NeuroToxicology 38 (2013) 33–41

Contents lists available at SciVerse ScienceDirect

NeuroToxicology

Urinary delta-ALA: A potential biomarker of exposure and neurotoxic effect in rats co-treated with a mixture of lead, arsenic and manganese

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ARTICLE INFO

Article history:
Received 19 April 2013
Accepted 3 June 2013
Available online 11 June 2013

Keywords:
Metal mixtures
Lead, arsenic and manganese
Neurotoxicity
Biomarkers
Delta-aminolevulinic acid

ABSTRACT

Lead (Pb), arsenic (As) and manganese (Mn) are neurotoxic elements that often occur in mixtures for which practically no information is available on biomarkers (BMs) for the evaluation of exposure/effects. Exposures to these metals may increase delta-aminolevulinic acid (delta-ALA), which in itself may potentiate neurotoxicity. The objective of this study was to investigate the utility of urinary delta-ALA (delta-ALA-U) levels as BM of exposure and/or neurotoxic effects induced by this mixture. Five groups of Wistar rats were treated for 8 days with Pb (5 mg/kg), As (60 mg/L), Mn (10 mg/kg), the 3-metal mixture (same doses of the single metals), and control group. Motor activity was evaluated and 24-h urine collected before and after the treatment. 24-hours (h) after the last dose, the rats were sacrificed and the brains removed for analyses. Delta-ALA and metal levels were determined in brain and urine. Co-treated rats showed a significant ($p < 0.05$) correlation between increased Pb, As, Mn and delta-ALA levels in the brain and decreased motor activity. Delta-ALA-U concentrations were higher in the mixture-treated group than the sum of the delta-ALA-U levels in each single-treated groups and discriminated ($p < 0.05$) between the mixture and untreated rats. Moreover, delta-ALA-U was correlated ($p < 0.05$) with brain delta-ALA levels. These results establish that treatments with this metal mixture exacerbate behavioral dysfunction, increasing most prominently brain Pb levels. This study is the first to establish that delta-ALA-U levels represent a sensitive BM of exposure/neurotoxic effect to this metal mixture.

1. Introduction

In today's industrialized world the sources of exposure to metals in occupational settings, polluted water, foodstuffs and in the environment, are ubiquitous (Ferrer, 2003). Human exposure to metals is a problem of global magnitude (Toscano and Guilarte, 2005). Lead (Pb), arsenic (As) and manganese (Mn) are among metals/metalloids that represent major public health concerns (ATSDR, 2007a,b,c). In fact, Pb remains the main environmental heavy metal pollutant (Charlet et al., 2012; Mameli et al., 2001) with estimates that combustion of leaded gasoline accounts for up to 90% of atmospheric Pb deposition. In addition, metal smelting activities contribute to Pb accumulation both in the atmosphere and soil (ATSDR, 2007c). Occupational exposures to Pb are common in the manufacturing of batteries, sheet lead and ceramic industries (Patrick, 2006). Pb poisoning from water supplies (Jemiola-Rzeminska et al., 2007) contaminated by mining and industrial waste (computer chips, wood preservatives and agrochemicals), as well as occupational settings (Rodríguez et al., 2003) is common. Increased As levels in the environment are mainly attributable to industrial products and wastes, agricultural pesticides and mine drainage. Occupational exposures to As also occur in industrial settings, particularly in nonferrous smelting, electronics, wood preservatives, glass manufacturing and application of arsenical pesticides (Kakkar and Jaffery, 2005). While an essential metal, excessive exposures to Mn may result from food and drinking water, as well as in clinical and occupational settings (Batterman et al., 2011; Bowler et al., 2006). The use of methylcyclopentadienyl manganese tricarbonyl (MMT), a substitute for leaded gasoline, the agricultural runoff with agrochemicals and activities, such as steeling and mining have been associated with increased Mn exposure (Martinez-Finley et al., 2012).

Given their co-existence in soil and atmosphere, exposure to Pb, As or Mn does not occur in isolation (Kordas et al., 2010). Indeed, in the real world, exposures to complex mixtures are the rule, rather than exception (Scherer, 2005). While the health sequalae of individual metal exposures may be known, there is a dearth of information on health outcomes subsequent to metal mixture exposures (Mowat and Bundy, 2002). Therefore, studying single

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http://dx.doi.org/10.1016/j.neuro.2013.06.003

metal exposures may fail to adequately predict health risks (Kordas et al., 2010).

Over the last several decades, the incidence of neurological diseases has increased (WHO, 2006). Given their environmental persistence and bioaccumulation (Mameli et al., 2001) and their propensity to accumulate in the brain (ATSDR, 2007a,b,c), metals have been implicated in the etiology of several neurodegenerative diseases (Weiss, 2010). Pb, As and Mn are established neurotoxic metals that readily cross the blood-brain barrier (BBB) (Balbuena et al., 2011; Martinez-Finley et al., 2012; Yokel, 2009). Chronic exposure to As or Pb induce peripheral motor nerve dysfunction (Blom et al., 1985; Mameli et al., 2001) with a broad range of behavioral disturbances (Gurer and Ercal, 2000; Halatek et al., 2009). Mn poisoning results in an irreversible condition known as "manganism," a neurodegenerative disorder that resembles Parkinson disease in both symptomatology and the underlying cellular mechanisms (Ellingsen et al., 2008; Martinez-Finley et al., 2012).

Neurological disorders induced by chronic metal exposure can be progressive and manifest clinically decades after the initial exposure (Gil and Pla, 2001). The onset of neurotoxic effects is largely subtle, insidiously manifested and unidentifiable as a clearly defined disease (Shy, 1993). Accordingly, biomarkers (BMs), as observable endpoints in a continuum of events from exposure to disease, have become increasingly important for the detection and diagnosis of early poisoning (Kakkar and Jaffery, 2005; Scherer, 2005). Studies on the interactions between metals, using BMs have been recognized as necessary and timely (Wang and Fowler, 2008). Nevertheless, despite of the existence of several BMs for Pb, As or Mn induced toxicity (Cowan et al., 2009; Heitland and Köster, 2003; Higashikawa et al., 2000), practically no information exists on the effects of co-exposure to these three metals and potential BMs of toxicity.

Useful BMs should reflect not only the exposure, but the onset of biochemical/physiological changes in cells and tissues (Scherer, 2005). With respect to metals, its detection in biological samples is the most common exposure BM (Phoon, 1998), with urinary analysis (a non-invasive procedure) representing the preferred media (Miller et al., 2004). Several attempts have been made to use urinary Pb as a surrogate of blood Pb levels, the traditional and preferred BM for the assessment of Pb exposure (Fukui et al., 1999; Patrick, 2006), but the approach is of limited value since it is not applicable on an individual basis (Fukui et al., 1999). Urinary As measurements are considered a reliable exposure BM, because urinary elimination is the major route for excretion of this metalloid (De Vizcaya-Ruiza et al., 2009). Urinary Mn levels are also of limited value, since they indicate an average level of exposure on a group basis, but are difficult to extrapolate on an individual basis (Batterman et al., 2011).

As for BMs of effect, a shared target for Pb, As and Mn toxicity is heme biosynthesis (Bhadauria and Flora, 2004; Maines, 1980; Patrick, 2006). Delta-ALA is the first precursor in heme synthesis (Ennis et al., 2003), formed from glycine and succinyl CoA, in a reaction catalyzed by deltaaminolevulinic acid synthetase (ALAS). Subsequently, delta-aminolaevulinic acid dehydratase (ALAD) catalyzes the condensation of two molecules of delta-ALA to form porphobilinogen, another heme precursor (Makino et al., 2000). Suboptimal condensation of two delta-ALA molecules leads to decreased heme formation, in turn, stimulating ALAS via a negative feedback loop, resulting in increased delta-ALA levels both in circulating blood and urine (Gurer and Ercal, 2000).

The best-known hematological effect of Pb is the interference with ALAD (ATSDR, 2007c; Patrick, 2006). ALAD is also a highly sensitive BM of As (Bhadauria and Flora, 2004). Little is known about the potential of Mn to interfere with heme biosynthesis; however Mn inhibits ALAS in both liver and brain (Maines, 1980). Increased plasma delta-ALA concentrations correlate with Pb-induced neurological disturbances (Gurer and Ercal, 2000; Olympio et al., 2009). ALA exerts a number of effects, including the inhibition of Na^+, K^+-ATPase and adenylate cyclase activities, free radical formation followed by oxidative damage and alterations on gamma-aminobutyric acid (GABA) and glutamate uptake and release (Demasi et al., 1996; Emanuelli et al., 2003; Juknat et al., 1995).

Several methodologies are used to select the most adequate BMs of exposure and/or effect in determining health risk in exposed populations. Correlation analysis is a common method, where it is determined whether a significant correlation exists between the levels of a biological parameter or endpoint(s) of disease and exposure to specific chemical(s). These parameter(s) may then be used as BMs of exposure and/or effect (Casarett and Doull's, 2007). An accurate BM must have the ability to adequately identify or differentiate one condition (or outcome) from another. In fact, the growing need for rigorous evaluation of new BMs is leading to the recognition that the use of appropriate statistical techniques is essential for the accurate evaluation of their clinical relevance. The diagnostic accuracy of a BM is most commonly measured by the calculation of its sensitivity and specificity. Sensitivity is the proportion of patients who are correctly categorized as exposed or having disease, among those who truly are exposed or have the disease. Similarly, specificity is the proportion of patients who are correctly categorized as non-exposed or not having the disease among all patients who truly do not have the exposure or disease (Soreide, 2008). In this context, receiver-operating characteristic (ROC) curve analysis is emerging as a useful tool to accurately assess BMs (Cai and Pepe, 2002; Doecke et al., 2012; Shin et al., 2009; Søreide, 2009; Wu et al., 2010). ROC has been previously applied in the assessment of BMs of exposure to Pb (Sakai, 2000).

Given that Pb, As and Mn interfere with delta-ALA metabolism and that an association exists between brain delta-ALA accumulation and neurotoxicity, the present study was designed to investigate if delta-ALA-U levels represent an adequate BM of exposure and/or neurotoxic effect in rats co-treated with Pb, As and Mn. The manifestation of shared neurotoxic endpoints by Pb, As and Mn led to the hypothesis that treatments with a mixture of these metals will result in interactive toxic responses. Specifically, we tested in an in vivo rat model, whether co-exposure to these 3 metals was interactive (additive and/or synergistic), and whether correlations could be established between several BMs and behavioral outcomes.

2. Experimental procedure

2.1. Chemicals

Chemicals were obtained from the following sources: Pb, As and Mn standards for Graphite Furnace Atomic Absorption Spectrometry (GFAAS) from Fluka, di-sodium hydrogen phosphate p.a. (Na_2HPO_4; ≥99%), hydrogen peroxide 30% (H_2O_2), magnesium matrix modifier for GFAAS ($Mg(NO_3)_2{\cdot}6H_2O$), nitric acid 65% suprapure (HNO_3), potassium dihydrogen phosphate (KH_2PO_4; 99.5%), delta-aminolevulinic acid standard, sodium acetate (CH_3COONa), and p-dimethylaminobenzaldehyde ($C_9H_{11}NO$) from Merck; acetic acid from Panreac; hydrohloric acid for ultratrace analysis (HCl); lead acetate trihydrate puriss. p.a. ($C_4H_6O_4Pb{\cdot}3H_2O$), manganese chloride tetrahydrate ($MnCl_2{\cdot}4H_2O$; 99.99%) and sodium (meta) arsenite purum p.a. (AsO_2Na; ≥99%) from Sigma.

2.2. In vivo assay

A sub-acute assay was performed in male Wistar rats (weighting 165–206 g) from Charles River Laboratories®, Barcelona. All experiments were performed in accordance with the guiding