February 2007
Volume 6

CENTER FOR CANCER RESEARCH
frontiers IN SCIENCE

CARCINOGENESIS

Effect of Estrogen on Arsenic-induced Urogenital Carcinogenesis

Waalkes MP, Liu J, Ward JM, Powell DA, and Diwan BA. Urogenital carcinogenesis in female CD1 mice induced by *in utero* arsenic exposure is exacerbated by postnatal diethylstilbestrol treatment. *Cancer Res* 66: 1337–45, 2006.

Environmental inorganic arsenic exposure from contaminated drinking water is a serious problem throughout the world. In humans, arsenic, a carcinogen, targets various tissues and is associated with urogenital system tumors, including urinary bladder cancers. Although arsenic is clearly carcinogenic in humans, in adult animals it has proven difficult to induce tumors with inorganic arsenic alone. Gestation, however, is a period of high sensitivity to chemical carcinogenesis in animals and probably humans. This is because of factors like rapid global proliferative growth during the fetal life stage.

We performed a series of transplacental carcinogenesis studies in which mice were exposed in the womb to arsenic via the maternal system (Waalkes MP et al. *Toxicol Appl Pharmacol* 186: 7–17, 2003; Waalkes MP et al. *Carcinogenesis* 25: 133–41, 2004; Waalkes MP et al. *J Natl Cancer Inst* 96: 466–74, 2004). This prior work showed that arsenic exposure *in utero* induced tumors and pretumorous lesions in several tissues of the offspring when they became adults. The targets of transplacental arsenic in mice included the ovaries, liver, adrenal glands, uterus, and oviducts, which are also potential targets of carcinogenic estrogens, such as synthetic estrogen diethylstilbestrol, in humans and rodents. Because arsenic showed an estrogen-like tumor spectrum in mice, we hypothesized that aberrant estrogen signaling plays a role in transplacental arsenic carcinogenesis. Estrogen receptor-α (ER-α), a key factor in estrogen signaling, helps control estrogen-induced cellular proliferative responses. ER-α overexpression increases sensitivity to estrogen carcinogenesis in mice. In our prior work, a marked overexpression of both ER-α and estrogen-related genes important in carcinogenesis was observed in adult mice bearing transplacental arsenic-induced tumors. Furthermore, in an arsenic-exposed human population with increased arsenic-associated cancers, ER-α was clearly overexpressed (Waalkes MP et al. *J Natl Cancer Inst* 96: 466–74, 2004). Thus, ER-α overexpression was associated with arsenic carcinogenicity.

In the present study, we directly tested the hypothesis that aberrant stimulation of estrogen response pathways plays a role in transplacental arsenic carcinogenesis. Specifically, the effects of postnatal diethylstilbestrol exposure on the carcinogenicity of *in utero* arsenic

exposure were explored. Pregnant mice received drinking water containing arsenic, and female offspring received diethylstilbestrol for several days after birth. In adulthood, arsenic alone induced adrenal adenomas and some urogenital tumors, including, mostly, benign tumors of the ovaries and uterus. Diethylstilbestrol alone induced some tumors (primarily cervical), but when given after *in utero* arsenic, it synergistically increased urogenital tumor incidence, multiplicity, and progression. For instance, compared with the incidence of urogenital malignancies in the control (0%), arsenic alone (9%) and diethylstilbestrol alone (21%) groups, arsenic plus diethylstilbestrol induced a 48% incidence of malignant urogenital tumors. Of the urogenital tumors induced by arsenic plus diethylstilbestrol, 80% were malignant, and 55% were in multiple sites, while 60% precipitated early death. Arsenic plus diethylstilbestrol increased ovarian, uterine, and vaginal tumors and urinary bladder proliferative lesions (tumors plus preneoplasias; Figure 1), including several transitional cell carcinomas, the urinary bladder tumor type seen in humans exposed to arsenic. Uterine and bladder carcinomas induced by arsenic plus diethylstilbestrol greatly overexpressed ER-α and *pS2,* an estrogen-regulated gene. In neonatal uteri, prenatal arsenic increased ER-α expression and enhanced estrogen-related gene expression induced by postnatal diethylstilbestrol. Thus, arsenic acts with estrogens to enhance production of female mouse urogenital cancers.

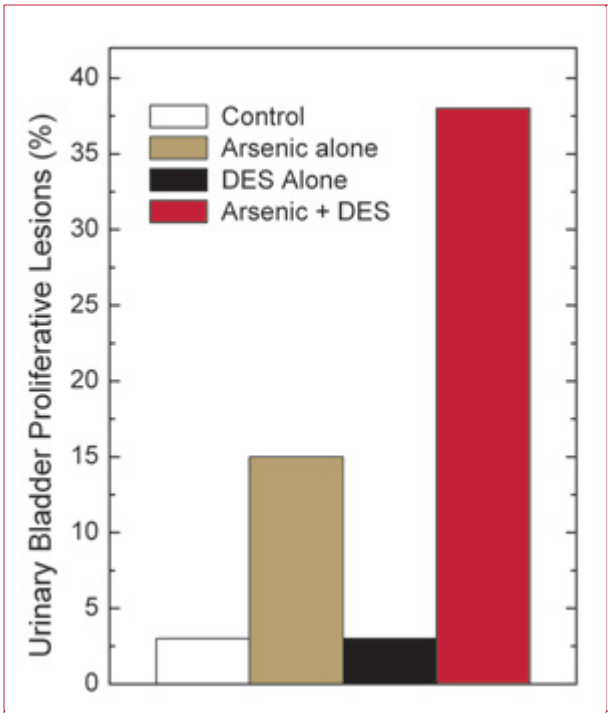


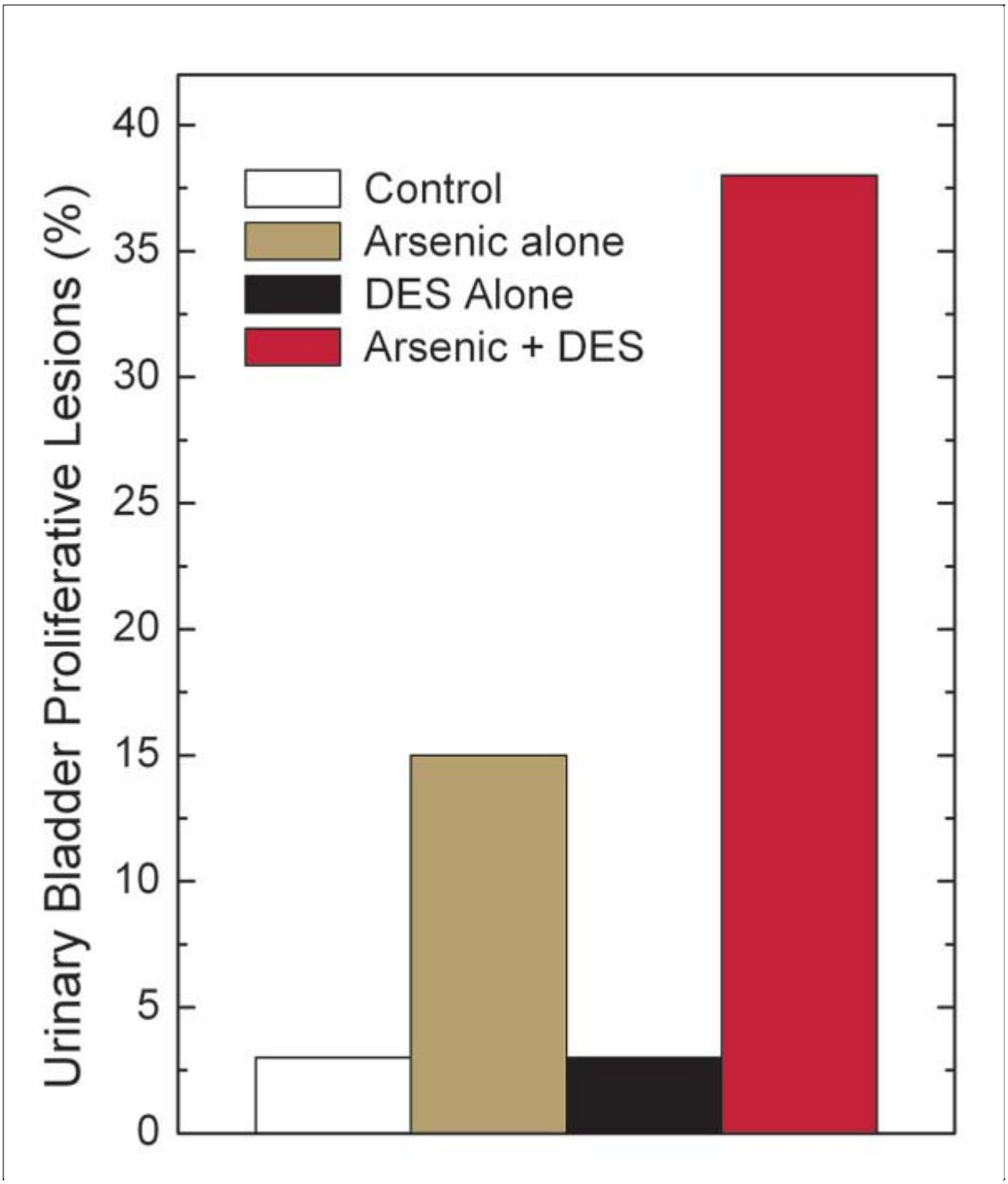
Figure 1. Urinary bladder proliferative lesions (tumors plus preneoplasias) in female mice after *in utero* arsenic exposure combined with postnatal diethylstilbestrol (DES). The urinary bladder proliferative lesions included three transitional cell carcinomas, the tumor type seen in humans exposed to arsenic.

The present data provide compelling evidence that arsenic can initiate or induce urogenital tract cancers, potentially including tumors of the urinary bladder, and that this response is exacerbated by estrogen. In this regard, prolonged arsenical exposure can produce urinary

bladder tumors in adult rats, but unlike the case with most other bladder carcinogens, females appear more sensitive than males (Wei M et al. *Carcinogenesis* 23: 1387–97, 2002; Shen J et al. *Toxicol Appl Pharmacol* 210: 171–180, 2006), which would be in keeping with a role for estrogens in this response. Furthermore, our assessment of early molecular events in transplacental arsenic carcinogenesis indicates that arsenic precipitates and can further facilitate aberrant estrogen signaling in urogenital target tissues of arsenic carcinogenesis, potentially leading to the reprogramming of critical signaling pathways. Estrogen levels during pregnancy are much higher than in other periods of adult life, which could provide an endogenous stimulus for *in utero* arsenic carcinogenesis. Because fetal arsenic exposure initiates cancer in so many sites within the female mouse urogenital system, we now hypothesize that arsenic *in utero* attacks a critical pool of progenitor cells in the urogenital system and induces aberrant genetic reprogramming as part of its carcinogenic mechanism, in a fashion similar to early life exposure to diethylstilbestrol (Cook JD et al. *Proc Natl Acad Sci U S A* 102: 8644–49, 2005).

These findings have important public health implications. For instance, pharmacological or environmental estrogen exposure could possibly enhance arsenic-initiated cancers, whereas prenatal arsenic exposure may predispose people to develop estrogen-related carcinogenesis. In addition, the fetal life stage is clearly a period of high sensitivity to arsenic carcinogenesis in mice and a comparable sensitivity in humans would be cause for great alarm. A transplacental component of human arsenic carcinogenesis may be difficult to prove because populations exposed to arsenic during gestation only do not appear to exist. However, in areas where chronic exposure to elevated environmental arsenic is common, all life stages are involved and significant *in utero* exposure inevitably occurs. Because of this, protection of pregnant women from excessive arsenic exposure may be a valid intervention strategy in preventing human cancer induced by environmental arsenic.

Michael P. Waalkes, PhD
Senior Investigator
Laboratory of Comparative Carcinogenesis
National Cancer Institute at NIEHS, Bldg. 101SC/Rm. F095
Tel: 919-541-2328
Fax: 919-541-3970
waalkes@niehs.nih.gov



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