease in apparent liver injury, based on ALT and AST levels, in MB- and MSG-treated animals is encouraging.

BUN concentrations, an indicator of renal dysfunction, were also elevated in exposed animals (Fig. 1C). Elevated BUN concentrations can be directly caused by renal failure or indirectly caused by cardiac dysfunction.[51] In exposed animals, overall BUN concentrations were significantly elevated. It is not clear how effective treatment was in returning BUN concentrations to normal because data is not consistent between the two cohorts. Treatment at 0.5 h returned BUN concentrations to normal, suggesting animals treated with MB and MSG have normal renal function despite exposure to 1080 (Table S1, online only). However, treatment at 0.5 and 2 h did not appear to reduce circulating BUN (Fig. 1C).

Prior data has shown 1080 diminishes respiratory function in exposed animals; however, as administered, MB and MSG did not improve respiratory parameters.[48] Minute volume, the product of tidal volume multiplied by the respiratory rate, was tracked for 24 h to evaluate the effects of therapeutics on respiration. Consistent with previously published data, animals exposed to 1080 showed signs of respiratory distress. MB and MSG treatment had no appreciable effect on respiratory parameters in 1080-exposed animals (Fig. 2). Following 1080 exposure, respiratory function decreased around 7 h postexposure and remained depressed throughout the remainder of the study.

Previously published a histologic analysis of lung tissue following exposure to 1080 has revealed evidence of pulmonary hemorrhage and inflammation.[48] To further evaluate the potential of MB and MSG to mitigate 1080-induced pulmonary toxicity, BALF was analyzed for evidence of heme (Fig. 3) and protein in the lungs (Fig. 4). An increase in BALF absorbance at 540 nm was observed in the sham-treated 1080-exposed animals of both cohorts (Fig. 3A and B). This is consistent with the presence of Hb, indicating hemorrhage in the lungs.[52] We observed that animals treated with MB and MSG postexposure appeared to have no increase in the amount of Hb in BALF over that, which was observed in sham-treated exposed animals. Higher BALF protein content was also observed in exposed animals (Fig. 4), consistent with pulmonary edema.[53] Treatment with MB and MSG had no significant effect on the elevated levels of protein when administered at 0.5 h only (Fig. 4A). In animals that were exposed to 1080 and received MB and MSG at 0.5 and 2 h, the protein content in BALF was elevated, but not statistically significant (Fig. 4B). These data suggest that while MB and MSG are able to reduce the hemorrhagic pulmonary edema, they are not able to improve overall respiratory function, possibly due to pulmonary edema.

Inflammation in response to acute pulmonary damage is often marked by increased counts of neutrophils and elevated concentrations of IL-17 and IL-6, both proinflammatory cytokines, and IL-10, an anti-inflammatory cytokine. Previous publications quantifying concentrations of cytokines in blood serum have shown elevated concentrations of the proinflammatory cytokine IL-17 in animals exposed to 1080.[48]

Transcriptomic analysis revealed changes in gene expression associated with the generation of an inflammatory response in multiple organs following 1080 exposure. The expression of genes regulating the acute phase response pathway was found to be altered in cardiac tissue at 2 h and 3 h postexposure (Fig. 5). This pathway was predicted to be activated (Z-score ≥ 2.0) in cardiac tissue at 2 h postexposure, as well as in hepatic and renal tissues 3 h postexposure (Table S2, online only). The IL-8 signaling pathway was predicted to be activated in cardiac tissue 2 h and 3 h postexposure, in hepatic tissue at 3 h postexposure, and in pulmonary tissue at 2 h postexposure (Table S3, online only). IL-8 is a potent chemotactic and neutrophil-activating factor that is an important contributor to the propagation of an inflammatory response. Additionally, the proinflammatory IL-6 signaling pathway was predicted to be activated in cardiac and hepatic tissue at 2 h and 3 h postexposure, as well as in pulmonary and renal tissue 3 h postexposure (Table S4, online only). Since IL-6 is one of the cytokines activated by IL-17, predicted data are consistent with the inflammatory response demonstrated in the literature.

A proinflammatory response by IL-6 may be complemented by elevated levels of IL-10, a protective anti-inflammatory cytokine. The severity of 1080 poisoning means inflammatory pathways may not be able to be activated before organ systems shut down. Thus, in an effort to elicit an inflammatory response before multiorgan failure, the third cohort of animals was exposed to $0.5 \times LD_{50}$ and treated with MB and MSG 0.5 h postexposure. Concentrations of IL-10 in serum collected from 1080-exposed animals were elevated compared with control animals but not statistically significant (Fig. S2, online only). Treatment with MB and MSG did not seem to affect concentrations of IL-10 in animals exposed to 1080. The treatment did, however, affect healthy animals: IL-10 concentrations were low in control animals dosed with MB and MSG. The antioxidant properties of MB may explain these data. The lack of protective anti-inflammatory cytokines suggests MB and MSG are not affecting this pathway; however, blood would need to be taken at multiple time points to make a firm determination.

White blood cell (WBC) counts further support an acute inflammatory response in the lungs. Decreases in total WBCs were driven by a reduction in lymphocytes. Elevated numbers of neutrophils, consistent with acute pulmonary injury, were observed in exposed animals and treated controls relative to sham controls (Fig. 6). Since neutrophils secrete both IL-17 and IL-6, these findings are consistent with both the transcriptomic analysis and previously published data on acute pulmonary inflammation.[54] These data are also consistent with transcriptomic analysis predicting activation of IL-8 that recruits neutrophils. The leucopenia observed in exposed animals is consistent with other models of 1080 intoxication.[18]

Discussion

Collectively, these data suggest MB and MSG are able to mitigate some signs of 1080 intoxication, though they are unable to prevent mortality. Overall, data from sham-treated animals and control animals were consistent with previously published results that list neurologic signs, multiorgan failure, respiratory distress, and an acute inflammatory response as signs of 1080 exposure. Data trends from biosample analysis suggest MB and MSG support neurologic, muscular, and organ function but cannot rescue respiratory function.

The metabolic panel suggests impaired organ function in sham-treated animals, consistent with previous results. Increased concentrations of AST and ALT in blood reflect impaired liver function, while elevated BUN concentrations are associated with renal damage and possible cardiac dysfunction. Overall, treatment with MB and MSG was able to shift averages toward normal levels, suggesting this course of treatment supports organ function either by directly mitigating toxic effects of 1080 or by indirectly rescuing organ function.

As highly metabolically active cells, hepatocytes may be especially susceptible to 1080-induced toxicity due to the mechanism of action. Mitochondrial dysfunction in hepatocytes would be expected to dramatically impair liver function. Blood panel results suggest treatment with MB and MSG may support hepatic mitochondrial function, potentially leading to improved liver function, protection against toxic effects of 1080, and reduced occurrence or severity of multiorgan failure, which is characteristic of 1080 poisoning.

Cardiomyocytes may also be particularly sensitive to mitochondrial dysfunction.[55] Cardiac cells have a high number of mitochondria to fulfill the energy demands of the heart muscle. The mechanism of toxicity for 1080 affects the citric acid cycle, directly leading to mitochondrial dysfunction and downstream to cardiac dysfunction.[48] Cardiac dysfunction is one possible explanation for elevated concentrations of BUN and pulmonary hemorrhagic edema. It is possible cardiac dysfunction may be one of the primary signs of 1080 poisoning, with renal damage and pulmonary edema being cardiogenic secondary effects. Treatment with MB and MSG reduced average BUN concentrations, suggesting improved cardiac function may be placing less stress on the kidneys. BALF from treated animals suggests treatment with MB and MSG reduced pulmonary hemorrhage, though pulmonary distress was still evident.

Improved cardiac function could also explain the decrease in pulmonary hemorrhage in treated animals. Analysis of BALF from treated animals showed less evidence of heme, suggesting MB and MSG reduced pulmonary hemorrhage. However, decreases in total protein concentrations in BALF showed minimal improvement, suggesting that although treatment can mitigate the more severe hemorrhage, treatment with MB and MSG fails to entirely prevent pulmonary edema. Minute volume was also depressed in exposed animals and unaffected by treatment strategies. Collectively, these data suggest MB and MSG can support pulmonary function but only to a limited extent. One possible explanation is that MB and MSG may indirectly support respiration through improving cardiac function, leading to a reduction in pulmonary hemorrhage. Collectively, these data suggest by

supporting the citric acid cycle, MB and MSG may support cardiac function sufficiently to reduce pulmonary hemorrhage, though not enough to restore respiratory function.

To understand the molecular mechanism of toxicity, transcriptomic analysis was used to further investigate the pulmonary inflammatory response. Increased neutrophils, IL-8, IL-17, and IL-6 are among the physiological markers consistent with acute pulmonary injury.[54] Previous publications have shown elevated IL-17 concentrations.[48] Transcriptomic analysis of animal tissues predicted activation of the IL-8 and IL-6 inflammatory pathways within pulmonary tissue. Consistent with unresolved acute pulmonary injury, increased circulating neutrophils suggest an inflammatory response. Expression of IL-10, an anti-inflammatory cytokine, would also be expected to be upregulated to protect pulmonary tissue from possible damage from proinflammatory cytokines; however, the transcriptomic data are inconclusive on whether the IL-10 pathway is activated following exposure at the time points tested, and serum concentrations of IL-10 were not elevated 24 h postexposure. This acute inflammatory response may reflect the inability of MB and MSG to resolve pulmonary toxicity. These data suggest that while MB and MSG support organ function, they do not resolve the pulmonary inflammatory response. Future studies may include candidate countermeasures to improve cardiac function and rescue pulmonary function. In these studies, the evaluation of inflammatory markers in BALF may provide more detailed information about the localized pulmonary effects of 1080.

Collectively, these data suggest that although treatment with MB and MSG is not sufficient to reduce lethality, these countermeasures do resolve some signs of 1080 poisoning. The results are consistent with the expected mechanisms of action of these therapeutics, neither of which is a true antidote. As an antioxidant, MB would be expected to support mitochondrial and overall cell function by neutralizing reactive oxygen species. MSG bypasses the citric acid cycle blockage and supports ATP production via the glutamine metabolic pathway. These results show that these therapeutics can indirectly support neurologic, muscular, and organ function, and suggest that these classes of therapeutics may be critical to countering the signs and symptoms caused by exposure to 1080. Future studies investigating alternative therapeutics from these classes may yield improved results.

Critically, these data suggest a path forward. 1080 poisoning seems to require a multi-pronged therapeutic approach. Although MB and MSG were unable to improve survival outcomes as applied, these candidate countermeasures have some important therapeutic benefits improving signs and symptoms and supporting organ function. However, respiratory parameters did not show significant improvement, and pairing MB and MSG with another therapeutic targeted at specifically addressing respiratory distress may resolve a broader range of signs and symptoms and shift lethality. Rather than seeking a single-strategy antidote, these results suggest 1080 poisoning may best respond to a pharmaceutical cocktail that counters signs and symptoms while supporting organ function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The research described was supported by an interagency agreement (AOD 18015–001-00000) between the NIH Office of the Director (OD) and the U.S. Army Medical Research Institute of Chemical Defense under the oversight of the Chemical Countermeasures Research Program (CCRP) within the Office of Biodefense Research (OBRS) at the National Institute of Allergy and Infectious Diseases (NIAID/NIH). Some project performers were supported in part by an appointment to the Postgraduate Research Participation Program at the U.S. Army Medical Research Institute of Chemical Defense administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and USAMRDC.

References

1. Marais JSC 1944 Monofluoroacetic acid, the toxic principle of “gifblaar” Dichapetalum cymosum. Onderstepoort J. Vet. Sci. Anim. Ind 20: 67.
2. Gratz NG 1973 “A criticle review of currently used single-dose rodenticides”. In Bull. W. H. O, Vol. 48: 469–477. World Health Organization. [PubMed: 4543551]
3. Kalmbach ER 1945 “Ten-eighty,” a war-produced rodenticide Science (New York, N.Y.). 102: 232–233. [PubMed: 17778513]
4. Egekeze JO & Oehme FW. 1979 Inorganic and organic fluoride concentrations in tissues after the oral administration of sodium monofluoroacetate (Compound 1080) to rats. Toxicology. 15: 43–53. [PubMed: 542959]
5. 2010 “Factsheet: The livestock protection collar”. In. W. Services, Ed.: 1–3. Washington, DC: Animal and Plant Health Inspection Service.
6. Sherley M 2007 Is sodium fluoroacetate (1080) a humane poison? Anim. Welfare. 16: 449–458.
7. Eason C 2002 Sodium monofluoroacetate (1080) risk assessment and risk communication. Toxicology. 181-182: 523–530.
8. Eason C, Miller A, Ogilvie S, et al. 2011 An updated review of the toxicology and ecotoxicology of sodium fluoroacetate (1080) in relation to its use as a pest control tool in New Zealand. N. Z. J. Ecol. 35: 1–20.
9. Twigg LE & Parker RW. 2010 Is sodium fluoroacetate (1080) a humane poison? The influence of mode of action, physiological effects, and target specificity. Anim. Welfare. 19: 249–263.
10. Eason CT, Shapiro L, Adams P, et al. 2010 Advancing a humane alternative to sodium fluoroacetate (1080) for wildlife management - welfare and wallaby control. Wildl. Res. 37: 497–503.
11. Sherley M 2004 The traditional categories of fluoroacetate poisoning signs and symptoms belie substantial underlying similarities. Toxicol. Lett. 151: 399–406. [PubMed: 15261984]
12. Brockmann JL, McDowell AV & Leeds WG. 1955 Fatal poisoning with sodium fluoroacetate; report of a case. J. Am. Med. Assoc. 159: 1529–1532. [PubMed: 13271109]
13. Taitelman U, Roy A & Hoffer E. 1983 Fluoroacetamide poisoning in man: the role of ionized calcium. Arch. Toxicol. Suppl. 6: 228–231. [PubMed: 6578726]
14. Goncharov N, Glashkina L, Savelieva E, et al. 2009 “Fluoroacetate”. In Handbook of Toxicology of Chemical Warfare Agents. R.C. Gupta, Ed.: 177–198. New York: Academic Press.
15. Shokry E, dos Santos FC, da Cunha PHJ, et al. 2017 Earwax: A clue to discover fluoroacetate intoxication in cattle. Toxicon. 137: 54–57. [PubMed: 28716647]
16. Giannitti F, Anderson M, Caspe SG, et al. 2013 An outbreak of sodium fluoroacetate (1080) intoxication in selenium- and copper-deficient sheep in California. Vet. Pathol. 50: 1022–1027. [PubMed: 23613492]
17. Brower A, Struthers J & Schmidt J. 2017 Sodium fluoroacetate toxicity: a case report of malicious poisoning in dogs across a Phoenix, Arizona neighborhood. Forensic Sci., Med., Pathol. 13: 450–453.
18. Collicchio-Zuanaze R, Sakate M, Langrafe L, et al. 2010 Hematological and biochemical profiles and histopathological evaluation of experimental intoxication by sodium fluoroacetate in cats. Hum. and Exp. Toxicol. 29: 903–913.

19. Parry E & Willison SA. 2018 Direct aqueous injection of the fluoroacetate anion in potable water for analysis by liquid chromatography tandem mass-spectrometry. Anal. Methods. 10: 5524–5531.
20. Wong YT, Law WK, Lai SSL, et al. 2018 Ultra-trace determination of sodium fluoroacetate (1080) as monofluoroacetate in milk and milk powder by GC-MS/MS and LC-MS/MS. Anal. Methods. 10: 3514–3524.
21. Holstege CP, Bechtel LK, Reilly TH, et al. 2007 Unusual but potential agents of terrorists. Emerg. Med. Clin. North Am. 25: 549–566; abstract xi. [PubMed: 17482032]
22. Hume T 2015 "'Eco-terrorism' threat to poison infant formula in New Zealand". In. https://www.cnn.com/2015/03/10/asia/new-zealand-dairy-threat/index.html: CNN.
23. Cooney TP, Varelis P & Bendall JG. 2016 High-Throughput Quantification of Monofluoroacetate (1080) in Milk as a Response to an Extortion Threat. J. Food Prot. 79: 273–281. [PubMed: 26818988]
24. CIA. (CIA). IIS undeclared research on poisons and toxins for assassination: Iraq's chemical warfare program. https://www.cia.gov/library/reports/general-reports-1/iraq_wmd_2004/chap5_annxA.html.
25. Rashbaum WK 2011 "12 held in sale of pest poisons, one 60 times as potent as the legal limit". In The New York Times. https://www.nytimes.com/2011/09/20/nyregion/12-arrested-in-sales-of-illegal-pesticides-in-chinatown.html.
26. Buffa P & Peters RA. 1949 The in vivo formation of citrate induced by fluoroacetate and its significance. J. Physiol. 110: 488–500. [PubMed: 15406444]
27. Buffa P & Pasquali-Ronchetti I. 1977 Biochemical lesions of respiratory enzymes and configurational changes of mitochondria in vivo. II. Early ultrastructural modifications correlated to the biochemical lesion induced by fluoroacetate. Cell Tissue Res. 183: 1–23. [PubMed: 922823]
28. Carrell HL, Glusker JP, Villafranca JJ, et al. 1970 Fluorocitrate inhibition of aconitase: relative configuration of inhibitory isomer by x-ray crystallography. Science (New York, N.Y.). 170: 1412–1414. [PubMed: 5481856]
29. Peters RA 1952 Lethal synthesis. Proc. R. Soc. London, Ser. B: Biol. Sci. 139: 143–170. [PubMed: 14911820]
30. Lauble H, Kennedy MC, Emptage MH, et al. 1996 The reaction of fluorocitrate with aconitase and the crystal structure of the enzyme-inhibitor complex. Proc. Natl. Acad. Sci. U. S. A. 93: 13699–13703. [PubMed: 8942997]
31. Gal EM, Drewes PA & Taylor NF. 1961 Metabolism of fluoroacetic acid-2-C-14 in the intact rat. Arch. Biochem. Biophys. 93: 1–14. [PubMed: 13702988]
32. Schaefer H & Machleidt H. 1971 Conversion of fluoroacetic acid to amino acids in the mammal. Biochim. Biophys. Acta. 252: 83–91. [PubMed: 5141830]
33. Soiefer AI & Kostyniak PJ. 1983 The enzymatic defluorination of fluoroacetate in mouse liver cytosol: the separation of defluorination activity from several glutathione S-transferases of mouse liver. Arch. Biochem. Biophys. 225: 928–935. [PubMed: 6625615]
34. Goncharov NV 2009 "Fluoroacetate". In Handbook of Toxicology of Chemical Warfare Agents. R.C. Gupta, Ed.: 177–198. New York: Academic Press.
35. Goncharov NV, Jenkins RO & Radilov AS. 2006 Toxicology of fluoroacetate: a review, with possible directions for therapy research. J. Appl. Toxicol. 26: 148–161. [PubMed: 16252258]
36. Hoyos CLA, Galvis MAC, Estrada DO, et al. 2018 Intravenous lipid emulsion and ethanol for sodium fluoroacetate poisoning. Am. J. Ther. 25: E756–E758. [PubMed: 29668489]
37. Pimentel MFA, Paula DAJ, Riet-Correa F, et al. 2019 Detection and characterization of bovine rumen microorganisms resistant to sodium fluoroacetate. Acta Scientiae Veterinariae. 47.
38. Leong LEX, Denman SE, Hugenholtz P, et al. 2016 Amino acid and peptide utilization profiles of the fluoroacetate-degrading bacterium synergistetes strain MFA1 under varying conditions. Microb. Ecol. 71: 494–504. [PubMed: 26111963]
39. Deakin JE, Cooper DW, Sinclair JJ, et al. 2013 Towards an understanding of the genetic basis behind 1080 (sodium fluoroacetate) tolerance and an investigation of the candidate gene ACO2. Aust. J. Zool. 61: 69–77.

40. Mead RJ, Moulden DL & Twigg LE. 1985 Significance of sulfhydryl compounds in the manifestation of fluoroacetate toxicity to the rat, brush-tailed possum, woylie and western grey kangaroo. Aust. J. Biol. Sci. 38: 139–149. [PubMed: 4051904]

41. Goncharov NV, Kuznetsov AV, Glashkina LM, et al., inventors. 2010 US patent Date of application: 2007.

42. Goncharov N, Savelieva E, Zinchenko V, et al. 2015 “Fluoroacetate”. In Handbook of Toxicology of Chemical Warfare Agents. Gupta RC, Ed.: 193–214. Elsevier.

43. Ginimuge PR & Jyothi SD. 2010 Methylene blue: revisited. J. Anaesthesiol. Clin. Pharmacol. 26: 517–520. [PubMed: 21547182]

44. F.D.A. 2016 PROVAYBLUE label. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/204630s000lbl.pdf.

45. Ryou M-G, Choudhury GR, Li W, et al. 2015 Methylene blue-induced neuronal protective mechanism against hypoxiareoxygenation stress. Neuroscience. 301: 193–203. [PubMed: 26047733]

46. Weissgerber LAJ 2008 Methylene blue for refactory hypotension: A case report. AANA Journal 76: 271–274. [PubMed: 18777811]

47. Haouzi P, McCann M, Tubbs N, et al. 2019 Antidotal effects of the phenothiazine chromophore methylene blue following cyanide intoxication. Toxicological Sciences. 107: 82–94.

48. McCranor BJ, Young TD, Tressler J, et al. 2019 The cardiopulmonary effects of sodium fluoroacetate (1080) in sprague-dawley rats. Cogent Biol. 5.

49. Rice NC, Rauscher NA, Langston JL, et al. 2018 Behavioral toxicity of sodium cyanide following oral ingestion in rats: Dose-dependent onset, severity, survival, and recovery. Food Chem. Toxicol. 114: 145–154. [PubMed: 29454866]

50. Mukai M, Bischoff K & Ramaiah SK. 2012 “Liver toxicity”. In Toxicology Veterinary. Gupta RC, Ed.: 246–263. Waltham: Elsevier.

51. Gwaltney-Brant SM 2012 “Renal toxicity”. In Veterinary Toxicology. Gupta RC, Ed.: 264–277. Waltham: Elsavier.

52. Beck-Shimmer B, Rosenberger DS, Neff SB, et al. 2005 Pumonary Aspiration Anesthesiology. 103: 556–566.

53. Aggarwal S, Jilling T, Doran S, et al. 2019 Phosgene inhalation causes hemolysis and acute lung injury Toxicol. Lett. 312: 204–213.

54. Ward PA & Lentsch AB. 1999 The acute inflammatory response and its regulation. AMA Arch. Surg. 134: 666–669. [PubMed: 10367878]

55. Zhou B & Tian R. 2018 Mitochondrial dysfunction in pathophysiology of heart failure. J. Clin. Invest. 128: 3716–3726. [PubMed: 30124471]

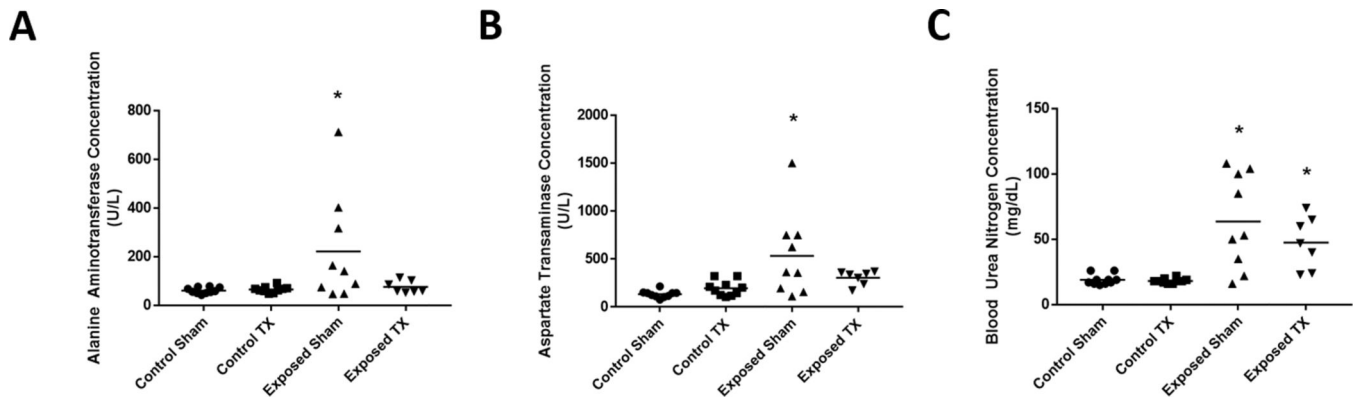

Figure 1.

Metabolic blood panel. The concentration of ALT, AST, and BUN 24 h after exposure to 1 × LD_{50} of 1080. Male rats were exposed to 1 × LD_{50} of 1080 and then treated with MB and MSG or sham injection. Treated animals were given MB (5 mg/kg, SC) and MSG (250 mg/kg, IP) twice at 0.5 and 2 h after exposure. Significant increases in all three makers were observed in the double sham-treated animals, and treatment with MB and MSG appears to restore alanine aminotransferase (A) and aspartate transaminase (B) concentrations to control levels. BUN levels were also elevated in exposed animals, but treatment did not appear to be of any benefit (C). Dash = average, $n = 6–10$, $*P < 0.05$ with a one-way ANOVA followed by Dunnett's multiple comparison test versus sham control.

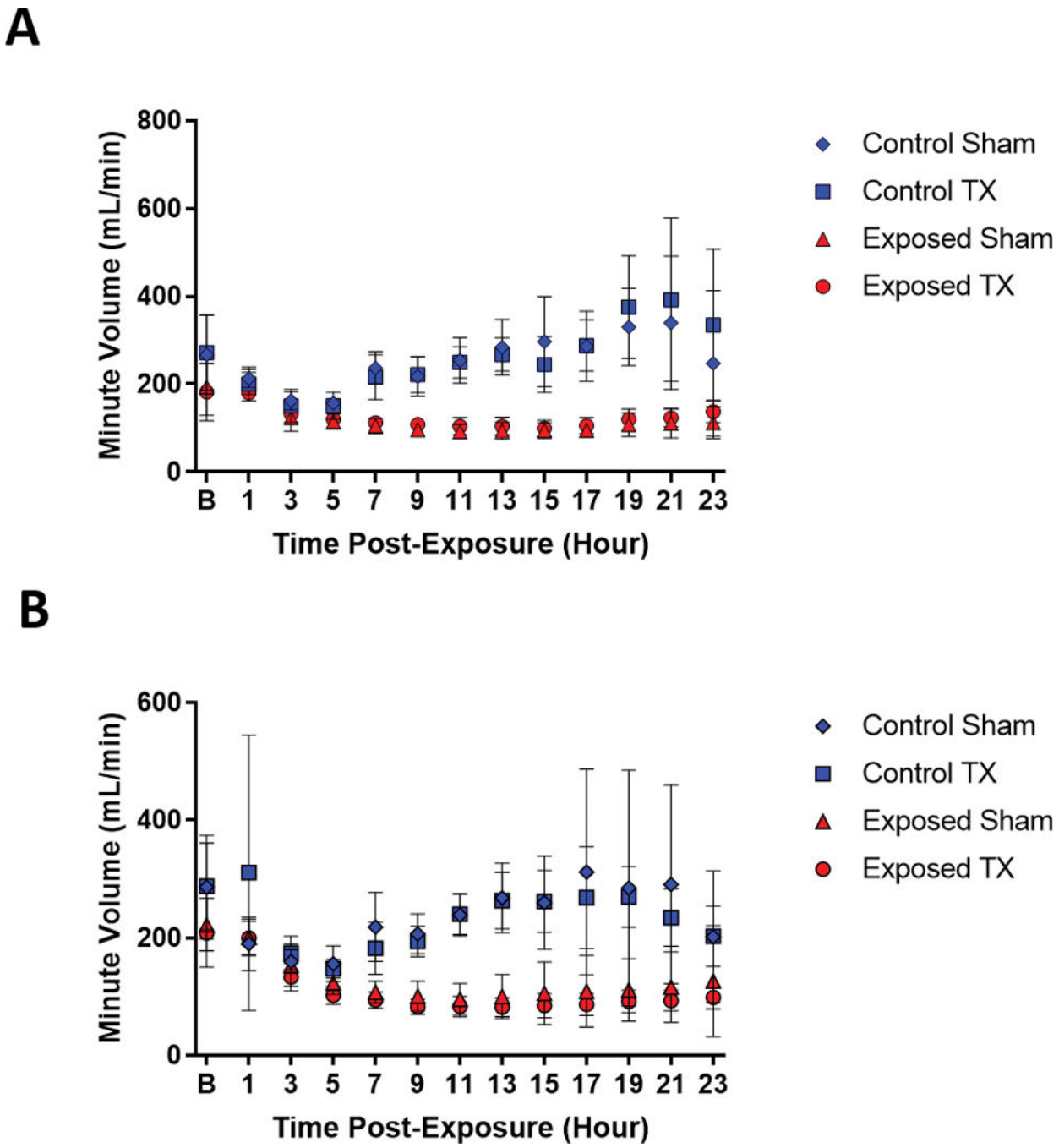

Figure 2.

Respiratory parameters following 1080 exposure. Minute volume was measured for animals exposed to 1 × LD_{50} of 1080 (red) and control animals (blue). Baseline respiration (B) and the first 23 h are shown. As relevant, MB (5 mg/kg, SC) and MSG (250 mg/kg, IP) were administered at either 0.5 (A) or 0.5 and 2 h (B) postexposure. Sham animals were treated with sterile water. All exposed animals showed a marked reduction in minute volume compared with the control animals starting around 7 h postexposure. Treatment with MB and MSG showed no effect on respiration. Error bars indicate standard deviation, $n = 9–10$.

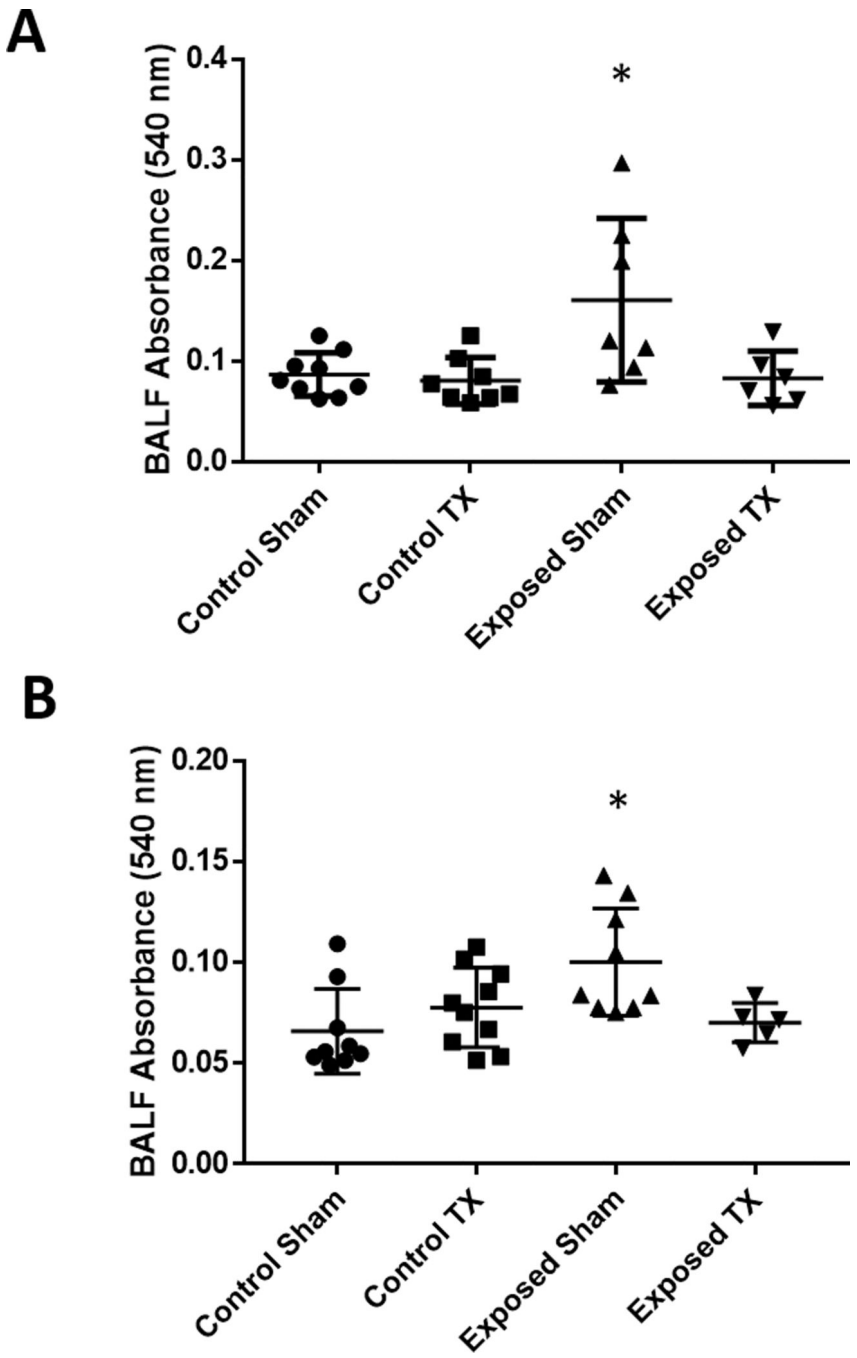

Figure 3.

Determination of heme in BALF. Animals were exposed to 1 × LD_{50} of 1080, and treated animals were given MB (5 mg/kg, SC) and MSG (250 mg/kg, IP) either once at 0.5 h (A) or at both 0.5 and 2 h (B). BALF was collected 24 h after exposure. In both cases, treating with MB and MSG was able to reduce the amount of absorbance at 540 nm, indicating a reduction in Hb in BALF. Error bars indicate standard deviation with average marked, $n = 5–8$, * $P < 0.05$ with a one-way ANOVA followed by Dunnett's multiple comparison test versus sham control.

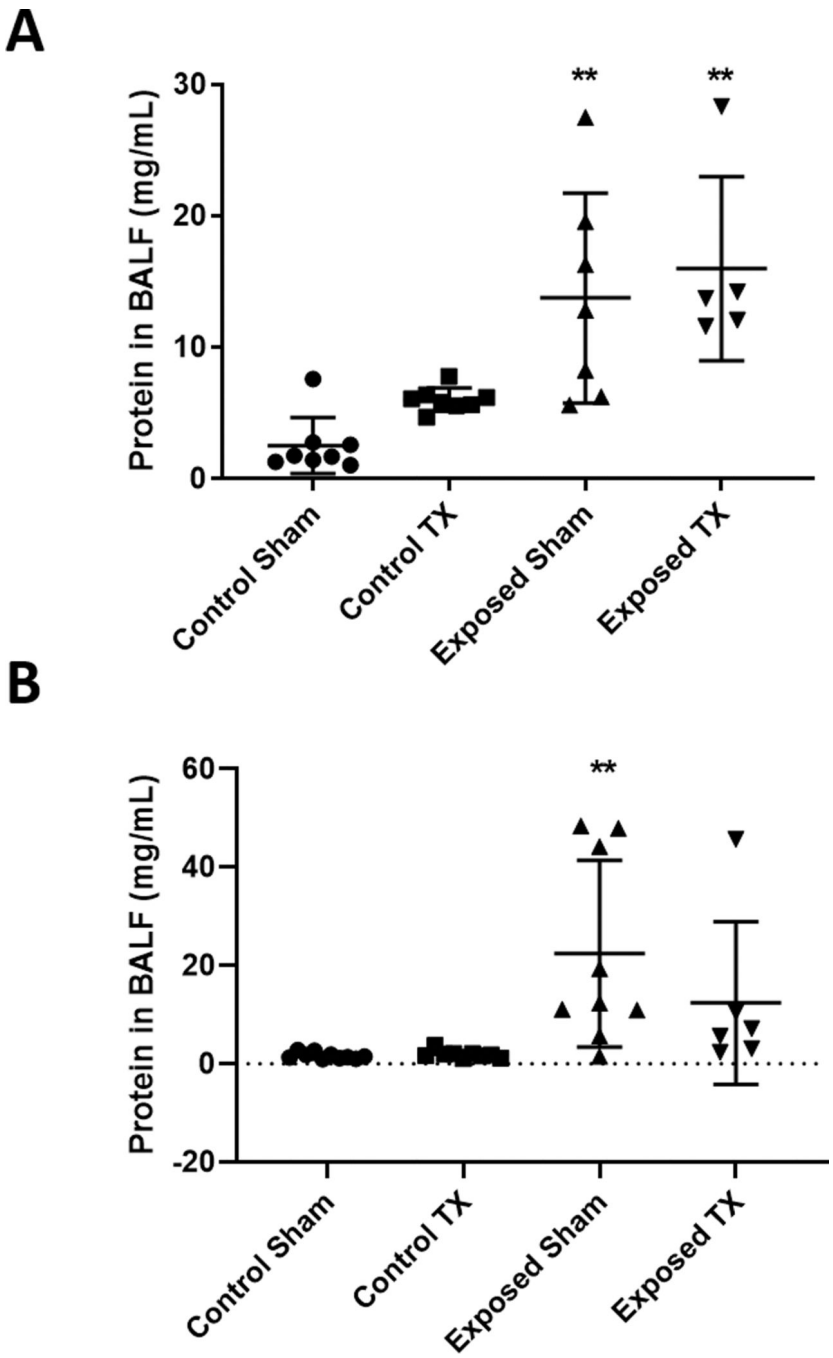

Figure 4.

Protein content in BALF. Animals were exposed to 1 × LD_{50} of 1080. Treated animals were given MB (5 mg/kg, SC) and MSG (250 mg/kg, IP) either at 0.5 h (A) or at 0.5 and 2 h (B). BALF was collected 24 h after exposure and total protein content was analyzed. Exposure to 1080 caused an increase in protein in the lungs, consistent with the development of pulmonary edema. A single administration of MB and MSG was unable to reduce the amount of protein in BALF (A), but 24 h after two administrations, the elevated protein concentration was no longer significant (B). Error bars indicate standard deviation with

average marked, $n = 5–10$, $^{**}P < 0.01$ with a one-way ANOVA followed by Dunnett's multiple comparison test versus sham control.

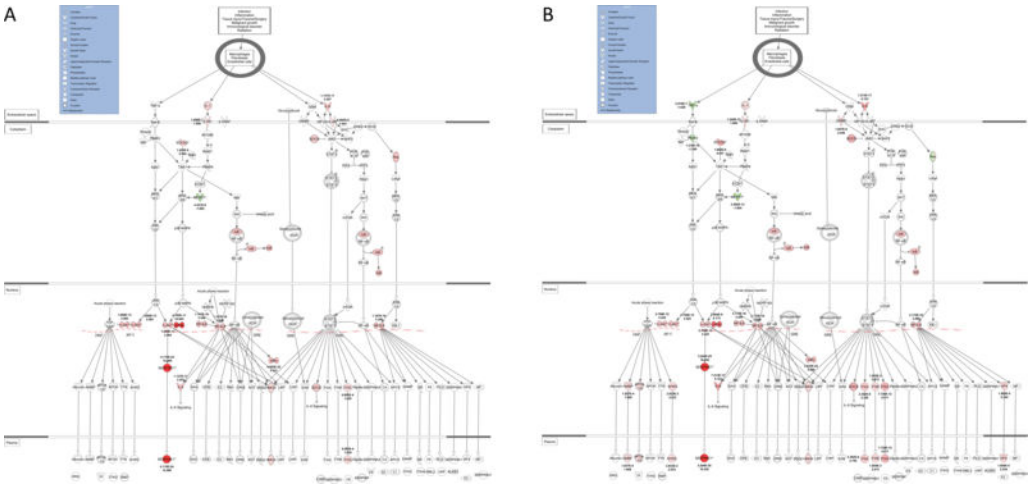

Figure 5.

Transcriptomic pathway analysis. Analysis of the acute phase response pathway in cardiac tissue at 2 (A) and 3 (B) h after 1080 exposure. Downregulated genes are shown in green and upregulated genes are in red. The *P* value and fold change, respectively, of significantly altered genes are shown adjacent to the affected genes. The acute phase response pathway was predicted to be activated at 2 h postexposure (Z-score: 2.31) but not at 3 h (Z-score: 1.79).

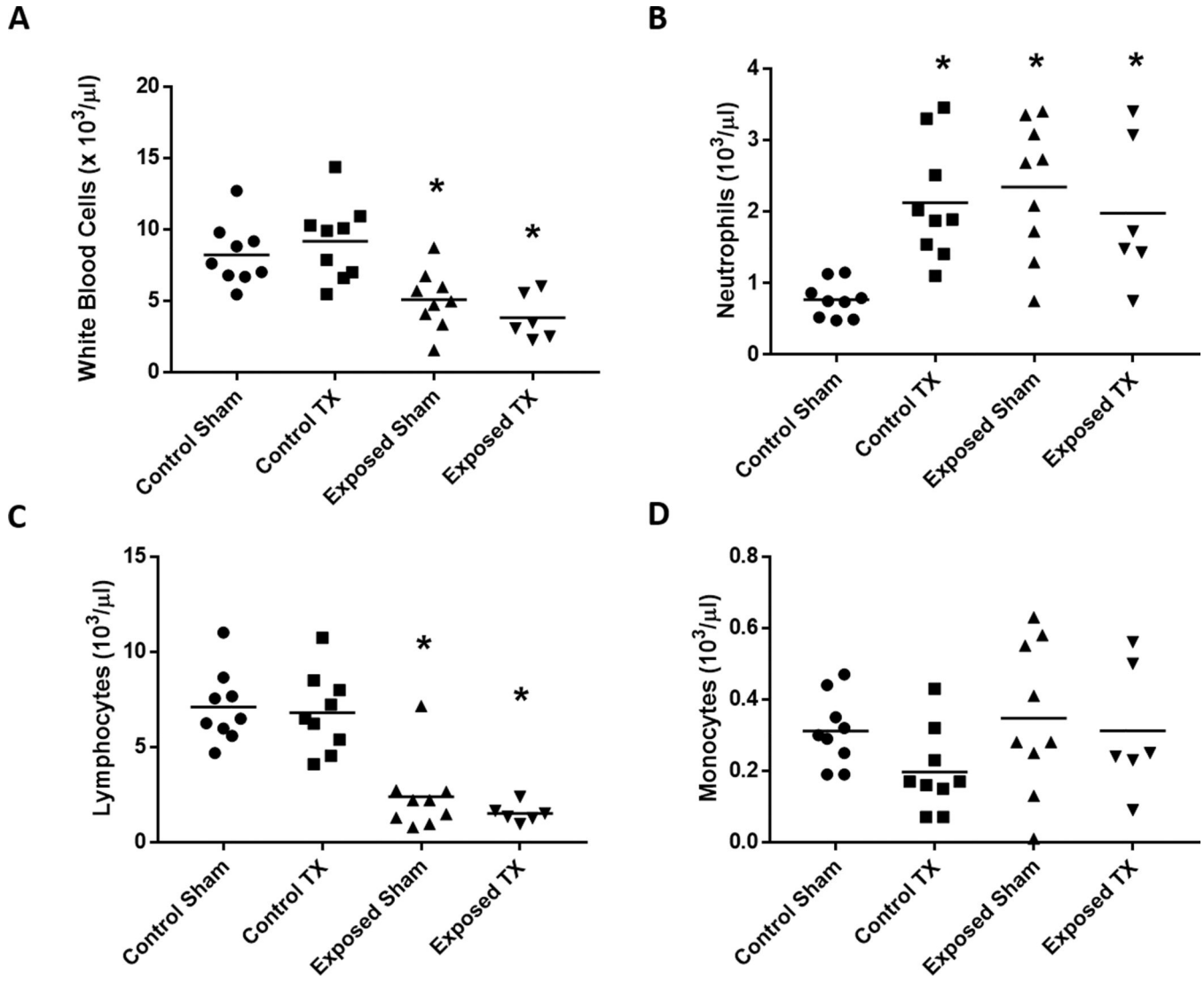

Figure 6.

White blood cell (WBC) count. Treated animals were given MB (5 mg/kg, SC) and MSG (250 mg/kg, IP). Injections were administered at 0.5 and 2 h after exposure. From animals that survived to 24 h, blood was collected from the descending aorta, and a complete blood count was performed with an automated hematology analyzer. Overall, the WBC counts were decreased in exposed animals (A), which was driven largely from a reduction in lymphocytes (C). Neutrophils were elevated in both exposed and treated control animals (B), while monocytes remained largely unchanged (D). Dash = average, $n = 6–110$, $*P < 0.05$ with a one-way ANOVA followed by Dunnett's multiple comparison test versus sham control.