Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.7, No.2, 77-83, 2020

The Japanese Society of Toxicology

Fundamental Toxicological Sciences
URL : http://www.fundtoxicolsci.org/index_e.html

Original Article

Arsenite inhibits gene expression of perlecan, syndecan-1, -2, -3 and biglycan in cultured vascular endothelial cells

Dong-pan Wu[1,2,*], Tsuyoshi Nakano[1,*], Yayoi Tsuneoka[1], Tsutomu Takahashi[1], Yo Shinoda[1], Toshiyuki Kaji[3] and Yasuyuki Fujiwara[1]

[1]Department of Environmental Health, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan
[2]Department of Pharmacy, Guang' anmen Hospital of China Academy of Chinese Medical Sciences, Beijing 100053, China
[3]Department of Environmental Health, Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan

(Received January 17, 2020; Accepted January 28, 2020)

ABSTRACT — Arsenic is an environmental pollutant and is a possible risk factor for vascular diseases such as atherosclerosis. Vascular proteoglycans (PGs) are key molecules in the initiation and progression of atherosclerosis. We previously demonstrated that arsenite, but not arsenate, decreases the synthesis of both heparan sulfate proteoglycans (HSPGs) and chondroitin/dermatan sulfate proteoglycans (CS/DSPGs) in cultured vascular endothelial cells. In the present study, we aimed to identify the PG molecules whose expression is decreased by arsenite, using a culture system of bovine aortic endothelial cells. The results indicate that a 24-hr treatment of arsenite significantly decreases the mRNA levels of a large HSPG perlecan, small HSPGs—syndecan-1, -2 and -3—, and a small CS/DSPG biglycan in vascular endothelial cells without nonspecific cell damage; the expression of syndecan-4 mRNA was unaffected by arsenite. The decreased expression of perlecan, syndecan-1 and biglycan genes began after 3 hr of arsenite treatment. However, arsenate did not change the mRNA expression levels of perlecan and biglycan in the cells. These results suggest that the inhibition of synthesis by arsenite occurs in particular types of proteoglycans, i.e. perlecan, syndecan-1, -2, -3, and biglycan in vascular endothelial cells.

Key words: Arsenite, Proteoglycan, Perlecan, Biglycan, Vascular endothelial cell

INTRODUCTION

Proteoglycans (PGs) are a heterogeneous group of molecules that are predominant constituents of both the extracellular matrix and the cell surface. PGs have the common structure of a core protein, to which one or more glycosaminoglycan chains are covalently attached (Ruoslahti, 1988). Vascular endothelial cells predominantly synthesize a large heparan sulfate proteoglycan (HSPG), perlecan, and a small chondroitin/dermatan sulfate proteoglycan (CS/DSPG), biglycan (Saku and Furthmayr, 1989; Kaji *et al*., 2000; Yamamoto *et al*., 2005). In addition, we showed that cultured bovine aortic endothelial cells express the small HSPGs, syndecan-1, -2, -3, and -4 (Hara *et al*., 2018) as well as perlecan and biglycan. These vascular PGs exhibit multiple functions, such as extracellular matrix assembly, lipid metabolism, permeability and thrombosis, in vascular tissue through

Correspondence: Toshiyuki Kaji (E-mail: t-kaji@rs.tus.ac.jp)
Yasuyuki Fujiwara (E-mail: yasuyuki@toyaku.ac.jp)
*These authors equally contributed to this work.

the interaction with diverse biologically active substances (Camejo, 1981; Berenson *et al*., 1984).

Arsenic is an environmental pollutant that exists widely around the world. Health impairment due to arsenic pollution of groundwater is currently noted among residents worldwide (Nordstrom, 2002). Epidemiological studies have shown that arsenic is a possible risk factor for vascular diseases related to atherosclerosis and blood circulation disorder (Moon *et al*., 2013; Tseng *et al*., 1995; Chen *et al*., 1988). It has been also demonstrated that exposure to several arsenic compounds, including arsenite, accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice, a model of human atherosclerosis (Simeonova *et al*., 2003; Srivastava *et al*., 2009; Negro Silva *et al*., 2017; Makhani *et al*., 2018). Atherosclerosis is initiated by functional damages in vascular endothelial cells followed by monocyte/macrophage invasion into under the endothelium tissue and vascular smooth muscle cell transformation and hyperplasia in the intima of the vascular wall (Ross, 1993).

We have previously shown that arsenite, but not arsenate, decreases the synthesis of both HSPGs and CS/DSPGs in cultured bovine aortic endothelial cells (Fujiwara *et al.*, 2005, 2012) using diethylaminoethyl-Sephacel column chromatography of radiolabeled PG molecules. However, type(s) of PGs of which synthesis is inhibited by arsenite is unclear. The purpose of the present study is to identify the PG types using a culture system of bovine aortic endothelial cells.

MATERIALS AND METHODS

Materials

Vascular endothelial cells derived from bovine aorta were purchased from Cell Applications (San Diego, CA, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were obtained from Nissui Pharmaceutical (Tokyo, Japan) and Biowest (Nuaille, France), respectively. TrueLine cell culture dishes and plates were purchased from Nippon Genetics (Tokyo, Japan). 3-(4,5-dimethylthiazol-2-thiazolyl)-2,5-diphenil-2H-tetrazolium bromide (MTT) was purchased from Dojindo Laboratories (Kumamoto, Japan). CytoTox96® non-radioactive cytotoxicity assay, a lactate dehydrogenase (LDH) assay kit, was purchased from Promega (Madison, WI, USA). ISOGEN II reagent was obtained from Nippon Gene (Tokyo, Japan). ReverTra Ace qPCR RT Master Mix and THUNDERBIRD SYBR qPCR Mix were purchased from Toyobo (Osaka, Japan). Sodium arsenite ($NaAsO_2$), sodium arsenate (Na_2HAsO_4), dimethyl sulfoxide (DMSO) and other reagents were obtained from Fujifilm Wako Pure Chemical (Osaka, Japan).

Cell culture

Vascular endothelial cells were cultured in DMEM supplemented with 10% fetal bovine serum in 100-mm dishes at 37°C in a humid atmosphere of 5% CO_2 in the air until confluence. They were then transferred into 24-well culture plates and cultured until confluence in DMEM supplemented with 10% fetal bovine serum. The medium was discarded and the cell layer was washed twice with fresh serum-free DMEM. The cell layer was then incubated with arsenite at 0, 1, 2, 5, 10, or 20 μM at 37°C for 3 or 24 hr in fresh serum-free DMEM. Separately, the cell layer was incubated with arsenate at 5 μM for 24 hr in fresh serum-free DMEM.

Cell viability assay

MTT assay was used to estimate cell viability, as described previously (Denizot and Lang, 1986). Briefly, cells were exposed to arsenite in a 24-well culture plate, then treated with 5 mg/mL MTT (1/20 volume) for another 2 hr at 37°C. After removing the medium, DMSO was added to dissolve MTT formazan. Absorbance at 570 nm was measured using a Multiskan FC microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

LDH leakage assay

LDH leakage assay was used to estimate cytotoxicity as described previously (Ichikawa *et al*., 2019). After incubation with arsenite, the conditioned medium was harvested, and an aliquot of the medium was used to measure LDH activity with a CytoTox96® non-radioactive cytotoxicity assay kit. The absorbance of each sample was measured at 490 nm using a Multiskan FC microplate reader (Thermo Fisher Scientific).

Real-time RT-PCR analysis

Total RNA extraction from cultured cells and subsequent real-time RT-PCR analysis were performed as described previously (Takahashi *et al.*, 2018). Briefly, after incubation with arsenite or arsenate, the culture medium was removed and the cell layer was washed twice with cold phosphate buffered saline and 300 μL cold ISOGEN II reagent was added to each culture well. Cells were collected by scraping and homogenized by pipetting. The RNA quality was ensured by spectrophotometric analysis (OD260/280) using NanoDrop Lite spectrophotometer (Thermo Fisher Scientific). Reverse transcriptome was performed using ReverTra Ace qPCR RT Master Mix and GeneAmp PCR system 9700 (Thermo Fisher Scientific). Real-time PCR was performed with the THUNDERBIRD

Table 1. Oligonucleotide primers used for real-time PCR.

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Product Size (bp)
HSPG2 (Perlecan)	AGCCGAGCCATGGATTTCAA	AACATATTGGAGCCGGTGCA	124
SDC1 (Syndecan-1)	CAGTCAGGAGACAGCATCAG	CCGACAGACATTCCATACC	153
SDC2 (Syndecan-2)	CCAGATGAAGAGGACACAAACG	CCAATAACTCCGCCAGCAA	101
SDC3 (Syndecan-3)	CAAGCAGGCGAGCGTC	GGTGGCAGAGATGAAGTGG	183
SDC4 (Syndecan-4)	TTGCCGTCTTCCTCGTGC	AGGCGTAGAACTCATTGGTGG	117
BGN (Biglycan)	GCTGCCACTGCCATCTGAG	CGAGGACCAAGGCGTAG	168
GAPDH	TGTTTGTGATGGGCGTGAAC	ACGATGCCAAAGTGGTCATG	124

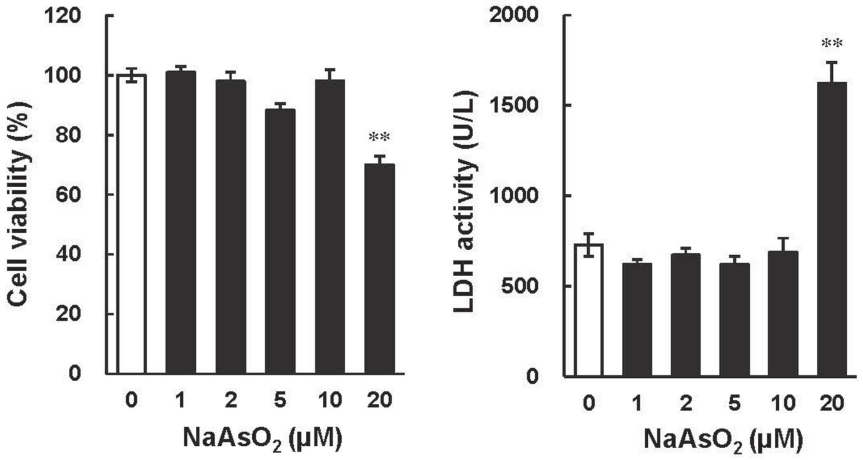

Fig. 1. Cell viability of vascular endothelial cells (left panel) and leakage of LDH into the medium from the cells (right panel) after exposure to sodium arsenite ($NaAsO_2$). Confluent cultures of bovine aortic endothelial cells were incubated for 24 hr with $NaAsO_2$ (1, 2, 5, 10, and 20 μM). Data are represented as mean ± SEM of four samples. Statistical significance when compared to 0 μM $NaAsO_2$: $^{**}p < 0.01$.

SYBR qPCR Mix using 0.5-μM primers and LightCycler 96 (Roche, Tokyo, Japan). The thermal treatment was 95°C for 10 min, and 45 cycles of 95°C for 10 sec and 60°C for 30 sec. The PCR primers (Table 1) were purchased from Eurofins Genomics (Tokyo, Japan). The fold change for each gene was assessed after normalization of the intensity value to that of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*).

Statistical analysis

All statistics were performed using Excel software (Microsoft, Redmond, WA, USA) with the Statcel3 add-in (OMS, Tokyo, Japan). Data are expressed as the mean ± SEM. The statistical significance of the data was determined using one-way ANOVA with the post-hoc Bonferroni/Dunn test or Student's *t*-test as appropriate. *P*-values less than 0.05 were considered statistically significant.

RESULTS

First, the cytotoxicity of arsenite to vascular endothelial cells was evaluated by the MTT assay and LDH leakage assay. As shown in Fig. 1, arsenite at 10 μM or less did not cause a significant decrease in the cell viability (left panel) or increase in the LDH activity (right panel), suggesting that arsenite at 10 μM and less does not exhibit cytotoxicity to vascular endothelial cells, although arsenite at 20 μM was cytotoxic.

Figure 2 shows the perlecan, syndecan-1, syndecan-2, syndecan-3, syndecan-4, and biglycan mRNAs in vascular endothelial cells after exposure to arsenite at 10 μM or less for 24 hr. Under this nontoxic experimental condition, arsenite significantly decreased the mRNA levels of *HSPG2* (perlecan), *SDC1* (syndecan-1), *SDC2* (syndecan-2), *SDC3* (syndecan-3), and *BGN* (biglycan). However, the *SDC4* (syndecan-4) mRNA level was unaffected by arsenite (Fig. 2A). In addition, the decrease in the mRNA levels of *HSPG2* (perlecan), *SDC1* (syndecan-1),

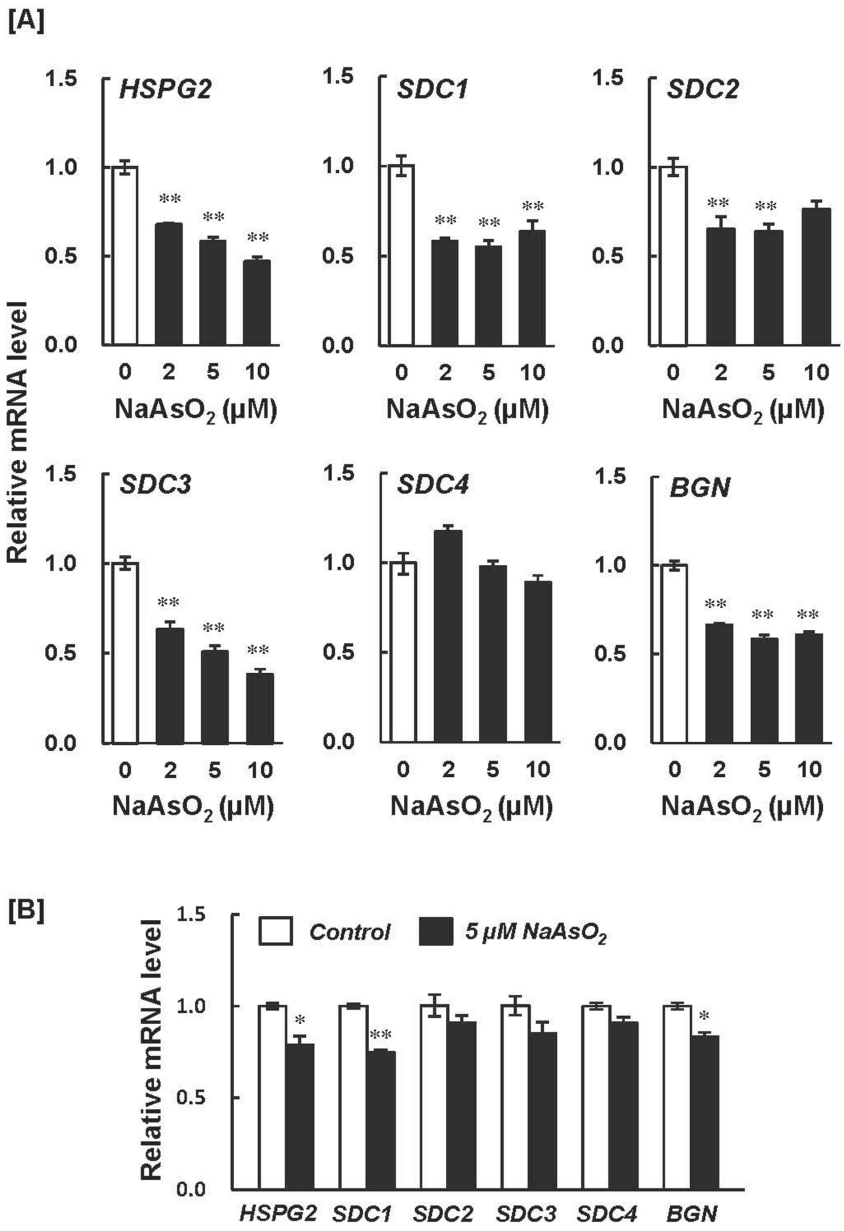

Fig. 2. Effect of sodium arsenite ($NaAsO_2$) on mRNA levels of PG molecules in vascular endothelial cells. (A) Confluent cultures of bovine aortic endothelial cells were incubated for 24 hr with $NaAsO_2$ (2, 5, and 10 μM). mRNA levels of PG molecules (*HSPG2*, *SDC1*, *SDC2*, *SDC3*, *SDC4*, and *BGN*) and *GAPDH* were measured by real-time RT-PCR. Data are represented as mean ± SEM of four samples. Statistical significance when compared to 0 μM $NaAsO_2$: $^{**}p < 0.01$. (B) Confluent cultures of the cells were incubated for 3 hr with $NaAsO_2$ (5 μM). mRNA levels of PG molecules and *GAPDH* were measured by real-time RT-PCR. Data are represented as mean ± SEM of three samples. Statistical significance when compared to corresponding control: $^{*}p < 0.05$; $^{**}p < 0.01$.

and *BGN* (biglycan) by arsenite at 5 μM began after a 3-hr treatment (Fig. 2B).

The effects of arsenite and arsenate on endothelial perlecan and biglycan mRNA expression were compared (Fig. 3). After a 24-hr incubation, arsenite at 5 μM significantly decreased both *HSPG2* (perlecan) and *BGN* (biglycan) mRNA levels in vascular endothelial cells. However, arsenate at 5 μM did not influence either *HSPG2*

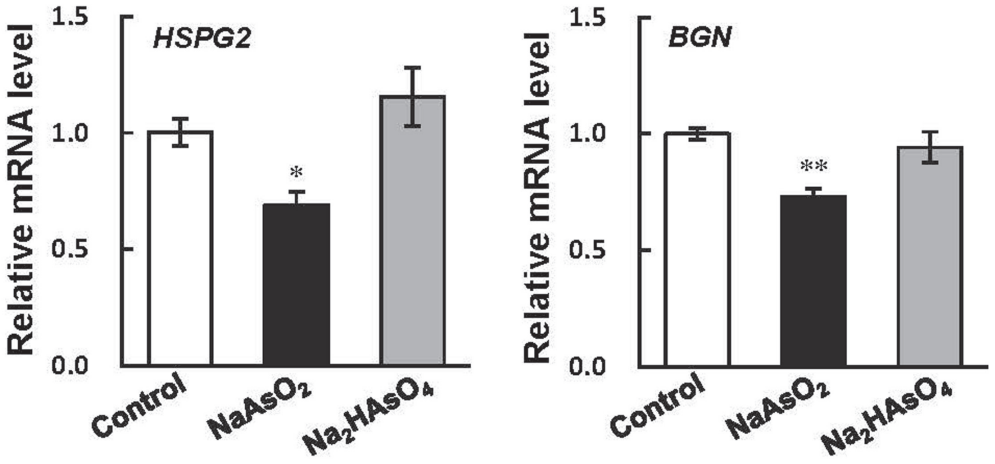

Fig. 3. Effects of sodium arsenite ($NaAsO_2$) and sodium arsenate (Na_2HAsO_4) on mRNA levels of *HSPG2* (perlecan) and *BGN* (biglycan) in vascular endothelial cells. Confluent cultures of bovine aortic endothelial cells were incubated for 24 hr with $NaAsO_2$ (5 μM) and Na_2HAsO_4 (5 μM). mRNA levels of *HSPG2, BGN,* and *GAPDH* were measured by real-time RT-PCR. Data are represented as mean ± SEM of three samples. Statistical significance when compared to control: $*p < 0.05$, $**p < 0.01$.

(perlecan) or *BGN* (biglycan) mRNA levels.

DISCUSSION

We have previously shown that arsenite decreases the synthesis of both HSPGs and CS/DSPGs in cultured bovine aortic endothelial cells (Fujiwara *et al.*, 2005, 2012). Although the cells predominantly synthesize perlecan and biglycan (Saku and Furthmayr, 1989; Kaji *et al*., 2000), the PG type(s) of PGs, of which synthesis is inhibited by arsenite, has not been identified. In the present study, it was shown that arsenite significantly decreases the gene expression of perlecan and biglycan in cultured bovine aortic endothelial cells without nonspecific cell damage. In addition, the gene expression of syndecan-1, -2, and -3 was also inhibited by arsenite, while that of syndecan-4 was not influenced. These results suggest that arsenite inhibits the synthesis of perlecan and syndecans (syndecan-1, -2, and -3) in HSPGs and biglycan in CS/DSPGs in vascular endothelial cells. However, pentavalent arsenate did not influence the gene expression of perlecan and biglycan, indicating that the trivalent form of arsenic is important for inhibition of endothelial PG synthesis. In contaminated water, arsenic is present in both the inorganic pentavalent form arsenate and trivalent form arsenite (Vahter, 2002). Pentavalent form arsenate ingested through drinking water is rapidly reduced in the blood to trivalent form arsenite, which can be further metabolized to mono- and dimethylated derivatives (Vahter, 2002; Abernathy *et al*., 1999; Ellinsworth, 2015). It is possible that trivalent methylated arsenicals, such as methylarsonous acid (MMA^{III}), which are intermediate metabolites in the methylation process of inorganic arsenic, may also influence vascular PG synthesis because MMA^{III} exhibits a more highly toxic effect than inorganic arsenite on vascular endothelial cells (Hirano *et al.*, 2004) and promotes atherosclerosis formation (Negro Silva *et al*., 2017).

Vascular endothelial cells have an anticoagulant property that contribute to the prevention of atherosclerosis. Perlecan and biglycan, which are major PGs in vascular endothelial cells, exhibit anti-thrombin activity through activation of antithrombin III and heparin cofactor II, respectively (Mertens *et al*., 1992; Whinna *et al*., 1993). Perlecan is also required to inhibit thrombosis after deep vascular injury (Nugent *et al.*, 2000). Fibroblast growth factor-2 and vascular endothelial growth factor are heparin-binding growth factors (Yayon *et al.*, 1991; Gitay-Goren *et al*., 1992) and important regulators of the vascular endothelial cell growth and repair process after vascular endothelium injury. Thus, arsenite may induce thrombosis and vascular endothelium dysfunction by the inhibition of endothelial perlecan, syndecan-1, -2, -3, and biglycan expression to promote the progression of atherosclerosis.

In cultured vascular endothelial cells, arsenite induces cellular redox modulation, transcription factor activation, and gene expression relevant to endothelial dysfunction through reactive oxygen species (ROS) (Simeonova and Luster, 2004). For example, it has been demonstrated

that arsenite activates activator protein-1 (AP-1), nuclear factor-κB (NF-κB), and NF-E2-related factor2 (Nrf2) (Kumagai and Sumi, 2007). We have recently shown that copper(II) bis(diethyldithiocarbamate) ($Cu(edtc)_2$), an activator of Nrf2 (Fujie *et al.*, 2016), induces the expression of syndecan-4 in bovine aortic endothelial cells (Hara *et al*., 2018). Additionally, syndecan-4 induction by $Cu(edtc)_2$ depends on the activation of p38 mitogen-activated protein kinase (MAPK) but not the Smad2/3, Nrf2, or epidermal growth factor receptor pathways. We reported that 1,10-phenanthroline (*o*-Phen) and dichloro(1,10-phenanthroline)zinc (Zn-Phen) also induce syndecan-4 expression in bovine aortic endothelial cells through activation of the hypoxia-inducible factor-1α/β pathway (Hara *et al*., 2017). In the present study, we examined the involvement of ROS production in the inhibitory effect of PG synthesis by arsenite using a ROS scavenger Trolox, but the scavenger did not attenuate the inhibitory effect of arsenite in at least the gene expression of perlecan and biglycan (data not shown). Further studies are needed to elucidate the mechanism of arsenite-induced inhibition of PG synthesis.

In the present study, we aimed to identify the PG molecules whose gene expression is decreased by arsenite in vascular endothelial cells. In conclusion, the present data suggested that the inhibitory effect of arsenite on the synthesis of HSPGs and CS/DSPGs may be due to decreased gene expression of perlecan, syndecan-1, -2, -3, and biglycan in vascular endothelial cells. Although the molecular mechanism of the arsenite-induced inhibitory effect is not still clear, the inhibition of endothelial PG synthesis by arsenite may cause functional damage of vascular tissue and may relate to the progression of vascular disease, including atherosclerosis.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Scientific Research (C) JP 16K08350 (to Y.F.) and a Grant-in-Aid for Scientific Research (C) JP 17K08391 (to T.T.) from the Japan Society for the Promotion of Science (JSPS).

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Abernathy, C.O., Liu, Y.P., Longfellow, D., Aposhian, H.V., Beck, B., Fowler, B., Goyer, R., Menzer, R., Rossman, T., Thompson, C. and Waalkes, M. (1999): Arsenic: health effects, mechanisms of actions, and research issues. Environ. Health Perspect., **107**, 593-597.

Berenson, G.S., Radhakrishnamurthy, B., Srinivasan, S.R., Vijayagopal, P., Dalferes, E.R. Jr. and Sharma, C. (1984): Recent advances in molecular pathology. Carbohydrate-protein macromolecules and arterial wall integrity-a role in atherogenesis. Exp. Mol. Pathol., **41**, 267-287.

Camejo, G. (1981): The interaction of lipids and lipoproteins with the intracellular matrix of arterial tissue: its possible role in atherosclerogenesis. Adv. Lipid Res., **19**, 1-53.

Chen, C.J., Wu, M.M., Lee, S.S., Wang, J.D., Cheng, S.H. and Wu, H.Y. (1988): Atherogenicity and carcinogenicity of high-arsenic artesian well water. Multiple risk factors and related malignant neoplasms of blackfoot disease. Arteriosclerosis, **8**, 452-460.

Denizot, F. and Lang, R. (1986): Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J. Immunol. Methods, **89**, 271-277.

Ellinsworth, D.C. (2015): Arsenic, reactive oxygen, and endothelial dysfunction. J. Pharmacol. Exp. Ther., **353**, 458-464.

Fujie, T., Murakami, M., Yoshida, E., Tachinami, T., Shinkai, Y., Fujiwara, Y., Yamamoto, C., Kumagai, Y., Naka, H. and Kaji, T. (2016): Copper diethyldithiocarbamate as an activator of Nrf2 in cultured vascular endothelial cells. J. Biol. Inorg. Chem., **21**, 263-273.

Fujiwara, Y., Nakase, Y. and Kaji, T. (2005): Sodium arsenite inhibits proteoglycan synthesis by vascular endothelial cells in culture. J. Health Sci., **51**, 461-468.

Fujiwara, Y., Yamamoto, C., Inagaki, T., Satoh, M. and Kaji, T. (2012): Bismuth protects against arsenite-induced inhibition of proteoglycan synthesis in cultured vascular endothelial cells. J. Toxicol. Sci., **37**, 837-843.

Gitay-Goren, H., Soker, S., Vlodavsky, I. and Neufeld, G. (1992): The binding of vascular endothelial growth factor to its receptors is dependent on cell surface-associated heparin-like molecules. J. Biol. Chem., **267**, 6093-6098.

Hara, T., Kojima, T., Matsuzaki, H., Nakamura, T., Yoshida, E., Fujiwara, Y., Yamamoto, C., Saito, S. and Kaji, T. (2017): Induction of syndecan-4 by organic-inorganic hybrid molecules with a 1,10-phenanthroline structure in cultured vascular endothelial cells. Int. J. Mol. Sci., **18**, 352.

Hara, T., Tatsuishi, H., Banno, T., Fujie, T., Yamamoto, C., Naka, H. and Kaji, T. (2018): Copper(II) bis(diethyldithiocarbamate) induces the expression of syndecan-4, a transmembrane heparan sulfate proteoglycan, via p38 MAPK activation in vascular endothelial cells. Int. J. Mol. Sci., **19**, 3302.

Hirano, S., Kobayashi, Y., Cui, X., Kanno, S., Hayakawa, T. and Shraim, A. (2004): The accumulation and toxicity of methylated arsenicals in endothelial cells: important roles of thiol compounds. Toxicol. Appl. Pharmacol., **198**, 458-467.

Ichikawa, M., Akimoto, J., Miki, Y., Maeda, J., Takahashi, T., Fujiwara, Y. and Kohno, M. (2019): Photodynamic therapy with talaporfin sodium induces dose- and time-dependent apoptotic cell death in malignant meningioma HKBMM cells. Photodiagn. Photodyn. Ther., **25**, 29-34.

Kaji, T., Yamada, A., Miyajima, S., Yamamoto, C., Fujiwara, Y., Wight, T.N. and Kinsella, M.G. (2000): Cell density-dependent regulation of proteoglycan synthesis by transforming growth factor-β1 in cultured bovine aortic endothelial cells. J. Biol. Chem., **275**, 1463-1470.

Kumagai, Y. and Sumi, D. (2007): Arsenic: signal transduction, transcription factor, and biotransformation involved in cellular response and toxicity. Annu. Rev. Pharmacol. Toxicol., **47**, 243-

262.

Makhani, K., Chiavatti, C., Plourde, D., Negro Silva, L.F., Lemaire, M., Lemarié, C.A., Lehoux, S. and Mann, K.K. (2018): Using the apolipoprotein E knock-out mouse model to define atherosclerotic plaque changes induced by low dose arsenic. Toxicol. Sci., **166**, 213-218.

Mertens, G., Cassiman, J.J., Van den Berghe, H., Vermylen, J. and David, G. (1992): Cell surface heparan sulfate proteoglycans from human vascular endothelial cells. Core protein characterization and antithrombin III binding properties. J. Biol. Chem., **267**, 20435-20443.

Moon, K.A., Guallar, E., Umans, J.G., Devereux, R.B., Best, L.G., Francesconi, K.A., Goessler, W., Pollak, J., Silbergeld, E.K., Howard, B.V. and Navas-Acien, A. (2013): Association between exposure to low to moderate arsenic levels and incident cardiovascular disease. A prospective cohort study. Ann. Intern. Med., **159**, 649-659.

Negro Silva, L.F., Lemaire, M., Lemarié, C.A., Plourde, D., Bolt, A.M., Chiavatti, C., Bohle, D.S., Slavkovich, V., Graziano, J.H., Lehoux, S. and Mann, K.K. (2017): Effects of inorganic arsenic, methylated arsenicals, and arsenobetaine on atherosclerosis in the mouse model and the role of As3mt-mediated methylation. Environ. Health Perspect., **125**, 077001.

Nordstrom, D.K. (2002): Public health. Worldwide occurrences of arsenic in ground water. Science, **296**, 2143-2145.

Nugent, M.A., Nugent, H.M., Iozzo, R.V., Sanchack, K. and Edelman, E.R. (2000): Perlecan is required to inhibit thrombosis after deep vascular injury and contributes to endothelial cell-mediated inhibition of intimal hyperplasia. Proc. Natl. Acad. Sci. USA, **97**, 6722-6727.

Ross, R. (1993): The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature, **362**, 801-809.

Ruoslahti, E. (1988): Structure and biology of proteoglycans. Annu. Rev. Cell Biol., **4**, 229-255.

Saku, T. and Furthmayr, H. (1989): Characterization of the major heparan sulfate proteoglycan secreted by bovine aortic endothelial cells in culture. Homology to the large molecular weight molecule of basement membranes. J. Biol. Chem., **264**, 3514-3523.

Simeonova, P.P., Hulderman, T., Harki, D. and Luster, M.I. (2003): Arsenic exposure accelerates atherogenesis in apolipoprotein $E^{-/-}$ mice. Environ. Health Perspect., **111**, 1744-1748.

Simeonova, P.P. and Luster, M.I. (2004): Arsenic and atherosclerosis. Toxicol. Appl. Pharmacol., **198**, 444-449.

Srivastava, S., Vladykovskaya, E.N., Haberzettl, P., Sithu, S.D., D'Souza, S.E. and States, J.C. (2009): Arsenic exacerbates atherosclerotic lesion formation and inflammation in ApoE-/- mice. Toxicol. Appl. Pharmacol., **241**, 90-100.

Takahashi, T., Suzuki, S., Misawa, S., Akimoto, J., Shinoda, Y. and Fujiwara, Y. (2018): Photodynamic therapy using talaporfin sodium induces heme oxygenase-1 expression in rat malignant meningioma KMY-J cells. J. Toxicol. Sci., **43**, 353-358.

Tseng, C.H., Chong, C.K., Chen, C.J., Lin, B.J. and Tai, T.Y. (1995): Abnormal peripheral microcirculation in seemingly normal subjects living in blackfoot-disease-hyperendemic villages in Taiwan. Int. J. Microcirc. Clin. Exp., **15**, 21-27.

Vahter, M. (2002): Mechanisms of arsenic biotransformation. Toxicology, **181-182**, 211-217.

Whinna, H.C., Choi, H.U., Rosenberg, L.C. and Church, F.C. (1993): Interaction of heparin cofactor II with biglycan and decorin. J. Biol. Chem., **268**, 3920-3924.

Yamamoto, C., Deng, X., Fujiwara, Y. and Kaji, T. (2005): Proteoglycans predominantly synthesized by human brain microvascular endothelial cells in culture are perlecan and biglycan. J. Health Sci., **51**, 576-583.

Yayon, A., Klagsbrun, M., Esko, J.D., Leder, P. and Ornitz, D.M. (1991): Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. Cell, **64**, 841-848.