Published in final edited form as:

Toxicol Lett. 2012 March 7; 209(2): 179–185. doi:10.1016/j.toxlet.2011.12.016.

Renal, hepatic, pulmonary and adrenal tumors induced by prenatal inorganic arsenic followed by dimethylarsinic acid in adulthood in CD1 mice

Erik J. Tokar¹, Bhalchandra A. Diwan², and Michael P. Waalkes^{1,*}

¹Inorganic Toxicology Group, National Toxicology Program Laboratory Branch, Division of the National Toxicology Program, the National Institute of Environmental Health Sciences, Research Triangle Park, NC, and the National Cancer Institute at Frederick, Frederick, MD

²Basic Research Program, SAIC-Frederick, National Cancer Institute at Frederick, Frederick, MD

Abstract

Inorganic arsenic, an early life carcinogen in humans and mice, can initiate lesions promotable by other agents in later life. The biomethylation product of arsenic, dimethylarsinic acid (DMA), is a multi-site tumor promoter. Thus, pregnant CD1 mice were given drinking water (0 or 85 ppm arsenic) from gestation day 8 to 18 and after weaning male offspring received DMA (0 or 200 ppm; drinking water) for up to 2 years. No renal tumors occurred in controls or DMA alone treated mice while gestational arsenic exposure plus later DMA induced a significant renal tumor incidence of 17% (primarily renal cell carcinoma). Arsenic plus DMA or arsenic alone also increased renal hyperplasia over control but DMA alone did not. Arsenic alone, DMA alone and arsenic plus DMA all induced urinary bladder hyperplasia (33–35%) versus control (2%). Compared to control (6%), arsenic alone tripled hepatocellular carcinoma (20%), and arsenic plus DMA doubled this rate again (43%), but DMA alone had no effect. DMA alone, arsenic alone, and arsenic plus DMA increased lung adenocarcinomas and adrenal adenomas versus control. Overall, DMA in adulthood promoted tumors/lesions initiated by prenatal arsenic in the kidney and liver, but acted independently in the urinary bladder, lung and adrenal.

Keywords

arsenic; mouse; cancer; kidney; liver; early life; lung; DMA

1. Introduction

Inorganic arsenic is a human carcinogen associated with skin, lung and urinary bladder cancer and potentially linked to cancer of the kidney, liver and prostate (IARC 2004, 2009). It is estimated that tens of millions of people world-wide are exposed to potentially toxic, naturally occurring levels of inorganic arsenic in the drinking water (IARC 2004). Recent evidence indicates that inorganic arsenic likely has multiple mechanisms of carcinogenic action, depending on the ability of target cells to biomethylate the metalloid, and these

*To whom all correspondence should be addressed: NTP, NIEHS, 111 Alexander Drive, P.O. Box 12233, MD E0-07, Research Triangle Park, NC, 27709; phone 919-541-2328; fax 919-541-3970; waalkes@niehs.nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

probably include both genotoxic and/or epigenetic components (Coppin et al., 2008; Kojima et al., 2009). Accumulating evidence also indicates that early life exposure to inorganic arsenic from the drinking water can subsequently cause cancer in adulthood in humans (Liaw et al., 2008; Smith et al., 2006; Yuan et al., 2010) and in mice (Tokar et al. 2010a; Waalkes et al., 2003, 2004, 2006a,b, 2007). In human populations, *in utero* or early life inorganic arsenic exposure from contaminated drinking water is now linked to pulmonary (Smith et al., 2006), hepatic (Liaw et al., 2008) and, most recently, renal (Yuan et al., 2010) cancers. Relatively brief, but high level human inorganic arsenic poisoning via contaminated baby food during infancy was recently also linked to increased liver cancers in adults (Yorifuji et al., 2011). In general, the lung, liver, and kidney are all sites that are consistent with the probable or possible target tissues of arsenic in humans as recently defined by International Agency for Cancer Research (IARC 2004, 2009). After fetal exposure to arsenic by way of the pregnant female consuming drinking water containing inorganic arsenic, pulmonary, hepatic, adrenal, ovarian and uterine tumors can occur in the offspring in adulthood and uterine, oviduct, renal and urinary bladder hyperplasias can occur depending on the strain used (Tokar et al. 2010a,b, 2011; Waalkes et al., 2003, 2004, 2006a,b, 2007). Furthermore, additional *post partum* treatment with tumor stimulants (phorbol ester, estrogens) after fetal arsenic exposure in mice can result in tumors of the skin (Waalkes et al., 2008), urinary bladder (Waalkes et al., 2006b), and vagina (Waalkes et al., 2006a) and stimulate hepatic and liver tumor response (Waalkes et al., 2004, 2006b) in adulthood compared to the separate treatments given alone.

Thus, most of the probable or possible tumors associated with inorganic arsenic in humans (i.e. lung, skin, urinary bladder, liver; IARC 2004, 2009) can be reproduced in mice by early life inorganic arsenic exposure either alone or with fetal arsenic exposure and additional treatments after birth (Tokar et al. 2010a,b, 2011; Waalkes et al., 2003, 2004, 2006a,b, 2007, 2008). The notable exceptions where human target sites for arsenic have not been replicated in mice are the prostate and the kidney (IARC 2004, 2009). There may be good reasons for the lack of an animal model for inorganic arsenic in the prostate, as mice are a poor choice as a human prostate cancer model and rats are a poor choice with inorganic arsenic because the biokinetics of inorganic arsenic are quite divergent from humans and mice (Benbrahim-Tallaa and Waalkes et al., 2008). Tissue concordance is an important factor in consideration of how well animal models duplicate human cancer response. Although renal cystic hyperplasia, considered a preneoplastic lesion, has been observed in male CD1 mice exposed to arsenic *in utero* (Waalkes et al., 2006b), overt significant increases in renal cancers have not been induced by early life arsenic exposure in this rodent model. In this regard, Yuan et al. (2010) very recently reported on renal cancer in a human population from Chile exposed to a rapid onset of high levels of arsenic in the drinking water then experiencing a steep decline several decades later with installation of water treatment plants. Some of the most stunning data from this work is that early life exposure to arsenic for persons born just before or during the high exposure period show a highly significant mortality rate ratio of 7.1 for kidney cancer in only their third decade of life (Yuan et al., 2010). This led to the conclusion that early life arsenic exposure caused a high rate of renal cancer mortality and that this mortality occurred early in this human population (Yuan et al., 2010). The concept that early life exposure to toxicants can impact development of disease in adulthood, such as cancer, is toxicologically akin with the nutritionally based “Barker hypothesis” (Barker 2007; Morley 2006), where fetal under-nutrition is associated with a change in susceptibility to a variety of disease states in humans after reaching adulthood. Implicit in the data of Yuan et al (2010) is that developmental inorganic arsenic exposure has set in place factors driving the formation of renal cancer at both a higher rate and earlier age of onset, both of which could be viewed as alterations in sensitivity or susceptibility. Clearly these data call for additional studies, including rodent work, to develop a model for this early life arsenic-initiated renal cancer in order to help define mode of action.

In this regard, dimethylarsinic acid (DMA) is a biomethylation product of arsenic in humans and mice (IARC, 2004) and has been used as a tumor promoter in rodents in several studies after various organic carcinogens (see IARC, 2004 and Tokar et al., 2010b for review). In one such study, Yamamoto et al. (1995) gave adult rats a low level of an organic carcinogen “cocktail” intended to initiate tumors in various organs, including the kidney. They followed this by chronic oral exposure to DMA in the drinking water (Yamamoto et al., 1995). Although malignant renal tumors were not observed with DMA alone, and only benign renal lesions were seen with the low level carcinogen cocktail alone, initiation with the organic carcinogen cocktail followed by oral DMA promotion caused a 35% rate of malignant renal tumors (Yamamoto et al., 1995). DMA is also a urinary bladder carcinogen by itself in adult rats (Arnold et al., 2006; Wei et al., 1999, 2002), but has not been shown to be similarly effective in adult mice (IARC 2004, 2009; Tokar et al., 2010a, b, 2011). Maternal exposure to inorganic arsenic in the drinking water during gestation in several strains of mice results in a significant level of urinary bladder hyperplasia in the offspring during adulthood (Tokar et al., 2010a; Waalkes et al., 2006a,b), including CD1 mice (Waalkes et al., 2006a,b), which can be promoted to advanced tumors by other treatments (Waalkes et al., 2006a,b). Chronic oral exposure to DMA in the drinking water will also act as a bladder tumor promoter in rats after treatment with low level organic carcinogens (Wanibuchi et al., 1996; Yamamoto et al., 1995).

Thus, we attempted to develop a model of renal and bladder cancer involving arsenical exposure in male CD1 mice. Therefore, in the present work male CD1 mouse offspring whose mothers had consumed drinking water containing inorganic arsenic during pregnancy, and which can develop renal hyperplasia and urinary bladder hyperplasia as adults (Waalkes et al., 2006b), were additionally treated with chronic DMA during adulthood in hopes of promoting renal and urinary bladder hyperplasias on to more advanced malignancies.

2. Materials and Methods

2.1 Animals and treatment

Animal care was provided in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources 1996). The animals were treated humanely and with regard for alleviation of suffering. Mice were housed under conditions of controlled temperature, humidity and light cycle. A basal diet (5L79; Ralston Purina, St. Louis, MO) and acidified water were provided *ad libitum*. The NCI-Frederick animal facility, where the biopsy portion of the present study was conducted, and its animal program are accredited by the American Association for Accreditation of Laboratory Animal Care.

For arsenic treatment, sodium arsenite (NaAsO_2 ; purity 98%) and dimethylarsinic acid (DMA; purity 98%) were obtained from Sigma Chemical Co. (St. Louis, MO) and added to the drinking water in all cases. The levels of inorganic arsenic used for exposure during *in utero* life were 0 (control) and 85 ppm arsenic. Sixty timed primigravid mice were randomly divided into groups of 10 or 20 and given drinking water with sodium arsenite at 0 (control or DMA alone; 30 pregnant mice), or 85 ppm arsenite *ad libitum* (arsenic alone or arsenic plus DMA; 30 pregnant mice) from day 8 to 18 of gestation. Treatment lasted through day 18 of gestation. Dam body weights (daily) water consumption (gestation days 7, 8, 14, 18), and average litter size at birth were unaltered by arsenic treatment. At birth litters were culled to no more than 8 pups. After birth, male offspring were weaned at 4 weeks and randomly put into groups (initial n of either 50 or 25) based on maternal exposure and then exposed to DMA (200 ppm) or unaltered drinking water for up to 104 weeks of age when the study was terminated. The initial group sizes were: control, n = 50; fetal arsenic alone n = 50; fetal

arsenic plus subsequent DMA from weaning through adulthood, n = 25; and DMA alone from weaning through adulthood, n = 25. Thus, all inorganic arsenic exposure occurred during gestation via maternal drinking water. All DMA exposure occurred from the time of weaning (4 weeks of age) until the study ended (104 weeks of age) or the individual animal required termination and was via the drinking water. Offspring weights were recorded weekly from birth until weaning at 4 weeks and then at 5 week intervals and were not impacted by treatments. Only terminal body weights are reported for brevity. Mice were checked daily and killed by CO₂ asphyxiation when significant clinical signs developed or at the intended end of the study at 104 weeks of age. Litters of origin were not followed in this study and, although litter effects could not be assessed, the use of 20 litters/group of 50 offspring or 10 litters/group of 25 offspring would have minimized any such impact (maximum 2–3 mice/litter going to any particular group). The doses of inorganic arsenic and DMA were selected based on prior study in rodents (Arnold et al., 2006; Waalkes et al., 2006b) and although higher than what would be expected for human exposure, it has been noted that it often takes up to 100 times the toxic dose of arsenic in humans for laboratory animals to show the same or similar toxic effect (Carter et al., 2003).

2.2 Pathological Assessment

The animal portion of this study was designed and performed entirely within the National Cancer Institute, and it does not reflect the standard procedures, depth or extent of a typical National Toxicology Program tumor endpoint study. A complete necropsy was performed on all moribund animals, animals found dead, or on mice at terminal sacrifice. The following tissues were routinely taken: liver, lung, kidneys, spleen, adrenals, urinary bladder, testes, gall bladder, thymus, skin, and grossly abnormal tissues. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin for histological analysis. The urinary bladder was inflated with fixative prior to being embedded. Pathological assessments were performed such that there was no pre-knowledge of the treatment.

2.3 Data Analysis

Data are expressed as tumor incidence or mean ± SEM for body weights. The decrease in number of mice available for assessment from the initial starting number represents animals found dead and considered too autolytic for appropriate histological evaluation. In cases where more than one separate tumor occurred in a single tissue, it is counted as a case of the highest grade tumor and the lower grade tumor is noted in the tables as co-occurring. This would apply to lung and liver where separate adenoma and carcinoma in the same tissue were observed. A mouse with multiple proliferative lesions (i.e. relevant hyperplasia, adenoma and carcinoma) was assigned only to the group dictated by the highest grade lesion. In no case was a liver hepatocellular carcinoma observed to arise in a liver adenoma. Incidence data were compared by one-sided Fischer's exact test. Body weight and average survival data were compared by ANOVA followed by a two-sided Dunnett's test. A $p \leq 0.05$ was considered statistically significant in all cases.

3. Results

3.1 Impact of treatments on survival and body weights

Pregnant CD1 mice were given drinking water with sodium arsenite at 0 or 85 ppm arsenite *ad libitum* from gestation day 8 to 18, and allowed to give birth. After birth and weaning at 4 weeks of age male offspring were randomly put into groups based on maternal gestational exposure and then either exposed to DMA (200 ppm) or unaltered water (arsenic alone) for up to 104 weeks of age when the study was terminated. Fetal exposure to arsenic during gestation via maternal consumption of drinking containing inorganic arsenic will henceforth

be called “fetal arsenic” or otherwise appropriately qualified. All DMA exposure was from weaning (4 weeks of age) until the study ended (104 weeks) or the animal required termination and will henceforth be called “adulthood DMA” or otherwise appropriately qualified. Thus, the four test groups of mice were: control, fetal arsenic alone, adulthood DMA alone and fetal arsenic plus adulthood DMA. The level of DMA used did not alter drinking water consumption (not shown). Table 1 shows that the average survival of the male offspring was unaltered by the prenatal, postnatal or combined arsenical treatments. Offspring weights were recorded weekly from birth until weaning at 4 weeks and then at 5 week intervals. The various treatments alone or in combination did not alter body weight of the male offspring at any time in the study. Only terminal body weights are reported for brevity (Table 1).

3.2 Effects of prenatal maternal inorganic arsenic followed by adulthood DMA exposure on renal and hepatic lesions

Table 2 shows the impact of developmental exposure via the maternal system to inorganic arsenic with or without additional DMA exposure during adult life on the incidence of renal and hepatic proliferative lesions. No proliferative lesions of any kind were seen in control kidneys. In mice exposed to prenatal arsenic alone renal cystic hyperplasias of the kidneys were significantly increased compared to control. One renal adenoma and one renal cell carcinoma (RCC) were observed in the arsenic alone mice, and, although not in statistical excess, the latter is noteworthy because of its rarity. DMA alone caused no renal tumors and did not significantly increase renal hyperplasia. However, when fetal arsenic exposure was combined with DMA exposure during adulthood a significant ($p < 0.05$) increase occurred in RCC incidence (13%) along with a significant increase in total renal tumor incidence (17%) when compared to control (0%). Furthermore, the incidence of renal hyperplasias was also increased in the arsenic plus DMA group (17%). Together, fully 35% of the mice exposed to arsenic *in utero* plus DMA in adulthood had a renal proliferative lesion (hyperplasia or tumor).

With regard to hepatic lesions (Table 2), the differences between groups were found primarily in malignant tumors, specifically hepatocellular carcinoma (HCC). Fetal arsenic exposure alone caused a significant 3.3-fold increased incidence of HCC to 20% when compared to control incidence (6%). DMA alone during adult life had no impact hepatocellular (HC) tumor incidence when compared to control. However, DMA given through adulthood after fetal arsenic exposure basically doubled the rate of HCC (43%) compared to arsenic alone (20%), which is a significant increase compared to both control and arsenic alone incidence. Arsenic plus DMA also caused an increase in total HC tumors not seen in other groups. Arsenic alone significantly increased the incidence of hepatic hemangiosarcoma (11%) over control, although this finding did not carry over to the arsenic plus DMA group, so its significance is unclear. Spontaneous liver tumors in this study with male CD1 mice were somewhat higher compared to our prior work with the same strain (Waalkes et al., 2006b).

To more fully appreciate the augmentation that DMA treatment during adulthood had on the effects of prenatal inorganic arsenic in liver and kidney, these data are shown visually in Figure 1. DMA exposure through adulthood when given after fetal arsenic exposure markedly increased RCC and HCC (Figure 1). In both cases, DMA given through adulthood after prenatal arsenic significantly increased incidence above control, and with HCC, this increase was also above the incidence of arsenic alone.

3.3 Effects of prenatal inorganic arsenic and adulthood DMA on lung, adrenal and urinary bladder lesions

Table 3 shows the effects of oral DMA exposure through adulthood after fetal inorganic arsenic exposure via the mother on proliferative lesions of the lung, adrenal, and urinary bladder. All three groups treated with arsenicals (arsenic alone, DMA alone and arsenic plus DMA) showed an increase in incidence of lung adenocarcinomas to similar levels (24–35%) compared to control (6%). In the mice treated during development (arsenic alone, arsenic plus DMA) this response was also reflected in an increase in the total lung epithelial tumor incidence compared to control. In addition, fetal arsenic exposure was associated with an increase in incidence where multiple lung tumor primaries occurred in a individual animal in both the arsenic alone and arsenic plus DMA groups. Although DMA exposure alone was associated with a significant increase in lung adenocarcinoma compared to control, it appeared to have little impact on the rate of tumor formation in mice exposed to fetal arsenic.

With regard to adrenal tumors, although none occurred in control mice, all three groups that received arsenicals showed a significant response, including arsenic alone, DMA alone and arsenic plus DMA (Table 3). Thus, although DMA treatment through adulthood alone was found to induce adrenal tumors, it did not impact the rate at which these were induced by developmental exposure to inorganic arsenic. All tumors induced by arsenicals listed in Table 2 were adrenal cortical adenomas.

Likewise, hyperplasia of the urinary bladder was induced in all arsenical treatment groups (arsenic alone, DMA alone, arsenic plus DMA) to a similar extent (32–35%) and were significantly increased compared to control (2%). Thus, in the urinary bladder DMA appeared to have no effect on the incidence or grade of *in utero* arsenic-induced proliferative lesions, although DMA alone increased urinary bladder hyperplasia. Four urinary bladder tumors occurred in arsenical treated groups that were of note, including two transitional cell carcinomas in the arsenic alone group and two papillomas in the arsenic plus DMA group. The hyperplastic lesions of the transitional epithelium of the urinary bladder in the present study with arsenical treated mice were similar in nature macroscopically to urinary bladder hyperplasia induced by prenatal arsenic and post natal estrogen male CD1 mice that was recently reported (Waalkes et al., 2006b).

3.4 Spontaneous Tumors not Associated with Treatments

Table 4 shows spontaneous tumors that were detected and found not to be associated with treatment. As is typical, lymphoma was common in all groups of these CD1 mice.

4. Discussion

In rodents, inorganic arsenic in the maternal drinking water initiates events in the fetus that can be stimulated by other agents given subsequently to form cancers in several organs (Waalkes et al., 2006a,b, 2008). In adult rats, DMA can be an effective tumor promoter in various tissues after treatment with low doses of organic carcinogens (IARC 2004; Tokar et al., 2010b; Wanibuchi et al., 1996; Yamamoto et al., 1995). In the present work, we tested the hypothesis that oral DMA given through adult life would stimulate events first set into place in the fetus after maternal gestational inorganic arsenic exposure. Since we had observed preneoplastic lesions of the kidney and urinary bladder in male CD1 mice in prior work with fetal arsenic exposure (Waalkes et al., 2006b), and since DMA was known to promote early renal lesions induced by organic carcinogens to renal malignancies (IARC 2004; Tokar et al., 2010b; Wanibuchi et al., 1996; Yamamoto et al., 1995) and was shown itself to be carcinogenic in the rat urinary bladder (Arnold et al., 2006; Wei et al., 1999,

2002), we expected the kidney and urinary bladder to develop tumors with the combination of prenatal arsenic exposure and DMA through adult life. Indeed, renal cancers, most prominently RCC, were increased with prenatal arsenic plus DMA through adulthood. However, though DMA alone increased urinary bladder hyperplasias, given after fetal arsenic exposure it did not stimulate the formation or advance the grade of urinary bladder proliferative lesions beyond those induced by fetal arsenic exposure alone. In contrast, DMA given through adulthood clearly simulated HCC development beyond that induced after *in utero* arsenic exposure alone, increasing both HCC and total liver tumor incidence. Finally, DMA given through adult life had no effect on fetal arsenic-induced lung or adrenal tumors, although the arsenical biomethylation product stimulated formation of both pulmonary adenocarcinoma and adrenal cortical tumors when given alone.

The results of the current study with male CD1 mice largely reproduce prior work where fetal exposure to inorganic arsenic alone induces HCC, lung adenocarcinoma, renal cystic hyperplasia, and adrenal adenoma in male CD1 mice (Waalkes et al., 2006b). Other prior studies using developmental arsenic exposure alone in C3H or Tg.AC mice show that the liver, adrenal, and lung are often targets of developmental inorganic arsenic exposure in other strains (Tokar et al. 2010a; Waalkes et al., 2003, 2004). It is also noteworthy in the present work that oral DMA in adulthood alone induced lung adenocarcinoma, adrenal adenoma, and urinary bladder hyperplasias in mice. The observation of increased lung adenocarcinoma incidence compared to control in the DMA alone group is blunted by the fact that total lung tumors were not increased, perhaps indicating a shift in tumor grade, although DMA did not act to enhance the stage of fetal arsenic induced pulmonary tumors. Nonetheless, DMA in the drinking water at doses similar to the present study can increase lung tumor incidence in adult A/J mice (Hayashi et al., 1998) and in adult *Ogg* $-/-$ mice (Kinoshita et al., 2007). The A/J mouse strain is highly susceptible to lung carcinogenesis while *Ogg* $-/-$ mice cannot repair certain types of oxidative DNA damage (Hayashi et al., 1998; Kinoshita et al., 2007). So the present data, where DMA alone in adult mice induces lung adenocarcinoma, support this prior work showing lung carcinogenesis in mice (Hayashi et al., 1998; Kinoshita et al., 2007). There is no prior evidence that DMA induces adrenal tumors in rodents, and therefore this is an additional target site that is of interest because it is consistent with a target tissue repeatedly seen with developmental exposure to inorganic arsenic in mice (Tokar et al. 2010a,b, 2011; Waalkes et al., 2003, 2004, 2006a,b, 2007). That oral exposure to DMA alone can induce urinary bladder hyperplasia in mice is, perhaps, not unexpected, as oral DMA alone is an effective carcinogen in the urinary bladder of rats (Arnold et al., 2006; Wei et al., 1999, 2002) and is a bladder tumor promoter in rats after low level organic carcinogen treatment (Wanibuchi et al., 1996; Yamamoto et al., 1995). The fact DMA-induced urinary bladder hyperplasias did not develop further into frank tumors may be an issue of the mouse simply not being sensitive to urinary bladder carcinogenesis induced by arsenicals. In fact, the study by Arnold et al. (2006) included both rats and mice (B6C3F1 strain) exposed to oral DMA (up to 500 ppm) and found no urinary bladder tumors or preneoplasia in the male or female mice treated for up to two years. Thus, although adulthood DMA treatment and prenatal inorganic arsenic exposures can induce hyperplastic lesions of the urinary bladder in mice, development of these lesions into overt tumors may require additional non-arsenical stimulation, as seen with prior work using prenatal arsenic exposure and postnatal tamoxifen treatments leading to urinary bladder tumors in male CD1 mice (Waalkes et al., 2006b).

In this present study, the finding of renal cancer induced by the combination of prenatal arsenic followed by DMA through adult life, although at a relatively low incidence (17%), is important for several reasons. Spontaneously, renal tumors are rare in mice, particularly RCC. Furthermore, there is now human evidence that early life exposure to inorganic arsenic followed by further exposure during adult life via contaminated drinking water

causes a remarkable increase in renal cancer mortality, increasing renal cancer mortality rate ratio to 7.1 in young adults aged 30–39 (Yuan et al., 2010). Thus, the human kidney cancers seen after early life exposure in this population not only occur with higher frequency but occur more rapidly (Yuan et al., 2010). In an extended abstract, Shirachi et al. (1983) reports that rats given a single injection of diethylnitrosamine and subsequently given oral sodium arsenite develop a 70% rate of renal tumors compared to 14% rate in animals given the organic carcinogen alone, indicating arsenic, or perhaps a metabolic product that would pass through the kidney, such as a biomethylation metabolite like DMA, could be promoting renal tumors in rodents. The IARC (IARC 2004, 2009) has considered the human kidney a possible target site for arsenic carcinogenesis irrespective of life stage of exposure. Thus, in addition to the recent human evidence (Yuan et al., 2010), rodent evidence now implicates the kidney as a target site of arsenic carcinogenesis when early life arsenic exposure is followed by further arsenical exposure through adulthood.

The liver tumor response after fetal arsenic exposure was also markedly enhanced by DMA treatment through adult life, although DMA alone had no impact on liver tumors or other lesions compared to control. Yamamoto et al. (1995) produced liver tumors in adult male rats by giving a low dose organic carcinogen cocktail containing compounds which would initiate liver tumors, followed by chronic oral DMA in doses that ranged up to 400 ppm. The organic carcinogen cocktail alone produced no HCC (0/20 rats) while the rats given the carcinogens followed by 200 ppm DMA (the dose used in the present study) showed a 40% HCC rate (8/20; Yamamoto et al., 1995). This is similar to the rate of HCC (43%) produced by combined prenatal arsenic followed by DMA through adulthood in the present study. Similarly, Yamamoto et al. (1995) found DMA alone had no effect on liver tumor formation. Thus, it appears that DMA can stimulate hepatic tumor production initiated by organic carcinogens as well as prenatal arsenic exposure. DMA is a major biomethylation product of inorganic arsenic and the liver is a major site of arsenic biomethylation. This could be very important in instances with arsenic exposure during gestation that may have “primed” hepatic events to be driven to carcinogenesis in adulthood by an inorganic arsenic biomethylation product produced locally. Thus, it could be envisioned with life-long inorganic arsenic exposure, as often happens in human populations, that fetal exposure may initiate events in the liver that during adulthood are then provided with a promotional product by hepatic metabolism.

5. Conclusions

Overall, the results of the present work indicate that prenatal arsenic plus DMA through adulthood enhanced liver and kidney tumor response compared with that produced by prenatal arsenic alone. Both tumor sites are important as early life arsenic exposures have now been associated with enhanced human liver and kidney cancers (Liaw et al., 2008; Yorifuji, et al., 2011; Yuan et al., 2010). Thus, the present work provides a model where arsenicals alone can produce kidney cancer in rodents, a further step towards full target site concordance with human data. DMA did not further stimulate fetal arsenic-induced lung or adrenal tumors or urinary bladder hyperplasias. DMA through adulthood alone caused lung adenocarcinoma, adrenal adenoma and urinary bladder hyperplasia.

Acknowledgments

The authors wish to thank Drs. Bucher, Walker, Morgan and Person for critical evaluation of this manuscript and Dan Logsdon for expert technical assistance. This research was supported in part by the National Toxicology Program, NIEHS and by the Intramural Research program of the NIH, National Cancer Institute, Center for Cancer Research. This article may be the work product of an employee or group of employees of the NIEHS, National Institutes of Health (NIH), however, the statements contained herein do not necessarily represent the statements, opinions or conclusions of the NIEHS, NIH or the United States Government. This project was also supported in

part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or the policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

References

- Arnold LL, Eldan M, Nyska A, van Gemert M, Cohen SM. Dimethylarsinic acid: results of chronic toxicity/oncogenicity studies in F344 rats and in B6C3F1 mice. *Toxicology*. 2006; 223:82–100. [PubMed: 16677751]
- Barker DJP. The origins of the developmental origins theory. *J Intern Med*. 2007; 261:412–417. [PubMed: 17444880]
- Benbrahim-Tallaa L, Waalkes MP. Inorganic arsenic and human prostate cancer. *Environ Health Perspect*. 2008; 116:158–164. [PubMed: 18288312]
- Carter DE, Aposhian HV, Gandolfi AJ. The metabolism of inorganic arsenic oxides, gallium arsenide, and arsine: A toxicochemical review. *Toxicol Appl Pharmacol*. 2003; 193:309–334. [PubMed: 14678742]
- Coppin JF, Qu W, Waalkes MP. Interplay between cellular methyl metabolism and adaptive efflux during oncogenic transformation from chronic arsenic exposure in human cells. *J Biol Chem*. 2008; 283:19342–19350. [PubMed: 18487201]
- Hayashi H, Kanisawa M, Yamanaka K, Ito T, Udaka N, Ohji H, Okudela K, Okada S, Kitamura H. Dimethylarsinic acid, a main metabolite of inorganic arsenics, has tumorigenicity and progression effects in the pulmonary tumors of A/J mice. *Cancer Lett*. 1998; 125:83–88. [PubMed: 9566700]
- IARC. Some drinking water disinfectants and contaminants, including arsenic; International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risk to Humans. Vol. 84. Lyon: IARC Press; 2004. Arsenic in drinking water; p. 269–477.
- Straif K, Benbrahim-Tallaa L, Baan R, Grosse Y, Secretan B, Ghissassi N, Freeman C, Galichet L, Coglian V, Heinrich U, Samet J, Demers PA, Gerin M, Siemiatycki J, Grandjean P, Aitio A, Kauppinen T, Goldberg M, Hartwig A, Muhle H, Fubini B, Merletti F, Ikeda M, Attfield MD, Cantor KP, Fowler BA, Henderson R, Infante PF, Kane AB, Landrigan PJ, Lunn R, Rossman TG, Stayner L, Waalkes MP, Ward EM, Ward JM. IARC. A review of human carcinogens - Part C: metals, arsenic, dusts, and fibers. *Lancet Oncol*. 2009; 10:453–455. [PubMed: 19418618]
- Institute of Laboratory Animal Resources. Guide for the Care and Use of Laboratory Animals. 7. Washington, DC: National Academy Press; 1996.
- Kinoshita A, Wanibuchi H, Morimura K, Wei M, Nakae D, Arai T, Minowa O, Noda T, Nishimura S, Fukushima S. Carcinogenicity of dimethylarsinic acid in Ogg1-deficient mice. *Cancer Sci*. 2007; 98:803–814. [PubMed: 17441966]
- Kojima C, Ramirez DC, Tokar EJ, Himeno S, Drobná Z, Stýblo M, Mason RP, Waalkes MP. Requirement of arsenic biomethylation for oxidative DNA damage. *J Natl Cancer Inst*. 2009; 101:1670–1681. [PubMed: 19933942]
- Liaw J, Marshall G, Yuan Y, Ferreccio C, Steinmaus C, Smith AH. Increased childhood liver cancer mortality and arsenic in drinking water in northern Chile. *Cancer Epidemiol Biomarkers Prev*. 2008; 17:1982–1987. [PubMed: 18708388]
- Morley R. Fetal origins of adult disease. *Semin Fetal Neonatal Med*. 2006; 11:73–78. [PubMed: 16368278]
- Shirachi DY, Johansen MG, McGowan JP, Tu SH. Tumorigenic effect of sodium arsenite in rat kidney. *Proc West Pharmacol Soc*. 1983; 26:413–415. [PubMed: 6688469]
- Smith AH, Marshall G, Yuan Y, Ferreccio C, Liaw J, von Ehrenstein O, Steinmaus C, Bates MN, Selvin S. Increased mortality from lung cancer and bronchiectasis in young adults after exposure to arsenic in utero and in early childhood. *Environ Health Perspect*. 2006; 114:1293–1296. [PubMed: 16882542]
- Tokar EJ, Diwan BA, Waalkes MP. Arsenic exposure in utero and nonepidermal proliferative response in adulthood in Tg.AC mice. *Int J Toxicol*. 2010a; 29:291–296. [PubMed: 20448261]

- Tokar EJ, Benbrahim-Tallaa L, Ward JM, Lunn R, Sams RL, Waalkes MP. Cancer in experimental animals exposed to arsenic and arsenic compounds. *Critical Rev Toxicol*. 2010b; 40:912–927. [PubMed: 20812815]
- Tokar EJ, Qu W, Waalkes MP. Arsenic, stem cells, and the developmental basis of adult cancer. *Toxicol Sci*. 2011; 120(Suppl 1):S192–203. [PubMed: 21071725]
- Waalkes MP, Ward JM, Liu J, Diwan BA. Transplacental carcinogenicity of inorganic arsenic in the drinking water: Induction of hepatic, ovarian, pulmonary and adrenal tumors in mice. *Toxicol Appl Pharmacol*. 2003; 186:7–17. [PubMed: 12583988]
- Waalkes MP, Ward JM, Diwan BA. Induction of tumors of the liver, lung, ovary and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: Promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers. *Carcinogenesis*. 2004; 25:133–141. [PubMed: 14514661]
- Waalkes MP, Liu J, Ward JM, Powell DA, Diwan BA. Urogenital carcinogenesis in female CD1 mice induced by in utero arsenic exposure is exacerbated by postnatal diethylstilbestrol treatment. *Cancer Res*. 2006a; 66:1337–1345. [PubMed: 16452187]
- Waalkes MP, Liu J, Ward JM, Diwan BA. Enhanced urinary bladder and liver carcinogenesis in male CD1 mice exposed to transplacental inorganic arsenic and postnatal diethylstilbestrol or tamoxifen. *Toxicol Appl Pharmacol*. 2006b; 215:295–305. [PubMed: 16712894]
- Waalkes MP, Liu J, Diwan BA. Transplacental arsenic carcinogenesis in mice. *Toxicol Appl Pharmacol*. 2007; 222:271–280. [PubMed: 17306315]
- Waalkes MP, Liu J, Germolec DR, Trempus CS, Cannon RE, Tokar EJ, Tennant RW, Ward JM, Diwan BA. Arsenic exposure in utero exacerbates skin cancer response in adulthood with contemporaneous distortion of tumor stem cell dynamics. *Cancer Res*. 2008; 68:8278–8285. [PubMed: 18922899]
- Wanibuchi H, Yamamoto S, Chen H, Yoshida K, Endo G, Hori T, Fukushima S. Promoting effects of dimethylarsinic acid on N-butyl-N-(4-hydroxybutyl)nitrosamine-induced urinary bladder carcinogenesis in rats. *Carcinogenesis*. 1996; 17:2435–2439. [PubMed: 8968060]
- Wei M, Wanibuchi H, Yamamoto S, Li W, Fukushima S. Urinary bladder carcinogenicity of dimethylarsinic acid in male F344 rats. *Carcinogenesis*. 1999; 20:1873–1876. [PubMed: 10469637]
- Wei M, Wanibuchi H, Morimura K, Iwai S, Yoshida K, Endo G, Nakae D, Fukushima S. Carcinogenicity of dimethylarsinic acid in male F344 rats and genetic alterations in induced urinary bladder tumors. *Carcinogenesis*. 2002; 23:1387–1397. [PubMed: 12151359]
- Yamamoto S, Konishi Y, Matsuda T, Murai T, Shibata MA, Matsui-Yuasa I, Otani S, Kuroda K, Endo G, Fukushima S. Cancer induction by an organic arsenic compound, dimethylarsinic acid (cacodylic acid), in F344/DuCrj rats after pretreatment with five carcinogens. *Cancer Res*. 1995; 55:1271–1276. [PubMed: 7882321]
- Yorifuji T, Tsuda T, Doi H, Grandjean P. Cancer excess after arsenic exposure from contaminated milk powder. *Environ Health Prev Med*. 2011; 16:164–170. [PubMed: 21431798]
- Yuan Y, Marshall G, Ferreccio C, Steinmaus C, Liaw J, Bates M, Smith AH. Kidney cancer mortality: fifty-year latency patterns related to arsenic exposure. *Epidemiol*. 2010; 21:103–108.

Highlights

Arsenicals produced renal cancer in mice, in accord with human data on arsenic and kidney cancer

Fetal arsenic-induced liver cancer response was greatly magnified by DMA exposure through adulthood.

DMA alone caused lung cancer in accord with human data on arsenic and pulmonary cancer.

Figure 1. Effect of Prenatal Exposure to Arsenic with or Without DMA Exposure Through Adulthood on Renal Cell Carcinoma and Hepatocellular Carcinoma

Data are expressed as incidence (%) of tumor bearing mice. Statistical differences are defined as follows: a = different from control; b = different from arsenic alone; c = different from DMA alone. Left panel: Renal cell carcinoma; Right panel: Hepatocellular carcinoma.

Table 1

Survival and Body Weights in Male CD1 Mice Exposed to Inorganic Arsenic *In Utero* and DMA In Adulthood ^a

Treatment Group:	<u>Control</u>	<u>Arsenic Alone</u>	<u>DMA Alone</u>	<u>Arsenic + DMA</u>
Initial Group Size:	50	50	25	25
Average Survival (wks):	75.6 ± 3.4	73.9 ± 2.7	83.5 ± 3.7	81.5 ± 2.7
Body Weight at Euthanasia (g):	55.3 ± 1.6	54.7 ± 1.7	55.0 ± 1.9	53.6 ± 2.4

^aSee Methods for treatment details. Arsenic was given in maternal drinking water (85 ppm) between gestation day 8 and 18. After weaning at 4 weeks of age, DMA was given in the drinking water (200 ppm) until the study ended at 104 experimental weeks. Data given as mean ± SEM. There were no significant differences from control by two-sided Dunnett multiple comparison test.

Table 2

The Incidence of Renal and Hepatic Tumors and Proliferative Lesions in Male CD1 Mice Exposed to Inorganic Arsenic *In Utero* and DMA In Adulthood ^a

Organ:	Treatment Group (n)			
	Control (49)	Arsenic (45)	DMA (25)	Arsenic + DMA (23)
Kidney				
Hyperplasia	0 (0%)	5 (11%) ^b	2 (8%)	4 (17%) ^b
Adenoma	0 (0%)	1 (2%)	0 (0%)	1 (4%)
RCC	0 (0%)	1 (2%)	0 (0%)	3 (13%) ^b
Total Tumors	0 (0%)	2 (4%)	0 (0%)	4 (17%) ^{b,d}
Hepatic				
Adenomas	5 (10%)	5 (11%)	1 (4%)	3 (13%)
HCC	3 (6%)	9 (20%) ^b	2 (8%)	10 (43%) ^{b,c,d}
Total HC Tumors	8 (16%)	14 (33%)	3 (12%)	13 (56%) ^{b,c,d}
Hemangioma	1 (2%)	2 (4%)	3 (12%)	1 (4%)
Hemangiosarcoma	0 (0%)	5 (11%) ^b	1 (4%)	2 (9%)

^a See Methods for treatment details. RCC = renal cell carcinoma. All renal hyperplasia was cystic in nature. HCC = hepatocellular carcinoma. HC = hepatocellular. When multiple liver primary tumors occurred the mouse was counted in the category of the highest stage only. The following incidence of mice with at least one HC adenoma and one HCC occurred: Control 2; Arsenic alone 3; DMA 1; Arsenic + DMA 4.

^b Significantly different ($p < 0.05$) from control by one-sided Fisher exact test. Total HC tumor incidence for Arsenic alone approached significance ($p = 0.07$) when compared to control.

^c Significantly different ($p < 0.05$) from Arsenic alone by one-sided Fisher exact test.

^d Significantly different ($p < 0.05$) from DMA alone by one-sided Fisher exact test.

Table 3

The Incidence of Pulmonary, Adrenal and Urinary Bladder Tumors or Hyperplasia in Male CD1 Mice Exposed to Inorganic Arsenic *In Utero* and DMA In Adulthood ^a

Organ:	Treatment Group (n):			
	Control (49)	Arsenic (45)	DMA (25)	Arsenic + DMA (23)
Lung:				
Adenoma	7 (14%)	6 (13%)	3 (12%)	3 (13%)
Adenocarcinoma	3 (6%)	14 (31%) ^b	6 (24%) ^b	8 (35%) ^b
Total Epithelial Tumors	10 (20%)	18 (40%) ^b	9 (36%)	10 (43%) ^b
Multiple	1 (2%)	7 (16%) ^b	1 (4%)	5 (22%) ^b
Adrenal Cortex:				
Adenomas	0 (0%)	17 (38%) ^b	6 (24%) ^b	11 (48%) ^b
Urinary Bladder:				
Hyperplasia	1 (2%)	15 (33%) ^b	8 (32%) ^b	8 (35%) ^b
Papilloma	0 (0%)	0 (0%)	0 (0%)	2 (9%)
TCC	0 (0%)	2 (4%)	0 (0%)	0 (0%)

^a See Methods for treatment details. When multiple lung primaries occurred the mouse was counted in the category of the highest stage only. The following incidence of mice with at least one lung adenoma and one lung adenocarcinoma occurred: Control 0; Arsenic alone 2; DMA 0; Arsenic + DMA 1. Multiple lung tumors are the incidence of mice with 2 or more lung tumors of any grade. TCC = transitional cell carcinoma. Of the hyperplastic lesions in the urinary bladder the following proportion showed dysplasia: control 0%; arsenic alone 30%; DMA alone 13%; arsenic + DMA 26%.

^b Significantly different ($p < 0.05$) from control by one-sided Fisher exact test.

Table 4

Incidental Tumors Unrelated to Treatment In Male CD1 Mice Exposed to Inorganic Arsenic *In Utero* and DMA In Adulthood^a

Group	n	Tumors
Control	49	3 lymphoma, 3 leukemia, 1 hemangioma (adrenal), 1 thyroid c-cell tumor, and a myoepithelioma
Arsenic alone	45	4 lymphoma, 2 lipoma, 2 hemangioma (lung, adrenal), 1 pheochromocytoma
DMA Alone	25	3 lymphoma, 1 leukemia, 1 osteosarcoma, 1 fibrosarcoma, 1 Harderian gland carcinoma, 1 thyroid adenoma
Arsenic plus DMA	23	3 lymphoma, 1 pheochromocytoma, 1 hemangioma (adrenal), 1 fibrosarcoma

^aSee Methods for treatment details. Arsenic was given in maternal drinking water (85 ppm) between gestation day 8 and 18. After weaning, DMA was given in the drinking water (200 ppm) until the study ended at 104 experimental weeks. None of the listed tumors were altered by treatment. This does not include hepatic hemangioma or hemangiosarcoma which are discussed in the text.