Original article

Chronic exposure to sodium arsenite causes fibrogenic changes in the skin of mice, and short-term exposure may show anti-fibrogenic effects on fibroblast cells

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Abstract

Arsenic (As) is distributed widely in food, water, and soil, and can cause a variety of diseases, including skin lesions, peripheral neuropathy, liver injury. In particular, skin may be very sensitive to As. The purpose of the present study is (1) to identify, *in vivo*, whether or not chronic intake of sodium arsenite (As(III))-containing water induces fibrogenic changes in skin tissues, and (2) to clarify the fibrogenic or anti-fibrogenic function of As(III) *in vitro*. Male C57BL/6N mice were given drinking water with As(III) (1.0 or 10 mg/L) for 50 days. Skin sections from these mice were used to examine the thickness of collagen fiber bundles. We found that more thickened collagen bundles were observed, as the concentration of As(III) increased. Next, we used normal human dermal fibroblasts (NHDF) and examined the effect of As(III) on α2(I) collagen (COL1A2) expression and matrix metalloproteinase-1 (MMP-1) expression, which is a principal degradative enzyme for COL1A2. Short-term treatment (12-24 h) with 0.5-10 μM As(III) reduced COL1A2 expression and, conversely, enhanced MMP-1 expression. Transient transfection of COL1A2 and MMP-1 promoter/luciferase constructs into NHDF cells revealed that these modifications by As(III) were induced at the transcriptional level. This is the first demonstration that chronic drinking of As(III)-contaminated water causes fibrogenic changes in skin tissue, while short-term exposure to As(III) may have an anti-fibrogenic effect.

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《**Key words**》 sodium arsenite, fibrosis, matrix metalloproteinase-1, type I collagen

Ⅰ. Introduction

Arsenic (As) is a common environmental contaminant and is associated with the occurrence of adverse health effects such as skin lesions, peripheral neuropathy, liver injury and cancers in skin, lung, urinary bladder and other organs[1,2]. In particular, it appears that skin is very sensitive to As, and skin lesions are some of the most common and earliest non-malignant effects related to chronic As exposure[3]. The well-known skin lesions caused by chronic As exposure consists of two

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types, dyspigmentation and hyperkeratosis.

Recently, on the other hand, some studies have reported sodium arsenite (As(III))-induced liver fibrosis in the murine model[4,5]. These findings are fascinating as, when using scissors to cut the mice of skin, we observed that the skin of mice which had been administered sodium arsenite via drinking water chronically is tougher than that of control. Therefore, we hypothesized that As(III) induces the fibrogenic changes not only in liver but also in skin, and this toughness of skin may be a new manifestation of As poisoning.

One of the main deposits involved in fibrosis in dermal tissues is type I collagen and, on the contrary, a principal degradative enzyme for type I collagen is the matrix metalloproteinase-1 (MMP-1). Type I collagen is composed of two α1(I) chains and one α2(I) chain, which together form a triple helix[6]. In subsequent studies, we focused on one type I collagen, α2(I) collagen (COL1A2). While the number of reports on the fibrogenic effects of As *in vivo* is increasing, there is no study to examine whether As modulate both the fibrogenic effect via COL1A2 and the anti-fibrogenic effect via MMP-1 *in vitro*.

In general, it is complicated to clarify the toxic effect of As, for the toxicity depends on the length of exposure to As (e.g. chronic vs. acute), the level of exposure (high dose vs. low dose), and/or the species of As (e.g. arsenite, arsenate etc.). Most cases of human toxicity from As have been associated with exposure to inorganic As, and inorganic trivalent arsenite (As(III)) is several times more toxic than inorganic pentavalent arsenate (As(V))[7]. Therefore, in the present study, we used As(III) and examined the effecs of As(III) on skin tissue and cells.

In this study, we examined whether chronic intake of As(III)-containing water induces fibrogenic changes in skin tissue, and whether exposure to a low dose of As(III), ranging from 0.5 to 10 μM, regulates COL1A2 and MMP-1 expression in normal human dermal fibroblasts (NHDFs).

Ⅱ. Materials and methods

1. In vivo animal study.

Male C57BL/6N mice were purchased from Charles River, Japan Inc. Mice were kept in steel microisolator cages at 22℃ with a 12/12 h light/dark cycle. Food and water were provided *ad libitum*. Mice were 4 weeks old at the initiation of the exposure study and As(III) was administered at 1.0 or 10 mg/L (ppm) As to mice via drinking water for 50 days. Exposure period was determined by reference to the previous experiments[8,9]. The concentration of 1.0 mg/L As was determined according to our previous observations in China[10]. Drinking water was prepared freshly twice a week. After the exposure to As(III), mice were sacrificed and taken blood samples from the heart. As in blood was determined by cold trap-hydrogenation-atomic absorption spectrophotomy[10]. Simultaneously, pieces of skin were excised, using scissors, from shaved ventral skin for histopathological analysis. Skin sections were fixed in 4 % paraformaldehyde in Dulbecco's phosphate-buffered saline (PBS), and embedded in paraffin. After deparaffinizing tissue sections, the samples were stained with Hematoxylin and Eosin (H&E). All the animal experiments were conducted in accordance with our Institutional guidelines for Animal Experiments and based on the guidelines issued by Science Council of Japan adapted for mice.

2. Cell culture and treatment.

Normal human dermal fibroblasts (Cambrex

Bio Science Walkersville, Inc. Walkersville, MD) were cultured in commercial medium for fibroblasts (Cambrex) containing 2 % of fetal bovine serum, 5 mg/L of insulin, 1 μg/L of human fibroblast growth factor (hFGF)-B, gentamicin sulfate and amphotericin-B at 37℃ in a humidified atmosphere with 5 % CO_2. Powdered $NaAsO_2$ (MERCK, Darmstadt, Germany) was dissolved in PBS and added directly to the culture medium. A fresh 1 M As(III) solution was prepared for each new experiment.

3. Antibodies.

The following primary antibodies were used: monoclonal mouse antibodies against MMP-1 and type I collagen (Daiichi Fine Chemical Co. Ltd, Takaoka, Japan). For the confirmation of equal loading, blots were reprobed with anti-β-actin antibody (Sigma, St. Louis, MO). HRP-conjugated secondary antibodies (DAKO, Glostrup, Denmark)) were used.

4. RNA isolation and reverse transcription polymerase chain reaction (RT-PCR).

Total RNA was isolated from NHDF cells using the GeneElute Mammalian Total RNA Kit (Sigma-Aldrich, Inc. St. Louis, MO) and semiquantitative RT-PCR was performed as described[9]. The initial denaturation was at 94℃ for 2 min, followed by each cycle of reaction at 94℃ for 30 sec, at the optimal annealing temperature for 30 sec, and at 72℃ for 30 sec, and followed by post-extension at 72℃ for 7 min. The sequences of primers used for PCR amplification are as follows: COL1A2 (forward, 5'-GAC CTC CAG GTG TAA GCG GT-3', and reverse, 5'-TTC AGG TTG GGC CCG GAT AC-3', 55℃, 23 cycles); MMP-1 (forward, 5'-GGT GCC CAG TGG TTG AAA AAT-3', and reverse, 5'-CAT CAC TTC TCC CCG AAT CGT-3', 57℃, 23 cycles). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was used as an internal control, was amplified for 25 cycles as described[11]. The PCR products were separated by electrophoresis on a 1.5 % agarose gel, stained with ethidium bromide.

5. Transient transfection and assessment of promoter activity.

Details about the constructions of -378 COL1A2 / LUC (kindly provided by Dr. Yutaka Inagaki) and -522 MMP-1/ LUC (-522/+72; 0.6kb) chimeric plasmids have been published[11, 12]. The promoter/luciferase constructs were transfected into NHDF cells using the calcium phosphate/DNA coprecipitation method. Transfection efficiency was normalized by using pRL-CMV vector (Promega) as an internal control. Before transfection, NHDF cells were cultured for 12 h in the presence of As(III). Five hours after transfection, the cells were treated with 10 % glycerol for 80 sec, then incubated for 40 h. Firefly and Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega). Cell transfections and luciferase assays were repeated independently more than 3 times, each of them was performed in duplicate.

6. Western blot analysis.

For the detection of MMP-1, culture media were concentrated using Amicon Ultra-4 (Millipore Corporation, Bedford, MA) according to the manufacturer's instructions. For the detection of other proteins, total and/or nuclear extracts were prepared from NHDF cells using NE-PER Nuclear and Cytoplasmic extraction kit (Pierce Biotechnology, USA) according to the manufacturer's protocol. The concentration of protein was measured using the DC Protein Assay Kit (Bio-Rad, Richmond, CA). Samples were mixed with 4×SDS sample buffer (0.25 M Tris-HCl, pH 6.8, 8 % SDS, 20 %

Glycerol, 5 % β-mercaptoethanol), and equal amounts of protein per lane were run on a 10 % SDS-PAGE and transferred onto a PVDF membrane (Amersham Biosciences, Buckinghamshire, UK). After blocking the non-specific binding sites with 5 % non-fat dry milk (Amersham Biosciences), blots were incubated with anti-MMP-1 (Daiichi Fine Chemical Co. Ltd.) at a dilution of 1: 1000 for 2 h or anti-COL1A2 (Daiichi Fine Chemical Co. Ltd.) at a dilution of 1: 500 overnight, followed by incubation with secondary HRP-conjugated anti-rabbit (1: 3000) or anti-mouse (1: 3,000) antibody (DAKO, Glostrup, Denmark) for 1 h at room temperature. The proteins were visualized by chemiluminescence by using the ECL Plus detection kit (Amersham Biosciences, Buchinghamshire, UK) according to the manufacturer's instruction. For confirmation of equal loading, blots were reprobed with anti-β-actin antibody (Sigma Chemical Co.) at a dilution of 1: 5,000.

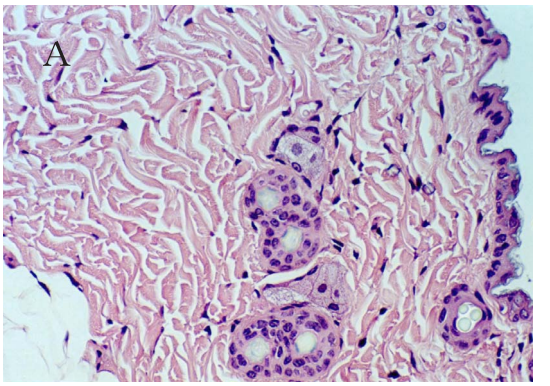
7. Statistical analysis.

The data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using the non-parametric Mann-Whitney test. *P*-values < 0.05 were considered statistically significant.

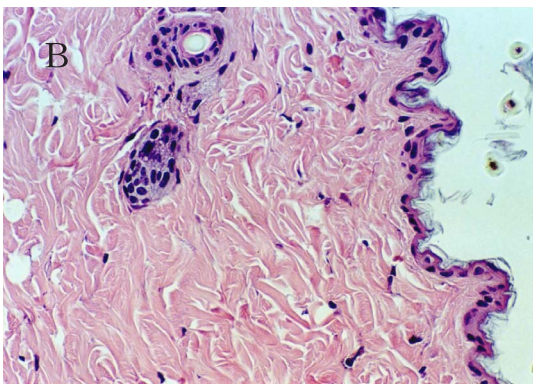
Ⅲ. Results

1. Effect of chronic intake of As(III) on skin tissue

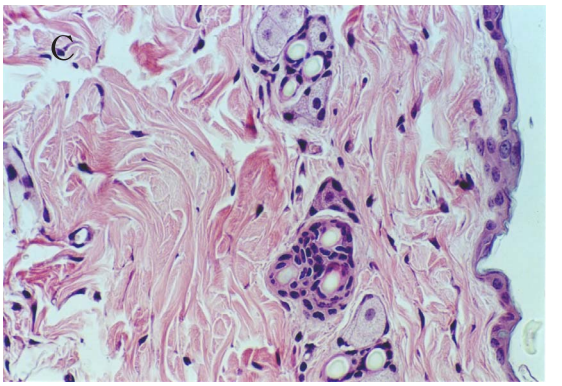
As(III) was administered at 1.0 or 10 mg/L to male C57BL/6N mice via drinking water for 50 days. After the exposure to As(III), mice were sacrificed and pieces of skin were excised, using scissors, from shaved ventral skin for histopathological analysis. The histology shows thickened collagen bundles in dermis of the exposed mice (Fig. 1). As the concentration of As(III) increased, more thickened



A (control)



B (1.0 mg/L As)



C (10 mg/L As)

Fig. 1 The skin histology of mice exposed to As (Ⅲ) for 50 days.

Photomicrographs present H & E stained paraffin sections of the ventral skin from the control group (A), exposure group (1.0 mg/L of As (B) and 10 mg/L of As (C)). Thickened collagen bundles in dermis of exposed mice were observed. As the concentration of As(III) increased, more thickened collagen bundles were observed (HE x100).

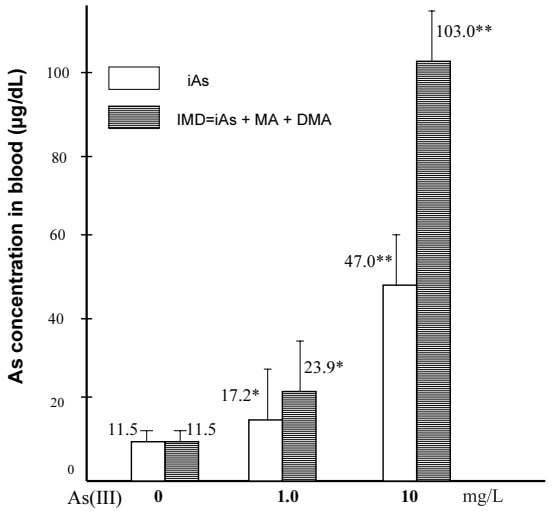


Fig. 2 Increase of the concentration of As in blood of arsenic exposed mice.

Mice were 4 weeks old at the initiation of the exposure study and As(III) was administered at 1.0 or 10 mg/L As(III) to mice via drinking water for 50 days. After the exposure to As(III), mice were sacrificed and taken blood samples from the heart. As in blood was determined by cold trap-hydrogenation-atomic absorption spectrophotomy. IMD represents three forms of As in blood: inorganic arsenic (iAs), mono-methylated arsenic (MA) and di-methylated arsenic (DMA). When the concentration of As(III) in drinking water was 0 mg/L (control), 1 mg/L, and 10 mg/L , the concentration of IMD in blood was 11.5 ± 3.8 μg/dL, 23.9 ± 12.5 μg/dL, and 103.0 ± 10.6 μg/dL (mean ± SD), respectively. The concentration of iAs was 11.5 ± 3.8 μg/dL, 17.2 ± 12.5 μg/dL, and 47.0 ± 12.9 μg/dL (mean ± SD), respectively. Statistical significance was defined as * $P< 0.05$ and ** $P< 0.01$.

collagen bundles were observed. This result showed that chronic intake of As(III)-containing water induces the thickness of collagen fiber bundles in the skin tissue of mice. Simultaneously, we determined the concentration of arsenic in blood of arsenic exposed mice by cold trap-hydrogenation-atomic absorption spectrophotomy. In Fig. 2, IMD represents three forms of As in blood: inorganic arsenic (iAs), mono-methylated arsenic (MA) and di-methylated arsenic (DMA). When the concentration of As(III) in drinking water was 0 mg/ L(control), 1.0 mg/L, and 10 mg/L , the total concentration of IMD in blood was 11.5 ± 3.8 μg/dL, 23.9 ± 12.5 μg/dL, and 103.0 ± 10.6 μg/dL (mean ± SD), respectively. The concentration of iAs was 11.5 ± 3.8 μg/dL, 17.2 ± 12.5 μg/dL, and 47.0 ± 12.9 μg/dL (mean ± SD), respectively.

2. Changes of COL1A2 and MMP-1 expression and transcriptional activity of their promoters after the exposure of fibroblasts to As(III)

The effect of As(III) on gene expression of normal human dermal fibroblasts (NHDF) was examined by RT-PCR (Fig. 3 (a)). NHDF expressed COL1A2 and MMP-1 mRNA without addition of As(III). After the exposure of NHDF cells to 0.5 μM, 1.0 μM, and 10 μM As(III) for 12 h, the bands of COL1A2 were weaker than those of the control, while the bands of MMP-1 were stronger. This result was confirmed by Western blot analysis (Fig. 3 (b)). These results indicated that short-term exposure (12 h) to As(III) suppressed COL1A2 expression and, on the contrary, induced MMP-1 expression in NHDF cells. In addition, we examined the effect of 24 h exposure to As(III), and the results were the same as those after exposure for 12 h (data not shown).

In order to clarify the contribution of transcriptional factors in NHDF cells, the -378 COL1A2 promoter construct was transfected into NHDF cells (Fig. 4 (a)). The COL1A2 promoter activity was decreased significantly by exposure to As(III). Next, the proximal -522 MMP-1 promoter construct which contains three AP-1 sites was transfected into NHDF cells. Unlike the COL1A2 promoter activity, the MMP-1 promoter activity was increased after the addition of As(III) (Fig. 4(b)). These results confirmed the analysis by RT-PCR and Western blotting.

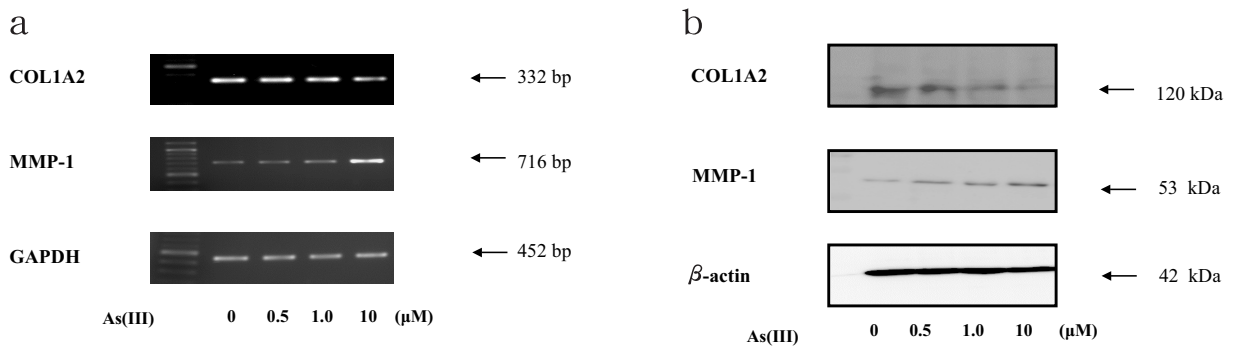


Fig. 3 COL1A2 and MMP-1 expressions in NHDF cells.

COL1A2 and MMP-1 expressions in NHDF cells were detected by RT-PCR (a) and Western blot analysis (b). After the exposure of NHDF cells to 0.5 μM, 1.0 μM, and 10 μM As(III) for 12 h, total RNA was extracted from semiconfluent NHDF cells and 1 μg of total RNA was used as a template for RT-PCR analysis. GAPDH (bottom) was used as an internal control. For Western blot analysis, proteins were extracted from NHDF cells treated with the indicated concentration of As(III) for 12 h and assessed by using an antibody to type I collagen. β-actin was used to confirm the equal protein loading. For detection of MMP-1, NHDF cells were cultured in serum-free medium for 12 h and the culture medium was concentrated using an Amicon Ultra (Millipore). Eight micrograms of proteins from each sample was used to detect MMP-1 by Western blot analysis. These are representative RT-PCR and Westerns from n=3-4 experiments.

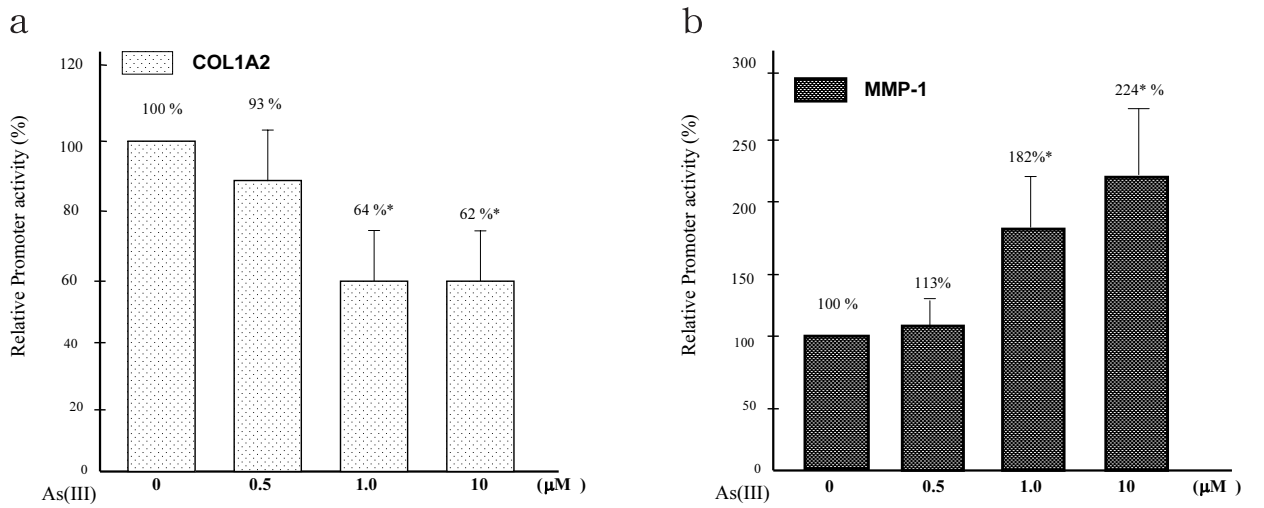


Fig. 4 Effect of sodium arsenite on COL1A2 and MMP-1 promoter activity in NHDF cells.

As described in Materials and Methods, NHDF cells were transfected with the -378 COL1A2 promoter/luciferase reporter gene construct or the -522 MMP-1 construct together with pRL-CMV vector. After transfection, NHDF cells were cultured for 12 h in the presence of As(III). After transfection, cells were incubated for 40 h. Relative luciferase activities (mean ± SD) were normalized for pRL-CMV activity. Transfections and assays were performed independently 3 or 4 times, each run in duplicate. Statistical significance was defined as * $P< 0.05$.

Ⅳ. Discussion

In the present study we identified *in vivo* that chronic intake of As(III)-containing water induces the thickness of collagen fiber bundles in the skin tissue of mice. In addition, we provide evidence that short-term exposure (12-24 h) of As(III) to NHDF cells induces MMP-1 expression, on the contrary, decreases

COL1A2 expression. This is the first report to show a fibrogenic effect of long-term exposure to As(III) *in vivo* and an anti-fibrogenic effect of short-term exposure to As(III) *in vitro*.

Chronic effects of As exposure via drinking water can cause many diseases[3]. In particular, dyspigmentation and hyperkeratosis are characteristic skin lesions. In this study we observed the thickness of collagen fiber bundles in skin tissues in male C57BL/6N mice administered at 1.0 or 10 mg/L As for 50 days. In addition, this change was induced dose-dependently. Moreover, we confirmed that the concentrations of both iAs and IMD (inorganic arsenic + mono-methylated arsenic + di-methylated arsenic) in blood of arsenic exposed mice were higher as the concentration of As(III) in drinking water increased. In this method, even when drinking water did not contain As(III), iAs and IMD were slightly detected. We suppose that this result may be due to food mice had as the previous report says[13]. Mazumder demonstrated that hepatic fibrosis was observed when male BALB/c mice were given drinking water contaminated with arsenic (3.2 mg/L) for 15 months, and fibrosis was not induced after 12 months of administration[4]. Both Mazumder's experiment and ours show that As(III) exposure induces fibrogenic changes in mice. However, the time difference at which As-induced lesions are revealed may reflect that skin is sensitive to As and As-induced skin lesions can be the earliest health effects related to chronic As exposure prior to malignant skin lesions. We show here that fibrogenic change is a manifestation of chronic As poisoning in addition to the previously observed skin lesions, dyspigmentation and hyperkeratosis. These three manifestations of fibrogenic change, dyspigmentation and hyperkeratosis, may be caused primarily by three different cells in skin such as fibroblast, melanocyte and keratinocyte, respectively. The differences of onset time and its severity of these three manifestations are supposed to reflect the possibility that these cells may exhibit differential sensitivity to As. While dyspigmentation and hyperkeratosis are very easy-to-find manifestations[14], fibrogenic change can not be detected without the samples of dermal tissue obtained by biopsy. Therefore, the investigation with the samples of skin tissue obtained from those who have drunk As-contaminated water chronically is needed to clarify the fibrogenic change in human skin.

Our next question was whether fibrogenic effect of As on skin lesion starts from the early time after exposure of As or not. Our experiment of As(III) *in vivo* showed that a longer period was needed to observe the thickness of collagen fiber bundles in skin tissues and it remained unknown that this fibrogenic change was occurring in skin tissues immediately after exposure of As(III). However, it is difficult to detect this early modulation *in vivo*. Therefore, we conducted *in vitro* analysis with NHDF cells and examined both fibrogenic and anti-fibrogenic change, focusing on COL1A2 and MMP-1 expression.

Interestingly, both semiquantitative RT-PCR analysis and Western blotting revealed the decrease of COL1A2 and the increase of MMP-1. These changes may induce an anti-fibrogenic effect of As(III) exposure. This anti-fibrogenic effect of short-term exposure to As(III) was also observed by examining the transcriptional activity of COL1A2 and MMP-1 promoters (Fig. 4).

These opposite results of As(III) may reflect the length of exposure and corresponding modulations of transcriptional factors

regulating COL1A2 and MMP-1 expressions. A growing body of evidence supports the role of As(III) in regulating NF-κB and AP-1 activity[15,16]. Hu et al. showed that short-term treatment (24 h) with 0.1–5 μM As(III) increased both AP-1 and NF-κB binding activity in human fibroblast cells, while chronic treatment (10-20 weeks) with 0.1 μM or 0.5 μM As(III) decreased the binding activity of both AP-1 and NF-κB[17]. In the human COL1A2 promote region, AP-1, NF-κB, SP-1, and Smad3/4 binding sites are contained and have been shown to participate in the regulatory programs activated by several cytokines to control type I collagen expression[18]. Moreover, NF-κB activation is essential for inhibition of basal COL1A2 expression. In the 4.4kb MMP-1 promoter region that covers the entire promoter region, nine AP-1 and one NF-κB binding motifs are included, and the transcriptional factors AP-1 and NF-κB enhance MMP-1 transcription[19]. Taken together, the modulation of AP-1 and NF-κB binding activity after short-term exposure of As may indicate that the enhancement of suppressor NF-κB caused the decrease of COL1A2 expression and that the enhancement of activator AP-1 and NF-κB was responsible for the increase of MMP-1 expression. Therefore our findings are essentially consistent with their results observed in short-term treatment. Moreover, if their observations in chronic exposure are adaptable, we can expect chronic exposure of fibroblasts to As(III) will produce a fibrogenic effect, because the corresponding molecular mechanism, the decreased binding activities of AP-1 and NF-κB may lead to enhancement of COL1A2 and decrease of MMP-1 expression.

In short, anti-fibrogeic change observed in vitro may represent the earlier change caused by As exposure, and fibrogenic change observed in vivo may show the later event developed by As exposure. These opposite effects of As can be caused by the modulation of the said transcription factors. Though the supportive investigation has already been reported by Hu et al., three further investigations may be needed in order to provide the precise molecular basis to our present observation: (1) to clarify the molecular mechanisms after short-term exposure of As(III) which regulate COL1A2 and MMP-1 expression, (2) to examine the molecular events in chronic exposure to As(III), and (3) when and how the suggestive molecular shift from increase of AP-1 and NF-κB binding activities to decrease of them may occur *in vivo* and *in vitro*.

In conclusion, the present study shows that chronic intake of As(III)-containing water induces the thickness of collagen fiber bundles in the skin tissue of mice and that anti-fibrogenic effect of As(III) may be producing at the early time via decrease of COL1A2 and increase of MMP-1 expression. These results and further investigations in the molecular mechanisms will be helpful to prevent the adverse health effects inducend by As exposure.

Acknowledgments

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References

1) Tchounwou P B, Patlolla A K, et al: Carcinogenic and systemic health effects associated with arsenic exposure—a critical review. Toxicol Pathol 31: 575–588, 2003
2) Rossman T G, Uddin A N, et al: Evidence that arsenite acts as a cocarcinogen in skin cancer. Toxicol Appl Pharmacol 198: 394–404, 2004

3) Yoshida T, Yamauchi H, et al: Chronic health effects in people exposed to arsenic via the drinking water: dose-response relationships in review. Toxicol Appl Pharmacol 198: 243–252, 2004
4) Mazumder D N: Effect of chronic intake of arsenic-contaminated water on liver. Toxicol Appl Pharmacol 206: 169–175, 2005
5) Das S, Santra A, et al: Implications of oxidative stress and hepatic cytokine (TNF-alpha and IL-6) response in the pathogenesis of hepatic collagenesis in chronic arsenic toxicity. Toxicol Appl Pharmacol 204: 18–26, 2005
6) Vuorio E, and de Crombrugghe B: The family of collagen genes. Annu Rev Biochem 59: 837–872, 1990
7) Vega L, Styblo M, et al: Differential effects of trivalent and pentavalent arsenicals on cell proliferation and cytokine secretion in normal human epidermal keratinocytes. Toxicol Appl Pharmacol 172: 225–232, 2001
8) Patterson R, Vegal L, et al.: Arsenic-induced alterations in the contact hypersensitivity response in Balb/c mice. Toxicol Appl Pharmacol 198: 434–443, 2004
9) Simeonova PP, Wang S, et al: Quantitative relationship between arsenic exposure and AP-1 activity in mouse urinary bladder epithelium. Toxicol Sci 60: 279–284, 2001
10) Yamauchi H, Takahiko Y, et al: Study on the metabolism of arsenic in the patients with chronic arsenic poisoning. Jpn J Clin Ecol 9: 68–74, 2000
11) Inagaki Y, Truter S, et al: Overlapping pathways mediate the opposing actions of tumor necrosis factor-alpha and transforming growth factor-beta on alpha 2(I) collagen gene transcription. J Biol Chem 270: 3353–3358,1995
12) Sugioka Y, Watanabe T, et al: c-Jun NH2-terminal kinase pathway is involved in constitutive matrix metalloproteinase-1 expression in a hepatocellular carcinoma-derived cell line. Int J Cancer109: 867–874, 2004
13) Roychowdhury T, Uchino T, et al: Survey of arsenic in food composites from an arsenic-affected area of West Bengal, India. Food Chem Toxicol 40: 1611–1621, 2002
14) Sun Guifan(eds): ENDEMIC ARSENICOSIS. A Clinical Diagnostic Manual with Photo Illustrations. UNICEF, 2004, pp31–59
15) Chen N Y, Ma W Y, et al; Inhibition of arsenite-induced apoptosis and AP-1 activity by epigallocatechin-3-gallate and theaflavins. J Environ Pathol Toxicol Oncol 19: 287–295, 2000
16) Felix K, Manna S K, et al: Low levels of arsenite activates nuclear factor-kappaB and activator protein-1 in immortalized mesencephalic cells. J Biochem Mol Toxicol 19: 67–77, 2005
17) Hu Y, Jin X, et al: Effect of arsenic on transcription factor AP-1 and NF-kappaB DNA binding activity and related gene expression. Toxicol Lett 133: 33–45, 2002
18) Ghosh A K: Factors involved in the regulation of type I collagen gene expression: implication in fibrosis. Exp Biol Med (Maywood) 227: 301–314, 2002
19) Vincenti M P, Coon C I, et al: Nuclear factor kappaB/p50 activates an element in the distal matrix metalloproteinase 1 promoter in interleukin-1beta-stimulated synovial fibroblasts. Arthritis Rheum 41: 1987–1994, 1998

要約

砒素曝露が皮膚の線維化に関与するとの報告は今日までなされていない。今回の論文の目的は、⑴亜砒酸ナトリウムを含む水をマウスに長期摂取させると皮膚組織に線維化が誘導されるか否か、⑵線維芽細胞への砒素曝露は線維化に関わる遺伝子変化を生じさせるか否かを明らかにすることである。C57BL/6N マウスに砒素（1.0と10 mg/L）を含む水を与え50日間飼育すると、皮膚組織で線維束の肥大が濃度依存的に認められた。次に、正常ヒト線維芽細胞において0.5-10 μM の砒素曝露により、12－24時間の短期曝露ではコラーゲン（COL1A2）の減少とその主な分解酵素マトリックスメタロプロテネース（MMP-1）の増加が、それぞれ RT-PCR 及びウエスタンブロッティング法で確認された。この変化はプロモーターアッセイ法でも確認され、砒素曝露による転写因子の関与を示唆した。

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