FORUM

Liver is a Target of Arsenic Carcinogenesis

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Inorganic arsenic is clearly a human carcinogen causing tumors of the skin, lung, urinary bladder, and possibly liver (IARC, 2004). At the time of construction of this monograph, the evidence for arsenic as a hepatocarcinogen in humans was considered controversial and in rodents considered insufficient. However, recent data has accumulated indicating hepatocarcinogenicity of arsenic. This forum reevaluates epidemiology studies, rodent studies together with in vitro models, and focuses on the liver as a target organ of arsenic toxicity and carcinogenesis. Hepatocellular carcinoma and hepatic angiosarcoma, have been frequently associated with environmental or medicinal exposure to arsenicals. Preneoplastic lesions, including hepatomegaly, hepatoportal sclerosis, fibrosis, and cirrhosis often occur after chronic arsenic exposure. Recent work in mice clearly shows that exposure to inorganic arsenic during gestation induces tumors, including hepatocellular adenoma and carcinoma, in offspring when they reach adulthood. In rats, the methylated arsenicals, dimethylarsenic acid promotes diethylaminoethylinduced liver tumors, whereas trimethylarsine oxide induces liver adenomas. Chronic exposure of rat liver epithelial cells to low concentrations of inorganic arsenic induces malignant transformation, producing aggressive, undifferentiated epithelial tumors when inoculated into the Nude mice. There are a variety of potential mechanisms for arsenical-induced hepatocarcinogenesis, such as oxidative DNA damage, impaired DNA damage repair, acquired apoptotic tolerance, hyperproliferation, altered DNA methylation, and aberrant estrogen signaling. Some of these mechanisms may be liver specific/selective. Overall, accumulating evidence clearly indicates that the liver could be an important target of arsenic carcinogenesis.

Key Words: arsenic; liver; carcinogenesis; epidemiology; animal models; mechanisms.

Arsenic (As) is a toxic and carcinogenic metalloid (IARC, 1980, 2004; NRC, 1999). Chronic arsenic poisoning, or arsenicosis, is typically defined by the classical dermal stigmata, together with internal disorders, such as liver injury, in the presence of known arsenic exposure. Probably, the most important concern with arsenic exposure is its carcinogenic potential. Epidemiologic studies have demonstrated an association between chronic arsenic exposure and cancer of the skin, lung, urinary bladder, and possibly liver, kidney, and prostate in humans (IARC, 2004; NRC, 1999). The epidemiological data for the skin, lung and urinary bladder are widely accepted as showing an etiological role for arsenic exposure, whereas other sites, such as liver, are considered more controversial. As a compounding factor in defining target sites of carcinogenesis, animal studies for many years have yielded negative results for inorganic arsenic as a carcinogen when given alone in adult mice, rats, hamsters, rabbits, dogs, and monkeys (IARC, 1980, 2004; NRC, 1999).

The association between environmental arsenic exposure and human liver cancers has been repeatedly reported (Centeno et al., 2002; Chen et al., 1992; Chiu et al., 2004; Morales et al., 2000; Zhou et al., 2002), but has not found general agreement (Chen et al., 2007; Guo, 2003). In its most recent monograph on arsenic, International Agency for Research on Cancer listed the liver as a potential organ for arsenic carcinogenesis, with cautionary notes on accuracy of the diagnosis of liver cancer in studies involving death certificates and potential confounding factors such as hepatitis (IARC, 2004). At the time of construction of this monograph the evidence for arsenic as a rodent liver carcinogen was also considered limited (IARC, 2004). However, recent data indicate that fetal exposure to inorganic arsenic in mice produces tumors in adulthood in a variety of organs, including the liver (Waalkes et al., 2007). Methylated arsenicals can influence or induce liver tumors (Nishikawa et al., 2002; Shen et al., 2003; Waniibuchi et al., 2004; Yamamoto et al., 1995). Thus, accumulating evidence indicates the liver is a frequent target of experimental arsenic carcinogenesis,fortifying the human data. This forum reevaluates the evidence associating arsenic exposure and liver cancer, and discusses the mechanisms potentially involved in arsenic hepatocarcinogenesis.

LIVER AS A TARGET OF ARSENIC TOXICITY IN HUMANS

Liver cancers can develop from specific chronic liver diseases. Epidemiology studies have clearly indicated an association between chronic arsenic exposure and abnormal liver function, hepatomegaly, hepatoportal sclerosis, liver fibrosis and cirrhosis (Table 1).
Abnormal Liver Function

Abnormal liver function, manifested by gastrointestinal symptoms such as abdominal pain, indigestion, loss of appetite and by clinical elevations of serum enzymes, frequently occurs from exposure to arsenic in the drinking water (Mazumder, 2005), or from environmental exposure to arsenic through burning high-arsenic coal in indoor stoves (Liu et al., 1992; Zhang et al., 2000).

Hepatomegaly

In hospitalized arsenicosis patients from West Bengal, India, the rate of hepatomegaly exceeds 75% and is positively correlated with the level of drinking water arsenic and hepatic arsenic content (Santra et al., 1999). In a large scale epidemiology survey in this region, the prevalence of hepatomegaly (10%) was significantly higher (Mazumder, 2005). In Southwest Guizhou, China, where arsenic exposure occurs from burning arsenic-containing coal in indoor stoves, the occurrence of hepatomegaly was 21% (Zhang et al., 2000), a rate much higher than other areas with elevated drinking water arsenic in China (Liu et al., 2002).

Hepatoportal Sclerosis (Noncirrhotic Portal Hypertension)

Hepatoportal sclerosis is a rare but relatively specific condition that may occur after chronic arsenic exposure (Nevens et al., 1990). Hospitalized Indian arsenicosis patients have very high rates of hepatoportal sclerosis developed from drinking water highly contaminated with arsenic (Mazumder, 2005; Santra et al., 1999). Chronic oral arsenic intoxication (from drinking water, traditional medicines, etc.) is thought to be an etiology factor for hepatoportal sclerosis in India populations (Datta et al., 1979). Hepatoportal sclerosis is often a result of damage to the local vasculature (Centeno et al., 2002; Nevens et al., 1990). Chronic arsenic exposure in animals can also produce liver endothelial cell damage, which subsequently damages parenchymal cells (Straub et al., 2007).

Liver Fibrosis and Cirrhosis

High hepatic arsenic levels can be associated with cirrhosis (Dhawan et al., 1983). This can be especially true in cirrhotic patients who consume “home-made brew” made with water highly contaminated with arsenic. Liver fibrosis is also common in arsenicosis patients from West Bengal, India (Mazumder, 2005; Santra et al., 1999), or in patients consuming high-arsenic contaminated food from burning arsenic coal in Guizhou, China (Liu et al., 1992; Zhou et al., 2002). Liver cirrhosis appears to be a primary cause of arsenic-related mortality in Guizhou, China, and is potentially associated with hepatocellular carcinoma (HCC) (Liu et al., 1992, 2002; Zhou et al., 2002).

Liver and Arsenic Biotransformation

Arsenic is well absorbed from the gastrointestinal tract, and first reaches the liver. Arsenate is reduced to arsenite in the liver (Gregus and Nemeti, 2002). Because the liver is rich in glutathione, it is a major site of arsenic detoxication, either from glutathione acting as an antioxidant, or by glutathione-arsenic conjugation for cellular efflux and biliary excretion (Liu et al., 2001a; NRC, 1999). The liver is also the major site of arsenic methylation, catalyzed by arsenic methyltransferase or AS3MT using S-adenosylmethionine (SAM) as the substrate (Thomas, 2007). Arsenic methylation capacity is often compromised in patients with liver diseases including cirrhosis (Buchet et al., 1984; Geubel et al., 1988). The role of compromised arsenic methylation in hepatic pathology or carcinogenesis is not well defined.

LIVER AS A TARGET OF ARSENIC CARCINOGENESIS IN HUMANS

HCC has been potentially linked to human arsenic exposure (Centeno et al., 2002; IARC, 1980, 2004; NRC, 1999), and will be the focus of this forum. Case reports of hepatic angiosarcoma in association with medicinal and environmental...
exposures have also been extensively reported (Centeno et al., 2002; IARC, 1980), but will not be discussed here. Table 2 summarizes the epidemiology studies associating HCC with environmental arsenic exposure.

 Liver Cancer (Primarily HCC) in Taiwan

The association between increased liver cancer mortality and elevated drinking water arsenic was first reported in a population from Southwest Taiwan. In the initial study, the standardized mortality ratio (SMR) was 1.7 for arsenic intoxicated men and 2.3 for similarly exposed women (Chen et al., 1985). Further studies in this area showed a significant association between duration of consumption of high-arsenic containing water and liver cancers, with an age- and sex-adjusted odds ratio of 2.67 (Chen et al., 1986, 1988). A dose-response relationship between arsenic levels in drinking water and age-adjusted liver cancer mortality was also observed (Chen and Wang, 1990; Chen et al., 1988; Wu et al., 1989). Arsenic-exposed men had higher liver cancer mortality than women in almost all age groups (Chen et al., 1992). Further analysis using EPA-adjusted arsenic concentrations in the drinking water (170, 470, and 800 ppb) demonstrated increased liver cancer mortality with increasing arsenic concentration with corresponding SMR’s of 1.2, 1.5, and 2.5 for men (> 20 years, dose-response for both sex*) and 2.1, and 3.6 for women (p < 0.001 for trend) (Smith et al., 1992). Risk assessments using different models showed a dose-response trend for liver cancer (Morales et al., 2000). These early data were later fortified by a separate study associating residence in four Taiwanese townships with endemic arsenicosis and liver cancer mortality during 1971–1994. The SMR for residence in these areas with prevalent arsenicosis was 1.98 for men and 2.14 for women, and was significantly elevated as compared with the regional rate and the rate for Taiwan as a whole (Tsai et al., 1999). A follow-up study on liver cancer mortality since 1983, after the termination of the consumption of arsenic water in 1970, showed a progressive reduction of mortality in women, suggesting a causal relationship between drinking water arsenic and liver cancer (Chiu et al., 2004). In a negative study, the liver cancer mortality from five townships with high arsenic in the drinking water (145,000 people) was compared with 238 control townships (11 million people) elsewhere in Taiwan, and showed no correlation (Guo, 2003). This nonweighed comparison may suffer from “ecological fallacy” as well as other study design limitations, including biases introduced by population, hepatitis, alcoholism, and other confounding factors. The overall series of Taiwanese studies strongly suggest an association between environmental elevated arsenic exposure and/or arsenicosis and an increased risk for developing liver cancers.

 Arsenic and Liver Cancers Worldwide

Other studies worldwide provide additional evidence for arsenic as a liver carcinogen. Increased liver cancer mortality in association with drinking water arsenic levels have been reported in populations from Inner Mongolia, China (Luo et al., 1995), Bangladesh (Chen and Ahsan, 2004), and Japan (Tsuda et al., 1995). In a study of a population from Argentina, using stratified arsenic doses, a positive trend for liver cancer was also found, although the individual associations with arsenic dose were not significant (Hopenhayn-Rich et al., 1998). Excess liver cancer mortality from environmental exposure to arsenic through use of high-arsenic containing coal in China has also reported (Zhou et al., 2002). On the other hand, arsenic exposure was not associated with liver cancer mortality in a population from Utah, USA (Lewis et al., 1999) or from Chile (Smith et al., 1998). A recent paper

### Table 2

Epidemiology Study of Liver Cancers Associated with Arsenic Exposure in Humans

<table>
<thead>
<tr>
<th>Exposure, location</th>
<th>Scope and endpoint</th>
<th>Result</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water, Taiwan</td>
<td>Eighty-four villages 1968–1982 mortality</td>
<td>SMR 1.7 for men and 2.3 for women*</td>
<td>Chen et al., 1985</td>
</tr>
<tr>
<td></td>
<td>Forty-two villages 1973–1986 mortality</td>
<td>All ages, dose-response for men*</td>
<td>Chen et al., 1988</td>
</tr>
<tr>
<td></td>
<td>Forty-two villages 1973–1986 mortality</td>
<td>&gt; 20 years, dose-response for both sex*</td>
<td>Wu et al., 1989</td>
</tr>
<tr>
<td></td>
<td>Three hundred and fourteen townships 1972–1983 mortality</td>
<td>Association of As for liver cancer*</td>
<td>Chen and Wang, 1990</td>
</tr>
<tr>
<td></td>
<td>Multistage model analysis</td>
<td>Liver cancer mortality, men &gt; women*</td>
<td>Chen et al., 1992</td>
</tr>
<tr>
<td></td>
<td>Above data analysis</td>
<td>Dose-response for liver cancer*</td>
<td>Morales et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Inner Mongolia 1971–1993 mortality</td>
<td>Reduction of liver cancer in women*</td>
<td>Chiu et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Eighty-four villages 1968–1982 mortality</td>
<td>Excess liver cancer mortality (rank #2)*</td>
<td>Luo et al., 1995</td>
</tr>
<tr>
<td>Drinking water, China</td>
<td>65,800 residents, age-sex adj. mortality</td>
<td>Lifetime liver cancer mortality doubled*</td>
<td>Chen and Ahsan, 2004</td>
</tr>
<tr>
<td>Drinking water, Japan</td>
<td>Four hundred and fifty-four resident cohort, 1959–1992 mortality</td>
<td>Excess mortality from liver cancer*</td>
<td>Tsuda et al., 1995</td>
</tr>
</tbody>
</table>

*Significantly different from unexposed population, p < 0.05.
claimed no association of liver cancer mortality with environmental arsenic exposure in Guizhou, China (Chen et al., 2007). However, in this rural region, many arsenicosis patients die from cirrhosis and ascites (~30%), lesions often linked to liver malignancy, but without autopsy to make a final diagnosis. The lack of valid death certificates makes this analysis questionable. Coexposures to hepatitis virus B or aflatoxin could be one potential cause of the variability in association between arsenic exposure and liver cancer worldwide.

Thus, an association of environmental arsenic exposure and liver cancers, either from Taiwan or from other places around the world, has been demonstrated.

**LIVER AS A TARGET OF ARSENIC CARCINOGENESIS IN EXPERIMENTAL MODELS**

In contrast to most other human carcinogens, it has proven difficult to corroborate the carcinogenic effects of inorganic arsenic under experimental conditions, including hepatocarcinogenicity (IARC, 2004; NRC, 1999). However, recent studies clearly show the liver can be a target of arsenic carcinogenesis in experimental models. Table 3 summarizes three lines of evidence in this regard, including: (1) transplacental arsenic hepatocarcinogenesis in mice; (2) liver cancers influenced or induced by methylated arsenicals in rats; and 3) In vitro malignant transformation of rat liver cells.

**Hepatocarcinogenic Effects of In Utero Inorganic Arsenic Exposure in Mice**

Gestation is a critical period of development and the fetus is highly sensitive to chemical carcinogenesis, including cancers induced by various inorganics (Waalkes et al., 2007). In this regard, inorganic arsenite was given in the maternal drinking water (0–85 ppm) to pregnant mice from gestation days 8–18. The doses chosen were nontoxic to the dam and pup (Waalkes et al., 2007). Maternal mice were allowed to give birth, and after weaning offspring were grouped according to gender and maternal exposure, and maintained for up to 2 years with no additional arsenic exposure. In C3H mice in utero arsenic exposure produced tumors of the liver, adrenal, ovary and lung of offspring as adults (Waalkes et al., 2003). A dose-dependent increase in HCC incidence (up to 4.7-fold) and a 5.5-fold increase in liver tumor multiplicity (tumors/liver) were observed in males (Waalkes et al., 2003). This finding of increased liver cancers was duplicated in a second study in which postnatal 12-O-teradecanoyl phorbol-13-acetate (TPA) was applied on the skin of the offspring after gestational arsenic exposure in an attempt to enhance skin cancer (Waalkes et al., 2004a). In this study the arsenic-exposed male offspring developed liver tumors in excess regardless of postnatal TPA, and combined arsenic plus TPA increased liver tumors in female mice (HCC or adenoma) in an arsenic dose-related fashion by over 260% and increased liver tumor multiplicity by 5.5-fold compared with TPA alone (Waalkes et al., 2004a). A third study used CD1 mice, which have a low rate of spontaneous liver tumor formation. In utero arsenic exposure in CD1 mice alone also increased liver tumor incidence and multiplicity in male offspring (Waalkes et al., 2006a). The basis for susceptibility of male mice to transplacental arsenic hepatocarcinogenesis is as yet unclear, but it is not uncommon for male to be in general more sensitive to chemically induced hepatocarcinogenesis. Although the external arsenic doses in these transplacental studies are high, they were well tolerated by the mice with no apparent toxic effects in either the dam or the offspring (Waalkes et al., 2003, 2004a, 2006b). This may be due to the fact that mice differ from humans in much more rapid arsenic clearance, and arsenic concentrations in blood from these transplacental exposures are comparable to those observed in humans exposed to environmental arsenic (Waalkes et al., 2007). Because of these biokinetic differences it is inappropriate to assume similar external doses would lead

### Table 3

<table>
<thead>
<tr>
<th>Model systems</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>Liver tumor induction by <em>in utero</em> inorganic arsenic exposure</td>
<td>Dose-dependent increase in HCC in males</td>
<td>Waalkes et al., 2003</td>
</tr>
<tr>
<td>Arsenite exposure at GD8–18 in C3H mice</td>
<td>TPA promotion of liver tumors in females</td>
<td>Waalkes et al., 2004b</td>
</tr>
<tr>
<td>Arsenite exposure at GD8–18 in CD1 mice</td>
<td>Induction of liver tumors in males</td>
<td>Waalkes et al., 2006a</td>
</tr>
<tr>
<td>DES enhancement of liver tumor formation in females</td>
<td>Waalkes et al., 2006b</td>
<td></td>
</tr>
<tr>
<td>Hepatocarcinogenesis by methylated arsenicals</td>
<td></td>
<td></td>
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<tr>
<td>DMA following DEN initiation in rats</td>
<td>Dose-response in liver tumor formation, including HCC</td>
<td>Yamamoto et al., 1995</td>
</tr>
<tr>
<td>TMAO exposure in rats for 2 years</td>
<td>Dose-dependent increase in liver adenoma and multiplicity</td>
<td>Shen et al., 2003</td>
</tr>
<tr>
<td>MMA, DMA, and TMAO</td>
<td>Preneoplastic and carcinogenic effects of methylated arsenicals</td>
<td>Nishikawa et al., 2002</td>
</tr>
<tr>
<td><em>In vitro</em> malignant transformation, rat liver cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic, low-dose arsenite exposure</td>
<td>Malignant transformation, aggressive tumors in Nude mice</td>
<td>Zhao et al., 1997</td>
</tr>
<tr>
<td>Genomic analysis using the transformed rat liver cell model</td>
<td></td>
<td>Chen et al., 2001a</td>
</tr>
</tbody>
</table>

*Note. GD, gestation day; DEN, diethylnitrosamine; DES, diethylstilbestrol.*
to similar internal organ dosimetry. A synergistic increase in liver tumor incidence and multiplicity in males was observed when in utero arsenic exposure was combined with postnatal treatment with the synthetic estrogen, diethylstilbestrol (Waalkes et al., 2006a). In utero arsenic plus postnatal diethylstilbestrol also additively increased liver tumor incidence in female CD1 mice (Waalkes et al., 2006b). As a corollary to these mouse studies, increased lung cancer mortality has been shown to occur in young adults following in utero or early life exposure to arsenic in humans (Smith et al., 2006). Furthermore, a variety of oncogenically relevant genes those are aberrantly expressed after exposure to a carcinogenic level of arsenic in mice (Liu et al., 2004, 2006b) show similarly distorted expression in the livers of chronic arsenicosis patients (Lu et al., 2001; Waalkes et al., 2004b). Thus, the developing fetus, including the human fetus, appears to be hypersensitive to arsenic carcinogenesis (Waalkes et al., 2007).

Hepatocarcinogenicity of Methylated Arsenicals

Hepatocarcinogenicity of trimethylarsine oxide (TMAO) given in the drinking water for up to 2 years was examined in F344 rats (Shen et al., 2003). A dose-dependent induction of hepatocellular adenoma occurred, and liver tumor multiplicity increased by up to 2,5-fold (Shen et al., 2003). In a separate study, F344 rats were preinitiated with treatment with multiple organic carcinogen cocktail, and subsequent received dimethylarsinic acid (DMA) in the drinking water (0, 50, 100, 200, 400 ppm) for 24 weeks (Yamamoto et al., 1995). DMA very efficiently promoted liver tumors, including HCC, in a DMA dose-dependent fashion (Yamamoto et al., 1995). At the highest doses of arsenic, HCC incidence after preinitiation with organic carcinogens was increased from 0 to 45%, and all of the rats in these groups showed hepatic preneoplastic lesions (Yamamoto et al., 1995). DMA alone was not hepatocarcinogenic in this study. Liver tumor promoting effects of monoarsononous acid (MMA), DMA, or TMAO were also examined in F344 rats after carcinogenic initiation with combined diethylnitrosamine and partial hepatectomy (Nishikawa et al., 2002). The number and area of hepatic glutathione S-transferase-pi (GST-pi) positive foci, indicative of early premalignant lesions, were significantly increased following 6 weeks of MMA, DMA, or TMAO treatment in the drinking water (Nishikawa et al., 2002). Although the doses of organic arsenicals used in these experiments are very high, these results suggest that the rat liver appears to be a target for direct carcinogenic and tumor promoting effects of various methylated arsenicals (IARC, 2004; Wanibuchi et al., 2004).

In Vitro Malignant Transformation

Chronic exposure of the nontumorigenic rat epithelial TRL1215 cells to nanomolar concentrations of inorganic arsenic induces malignant transformation (Zhao et al., 1997). These transformed cells produced aggressive tumors capable of invasion and distant metastasis upon inoculation into Nude mice (Zhao et al., 1997). Liver cell transformation with the long-term, environmental-relevant arsenic exposures is of significance. Thus, a series of studies were performed to explore the molecular events potentially involved in arsenic hepatocarcinogenesis. Altered DNA methylation status is clearly associated with arsenic-induced liver cell transformation (Chen et al., 2001a; 2001b; Liu et al., 2006a; Zhao et al., 1997). Acquired tolerance to apoptosis with increased cellular arsenic efflux is also evident (Liu et al., 2001a; Qu et al., 2002). Arsenic-induced cell cycle dysregulation, hyperproliferation and aberrant gene expression related to oncogenesis occurred with arsenic carcinogenesis (Chen et al., 2001b, Liu et al., 2006a). Many of these aberrantly expressed genes have been observed in intact animals or in humans (Liu et al., 2006b; Lu et al., 2001), fortifying the evidence for the liver as a direct target of arsenic carcinogenesis.

POTENTIAL MECHANISMS OF ARSENIC HEPATOCARCINOGENESIS

The mechanism of arsenic carcinogenesis, including hepatocarcinogenesis, is not completely understood, and various potential mechanisms have been proposed (IARC, 2004; Rossman, 2003). Table 4 lists several mechanisms possibly involved in arsenic-induced hepatocarcinogenesis. These include oxidative DNA damage, acquired tolerance to apoptosis and enhanced cell proliferation, altered DNA methylation and genomic instability, and aberrant estrogen signaling.

Oxidative DNA Damage

Arsenicals are known to produce oxidative stress as a mechanism of hepatotoxicity and, possibly, carcinogenicity (IARC, 2004; NRC, 1999). Hepatic lipid peroxidation and glutathione depletion are observed in chronic arsenic-treated animals (Mazumder, 2005). A number of oxidative stress-related genes, such as heme oxygenase-1 and metallothionein, are often increased following acute, high-dose arsenic exposure (Liu et al., 2001b). However, expressions of these stress-related genes were not increased during low-dose, chronic exposures (Liu et al., 2006b; Xie et al., 2004). Various adaptive mechanisms that reduce acute arsenic toxicity are often induced to protect against arsenic-induced oxidative stress (Xie et al., 2004). One of these adaptive mechanisms is the induction of hepatic glutathione S-transferase, which in turn plays a key role in ameliorating arsenic-induced oxidative damage and helping transport arsenic out of the liver cell (Liu et al., 2001a, 2006a). Increases in hepatic DNA 8-hydroxydeoxyguanosine levels, a biomarker for oxidative DNA damage, have been associated with hepatocarcinogenesis induced by methylated arsenicals (Kinoshita et al., 2007; Shen et al., 2003; Wanibuchi et al., 2004).

Impaired DNA Repair

Arsenic-induced oxidative DNA damage, like any such damage, can be repaired by specific DNA repair machinery.
TABLE 4
Potential Mechanisms for Arsenic Hepatocarcinogenesis

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>Outcomes</th>
<th>Representative reference</th>
</tr>
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<tbody>
<tr>
<td>Oxidative damage</td>
<td></td>
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<tr>
<td>Oxidative stress</td>
<td>Lipid peroxidation, GST-π positive foci</td>
<td>Mazumder, 2005; Nishikawa et al., 2002</td>
</tr>
<tr>
<td>Oxidative-related gene expression</td>
<td>Induction of HO-1, MT, and GSH-related enzymes</td>
<td>Liu et al., 2001b, 2006a</td>
</tr>
<tr>
<td>Oxidative DNA damage</td>
<td>Increase in hepatic DNA 8-OHdG levels</td>
<td>Kinoshita et al., 2007; Shen et al., 2003</td>
</tr>
<tr>
<td>Impaired DNA repair</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired repair of DNA damage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interact with zinc finger proteins</td>
<td>Interfere with PARP-1, Fpp, XPA, etc</td>
<td>Inhibition of NER and BER</td>
</tr>
<tr>
<td>Apoptosis tolerance and cell proliferation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-α overexpression</td>
<td>Intense staining of ER-α in arsenic induced tumors</td>
<td>Waalkes et al., 2004a, 2006b</td>
</tr>
<tr>
<td>Estrogen linked gene expression</td>
<td>ER linked gene expression in adult and fetal livers</td>
<td>Liu et al., 2004, 2006b</td>
</tr>
<tr>
<td>Altered steroid metabolism</td>
<td>Altered steroid metabolism and liver feminization</td>
<td>Liu et al., 2007</td>
</tr>
<tr>
<td>Synergistic effects with DES</td>
<td>Increased liver tumors following postnatal DES</td>
<td>Waalkes et al., 2006a, b</td>
</tr>
</tbody>
</table>

*Note.* The details of the experimental systems are described in the text. HO-1, heme oxygenase-1; MT, metallothionein; DNMT, DNA methyltransferase; DES, diethylstilbestrol.

However, arsenic has been shown to impair nucleotide excision repair and base excision repair processes, probably through interaction with zinc finger proteins involved in DNA repair enzymes (Hartwig et al., 2003; Rossman, 2003). Zinc finger proteins are often involved in DNA binding and protein-protein interactions and appear to be sensitive intracellular targets for arsenic. Well-studied examples are poly(ADP-ribose) polymerase-1 (PARP-1), the formamidopyrimidine-DNA glycosylase (Fpg), and zinc finger protein structure xeroderma pigmentosum complementation group A (XPA) (Hartwig et al., 2003). Although arsenic is known to inhibit many enzymes, the impairment of DNA repair may not be necessarily through direct inhibition of DNA repair enzymes (Rossman, 2003). Arsenic exposure in humans has been associated with decreased expression of the DNA excision repair cross-complement 1 in the United States and Mexico populations (Andrew et al., 2006).

**Apoptotic Tolerance and Hyperproliferation**

Arsenic intoxicated cells can be eliminated through apoptosis, if the damage is severe enough. However, during chronic arsenic exposure, adaptation to the effects of arsenic occurs, including apoptosis, and this frequently results in a generalized tolerance to apoptosis. Apoptotic resistance is a common phenomenon in cells malignantly transformed by arsenic, including rat liver epithelial cells (Qu et al., 2002). Tolerance to apoptosis maybe an important factor for arsenic carcinogenesis, because it may allow the damaged cells that otherwise would be eliminated to survive and to transmit genetic or epigenetic lesions (Waalkes et al., 2000). In fact, acquired resistance toward apoptosis is a hallmark of most cancers, including liver cancers. Apoptotic tolerance is often associated with increased cell proliferation, as evidenced by proliferative lesions in vivo frequently seen with chronic arsenic exposure (Chen et al., 2004; Waalkes et al., 2000). Arsenic often induces overexpression of cell proliferation-related genes, such as cyclin D1 and proliferating cell nuclear antigen (PCNA), as seen in arsenic-treated mouse liver (Chen et al., 2004; Waalkes et al., 2000, 2004b), in arsenite-transformed liver cells (Chen et al., 2001b; Liu et al., 2006a), or in methylated arsenical-induced liver preneoplasia (Kinoshita et al., 2007; Shen et al., 2003; Wanibuchi et al., 2004).

**DNA Methylation and Genomic Instability**

Long-term low-dose arsenic exposure induces global loss of DNA methylation in cultured rat liver cells (Zhao et al., 1997) and in human HaCaT keratinocytes (Reichard et al., 2007). Arsenic-induced global DNA hypomethylation was also seen in mouse livers chronically exposed to inorganic arsenic (Chen et al., 2004; Okoji et al., 2002; Xie et al., 2004, 2007). Arsenic-induced global DNA hypomethylation is associated with the depletion of SAM pool (Zhao et al., 1997) and suppression of DNA methyltransferases DNMT1 and DNMT3A (Reichard et al., 2007). Specific hypomethylation of the estrogen receptor-α (ER-α) gene promoter is seen in arsenic-exposed mouse livers and may result in aberrant ER-α
expression and aberrant estrogen signaling (Chen et al., 2004; Waalkes et al., 2004b, 2007), which is potentially involved in arsenic hepatocarcinogenesis. Liver steatosis (fatty liver, a preneoplastic change associated with methyl deficiency) is also a frequent observation following chronic arsenic exposure and associated with methyl insufficiency and DNA methylation loss in cells or animals (Chen et al., 2004; Okoji et al., 2002; Reichard et al., 2007; Xie et al., 2004, 2007). Arsenic-induced alterations in DNA methylation could enhance genomic instability (Rossman, 2003), such as chromosomal instability in mammalian cells (Sciandrello et al., 2004). Of note is that individual gene hypermethylation can occur concomitantly with global DNA hypomethylation. In this regard, the loss of p16 expression is observed in arsenic-transformed liver cells, which could be due to both the hypermethylation of the p16 gene and the homozygous deletion of p16 (Liu et al., 2006a). Both inorganic arsenite and arsenate produced hypermethylation of the p53 gene in human lung adenocarcinoma A549 cells (Mass and Wang, 1997). Thus, altered DNA methylation status could affect genetic stability and gene expression, and could be a key factor in arsenic hepatocarcinogenesis.

**Aberrant Estrogen Signaling**

Studies of the transplacental carcinogenic potential of arsenic in mice (Waalkes et al., 2007) showed consistent targets (i.e., liver, ovary, adrenal, uterus, oviduct), which are also targets of broad range or tissue-selective carcinogenic estrogens. The estrogen signaling system is a likely factor in induction or promotion of hepatocarcinogenesis after exposure to estrogenic carcinogens and is linked to HCC (Dickson and Stancel, 2000). Intense expression of ER-α is observed in liver tumors and tumor-surrounding normal tissues after gestational arsenic exposure in mice (Waalkes et al., 2004b, 2006a). ER-α overexpression also occurred in uterine tumors, urinary bladder carcinoma (Waalkes et al., 2006b). In contrast, little or no ER-α staining was found in diethylnitrosamine-induced liver tumors (Waalkes et al., 2006a). Expression of estrogen-regulated/linked genes is observed in adult mouse livers bearing in utero arsenic-induced HCC, and the feminized expression pattern for several cytochrome P450s concomitantly occurred, including overexpression of female-dominant Cyp2a4 and Cyp2b9 and reduced expression of male-dominant Cyp7b1 (Liu et al., 2004, 2006b; Waalkes et al., 2004b). Aberrant expression of estrogen-linked genes and steroid metabolic genes was also observed in fetal mouse livers immediately following gestational arsenic exposure (Liu et al., 2007). Overexpression of ER-α and ER-linked cyclin D1 was also evident in liver biopsy samples of arsenicosis patients in Guizhou, China (Waalkes et al., 2004b). Perhaps the most important evidence for aberrant estrogen signaling comes from the study of the carcinogenic effects of in utero arsenic exposure followed postnatal diethylstilbestrol, a synthetic estrogen treatments in CD1 mice (Waalkes et al., 2006a, b). The combined treatment synergistically increased liver tumor in male offspring, and increases liver tumor incidence in females (Waalkes et al., 2006a). Of note, urogenital system tumors were also synergistically increased when in utero arsenic was combined with postnatal DES (Waalkes et al., 2006a, b). Although aberrant ER signaling pathways may be important in liver carcinogenesis induced by arsenic, it may not apply to all targets of arsenic carcinogenesis.

**CONCLUSIONS AND PERSPECTIVES**

Epidemiologic data, fortified by data from case reports and data from rodent and cell model systems, clearly indicate that the liver is a potential target of arsenic carcinogenesis. It is probable that multiple mechanisms are involved in arsenic-induced hepatocarcinogenesis, some of which may be specific to the liver.

Environmental exposure to arsenic is unavoidable and medicinal use of arsenicals in the treatment of certain cancers is increasing. Attention should be paid to arsenic-induced liver dysfunction, hepatomegaly and liver fibrosis, as these preneoplastic changes could advance to malignancy. Special caution should be paid to early life arsenic exposure, as gestation and early life appear highly sensitive to arsenic carcinogenesis occurring much later in life. A better understanding of these mechanisms will be critical in the prevention and treatment of liver cancers associated with arsenic exposure.

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