—Minireview—

Inhibition of the Repair of Injured Endothelial Cell Monolayers by Lead and Its Possible Mechanisms

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Lead is a heavy metal which has been experimentally and epidemiologically shown to induce vascular lesions such as atherosclerosis, however, little is known about the mechanism. Since damage of endothelial cell layers is a key event in the initiation of atherosclerosis, the effect of lead on the maintenance of cell layers has been studied using a cell culture system in our laboratory. The following results were obtained: (1) Endothelial cell monolayers are not destroyed but the repair after injury is markedly inhibited by lead through inhibition of the proliferation. (2) Lead suppresses the proliferative response of endothelial cells to basic fibroblast growth factor (bFGF). (3) Lead reduces the amount of endogenous bFGF bound to heparan sulfate proteoglycans through inhibition of the synthesis of perlecan, a large heparan sulfate proteoglycan. Since heparan sulfate chains of perlecan promote the binding of bFGF to its receptor, our results indicate that inhibition of the repair of injured endothelial monolayers by lead is due to a lower proliferative response of the cells to endogenous bFGF, caused by a suppression of perlecan synthesis.

Key words —— lead, fibroblast growth factor, perlecan, endothelial cell, proteoglycan, proliferation

Vascular endothelial cells form a monolayer on the inner surface of the blood vessel wall. This morphological characteristic is believed to prevent vascular lesions such as atherosclerosis by blocking direct contact between blood and underlying tissue. When the vascular endothelium is injured, basic fibroblast growth factor (bFGF) is released from damaged endothelial cells and the growth factor then promotes the repair of the vascular wall *via* stimulation of proliferation and migration of the cells near the damaged site in an autocrine fashion.[1] Any retardation of the repair of endothelial monolayers after damage may result in formation of a de-endothelialized area and induce platelet aggregation[2] which is an initial event in atherosclerosis.[3] Since endothelial cell growth strongly depends on endogenous bFGF, the rapid repair of de-endothelialized areas by the growth factor is important for the prevention of atherosclerosis. Accordingly, inhibition of bFGF-induced repair of endothelial cell monolayers after injury is one factor in the pathogenesis of atherosclerosis.

Proteoglycans are a component of the extracellular matrix and are widely distributed in mammalian tissues.[4] The macromolecules are a heterogeneous group of proteins that have anionic glycosaminoglycan chains covalently bound to core proteins. Although proteoglycans have various functions, one of the most important properties of heparan sulfate proteoglycans (HSPGs) is their interaction with bFGF and its receptor.[5] Heparan sulfate is the major glycosaminoglycan in vascular endothelial cell layers[6] and HSPGs contain two subclasses: one is the high M_r subclass which includes the large HSPG perlecan[7] and the other is the low M_r subclass which includes small HSPG species such as syndecan, glypican, fibroglycan[8] and ryudocan.[9] It has been shown that perlecan promotes the binding of bFGF to its receptor[10] whereas the other small HSPGs act competitively on this reaction,[11,12] although all HSPG species exhibit heparin-like activity.[8] Since there is a strong interrelationship between atherosclerosis and thrombosis, endothelial HSPGs are

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postulated to be involved in the pathogenesis of atherosclerosis by virtue of their heparin-like antithrombogenic activity as well as the regulation of endothelial cell functions mediated by bFGF.

Lead is an environmental pollutant which has been epidemiologically and experimentally shown to cause vascular disorders such as atherosclerosis and hypertension.[13-15] We hypothesized that lead-induced vascular lesions, at least partly, may result from dysfunction of vascular endothelial cells. Cell culture studies showed that lead inhibits the secretion of tissue plasminogen activator[16] but not its inhibitor type 1[17]; as a result, the fibrinolytic activity in the liquid phase is reduced. Lead also inhibits the synthesis of heparan sulfate chains by endothelial cells,[18] suggesting that the cells may have lower surface anticoagulant activity after exposure to the metal. Although these results supported our hypothesis, we also considered the effect of lead on the maintenance of vascular endothelial cell monolayers.

We have shown that lead does not destroy vascular endothelial cell monolayers[19] but is toxic in the repair process of injured endothelial cell layers. The present report reviews our recent research on this toxic effect of lead.

Lead Inhibits the Repair of Injured Monolayers of Vascular Endothelial Cells[20,21]

When the vascular endothelial cell layer is damaged, the cells migrate to the damaged area and proliferate to repair. Confluent cultures of bovine aortic endothelial cells, half area of which had been injured, were incubated in the presence or absence of lead nitrate and the repair of the endothelial cell monolayers was evaluated by monitoring the cell numbers appearing in the injured area. Both morphologically and quantitatively, it was found that lead inhibits the repair of wounded endothelial cell monolayers. Although mercury weakly inhibited the repair with nonspecific cell damage, other cations including zinc, bismuth, cobalt, manganese and nickel failed to exhibit such inhibition, indicating that lead is a unique metal, able to inhibit endothelial repair without nonspecific cell damage. Lead also inhibited the repair stimulated by exogenous bFGF, suggesting that the metal may disturb endothelial repair even when there is marked release of bFGF from dead cells at the injury sites. Thus it is suggested that lead exhibits its toxic effect during the repair of endothelial monolayers in the process of physiological remodelling of the endothelium or after de-endothelialization by pathological factors such as hypertension, environmental pollutants and other nutritional conditions.

Lead Inhibits the Proliferation of Vascular Endothelial Cells[22,23]

Since vascular endothelial cell proliferation is one of the most important events in the repair of the injured endothelium, we next investigated the effect of lead on cell proliferation. We showed that lead significantly reduced the incorporation of [^{3}H]thymidine into the acid-insoluble fraction in growing cultures of vascular endothelial cells, indicating that the metal inhibits proliferation. Inhibition of the proliferation by lead was also observed when the cells were stimulated by bFGF. On the other hand, the proliferation of vascular smooth muscle cells was promoted by lead. These results led us to believe that the inhibitory effect of lead on the cell proliferation may be unique to endothelial cells and is due to a lower response of the cells to endogenous bFGF, resulting in a delay repair of wounded endothelial monolayers.

Inhibition of Endothelial Cell Proliferation by Lead is Due to a Lower Response to Endogenous bFGF[24]

To address the question whether inhibition of endothelial cell proliferation by lead depends on bFGF or not, we investigated the response of the growing cells to the growth factor after exposure to the metal. In the presence of bFGF-neutralizing antibody, lead failed to inhibit [^{3}H]-thymidine incorporation by the cells. Pretreatment of endothelial cells with lead resulted in a reduction in the stimulation of the [^{3}H]thymidine incorporation by exogenous bFGF, indicating that exposure to the metal induces a lower proliferative response of cells to the growth factor. The lower response can be induced by the following mechanisms: (i) Lead inhibits the synthesis of bFGF. (ii) Lead reduces bFGF bound to its receptor through inhibition of receptor synthesis and/or direct action on the binding. (iii) Lead reduces bFGF bound to cell-surface heparan sulfate through inhibition of HSPG synthesis and/or direct action on binding. It was also shown that

lead significantly reduces endogenous bFGF bound to cell surface HSPGs but not to high affinity FGF receptor without a change in its accumulation within the cells. Based on this result, it was postulated that the reduced response of endothelial cells to bFGF after exposure to lead results from a lower availability of growth factor. Although inhibition of the binding of [^{125}I]bFGF to cell surface HSPGs by lead was only weak, the metal markedly suppressed the incorporation of [^{35}S]sulfate into heparan sulfate accumulated in the cell layer and conditioned medium. This indicates that lead strongly inhibits the synthesis of glycosaminoglycans such as heparan sulfate but no effect on the binding of bFGF to its receptor. This hypothesis was supported by the fact that inhibition of [^{3}H]-thymidine incorporation by lead was restored by addition of heparin to the medium. Taken together, these findings suggest that inhibition of vascular endothelial cell proliferation by lead is due to a lower response to endogenous bFGF through a suppression of heparan sulfate synthesis.

Lead Inhibits the Synthesis of Perlecan, a Large HSPG in Growing Vascular Endothelial Cells[25]

The reduction in heparan sulfate chains can be caused by a reduction in (i) HSPG core proteins, (ii) the number of heparan sulfate chains bound to a HSPG core protein and (iii) the length of heparan sulfate chains. We characterized HSPGs synthesized by growing endothelial cells after exposure to lead by biochemical techniques. When [^{35}S]sulfate-labeled HSPGs were separated from highly charged chondroitin/dermatan sulfate proteoglycans by DEAE-Sephacel ion exchange chromatography, it was found that lead markedly reduced HSPGs but not chondroitin/dermatan sulfate proteoglycans. The HSPGs were separated on the basis of their hydrodynamic size by a Sepharose CL-4B column. It was found that lead markedly reduced the radioactivity in the high M_r subclass rather than the low M_r subclass of HSPGs, indicating that the metal reduces the number of heparan sulfate chains bound to large HSPGs such as perlecan. On the other hand, the length of heparan sulfate chains was approximately M_r 50000 in both the control and lead-treated cells when estimated by the elution position of [^{35}S]sulfate radioactivity after digestion of core proteins with papain and Sepharose CL-6B chromatography. Analysis of core proteins labeled with [^{35}S]amino acids by SDS-PAGE showed that lead markedly reduced the amount of a large HSPG core protein with a molecular weight of approximately 400 kDa; this core protein was identified as perlecan by Western blot analysis. Thus it is suggested that the characteristic change in endothelial proteoglycans after exposure to lead is a reduction in the number of perlecan molecules without any changes in length of the heparan sulfate chains.

CONCLUSION

We propose that the pathogenesis of lead-induced atherosclerosis[13] involves inhibition of the repair process of injured endothelial cell layers. Our results suggest that inhibition of the repair by lead is caused by inhibition of vascular endothelial cell proliferation. Also, the inhibition is postulated to be due to a lower response of the cells to endogenous bFGF due to a reduction in the availability of growth factor through inhibition of perlecan synthesis. Disrupted regulation of the endothelial extracellular matrix by lead may be unique and is considered to be helpful in understanding the toxic effect of the metal in vascular tissue.

REFERENCES

1) Sato Y., Rifkin D. B., *J. Cell Biol.*, **107**, 1199—1205 (1988).
2) Matsuoka I., Nakahata N., Nakanishi H., *Biochem. Pharmacol.*, **38**, 1841—1847 (1989).
3) Ross R., Glomset J. A., *N. Engl. J. Med.*, **295**, 420—425 (1976) .
4) Hascall V., Kimura J. H., *Methods Enzymol.*, **82**, 769—800 (1982).
5) Yayon A., Klagsbrun M., Esko J. D., Leder P., Ornitz D. M., *Cell*, **64**, 841—848 (1991).
6) Gamse G., Fromme H. G., Kresse H., *Biochim. Biophys. Acta*, **544**, 514—528 (1978).
7) Iozzo R. V., Cohen I. R., Grassel S., Murdoch A. D., *Biochem. J.*, **302**, 625—639 (1994).
8) Mertens G., Cassiman J. J., van den Berghe H., Vermylen J., David G., *J. Biol. Chem.*, **267**, 20435—20443 (1992).
9) Kojima T., Shworak N. W., Rosenberg R. D., *J.*

Biol. Chem., **267**, 4870—4877 (1992).
10) Aviezer D., Hecht D., Safran M., Eisinger M., David G., Yayon A., *Cell*, **79**, 1005—1013 (1994).
11) Aviezer D., Levy E., Safran M., Svahn C., Buddecke E., Schmidt A., David G., Vlodavsky I., Yayon A., *J. Biol. Chem.*, **269**, 114—121 (1994).
12) Mali M., Elenius K., Miettinen H. M., Jakanen M., *J. Biol. Chem.*, **268**, 24215—24222 (1993).
13) Revis N. W., Zinsmeister A. R., Bull R., *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 6494—6498 (1981).
14) Dingwall—Fordyce I., Lane R. E., *Br. J. Ind. Med.*, **20**, 313—315 (1963).
15) Schwartz J., *Environ. Health Perspect.*, **91**, 71—75 (1991) .
16) Kaji T., Yamamoto C., Sakamoto M., Kozuka H., *Toxicology*, **73**, 219—227 (1992).
17) Yamamoto C., Kaji T., *J. Health Sci.*, **45**, 119—125 (1999).
18) Kaji T., Yamamoto C., Sakamoto M., *Toxicology*, **68**, 249—257 (1991).
19) Kaji T., Suzuki M., Yamamoto C., Mishima A., Sakamoto M., Kozuka H., *Arch. Environ. Contam. Toxicol.*, **28**, 168—172 (1995).
20) Fujiwara Y., Kaji T., Sakurai S., Sakamoto M., Kozuka H., *Toxicology*, **117**, 193—198 (1997).
21) Kaji T., Fujiwara Y., Sakurai S., Yamamoto C., Kozuka H., Koizumi F., *Res. Commun. Mol. Pathol. Pharmacol.*, **89**, 189—198 (1995).
22) Kaji T., Fujiwara Y., Hoshino M., Yamamoto C., Sakamoto M., Kozuka H., *Toxicology*, **95**, 87—92 (1995).
23) Fujiwara Y., Kaji T., Yamamoto C., Sakamoto M., Kozuka H., *Toxicology*, **98**, 105—110 (1995).
24) Fujiwara Y., Kaji T., *Toxicology*, **133**, 147—157 (1999).
25) Fujiwara Y., Kaji T., *Toxicology*, **133**, 159—169 (1999).