

High Dietary Fat Exacerbates Arsenic-Induced Liver Fibrosis in Mice

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Many factors could potentially affect the process of arsenic-induced liver fibrosis. The present study was undertaken to examine the effect of high fat diet on arsenic-induced liver fibrosis and preneoplastic changes. Mice were given sodium arsenite (As^{3+} , 200 ppm) or sodium arsenate (As^{5+} , 200 ppm) in the drinking water for 10 months, and provided a normal diet or a diet containing 20% added fat. Serum aspartate aminotransferase (AST), indicative of liver injury, was elevated in both arsenite and arsenate groups, and a high fat diet further increased these levels. Histopathology (H&E and Masson stain) showed that liver inflammation, steatosis (fatty liver), hepatocyte degeneration, and fibrosis occurred with arsenic alone, but their severity was markedly increased with the high fat diet. Total liver RNA was isolated for real-time RT-PCR analysis. Arsenic exposure increased the expression of inflammation genes, such as $\text{TNF-}\alpha$, IL-6, iNOS, chemokines, and macrophage inflammatory protein-2. The expression of the stress-related gene heme oxygenase-1 was increased, while metallothionein-1 and GSH S-transferase-pi were decreased when arsenic was combined with the high fat diet. Expression of genes related to liver fibrosis, such as procollagen-1 and -3, SM-actin and $\text{TGF-}\beta$, were synergistically increased in the arsenic plus high fat diet group. The expression of genes encoding matrix metalloproteinases (MMP2, MMP9) and tissue inhibitors of metalloproteinases (TIMP1, TIMP2) was also enhanced, suggestive of early oncogenic events. In general, arsenite produced more pronounced effects than arsenate. In summary, chronic inorganic arsenic exposure in mice produces liver injury, and a high fat

diet markedly increases arsenic-induced hepatofibrogenesis. *Exp Biol Med* 233:377–384, 2008

Key words: inorganic arsenic; chronic exposure; fat diet; liver injury; hepatofibrogenesis; gene expression

Introduction

Arsenic (As) is a toxic and carcinogenic metalloid. Inorganic arsenic exists in trivalent (As^{3+}) and pentavalent (As^{5+}) forms that are widely distributed in nature. Environmental arsenic exposure mainly occurs from arsenic-contaminated drinking water (1), but also occurs from burning high arsenic coal (2). Liver injury is a major health problem in arsenicosis patients (3, 4) and manifests clinically as abdominal pain, hepatomegaly, and abnormal liver function (1, 3, 5). In liver biopsy samples, triad inflammation, hepatocellular balloon-like vacuolation, endothelial cell degeneration, focal apoptosis, necrosis, and fibrosis were observed (4–6). Arsenic-induced liver pathology could progress to fibrosis and cirrhosis, even to hepatocellular carcinoma or liver angiosarcoma (7, 8). Exposure to arsenic through contaminated drinking water in Taiwan, Bangladesh, and India caused liver toxicities and increased liver tumors (1, 5, 6).

In experimental animals, chronic administration of inorganic arsenic through the drinking water in mice causes inflammation and oxidative damage to the liver (5, 9). Arsenic-induced oxidative liver damage is often associated with increases in inflammatory cytokines and the depletion of glutathione-related antioxidants (9, 10). The toxic insult to the liver caused by arsenic in the drinking water eventually results in fatty liver (steatosis) (5, 11) and hepatic fibrosis (12, 13).

Liver fibrosis occurs as a later stage of chronic arsenic hepatotoxicity, and its persistence may progress to cirrhosis and even to liver cancers (7). In humans chronically exposed to arsenic through domestic burning arsenic coal in unvented stoves for heating and cooking, liver cirrhosis,

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Table 1. Primer Sequences for Real-Time RT-PCR Analysis

Gene	Number	GenBank	
		Forward	Reverse
β -actin	M12481	GGCCAACCGTGAAAAGATGA	CAGCCTGGATGGCTACGTACA
GST- π	D30687	TGGGCATCTGAAGCCTTTTG	GATCTGGTCACCCACGATGAA
HO-1	M33203	CCTCACTGGCAGGAAATCATC	CCTCGTGGAGACGCTTTACATA
IL-6	J03783	GCCCACCAAGAACGATAGTCA	GAAGGCAACTGGATGGAAGTCT
iNOS	M87039	ACATCAGGTCCGGCCATCACT	CGTACCGGATGAGCTGTGAATT
mKC	NM_008176	TGGCTGGGATTACCTCAAG	GTGGCTATGACTTCGGTTTGG
MIP2	NM_009140	CCTCAACGGAAGAACCAAGAG	CTCAGACAGCGAGGCACATC
MMP2	M84324	CACCTGGTTTACCCTTTCTG	AACGAGCGAAGGGCATACAA
MMP9	NM_013599	GCTCATGTACCCGCTGTATAGCT	CAGATACTGGATGCCGTCTATGTC
MT-1	BC027262	AATGTGCCCAGGGCTGTGT	GCTGGGTTGGTCCGATACTATT
Procollagen-1	BC007258	GCAGGGTTCCAACGATGTTG	GCAGCCATCGACTAGGACAGA
Procollagen-3	XM_129745	GGTGGTTTTTCAGTTCAGCTATGG	CTGGAAAGAAGTCTGAGGAATGC
SM-actin	NM_007392	GCTGTCTACCTTCCAGCAGATGT	GCATTTGCGGTGGACGAT
TGF- β	M13177	GACCCTGCCCCTATATTTGGA	GCCCGGGTTGTGTTGGT
TIMP1	NM_011593	TGGGAAATGCCGCAGATATC	TGGGACTTGTGGGCATATCC
TIMP2	M82858	ACGCTTAGCATCACCCAGAAG	GGACAGCGAGTGATCTTGCA
TNF- α	XM_110221	GACCCTCACACTCAGATCATCTTCT	CCTCCACTTGGTGGTTTGCT

ascites, and liver cancers are the main causes of arsenic-related mortality (8).

Thus, the prevention of arsenic-induced liver fibrosis is critical for human health (14). Unfortunately, models of arsenic-induced liver fibrosis in animals are unavailable and would be key for defining molecular events and potential intervention strategies. The initial goal of the current study was to develop a liver fibrosis model in mice using inorganic arsenicals in the drinking water for the long-term exposures. Since steatosis appears to be a hallmark of arsenic-induced liver pathology and a high fat diet is known to facilitate chemical-induced fibrosis (19), we hypothesized that a high fat diet would facilitate arsenic hepatofibrogenesis, and a high fat diet was added to additional groups with drinking water arsenic. A secondary goal was to examine molecular events associated with chronic arsenic-induced liver injury to define mechanisms and potentially assist in clinical intervention. This paper reports on data from mice receiving a maximum tolerated dose of inorganic arsenite (As^{3+} , 200 ppm) or arsenate (As^{5+} , 200 ppm) for 10 months, with a normal or a high fat diet. The results clearly demonstrated that the fat diet enhanced chronic arsenic-induced liver injury and hepatofibrogenesis, as well as aberrant gene expressions consistent with this pathology.

Materials and Methods

Chemicals and Diets. Sodium arsenite (NaAsO_2) and sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) were obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in the drinking water. The high fat diet was composed of 20% pork fat and 0.5% cholesterol (Nan-Ji Sciences, Shanghai, China) in normal rodent chow. All other chemicals were of reagent grade.

Animal Treatment and Sample Collection. Adult Kunming white mice were obtained from Animal Care

Facilities of Guiyang Medical College and were given tap water (control) or water containing 200 ppm arsenic as sodium arsenite (As^{3+}) or sodium arsenate (As^{5+}) *ad libitum* for 10 months, together with the normal diet or the high fat diet. At the end of the experiment, the mice were killed by CO_2 asphyxiation, and blood and livers were collected. Animal care was provided in accordance with the World Health Organization Guidelines for Care and Use of Animals.

Histopathology. A portion of the left lobe of the liver from each animal was removed, fixed in 10% formalin, processed with standard pathology, stained with H&E or the fibrosis-specific Masson stain, and evaluated by the pathology department of Guiyang Medical College. The severity ratings for hepatic fibrosis were: 0, no fibrosis; 1, a few fibrotic fibers; 2, fibrotic fiber extension, partial septa formation; 3, fibrotic fibers are sufficient enough to form septa (pseudo lobe); and 4, extensive fibrosis with pseudo lobe formation and proliferative lesions. The average severity was calculated with Ridit test for each group based on individual ratings.

Real-Time RT-PCR Analysis. Total RNA was extracted from liver tissues with Trizol reagent and purified with RNeasy columns (Qiagen, Valencia, CA), followed by reverse transcription with MMLV reverse transcriptase and oligodT primers. The PCR primers were designed with Primer Express software and synthesized by Sigma-Genosys (Woodlands, TX) (Table 1). The SYBR Green DNA PCR kit (Applied Biosystems, Foster City, CA) was used for real-time RT-PCR analysis. Differences in gene expression between groups were calculated using cycle time (Ct) values, which were normalized against β -actin and expressed as relative to controls.

Statistics. Data are expressed as mean $\pm$ SEM. For comparisons between two groups, the Student's *t* test was

used. For comparisons among three or more groups, data were analyzed using a two-way analysis of variance, followed by Duncan's multiple range test. The level of significance was set at $P < 0.05$ in all cases.

Results

Serum Aspartate Aminotransferase (AST) Activities. Long-term (10 months) arsenic exposure at the dose of 200 ppm produced clinical signs of liver injury in mice, as manifested in increases in serum AST levels (Fig. 1). Serum AST was increased 5-fold in mice treated with arsenite (As^{3+}) alone and 2.5-fold in the arsenate (As^{5+}) alone group. The high fat diet alone did not alter serum AST levels. However, when the high fat diet was combined with arsenite or arsenate, serum AST was increased up to 10-fold (Fig. 1). In both cases when the high fat diet was combined with arsenite or arsenate, serum levels of AST were higher than with arsenic alone. The body weight, feed, and water intake were reduced in both arsenite and arsenate groups as compared with controls. The arsenic content in the liver was undetectable in controls and was 3.99 mg/g for arsenite and 2.61 mg/g for arsenate, respectively.

Liver Fibrosis Scores. Consistent with clinical evidence of liver injury, long-term (10 months) arsenic exposure at 200 ppm produced a variety of pathological changes in the liver, including inflammation, steatosis, hepatocyte degeneration, and liver fibrosis after H&E staining (not shown). Fibrosis was clearly evident after use of the fibrosis-specific Masson stain (Fig. 2). Control mouse livers were negative, while livers from mice given either arsenite or arsenate alone produced mild fibrosis. Fatty accumulation was more evident in the arsenate group. The high fat diet alone produced widespread fatty accumulation and mild fibrosis. However, when the high fat diet was combined with either arsenite or arsenate, fibrotic lesions clearly became more severe. Quantitative analysis of fibrosis scores with criteria described (see Materials and Methods) showed average hepatic fibrosis severity scores were increased 5-fold with arsenite alone and 4-fold with arsenate alone (Fig. 3). The high fat diet alone doubled the hepatic fibrosis scores. However, when the high fat diet was combined with either arsenite or arsenate, hepatic fibrosis scores were increased dramatically to levels above any of the treatment alone.

Gene Expression Analysis by Real-Time RT-PCR. To explore the molecular events associated with chronic arsenic-induced liver fibrosis, expression analysis of selected genes was performed (Table 2). In general, hepatic gene expression changes are in agreement with observed pathological lesions and clinical chemistry, in that arsenite treatment alone produced more pronounced effects than arsenate treatment alone, and the high fat diet in combination with the inorganic arsenicals exaggerated the gene expression changes.

Consistent with the literature (9, 10), chronic arsenic

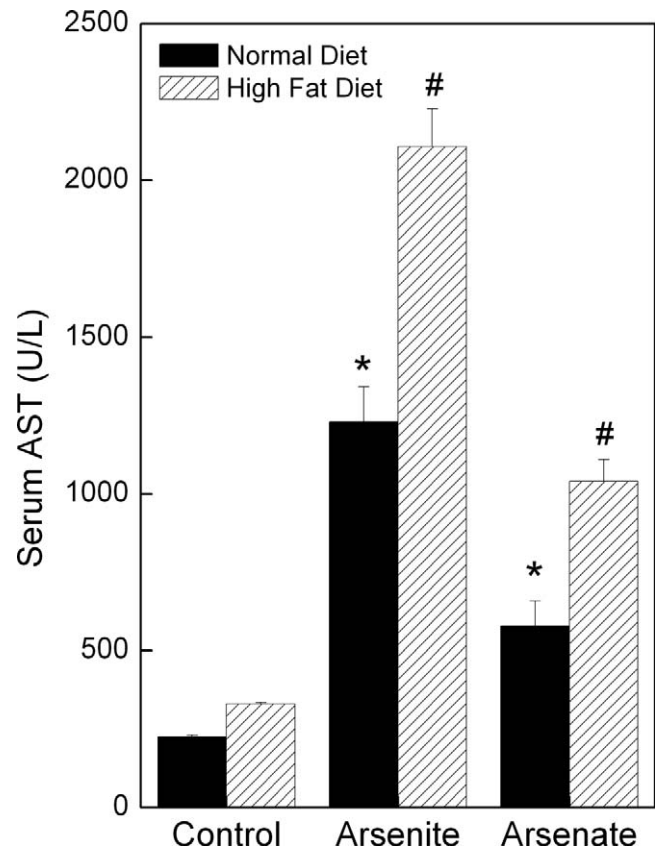


Figure 1. Serum aspartate aminotransferase (AST) activities in mice exposed to sodium arsenite (As^{3+} , 200 ppm) or sodium arsenate (As^{5+} , 200 ppm) for 10 months in the presence or absence of high dietary fat. Data are mean $\pm$ SEM ($n = 10$). *Significantly different from untreated control, $P < 0.05$; #Significantly different from corresponding arsenic group without fat diet, $P < 0.05$.

exposure increased the expression of genes related to hepatic inflammation. For example, arsenite increased the expression of $\text{TNF-}\alpha$ by 6-fold, which was then enhanced by consumption of the high fat diet to 18-fold. Similarly, arsenite and arsenate increased the IL-6 expression 7.5- and 2.5-fold, which was dramatically increased by consumption of the high fat diet to 44- and 5-fold, respectively. Arsenite alone increased the expression of inducible nitric oxide synthase (iNOS), mouse chemokine (mKC), and macrophage inflammatory protein-2 (MIP2) by 400% or more, and the increases were further enhanced by feeding the high fat diet to 700% or more. In comparison, arsenate alone only doubled the expression of mKC but had no effect on the expression of iNOS and MIP2, regardless of fat diet.

The expression of the stress-related gene heme oxygenase-1 (HO-1) was increased 4.7-fold by arsenite alone, but with the high fat diet increased to 8.7-fold. In contrast to acute arsenic exposure, after 10-month chronic exposure, the expressions of metallothionein-1 (MT-1) and GSH S -transferase-pi (GST-pi) were unchanged by arsenicals alone and decreased when arsenicals were combined with a high fat diet. Both MT-1 and GST-pi are important adaptive mechanisms for arsenic tolerance during chronic exposures

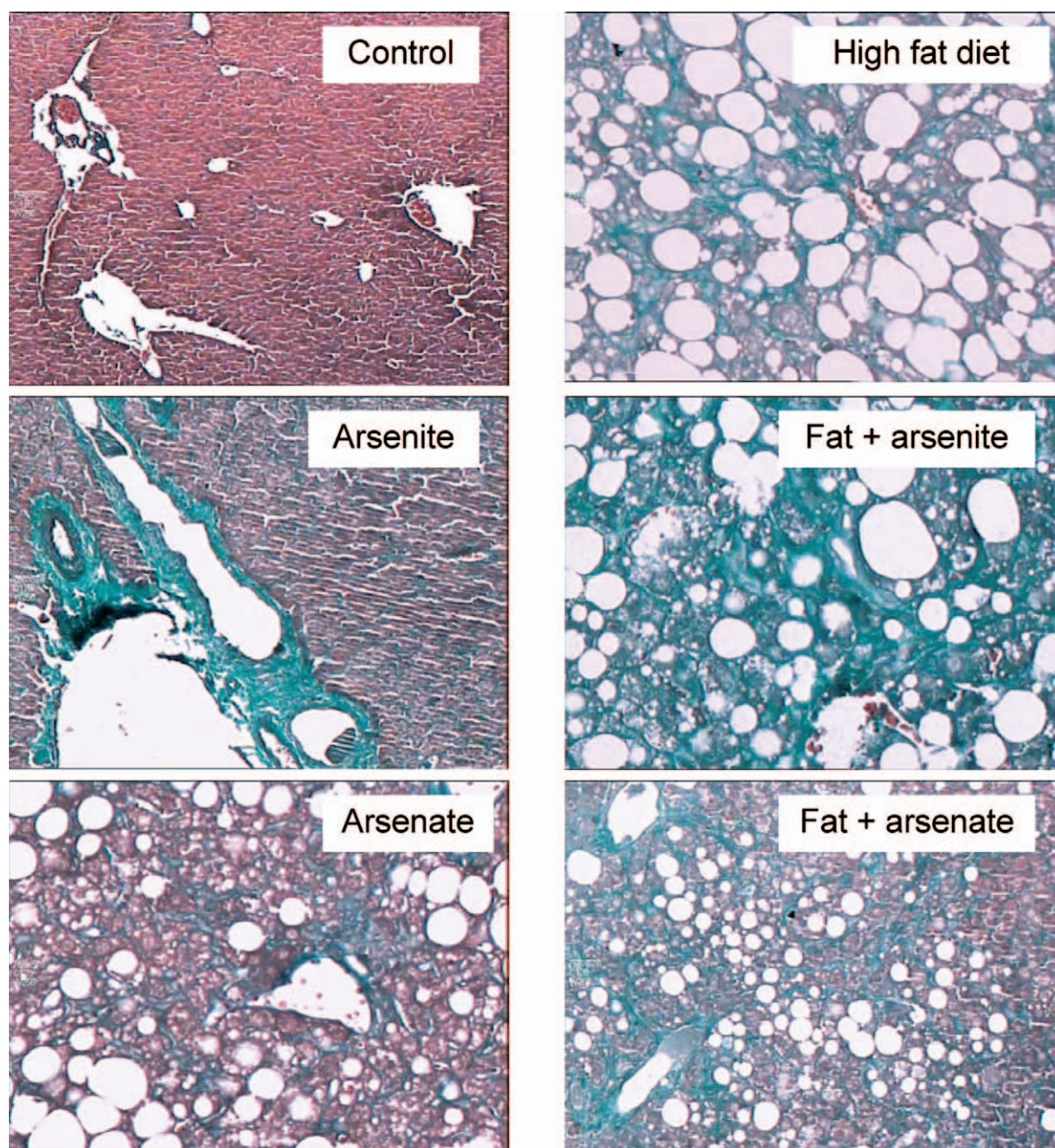


Figure 2. Representative Masson-stained photo-pictures in livers of mice exposed to sodium arsenite (As^{3+} , 200 ppm) or sodium arsenate (As^{5+} , 200 ppm) for 10 months in the presence or absence of high dietary fat. Magnification $\times 200$. Color figure is available in the online version of the journal.

(15, 16), and their compromised expression may further predispose animals to arsenic toxicity, similar to our recent observation of decreased blood MT levels in humans chronically exposed to high levels of environmental arsenic (17).

Perhaps the most important findings are the increased expression of genes related to liver fibrogenesis following exposure to arsenic and high fat diets (Fig. 4 and Table 2). For example, the expression of procollagen 1 was increased 4-fold by arsenite but 7.5-fold by arsenic plus the high fat diet. The expression of procollagen 3 was doubled by

arsenite, arsenate, or the high fat diet alone, but when the arsenicals were combined with the high fat diet, this enhanced the expression of procollagen 3 to 8- to 15-fold above control. SM-actin, a biomarker for liver fibrosis, was increased 5-fold by arsenite and 10-fold by arsenite combined with the high fat diet. The high fat diet also greatly enhanced the arsenic-induced expression of TGF- β , an important signal transduction pathway for fibrogenesis, as compared with arsenicals alone.

Another important gene expression category is extracellular matrix metalloproteinases (MMPs) and the tissue

metalloproteinase inhibitors (TIMPs). Arsenite alone increased both MMP2 and MMP9 expression over 300%, and expression was further enhanced by the high fat diet. Arsenate alone had no effect on MMP2 and MMP9 expression, and the high fat diet enhanced MMP2 but had no effect on MMP9 expression. The expression of TIMP1 was increased 200–500% by arsenite, arsenate, or the high fat diet alone, but their combination greatly increased its expression up to 19-fold. The high fat diet also enhanced the expression of TIMP2, and in contrast to other genes, arsenate produced more pronounced effect on TIMP2 expression than arsenite.

Discussion

The current study showed that the high fat diet clearly exaggerated chronic arsenic-induced liver injury in mice. The validity of the central hypothesis, that the high fat diet could enhance chronic arsenic-induced hepatofibrogenesis, was thus clearly demonstrated in the present study. Liver is a major target organ in arsenic toxicity and carcinogenesis (Liu and Waalkes, submitted). Chronic exposure of experimental animals to inorganic arsenic has been shown to produce various liver lesions, including inflammation and oxidative damage (5, 9, 10), fatty accumulation, parenchymal cell degeneration (5, 11), hepatic fibrosis (5, 12), and liver proliferative lesions (11, 18). Fibrosis is considered the critical stage of liver injury, as it may progress to cirrhosis, and even on to liver cancers (7, 13). In Guizhou where chronic arsenic exposure mainly comes from burning coal containing high levels of arsenic (2), liver cirrhosis, ascites, and liver cancers are predominate forms of arsenic-related mortality (8), and anti-fibrosis treatment with the Chinese medicine preparations has been shown to be beneficial in arseniosis patients (14) and with CCl₄-induced liver fibrosis in rats (19). However, it has been difficult to produce liver fibrosis in animals with arsenic alone (11, 20). In reality, people are not only exposed to environmental arsenic alone, and many confounding factors, including dietary factors, could well contribute to arsenic-induced liver injury. A high fat diet has been classically used to exaggerate liver injuries induced with various chemicals, such as CCl₄ (19), and we suspected it could enhance the hepatofibrogenic effect of inorganic arsenic. Indeed, the present study clearly demonstrated that the high fat diet in mice greatly enhanced arsenic-induced liver injury, liver fibrogenesis, and alterations of gene expression associated with these processes.

The high fat diet alone only modestly elevated serum AST levels (50%), but synergistically elevated serum AST levels in mice drinking water containing arsenite (from 5- to 10-fold) or arsenate (from 2.5- to 5-fold), indicative of aggravated liver injury. The high fat diet alone increased liver fibrosis scores 2-fold over control, but additively increased hepatic fibrosis with arsenite (from 5- to 7-fold), or with arsenate (from 5.5- to 7-fold). Thus, both serology and histopathology indicate significant exacerbation of liver

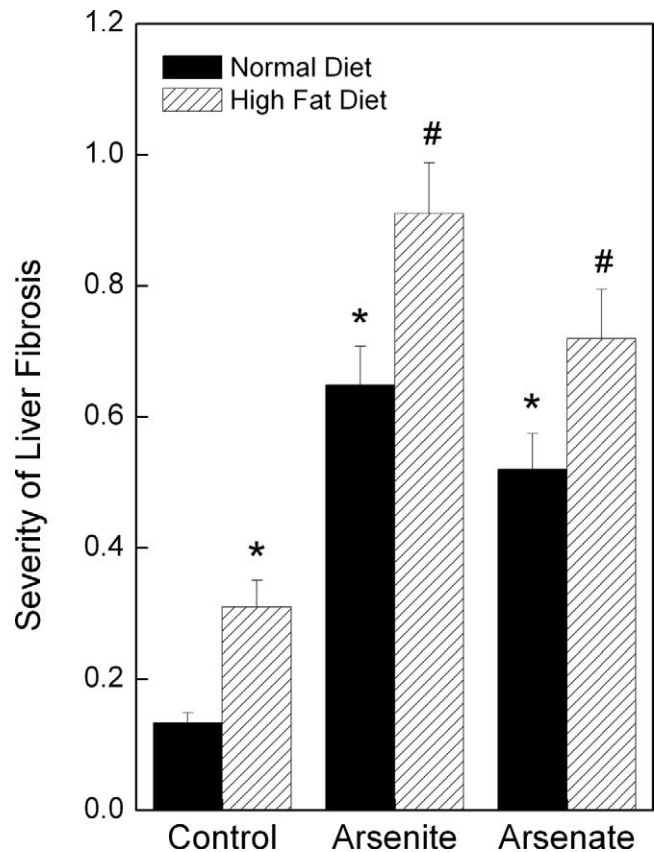


Figure 3. Liver fibrosis scores in mice exposed to sodium arsenite (As³⁺, 200 ppm) or sodium arsenate (As⁵⁺, 200 ppm) for 10 months in the presence or absence of high dietary fat. Data are mean $\pm$ SEM ($n = 10$). *Significantly different from untreated control, $P < 0.05$; #Significantly different from corresponding arsenic group without fat diet, $P < 0.05$.

fibrogenesis occurred with a high fat diet together with inorganic arsenic exposure. In general, arsenite produced more pronounced effects than arsenate, and the combination with the high fat diet enhanced toxicity further. A high fat diet is often used to promote liver fibrosis induced by various hepatotoxicants (19), and a high fat diet also acts additively or synergistically with inorganic arsenic to produce liver fibrosis.

Consistent with the literature (9, 10), chronic arsenic exposure also increased the expression of genes related to hepatic inflammatory response, such as TNF- α and IL-6, and the high fat diet further enhanced this expression. Little is known about the effects of chronic arsenic on the expressions of iNOS, mKC, and MIP2, all inflammatory mediators. This study reveals the increased expression of inflammatory genes consistent with arsenic-induced triad inflammation, and these inflammatory mediators could actively participate in arsenic hepatofibrogenesis.

The high fat diet slightly induced the expression of oxidative stress-related gene HO-1 (2-fold), but synergistically enhanced HO-1 expression when combined with arsenite (from 4.5- to 9-fold). However, arsenate had no effect on HO-1 expression, with or without a fat diet. HO-1

Table 2. Hepatic Gene Expression Changes Following Chronic Exposure of Mice to Arsenite or Arsenate in the Drinking Water, with a Normal or a High Fat Diet

Genes	Control	Fat diet	Arsenite	Fat diet + arsenite	Arsenate	Fat diet + arsenate
Inflammation genes						
TNF- α	100 $\pm$ 12	582 $\pm$ 90 ^a	580 $\pm$ 94 ^a	1790 $\pm$ 85 ^b	230 $\pm$ 22 ^a	270 $\pm$ 25
IL-6	100 $\pm$ 15	250 $\pm$ 35 ^a	750 $\pm$ 135 ^a	4420 $\pm$ 740 ^b	250 $\pm$ 25 ^a	485 $\pm$ 40 ^b
iNOS	100 $\pm$ 19	620 $\pm$ 160 ^a	130 $\pm$ 10	1425 $\pm$ 230 ^b	101 $\pm$ 10	95 $\pm$ 12
mKC	100 $\pm$ 8	120 $\pm$ 20	410 $\pm$ 20 ^a	770 $\pm$ 80 ^b	200 $\pm$ 30 ^a	215 $\pm$ 30
MIP2	100 $\pm$ 11	102 $\pm$ 12	450 $\pm$ 75 ^a	690 $\pm$ 50 ^b	80 $\pm$ 13	67 $\pm$ 16
Stress-related genes						
HO-1	100 $\pm$ 18	190 $\pm$ 13 ^a	469 $\pm$ 110 ^a	870 $\pm$ 70 ^b	70 $\pm$ 23	85 $\pm$ 21
MT-1	100 $\pm$ 8	44 $\pm$ 4 ^a	88 $\pm$ 4	30 $\pm$ 3 ^b	125 $\pm$ 40	105 $\pm$ 14
GST- π	100 $\pm$ 8	103 $\pm$ 5	75 $\pm$ 4	10 $\pm$ 1 ^b	75 $\pm$ 5	53 $\pm$ 15 ^b
Liver fibrosis-related genes						
Procollagen-1	100 $\pm$ 5	200 $\pm$ 10 ^a	435 $\pm$ 60 ^a	765 $\pm$ 40 ^b	102 $\pm$ 15	180 $\pm$ 12 ^b
Procollagen-3	100 $\pm$ 9	270 $\pm$ 15 ^a	230 $\pm$ 10 ^a	760 $\pm$ 120 ^b	225 $\pm$ 30 ^a	1520 $\pm$ 90 ^b
SM-actin	100 $\pm$ 8	220 $\pm$ 10 ^a	500 $\pm$ 25 ^a	990 $\pm$ 150 ^b	102 $\pm$ 12	190 $\pm$ 20 ^b
TGF- β	100 $\pm$ 25	580 $\pm$ 75 ^a	230 $\pm$ 34 ^a	450 $\pm$ 30 ^b	85 $\pm$ 20	440 $\pm$ 40 ^b
Matrix metalloproteinase-related genes						
MMP2	100 $\pm$ 7	730 $\pm$ 140 ^a	350 $\pm$ 50 ^a	450 $\pm$ 82	85 $\pm$ 10	920 $\pm$ 110 ^b
MMP9	100 $\pm$ 15	100 $\pm$ 10	370 $\pm$ 15 ^a	640 $\pm$ 25 ^b	65 $\pm$ 10	60 $\pm$ 15
TIMP1	100 $\pm$ 13	380 $\pm$ 55 ^a	520 $\pm$ 140 ^a	1950 $\pm$ 450 ^b	175 $\pm$ 15 ^a	555 $\pm$ 36 ^b
TIMP2	100 $\pm$ 11	820 $\pm$ 70 ^a	50 $\pm$ 17	150 $\pm$ 20 ^b	210 $\pm$ 30 ^a	420 $\pm$ 30 ^b

Mice were given the drinking water containing sodium arsenite (As³⁺, 200 ppm), sodium arsenate (As⁵⁺, 200 ppm), or tap water (Control) for 10 months in the presence or absence of a high fat diet (20% fat, 0.5% cholesterol). Data are mean $\pm$ SEM ($n = 3-6$).

^a Significantly different from controls, $P < 0.05$.

^b Significantly different from groups without a high fat diet, $P < 0.05$.

is a well-known biomarker for arsenic-induced oxidative stress (1), and arsenite is more effective than arsenate in the induction of HO-1 (15). Lack of HO-1 induction by arsenate suggests that oxidative stress may not be required for hepatofibrogenesis. In comparison to iron overload, a high fat diet produces hepatic fibrogenesis in rats without eliciting oxidative stress, supporting the notion that oxidative stress alone is not sufficient to elicit liver fibrosis (21). It should be noted that the stress-related gene expression, such as MT-1 and GST- π , were not increased, and their expressions were significantly decreased when the fat diet was combined with arsenic. This observation suggests that arsenic-induced oxidative stress-related gene expression may be involved at the early phase, but not be evident during chronic fibrogenic process, even during hepatocarcinogenic process (22). In this regard, we have found that the expression of MT-1, an acute phase protein inducible by arsenic (9, 15) is actually decreased in transplacental arsenic-induced liver tumors (22), and reduced blood MT-1 expression is also observed in arsenicosis patients in Guizhou, China, although this could be a predisposing factor (17). Enhanced GST- π expression is considered an adaptive mechanism for chronic arsenic exposure, which facilitates arsenic efflux from the liver (16). The high fat diet itself had no effect on GST- π expression but significantly attenuated GST- π expression in mice treated with arsenite (reduced to 10% of control) and

arsenate (reduced to 50% of control). The significance of such a decrease is not immediately clear, but may be related to compromised adaptive responses, or to other enhanced compensatory mechanisms, which requires further investigation.

The most dramatic effects of a high fat diet are on the expression of genes related to liver fibrogenesis. The high fat diet alone increased the expressions of procollagen 1 and 3 about 2-fold, but synergistically enhanced arsenite and arsenate induced increases in procollagen expression (8- to 15-fold). Collagen production is important in liver fibrosis, and a high fat diet is known to increase collagen expression with chemicals such as CCl₄ (19). Similar to collagen, SM-actin, a biomarker for liver fibrosis, was synergistically increased when the high fat diet was combined with inorganic arsenic (increased up to 10-fold). Little is known about collagens and SM-actin in arsenic-induced liver fibrosis, and these findings are novel and significant. A high fat diet also greatly enhanced the expression of TGF- β , an important signal transduction pathway mediator for fibrogenesis, especially for arsenite (up to 13-fold). Arsenic is known to induce TGF- α in skin lesions (23), and the present study demonstrated that arsenic is also effective in enhancing the expression of TGF- β in mouse liver fibrogenesis. TGF- β is the most powerful profibrogenic mediator, plays a major role in the development of liver cirrhosis, and regulates ECM gene expression and matrix

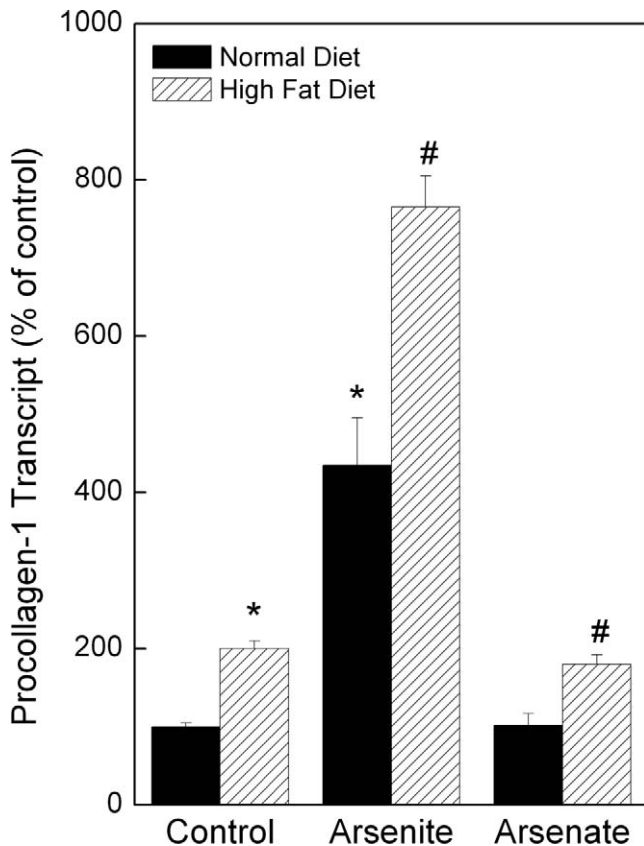


Figure 4. Expression of hepatic procollagen 1 transcript. Total RNA was isolated from livers of mice exposed to sodium arsenite (As^{3+} , 200 ppm) or sodium arsenate (As^{5+} , 200 ppm) for 10 months in the presence or absence of high dietary fat. Data are mean $\pm$ SEM ($n=10$). *Significantly different from untreated control, $P < 0.05$; #Significantly different from corresponding arsenic group without fat diet, $P < 0.05$.

degradation (24). The TGF- β pathway was clearly involved in arsenic hepatofibrogenesis in the present study.

Another important finding in gene expression is the increased expression of extracellular matrix metalloproteinase MMP2 and MMP9 by arsenic. Increased expression of MMP9 in cellular models of arsenic carcinogenesis is clearly one of the most sensitive biomarkers of malignant transformation (25, 26), and increased MMP2 and MMP9 expression are frequently associated with hepatocellular carcinomas and correlate with aggressive tumor behavior and poor prognosis (27, 28). Although no liver tumors were observed in the present study, the increased MMP2 and MMP9 indicated that preneoplastic molecular changes occurred in chronic arsenic exposed mouse livers, and the enhanced expression of MMP2 and MMP9 could play an important role in arsenic hepatocarcinogenesis. In liver fibrosis, the increased MMPs could facilitate collagen fiber degradation (13, 28), but the concomitantly increased expression of the tissue inhibitors of metalloproteinases (TIMPs) by arsenic was so dramatic that it could efficiently offset the fibrolysis effects of MMPs, resulting in the increased hepatic fibrosis observed in the present study.

These MMPs and TIMPs regulate the extracellular matrix turnover and remodeling during normal development and pathogenesis and play important roles in liver fibrogenesis, but are also involved in cell signaling, angiogenesis, and tumor cell metastasis (28, 29). It has been recently shown that the elevated stromal expression of TIMP1 promotes liver metastasis (30). The interplay between MMPs and TIMPs, as well as enhanced liver steatosis, a preneoplastic lesion (11), could be important in arsenic-induced liver fibrosis and hepatocarcinogenesis and are worth further investigations.

In summary, chronic inorganic arsenic exposure through the drinking water in mice produced liver pathology, and a high fat diet greatly enhanced arsenic-induced liver fibrosis. An array of aberrant gene expression changes are associated with liver fibrogenesis produced by arsenic, and these changes appear to be enhanced by consumption of a high fat diet. These molecular events could be critical to clinical intervention to protect against or prevent arsenic-induced liver pathology in humans.

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