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Disaccharide Composition of Glycosaminoglycan Chains in Growing Vascular Endothelial Cells in Culture after Exposure to Lead

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Heparan sulfate (HS) chains bind and activate fibroblast growth factor-2 (FGF-2) depending on their microstructure. The glycosaminoglycan chains present particularly as a large HS proteoglycan perlecan in vascular endothelial cells. A heavy metal lead induces a lower response to FGF-2 by inhibition of perlecan synthesis and inhibits the proliferation of the cells. The present study was undertaken to address the question whether lead influences the formation of HS chains in vascular endothelial cells. The data indicate that lead decreases the amount of disaccharide units including hexuronic acid-*N*-acetylglucosamine, hexuronic acid-*N*-sulfated glucosamine and 2-*O*-sulfated hexuronic acid-*N*-sulfated glucosamine in HS chains accumulated in the cell layer and the conditioned medium. However, no percentage of any disaccharide unit was affected by the metal. The percentage of disaccharide units in chondroitin/dermatan sulfate was also unaffected by lead. The present data support the hypothesis that lead-induced lower response of vascular endothelial cells to endogenous FGF-2 mainly results from a decrease in perlecan molecules of extracellular matrix as a result of selective inhibition of perlecan core protein synthesis rather than a change in the HS structure.

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INTRODUCTION

Vascular endothelial cells proliferate strongly depending on endogenous fibroblast growth factor-2 (FGF-2) when they repair damaged cell layers. The growth factor acts on the cells primarily through cell surface high affinity tyrosine kinase receptors;[1] however, their activity is modulated by heparan sulfate proteoglycans (HSPGs).[2–4] Proteoglycans (PGs) are a heterogeneous group of proteins that have anionic glycosaminoglycan (GAG) side chains such as heparan sulfate (HS) and chondroitin/dermatan sulfate (CS/DS) that consist of alternating hexuronic acid and hexosamine covalently bound to a core protein.[5] FGF-2 is bound to a specific HS sequence of a hexasaccharide containing 2-*O*-sulfated iduronic acid [IdoA(2S)] and *N*-sulfated glucosamine (GlcNS).[6] A dodecasaccharide containing 6-*O*-sulfated GlcNS [GlcNS(6S)] residues as well as the binding region is required for receptor signaling.[7] The PG whose HS chains regulate the activity of FGF-2 *in vitro*[8] and *in vivo*[9,10] is a large HSPG perlecan. However, the ability of HS chains to activate FGF-2, which relies on the primary sequence, structure and organization of HS chains, is changeable depending on the cell type and differentiation state.[10,11]

Lead is a toxic heavy metal that has been shown to induce vascular disorders such as atherosclerosis in epidemiological and experimental studies.[12–16] Our cell culture studies indicate that the metal influences fibrinolytic activity and the monolayer maintenance of vascular endothelial cells,[17] important events in the initiation of atherosclerosis.[18] In addition, lead inhibits the repair of wounded monolayers of endothelial cells by induction of a lower cell response to endogenous FGF-2 as a result of inhibition of perlecan core protein synthesis without a change of HS chain length.[19] However, it is unclear whether or not lead changes the composition of HS chains in perlecan molecules of vascular endothelial cells.

In the present study, growing cultures of bovine aortic endothelial cells were exposed to lead, and the disaccharide units of HS and CS/DS chains obtained from the cell layer and the conditioned me-

dium were separately analyzed by fluorophore-assisted carbohydrate electrophoresis (FACE) after enzymatic digestion.

MATERIALS AND METHODS

Materials —— Vascular endothelial cells derived from bovine aorta were purchased from Dainihon Pharmaceutical (Osaka, Japan). Dulbecco's modified Eagle medium (DMEM) and ASF 301 medium were purchased from Nissui Pharmaceutical (Tokyo, Japan) and Ajinomoto (Tokyo, Japan), respectively. Fetal bovine serum was obtained from MP Biomedicals (Invine, CA, U.S.A.). Tissue culture dishes and plates were obtained from Asahi Techno Glass (Tokyo, Japan). Microcon 3 (3000 MW cut-off) ultrafiltration devices were purchased from Millipore (Billerica, MA, U.S.A.), and 2-aminoacridone hydrochloride was from Molecular Probes (Eugene, OR, U.S.A.). Heparitinase I (EC 4.2.2.8 derived from *Flavobacterium heparinum*), heparitinase II (derived from *Flavobacterium heparinum*), protease-free chondroitinase ABC (EC 4.2.2.4 derived from *Proteus vulgaris*), chondroitinase AC II (EC 4.2.2.5 derived from *Arthrobacter aurescens*) and Δ-disaccharide standards were from Seikagaku (Tokyo, Japan). Proteinase K (fungal) was purchased from Invitrogen (Carlsbad, CA, U.S.A.). DEAE-Sephacel, benzamidine, phenylmethanesulfonyl fluoride, Tris base, benzamidine, sodium cyanoborohydride (95% pure), sodium acetate, ammonium hydroxide, acetic acid, dimethylsulfoxide, chondroitin disaccharide Δdi-6S and D-galactose 6-sulfate (gal6S) were from Sigma Aldrich (St. Louis, MO, U.S.A.). Lead nitrate and other reagents were from Nacalai Tesque (Kyoto, Japan).

Cell Culture and PG Isolation —— 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 air until confluence. They were then transferred into 100 mm dishes at 1×10^4 cells/cm^2 and cultured for 24 hr in DMEM supplemented with 10% fetal bovine serum ("growing cultures"). The medium was discarded and the cell layer was washed twice with serum-free ASF 301 medium. The growing cultures of endothelial cells were then incubated at 37°C for 48 hr in fresh serum-free ASF 301 medium with lead nitrate (2 μM). After incubation, the conditioned medium was harvested and solid urea was added to a concentration of 8 M ("the medium extract"). The cell layer was washed twice with ice-cold Ca, Mg-free phosphate-buffered saline and extracted with 8 M urea solution containing 0.1 M 6-aminohexanoic acid, 5 mM benzamidine, 10 mM *N*-ethylmaleimide, 2 mM EDTA, 0.1 M phenylmethanesulfonyl fluoride, 0.1 M NaCl, 50 mM Tris base and 2% Triton X-100 (pH 7.5) at 4°C for 15 min; the cell layer was harvested by scraping with a rubber policeman ("the cell extract"). The medium and cell extracts were purified and concentrated by ion exchange chromatography on DEAE-Sephacel in 8 M urea buffer (8 M urea, 2 mM EDTA, 0.5% Triton X-100 and 50 mM Tris base, pH 7.5) containing 0.25 M NaCl and eluted with 8 M urea buffer containing 3 M NaCl.

Enzymatic Digestion and FACE —— DEAE-Sephacel-purified PGs were precipitated with 3.5 volumes of 1.3% potassium acetate in 95% ethanol. Core proteins of the PGs were digested at 60°C for 18 hr with 0.8 mg/ml proteinase K in 0.1 M sodium acetate buffer (pH 7.2). After inactivation of the proteinase at 100°C for 10 min, buffer salt and protein digests were removed by centrifugation of the digests in Microcon 3. The GAGs on the filter were recovered in 0.1 M ammonium acetate (pH 7.3) and digested at 37°C for 18 hr with both 0.2 U/ml chondroitinase ABC and 1 U/ml of chondroitinase AC II. The CS/DS hydrolase products were recovered with Microcon 3 and dried for further processing. The undigested materials on the filter (HS chains) were recovered in 0.1 M ammonium acetate (pH 7.0) containing 0.01% bovine serum albumin and digested at 37°C for 8 hr with both 0.03 U/ml heparitinase I and II. The HS hydrolase products were recovered with Microcon 3 and then dried. The dried HS and CS/DS samples were fluorotagged with 0.1 M 2-aminoacridone hydrochloride. The fluorotagged-CS/DS hydrolase products were separated on the separating gels (20% acrylamide, 2.5% glycerol and 45 mM Tris acetate, pH 7.0) with a stacking gel (7.7% acrylamide, 1.7% agarose, 5% glycerol and 45 mM Tris acetate, pH 7.0). The fluorotagged-HS hydrolase products were separated on the separating gels (20% acrylamide, 2.5% glycerol, 0.1 M Tris base and 90 mM boric acid) with a stacking gel (7.7% acrylamide, 1.7% agarose, 5% glycerol, 0.1 M Tris base and 90 mM boric acid). Electrophoresis was carried out in the running buffer (0.1 M Tris-borate, pH 8.3) at 4°C. The fluorescent images were displayed in a gel documentation system (Atto, AE-6914) and the bands of HS and CS/DS Δ-disaccharides were quantitatively analyzed by NIH Image

Analyses Software using the bands of Δdi-6S and gal6S, respectively, as standards. Separately, growing cultures were treated with lead under the same conditions and the DNA content was determined by fluorometric methods[20] to express the amount of Δ-disaccharides as pmol/μg DNA.

Statistical Analysis ——— Data were analyzed for statistical significance by analysis of variance (ANOVA) and Bonferroni's multiple *t*-test. *p*-Values of less than 0.05 were considered to indicate statistically significant differences.

RESULTS AND DISCUSSION

Figure 1 shows the morphological appearance of growing endothelial cells after exposure to lead at 2 μM for 48 hr. Lead caused a decrease in the cell number with an appearance of spindle-shaped cells; however, no degenerative change was observed. The DNA content was also decreased by lead (from 163 ± 3.29 ng/cm^2 to 137 ± 4.62 ng/cm^2, $p < 0.01$). These results agree with our previous observation that lead inhibits the proliferation of vascular endothelial cells without nonspecific cell damage.[21]

Figure 2 shows typical gel images of FACE of disaccharide components derived from HS (Fig. 2A) and CS/DS (Fig. 2B) isolated from the cell layer and the conditioned medium of vascular endothelial cells before and after exposure to lead. In both the cell layer and the medium, disaccharide units of Δ-glucuronic acid (GlcA)/iduronic acid (IdoA)-*N*-acetylglucosamine (GlcNAc), ΔGlcA/IdoA-GlcNS and ΔIdoA(2S)-GlcNS were generated by digestion with heparitinase I and II; ΔGlcA/IdoA-GlcNS(6S) and ΔIdoA(2S)-GlcNS(6S) were also detected in the medium. These HS disaccharide units would be derived mainly from HS chains of perlecan, because bovine aortic endothelial cells predominantly synthesize and secrete perlecan as HSPGs.[22] On the other hand, ΔGlcA/IdoA-*N*-acetylgalactosamine (GalNAc), ΔGlcA/IdoA-6-*O*-sulfated GalNAc [GalNAc(6S)] and ΔGlcA/IdoA-4-*O*-sulfated GalNAc [GalNAc(4S)] disaccharide units were generated by digestion with chondroitinase ABC and AC II in both the cell layer and the medium; ΔGlcA/IdoA-4-*O*-, 6-*O*-sulfated GalNAc [GalNAc(4S,6S)] was also detected in the medium. These CS/DS disaccharide units would be derived mainly from CS/DS chains of biglycan, because only biglycan accumulates in both the cell layer and the medium as CS/DSPGs.[22]

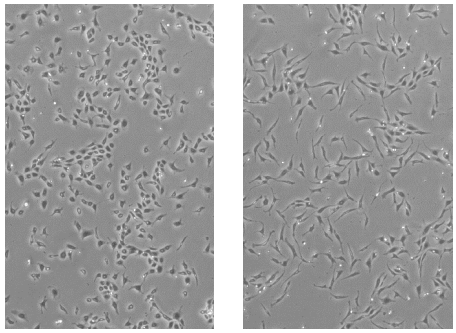

Fig. 1. Morphological Appearance of Growing Vascular Endothelial Cells after Exposure to Lead (× 40)

Growing cultures of bovine aortic endothelial cells were incubated at 37°C for 48 hr with (right panel) or without (left panel) lead nitrate (2 μM).

Table 1 shows the disaccharide composition of HS derived from vascular endothelial cells before and after exposure to lead. Endothelial cell HS chains accumulated in the cell layer were composed of GlcA/IdoA-GlcNAc (54.1%), GlcA/IdoA-GlcNS (33.3%) and IdoA(2S)-GlcNS (12.6%), while those accumulated in the conditioned medium were composed of GlcA/IdoA-GlcNAc (50.8%), GlcA/IdoA-GlcNS (30.8%), IdoA(2S)-GlcNS (15.1%), a trace of GlcA/IdoA-GlcNS(6S) and IdoA(2S)-GlcNS(6S). The high proportion of the *N*-acetylated disaccharide units (GlcA/IdoA-GlcNAc) together with the low proportion of IdoA(2S)-GlcNS(± 6S) disaccharides is consistent with previous reports[7,23,24] where endothelial cell HS was analyzed by strong anion exchange HPLC. Lead significantly decreased the total number of HS disaccharides accumulated in both the cell layer and the conditioned medium as a result of decrease in the disaccharides of GlcA/IdoA-GlcNAc, GlcA/IdoA-GlcNS and IdoA(2S)-GlcNS. Although *N*- and 2-*O*-sulfation was slightly inhibited by lead, no proportion of any disaccharide unit was affected by the metal in either the cell layer or the medium. Thus, lead-induced decrease in the amounts of disaccharide units from HS chains is regarded as a reflection of reduced perlecan synthesis by the metal.[25] In other words, lead specifically inhibits the synthesis of perlecan core protein but does not influence the HS disaccharide composition in growing vascular endothelial cells. This indicates that lead exhibits toxicity on the core protein synthesis rather than the HS formation in the process of perlecan synthesis, resulting in an induction of lower ability of endothelial cells to respond to endogenous

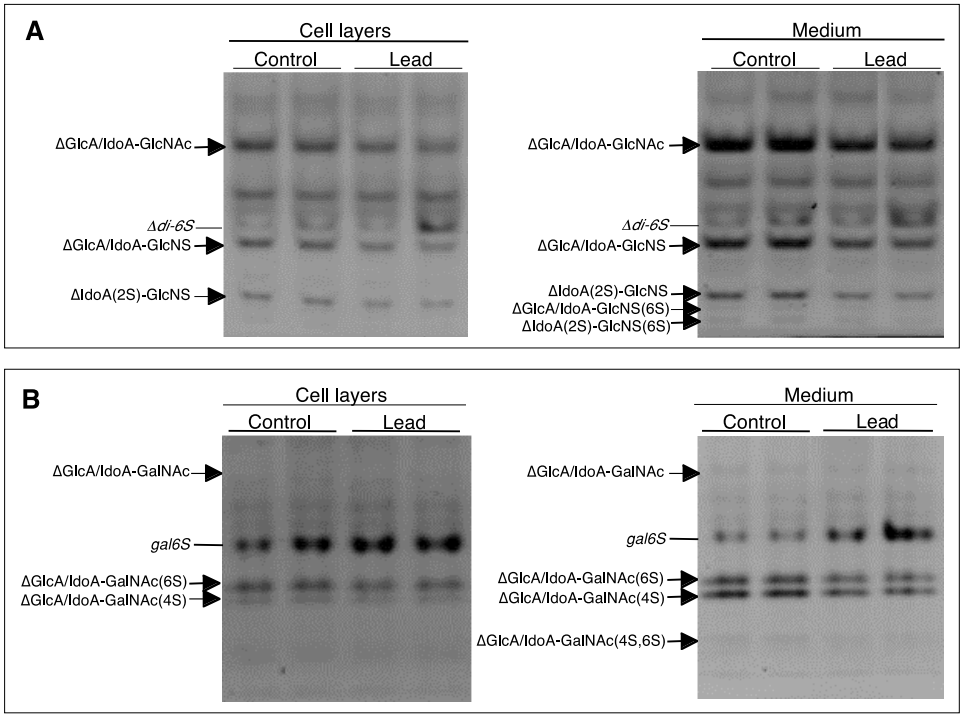

Fig. 2. FACE Analysis of HS (Panel A) and CS/DS (Panel B) from the Cell Layer and the Conditioned Medium of Growing Vascular Endothelial Cells after Exposure to Lead

Growing cultures of bovine aortic endothelial cells were incubated at 37°C for 48 hr with or without lead nitrate (2 μM).

Table 1. Disaccharide Composition of HS Chains in Sparse Vascular Endothelial Cells after Exposure to Lead

Disaccharide	Disaccharide amount			
	Control		2 μM Lead	
	pmol/μg DNA	(%)	pmol/μg DNA	(%)
Cell layer				
Total	164 ± 4.33		131 ± 5.10*	
GlcA/IdoA-GlcNAc	88.7 ± 3.03	(54.1)	76.3 ± 3.13*	(58.0)
GlcA/IdoA-GlcNS	54.5 ± 0.75	(33.3)	40.2 ± 1.54**	(30.6)
IdoA(2S)-GlcNS	20.7 ± 0.75	(12.6)	15.0 ± 1.08**	(11.4)
GlcA/IdoA-GlcNS(6S)	—		—	
IdoA(2S)-GlcNS(6S)	—		—	
% *N*-sulfattion	45.9		42.0	
% 2-*O*-sulfation	12.6		11.4	
Medium				
Total	331 ± 7.36		244 ± 12.9**	
GlcA/IdoA-GlcNAc	168 ± 4.28	(50.8)	131 ± 6.87*	(53.7)
GlcA/IdoA-GlcNS	102 ± 3.07	(30.8)	72.2 ± 3.66**	(29.6)
IdoA(2S)-GlcNS	50.0 ± 1.86	(15.1)	33.6 ± 1.68**	(13.8)
GlcA/IdoA-GlcNS(6S)	5.15 ± 0.27	(1.6)	3.40 ± 0.56	(1.4)
IdoA(2S)-GlcNS(6S)	5.54 ± 0.30	(1.7)	3.66 ± 0.69	(1.5)
% *N*-sulfattion	49.2		46.3	
% 2-*O*-sulfation	16.8		15.3	

Sparse cultures of bovine aortic endothelial cells were incubated at 37°C for 48 hr with lead nitrate (2 μM). Digestion products separated by FACE gels (see Fig. 2) were quantitated using NIH image software. Values are means ± S.E. of four samples. Significantly different from the corresponding control, *$p < 0.05$; **$p < 0.01$. —, not detected.

Table 2. Disaccharide Composition of CS/DS Chains in Sparse Vascular Endothelial Cells after Exposure to Lead

Disaccharide	Disaccharide amount			
	Control		2 μM Lead	
	pmol/μg DNA	(%)	pmol/μg DNA	(%)
Cell layer				
Total	46.0 $\pm$ 1.41		46.1 $\pm$ 1.52	
GlcA/IdoA-GalNAc	2.94 $\pm$ 0.68	(6.4)	2.45 $\pm$ 0.80	(5.3)
GlcA/IdoA-GalNAc(6S)	28.9 $\pm$ 1.21	(62.9)	29.4 $\pm$ 1.44	(63.8)
GlcA/IdoA-GalNAc(4S)	14.1 $\pm$ 0.48	(30.7)	14.2 $\pm$ 0.33	(30.8)
GlcA/IdoA-GalNAc(4S,6S)	—		—	
Medium				
Total	133 $\pm$ 1.41		113 $\pm$ 1.52*	
GlcA/IdoA-GalNAc	6.89 $\pm$ 0.19	(5.2)	6.59 $\pm$ 0.33	(5.8)
GlcA/IdoA-GalNAc(6S)	53.9 $\pm$ 0.73	(40.3)	45.7 $\pm$ 1.14*	(40.2)
GlcA/IdoA-GalNAc(4S)	68.6 $\pm$ 1.00	(51.3)	57.6 $\pm$ 2.29*	(50.7)
GlcA/IdoA-GalNAc(4S,6S)	4.22 $\pm$ 0.86	(3.2)	3.74 $\pm$ 0.11	(3.3)

Sparse cultures of bovine aortic endothelial cells were incubated at 37°C for 48 hr with lead nitrate (2 μM). Digestion products separated by FACE gels (see Fig. 2) were quantitated using NIH image software. Values are means $\pm$ S.E. of four samples. Significantly different from the corresponding control, *$p < 0.01$. —, not detected.

FGF-2.

Table 2 shows the disaccharide composition of CS/DS derived from growing endothelial cells before and after exposure to lead. Although no marked change was observed in the cell layer, lead significantly decreased the amount of GlcA/IdoA-GalNAc(6S) and GlcA/IdoA-GalNAc(4S) in the conditioned medium, resulting in a moderate decrease in the total number of CS/DS disaccharides. However, the proportion of each disaccharide unit was similar before and after exposure to lead, indicating that the metal does not influence the sulfation of GlcA/IdoA-GalNAc in the process of CS/DS chain formation. It is suggested that lead does not change the association of biglycan with endothelial cell layers, although it may moderately inhibit the biglycan synthesis.

The present data support the hypothesis that lead specifically acts on the perlecan core protein synthesis without influence upon HS chain formation in endothelial PG metabolism. Although the possibility that lead may reduce a proportion of sulfated-disaccharides in the endothelial cell perlecan *via* inhibition of HS sulfotranferases cannot be excluded, our conclusion is that lead-induced lower response of vascular endothelial cells to endogenous FGF-2 mainly results from a decrease in perlecan molecules of extracellular matrix as a result of selective inhibition of perlecan core protein synthesis rather than a change in the HS structure.

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REFERENCES

1) Givol, D. and Yayon, A. (1992) Complexity of FGF receptors: genetic basis for structural diversity and functional specificity. *FASEB J*., **6**, 3362–3369.
2) 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.
3) Rapraeger, A. C., Krufka, A. and Olwin, B. B. (1991) Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. *Science*, **52**, 1705–1708.
4) Ornitz, D. M., Yayon, A., Flanagan, J. G., Svahn, C. M., Levi, E. and Leder, P. (1992) Heparin is required for cell-free binding of basic fibroblast growth factor to a soluble receptor and for mitogenesis in whole cells. *Mol. Cell. Biol*., **12**, 240–247.
5) Wight, T. N. (1980) Vessel proteoglycans and thrombogenesis. *Prog. Hemost. Thromb*., **5**, 1–39.
6) Guimond, S., Maccarana, M., Olwin, B. B., Lindahl, U. and Rapraeger, A. C. (1993) Activating and inhibitory heparin sequences for FGF-2 (basic FGF). Distinct requirements for FGF-1, FGF-2, and FGF-

4. *J. Biol. Chem.*, **268**, 23906–23914.
7) Pye, D. A. and Kumar, S. (1998) Endothelial and fibroblast cell-derived heparan sulphate bind with differing affinity to basic fibroblast growth factor. *Biochem. Biophys. Res. Commun.*, **248**, 889–895.
8) Iozzo, R. V. (1994) Perlecan: a gem of a proteoglycan. *Matrix Biol.*, **14**, 203–208.
9) Nugent, M. A. and Iozzo, R. V. (2000) Fibroblast growth factor-2. *Int. J. Biochem. Cell Biol.*, **32**, 115–120.
10) Aviezer, D., Hecht, D., Safran, M., Eisinger, M., David, G. and Yayon, A. (1994) Perlecan, basal lamina proteoglycan, promotes basic fibroblast growth factor-receptor binding, mitogenesis, and angiogenesis. *Cell*, **79**, 1005–1013.
11) Jayson, G. C., Lyon, M., Paraskeva, C., Turnbull, J. E., Deakin, J. A. and Gallagher, J. T. (1998) Heparan sulfate undergoes specific structural changes during the progression from human colon adenoma to carcinoma in vitro. *J. Biol. Chem.*, **273**, 51–57.
12) Dingwall-Fordyce, I. and Lane, R. E. (1963) A follow-up study of lead workers. *Br. J. Ind. Med.*, **20**, 313–315.
13) Revis, N. W., Zinsmeister, A. R. and Bull, R. (1981) Atherosclerosis and hypertension induced by lead and cadmium ions: an effect prevented by calcium ion. *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 6494–6498.
14) Lal, B., Murthy, R. C., Anand, M., Chandra, S. V., Kumar, R., Tripathi, O. and Srimal, R. C. (1991) Cardiotoxicity and hypertension in rats after oral lead exposure. *Drug Chem. Toxicol.*, **14**, 305–318.
15) Schwartz, J. (1991) Lead, blood pressure, and cardiovascular disease in men and women. *Environ. Health Perspect.*, **91**, 71–75.
16) Houtman, J. P. (1996) Trace elements and cardiovascular diseases. *J. Cardiovasc. Risk*, **3**, 18–25.
17) Kaji, T. (2004) Cell biology of heavy metal toxicity in vascular tissue. *Yakugaku Zasshi*, **124**, 113–120.
18) Ross, R., Glomset, J. and Harker, L. (1997) Response to injury and atherosclerosis. *Am. J. Pathol.*, **86**, 675–684.
19) Fujiwara, Y. and Kaji, T. (2000) Inhibition of the repair of injured endothelial cell monolayers by lead and its possible mechanisms. *J. Health Sci.*, **46**, 1–4.
20) Kissane, J. M. and Robins, E. (1958) The fluorometric measurement of deoxyribonucleic acid in animal tissues with special reference to the central nervous system. *J. Biol. Chem.*, **233**, 184–188.
21) Kaji, T., Fujiwara, Y., Hoshino, M., Yamamoto, C., Sakamoto, M. and Kozuka, H. (1995) Inhibitory effect of lead on the proliferation of cultured vascular endothelial cells. *Toxicology*, **95**, 87–92.
22) 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-β_1 in cultured bovine aortic endothelial cells. *J. Biol. Chem.*, **275**, 1463–1470.
23) Knox, S., Merry, C., Stringer, S., Melrose, J. and Whitelock, J. (2002) Not all perlecans are created equal: interactions with fibroblast growth factor (FGF) 2 and FGF receptors. *J. Biol. Chem.*, **277**, 14657–14665.
24) Feitsma, K., Hausser, H., Robenek, H., Kresse, H. and Vischer, P. (2000) Interaction of thrombospondin-1 and heparan sulfate from endothelial cells. Structural requirements of heparan sulfate. *J. Biol. Chem.*, **275**, 9396–9402.
25) Fujiwara, Y. and Kaji, T. (1999) Lead inhibits the core protein synthesis of a large heparan sulfate proteoglycan perlecan by proliferating vascular endothelial cells in culture. *Toxicology*, **133**, 159–169.