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Inorganic arsenic exposure affects pain behavior and inflammatory response in rat

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Abstract

Inorganic arsenic (iAs) contamination of drinking water is a worldwide problem associated with an increased risk for the development of various types of cancer and noncancerous damage. *In vitro* studies have suggested that iAs can modulate the activity of macrophages producing an over-expression of cyclooxygenase-2 (COX-2) and resulting in an increase in prostaglandin E_2 (PGE_2) concentrations in endothelial cells. These effects may lead to an *in vivo* enhancement of inflammatory and pain responses. Our aim was to determine the effect of a single dose of arsenic or subchronic exposure to arsenic on pain behavior and tissue inflammation in rats. Rats were given a single dose of sodium arsenite (0.1, 1 and 10 mg/kg i.p.) or submitted to subchronic exposure to arsenic added to the drinking water for 4 weeks (0.1, 1, 10 and 100 ppm). Inflammatory pain was assessed by using the formalin and tail-flick tests, while inflammation was evaluated with the carrageenan model. Arsenite did not induce pain or significant inflammation by itself. In contrast, arsenite in both single dose administration and subchronic exposure increased not only the inflammatory process and the underlying hyperalgesic pain, but also induced a decrease in the pain threshold. Alterations in pain processing were dependent on the arsenic dose and the length of exposure, and the underlying mechanism involved an increased release of local PGE_2. These results suggest that inorganic arsenic exposure enhances pain perception and exacerbates the pathological state of inflammatory diseases.

Keywords: Arsenic; Arsenic in muscle; Inflammatory pain; Tissue inflammation; Experimental pain

Introduction

Inorganic arsenic (iAs) is a ubiquitous metalloid found in small amounts in soils and underground water throughout the world. Drinking water containing high levels of iAs is the major source of exposure to arsenic for millions of people worldwide. Epidemiologic studies carried out in these populations have linked chronic exposure to iAs with an increased prevalence of various types of cancer (bladder, lung and skin) as well as non-cancerous diseases such as peripheral vascular disorders, including so-called "blackfoot disease" (Bates et al., 1992; Morales et al., 2000; Yang, 2006), and atherosclerosis (Simeonova and Luster, 2004). Meanwhile, acute toxic symptoms of iAs poisoning are due to severe inflammation of the mucous membranes and increased permeability of the capillaries (Pakulska and Czerczak, 2006). Most regulatory activities are focused on the potential of iAs to cause cancer; however, risk assessment of non-carcinogenic effects such as cardiovascular and inflammatory diseases is also needed to assure adequate protection of public health (Chapell et al., 1997). Although there are no epidemiologic studies relating iAs exposure to inflammatory diseases, an increased risk of mortality associated with cardiovascular disease has been reported (Engel et al., 1994; Hertz-Picciotto et al., 2000).

Inflammation plays a pivotal role in several diseases, including atherosclerosis, asthma and arthritis; all of them share at least one common mechanism, release of prostaglandins (PGs) (Schaible et al., 2002; Tsai et al., 2002). In that sense, there is clinical and experimental evidence that iAs produce or

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exacerbate inflammatory states (Bunderson et al., 2004; Chen et al., 2007; Simeonova and Luster, 2004; Tseng, 2005). For instance, iAs exposure has been well documented as a major risk factor in the development of blackfoot disease, a unique peripheral vascular disease characterized by hypercoagulability, endothelial injury, smooth muscle cell proliferation, somatic mutation, oxidative stress, and apoptosis (Tseng, 2005; Yang, 2006). A possible mechanism explaining the exacerbated inflammation state is the over-expression of inducible cyclooxygenase 2 (COX-2), the enzyme responsible for PGs biosynthesis (Gimbrone et al., 2000). For instance, thromboxane (TXB_A) over-production has been related to enhanced platelet aggregation and thrombus formation after iAs ingestion in drinking water (Lee et al., 2002). Moreover in vitro experiments have evidenced that the expression of the inflammatory mediator cyclooxygenase-2 (COX-2) was upregulated in response to arsenite exposure as demonstrated by Western blot in bovine aortic endothelial cells exposed to sodium arsenite at concentrations equivalent to 133 μM (Bunderson et al., 2004). It is likely that arsenite exposure can stimulate COX-2 expression through activating the NFκB pathway in endothelial cells (Tsai et al., 2002).

On the other hand, despite the recognition that PGs are potent mediators of inflammation and immunomodulators in inflamed arthritic tissues as well as responsible for the generation and amplification of pain signals at the peripheral and spinal level (Burian and Geisslinger, 2005; Vane et al., 1994), evidence regarding the role of iAs exposure on the PG production and inflammatory pain is lacking. Along these lines, we previously reported that acute intoxication with sodium arsenite exacerbated the nociceptive behavior of licking in mice using the formalin test, an experimental model of inflammatory pain (Aguirre-Banuelos et al., 2004).

This study was designed to extend the evidence for the modulatory role of acute and also subchronic exposure to arsenite in pain behavior as well as tissue inflammation in rats using the carrageenan-induced paw edema model. Two different experimental models of nociception, namely the formalin test and tail-flick test, were used to initially identify the mechanism underlying the arsenic-induced exacerbated pain, i.e., whether iAs amplifies peripheral hyperalgesia (formalin) or reduces the pain threshold at the spinal level (tail-flick). This is the first report to profile acute and subchronic arsenic toxicity with respect to in vivo inflammatory pain response.

Materials and methods

Chemicals

Sodium arsenite ($NaAs^{III}O_2$), antifoam B, dimethylarsenic acid (DMA^V, $(CH_3)_2As^V$ O(OH), formalin and the lambda fraction of carrageenan were purchased from Sigma-Aldrich (St. Louis, MO). Methylarsonic acid, disodium salt ($MMAs^V$), and $CH_3As^VO(ONa)_2$ were obtained from Ventron (Danvers, MA). Sodium borohydride ($NaBH_4$), and sodium hydroxide used in arsenic analysis were obtained from Merck (Mexico). Ultrapure phosphoric acid was purchased from J.T. Baker (Phillipsburg, NJ) and prostaglandin E_2 EIA kit and prostaglandin E_2 affinity purification kit were obtained from Cayman Chemical Co. (Ann Arbor, MI). All other chemicals used were from the highest purity commercially available.

Animals

Female Wistar rats ($n=270$) of 10–12 weeks old were provided by the Universidad Autonoma de San Luis Potosi animal house facilities. Rats were weighed, randomly assigned to experimental groups, and initially housed in acrylic cages in groups of 6 animals per cage. Housing conditions were a 12/12-h light/dark cycle and temperature and humidity at 18–23 °C and 40–70%, respectively. The animals were provided with food (LabDiet® 5053 containing <1 ppm arsenic; St. Louis, MO) and deionized water ad libitum during experimental protocols. All experimental protocols were approved by the local ethics and research committee and followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). All efforts were made to minimize animal suffering. Experiments were performed between 7:00 and 13:00.

The experimental design divided exposure to arsenite into acute (single-dose) and subchronic groups. In both types of exposure, rats were assigned to groups of 6 to independently test the nociceptive behavior in two experimental models, namely the formalin test and tail-flick test, as well as to evaluate inflammation with the carrageenan-induced edema test.

Acute single-dose exposure

Experimental doses were chosen based on pilot experiments which showed that arsenic was able to modify pain perception in rats, as well as based on previous reports indicating that such levels are responsible for systemic injury following occupational and environmental exposure (Kapaj et al., 2006; Martínez-Barbeito et al., 2007). Animals were injected intraperitoneally with 0.1, 1 or 10 mg/kg sodium arsenite in PBS or with PBS alone for the control group, and after 24 h the nociceptive (using the formalin or the tail-flick test) and inflammatory responses were evaluated.

Subchronic exposure

It is well known that rats are more resistant than humans regarding arsenic poisoning. Therefore, the experimental doses for these protocols were chosen over the internationally recommended guidelines for drinking water but in the range found in highly As-contaminated groundwater locations worldwide (Kapaj et al., 2006). Control animals received deionized drinking water for four weeks. Experimental groups received sodium arsenite at doses of 0.1, 1, 10 and 100 ppm in drinking water or deionized water as control group for four weeks. Arsenite solutions were prepared freshly twice a week to minimize oxidation to arsenate. On the last day of arsenic intoxication, rats were housed one per cage in Nalgene metabolism cages (Nalge Co., Rochester, NY) to collect 24-h urine for arsenic analysis. Thereafter, animals were placed in open Plexiglas observation chambers for 30 min to allow them to acclimate to their surroundings, and they were then removed for testing the nociceptive or inflammatory responses as described below.

Measurement of nociceptive activity

Formalin test. To examine the effects of arsenic on formalin-induced flinching behavior, formalin was diluted to 5% from a stock solution of 100% (formaldehyde solution, 37% w/w) and injected subcutaneously into the right hind paw in a volume of 50 μl. Immediately after the formalin injection, the rat was placed in a test chamber and was observed continuously by a blinded observer. Nociceptive behavior was quantified as the number of flinches of the injected paws during 1-min periods every 5 min up to 60 min after injection (Wheeler-Aceto and Cowan, 1991). Flinching was readily discriminated and characterized as rapid and brief withdrawal or flexing of the injected paw. The typical biphasic pattern of formalin-induced flinching is identified as an initial acute phase (0–10 min, first phase) followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (15–60 min, second phase of the experimental model) (Yaksh and Malmberg, 1994). Nociceptive response was expressed as the total number of flinches in 5-min periods.

Tail flick test. The effect of iAs on spinal reflexes was assessed by the tail-flick test. Rats were placed in a ventilated glass tube with the tail laid across a