Regulatory Toxicology and Pharmacology 73 (2015) 378–390

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

In utero arsenic exposure in mice and early life susceptibility to cancer

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ARTICLE INFO

Article history:
Received 1 December 2014
Received in revised form
14 July 2015
Accepted 28 July 2015
Available online 31 July 2015

Keywords:
Adrenal
Arsenic
Cancer
In utero
Mice
Liver
Prenatal
Transplacental carcinogenesis

ABSTRACT

In its review of the U.S. Environmental Protection Agency's toxicological review of inorganic arsenic (iAs), the National Academy of Sciences identified carcinogenic endpoints among the highest priority health effects of concern and stated the need to consider evidence that early life exposures may increase the risk of adverse health effects. Recent studies in mice suggest that *in utero* exposure to arsenic increases susceptibility to cancer later in life. These data are striking in light of the general lack of evidence for carcinogenicity in rodents exposed to iAs. To evaluate the transplacental carcinogenic potential of iAs, a detailed analysis of the toxicology literature evaluating the role of *in utero* arsenic exposure in carcinogenesis was conducted. Bladder, lung, and skin tumors, which are the tumor types most consistently reported in humans exposed to high arsenic levels, were not consistently increased in mouse studies. There was also a lack of concordance across studies for other tumor types not typically reported in humans. Therefore, we considered methodological and other critical issues that may have contributed to variable results and we suggest additional studies to address these issues. It was concluded that the available data do not provide evidence of a causal link between *in utero* arsenic exposure and cancer or indicate early life-stage susceptibility to arsenic-induced cancer, particularly at environmentally relevant doses.

1. Introduction

In its review of the U.S. Environmental Protection Agency's updated Integrated Risk Information System (IRIS) review of inorganic arsenic (iAs), the National Academy of Sciences (NAS) identified carcinogenic endpoints (skin, bladder, and lung) among the highest priority Tier I health endpoints of concern (NRC, 2013). NAS also stated that efforts should be made to consider "the growing evidence from human and animal studies that suggests that early life exposure to arsenic may increase the risk of adverse health effects."

In human populations chronically exposed to high levels of iAs in drinking water, the most consistent evidence indicates that oral iAs exposure causes skin, bladder, and lung cancer (Cohen et al., 2013; IARC, 2012; NRC, 2013). Although some studies have identified tumors at other sites, such as liver and kidney, the evidence is inconclusive for these and other cancers (IARC, 2012; NRC, 2013). Some evidence from human populations suggests early life-stage sensitivity to iAs-induced carcinogenicity. Specifically, a population in Antofagasta, Chile (and surrounding towns using the same water source) exposed to high levels of iAs in drinking water (0.87 ppm) for a discrete period of time (12 years, beginning in 1958) had higher rates of lung, bladder, laryngeal, kidney and liver cancer mortality as adults when exposure included early childhood or *in utero* plus early childhood period, compared to the same age group in the rest of Chile (Smith et al., 2012, 2006). A major limitation of the studies by Smith and colleagues is the ecologic study design; residence was identified based on location at the time of death, so residence during the exposure period is unknown, as is the magnitude of actual iAs exposure. As the authors note, however,

Abbreviations: DES, diethylstilbestrol; DMA, dimethylarsinic acid; EPA, Environmental Protection Agency; GD, gestational day; HCC, hepatocellular carcinoma; IARC, International Agency for Research on Cancer; iAs, inorganic arsenic; IRIS, Integrated Risk Information System; MMA, monomethylarsonic acid; NAS, National Academies of Science; NIEHS, National Institute of Environmental Health Sciences; NRC, National Research Council; NTP, National Toxicology Program; OECD, Organisation for Economic Cooperation and Development; PND, postnatal day; ppb, parts per billion; ppm, parts per million; RCC, renal cell carcinoma; TPA, 12-O-tetradecanoyl phorbol-13-acetate.

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http://dx.doi.org/10.1016/j.yrtph.2015.07.023
0273-2300/

the strength of association is too high to be explained entirely by migration of high risk individuals into the study area (Smith et al., 2006). In addition, because there was essentially one source of drinking water, people who lived in the region during the high iAs period likely drank the affected water. The specific issue of early life-stage susceptibility could be better evaluated if the authors had also reported mortality rates for individuals from the study area that were past childhood during the high iAs exposure period or born after the high iAs exposure period. As it is, the question remains whether the higher mortality rates are associated with iAs exposure irrespective of life-stage, or if there is some other confounding factor associated with living in that region. When the study was conducted, individuals born after the high exposure period would have been too young (<30 years of age) to provide a meaningful basis for evaluation of cancer. However, follow-up studies should be able to address this issue.

Evidence developed over the last 10 years in studies conducted in mice suggests that *in utero* exposure to iAs increases susceptibility to developing cancer later in life (Tokar et al., 2010, 2012; Waalkes et al., 2006a, 2006b, 2004, 2003). These data are particularly striking in light of the general lack of evidence for carcinogenicity in rodents exposed to iAs in later life. In order to more fully evaluate the transplacental carcinogenic potential of iAs, we conducted a detailed analysis of the toxicology literature evaluating the role of *in utero* iAs exposure and carcinogenesis.

2. Methods

To identify studies performed in animals involving *in utero* exposure to iAs and later examination for tumor development, an initial literature search was conducted in April 2013 in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) using the following search terms: [(arsenic) and (cancer or tumor) and (*in utero* or transplacental)]. An updated literature search was conducted using the same search terms in June 2014 to identify potential new data. Based on examination of titles and abstracts, a total of 11 publications that included *in utero* exposure of laboratory animals to iAs and subsequent follow-up to examine tumor incidences in later life were identified. The resulting studies were reviewed in detail and the reported results compared across studies as well as with data available in the open literature regarding background spontaneous tumor rates and findings in humans with iAs exposure in the drinking water. Potential methodological issues were identified and discussed.

3. Results

A literature search was conducted to identify all animal studies involving *in utero*-only exposure to iAs and evaluation for the development of tumors in later life. Eleven publications were identified that described a total of nine separate studies. All of these were conducted in mice, including three separate strains; no studies conducted in other animal species were identified in the search. All but two of the studies were conducted by a single laboratory at the National Institutes of Environmental Health Sciences (NIEHS). The seven studies that included one or more *in utero*-only exposure groups were included in the primary analysis of evidence for early-life susceptibility. Studies that included *in utero* exposure in the dosing regimen, but without an *in utero*-only exposure group, were excluded from the primary analysis, but evaluated separately as additional supporting data. Studies that evaluated endpoints other than tumor development (e.g., parameters related to gene expression) were not included in this analysis. The studies identified from the literature search are summarized in Table 1 and discussed below.

3.1. Mouse transplacental carcinogenesis model

At NIEHS, Waalkes et al. (2003) developed a carcinogenesis model for transplacental iAs exposure in mice whereby the pregnant animals were administered sodium arsenite in drinking water during gestation *ad libitum*. Offspring from the dams received no additional iAs exposure (although some exposure would also occur during lactation, particularly in the first few postnatal days) and were followed through adulthood, at which time they were sacrificed and examined for tumor development. This model was also used in four subsequent studies from the same laboratory (Tokar et al., 2010, 2012; Waalkes et al., 2006a, 2006b, 2004) (Waalkes et al., 2006a and 2006b report data from the same study for males and females, respectively), as well as in one additional study by Nohara et al. (2012). All of the NIEHS studies were conducted at the National Cancer Institute's Frederick, MD animal facility. Ahlborn et al. (2009) conducted a similar study that included an *in utero*-only exposure group, in addition to groups with longer exposure durations during later life-stages. In some cases the mice also received co-exposures and/or administration of different forms of arsenic. A comparison of tumor sites from the five studies conducted at NIEHS is presented in Table 2.

The selection of the mouse strain used in the initial study was based on data from a preliminary investigation in which pregnant C3H/HeNCr (C3H), C57BL/6NCr, and B6C3F1/NCr mice were administered 75 ppm or 100 ppm sodium arsenite in drinking water during gestational days (GD) 8–21 (Waalkes et al., 2003). The higher dose resulted in reduced maternal water consumption (~20%) in all three strains. Decreased fetal body weight (9.9%) and crown-rump distance (4.9%) was observed in the C3H mice. No adverse effects were reported in any strain at the 75 ppm dose level. Based on these data, the investigators selected the C3H strain as the most sensitive and selected sodium arsenite dose levels of 42.5 ppm and 85 ppm for this and subsequent studies.

The general protocol of the *in utero*-only studies involved sodium arsenite exposure to pregnant mice *via* drinking water, typically 10 mice per dose group, during GD 8-18. After cessation of exposure, dams were allowed to give birth, and at 4 days postpartum, each litter was culled to no more than 8 pups. Pups were weaned at 4 weeks postpartum, with 25 animals per sex per dose randomly selected for continued observation, typically until 1.5–2 years of age, and subsequent evaluation for tumors. Thus, effects were evaluated on an individual animal basis and not by litter, a point that will be discussed further below.

3.1.1. Transplacental carcinogenesis studies at NIEHS

In their initial study, Waalkes et al. (2003) administered 0, 42.5, or 85 ppm sodium arsenite to pregnant C3H mice ($n = 10$/group) in drinking water *ad libitum* during GD 8-18. Based on measured body weights and water consumption values, the investigators estimated mean iAs intakes to be 9.55 and 19.13 mg/kg-day arsenite for the two dose groups, respectively. Male mice, in particular, experienced high mortality (≥40%) in all dose groups, including the control group. Because of significantly higher mortality in the high dose group (60% after 52 weeks), the study was terminated for males at 74 weeks of age. Mortality in female mice reached 30–40% but did not differ among groups, so the observation period was continued until the originally planned termination at 90 weeks. The excessive mortality in the high dose males was stated to be due mainly to the development of malignant hepatic tumors. Although the cause of high mortality in the control and low dose group was not specifically reported, these animals also had high liver tumor incidences. Transplacental iAs exposure resulted in significantly higher tumor incidence in both male and female mice, but the tumor types differed by sex. The incidences of liver tumors (hepatocellular