— **Minireview** —

The Biological Effects of Depolymerized Sodium Spirulan and Sulfated Colominic Acid on Vascular Cells are Beneficial in Preventing Atherosclerosis

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Atherosclerosis is a vascular lesion that is a common health problem in advanced countries. Functional damage of the vascular endothelial cells, vascular smooth muscle cell hyperplasia, and procoagulant/antifibrinolytic state of blood are generally observed in the lesion. Since polysaccharides such as heparin modulate vascular cell behavior through interaction with cytokines/growth factors, we hypothesized that polysaccharides from natural sources may possess beneficial biological activities that prevent atherosclerosis. Changes in cultured aortic endothelial and smooth muscle cells were investigated after treatment with the polysaccharides sodium spirulan (Na-SP) — a sulfated polysaccharide obtained from a hot water extract of the blue-green alga *Spirulina platensis* — and colominic acid (CA)-prepared as a homopolymer of *N*-acetylneuraminic acid produced by *Escherichia coli* K1. The experiments suggest that depolymerized Na-SP and sulfated CA can function as precursors of the agents that prevent atherosclerosis. In particular, both the chemically modified polysaccharides significantly inhibit the proliferation of the arterial smooth muscle cells without exhibiting any toxic effects on the integrity of the vascular endothelial cell layers. The results also indicate that chemical modifications, for example, depolymerization of Na-SP and sulfation of CA, can control the biological effects of these polysaccharides on vascular cells.

Key words —— sodium spirulan, colominic acid, atherosclerosis, vascular smooth muscle cell, vascular endothelial cell, polysaccharide

INTRODUCTION

Atherosclerosis is the principal vascular lesion involved in the pathogenesis of myocardial and cerebral infarction, which are common health problems in advanced countries. The lesion is initiated by functional damage of the vascular endothelial cells followed by an intimal hyperplasia of arterial smooth muscle cells.[1] When a monolayer of vascular endothelial cells is damaged slightly, the cells migrate and proliferate to reendothelialize. However, repeated injuries to vascular endothelial cells and insufficient repair of the damaged endothelium lead to arterial smooth muscle cell hyperplasia and eventually to the development of atherosclerosis. As a result, the endothelial cell monolayer becomes thrombogenic.[2] On the other hand, the arterial smooth muscle cells alter their phenotype from a contractile to a synthetic state and actively proliferate, resulting in intimal thickening.[3] Although the mechanism of expansion of the atherosclerotic intima is not simple, inhibition of the arterial smooth muscle cell proliferation without damaging the endothelial cell monolayers is one of the effective strategies to prevent atherosclerosis.

It has been shown that polysaccharides inhibit the proliferation of arterial smooth muscle cells. For example, heparin inhibits the proliferation *in vivo* and *in vitro*.[4] The inhibitory effect does not depend on the anticoagulant activity[5] but is influenced by the molecular weight[6] and the degree of *O*-sulfation.[7] In addition, a highly sulfated semisynthetic polysaccharide, pentosan polysulfate,[8] and a natural sulfated fucopolysaccharide, fucoidan,[9] also

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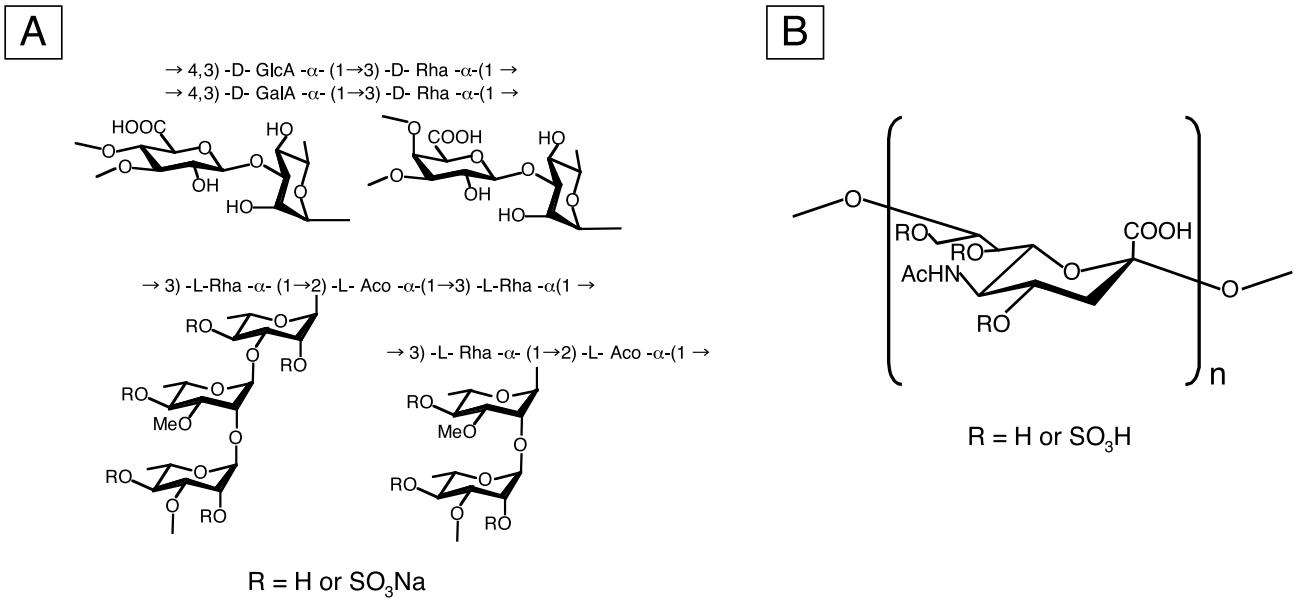

Fig. 1. Structure of Na-SP (A) and CA/SCA (B)

inhibit the proliferation. On the other hand, with respect to the vascular endothelial cell proliferation, it has been shown that polysaccharides, including heparin,[10,11] heparan sulfate,[12] and high concentrations of unfractionated chondroitin sulfate,[13] have inhibitory effects. These results indicate that polysaccharides can modulate the arterial endothelial and smooth muscle cell proliferations; sulfation and depolymerization may influence the biological activities of these polysaccharides that affect the proliferation.

Sodium spirulan (Na-SP) is a sulfated polysaccharide with M_r ~220000 that was isolated from a hot water extract of the blue-green alga *Spirulina platensis* as an antiviral agent.[14,15] The polysaccharide consists of two types of disaccharide repeating units, *O*-hexuronosyl-rhamnose and *O*-rhamnosyl-3-*O*-methylrhamnose with sulfate groups, other minor saccharides, and sodium ions (Fig. 1A).[16,17] Interestingly, Na-SP not only exhibits the antiviral activity but also activates heparin cofactor II, a physiological inhibitor of thrombin, by a mechanism that is different from that of heparin.[18,19] In addition, Na-SP induces the synthesis of tissue plasminogen activator (t-PA) that activates the fibrinolytic system in cultured human fetal lung fibroblasts.[20] Replacement of the sodium ion with calcium ion generally maintains the biological activities of Na-SP; however, removal of the sodium ion or desulfation markedly reduces its activities.

Colominic acid (CA) is an α2,8-linked polymer of sialic acid (Fig. 1B), originally isolated from *Escherichia coli* K1.[21] Although little is known about the biological activity of CA, *O*-sulfated CA (SCA), prepared by chemical sulfation of the CA chain,[22] exhibits several biological activities, including antiviral activities,[23–25] inhibition of the cytotoxic action of bee and snake venom,[26] inhibition of P-selectin-dependent macrophage infiltration in the glomeruli in experimental rats with crescentic glomerulonephritis,[27] and inhibition of the fertilization of mouse gametes.[28] However, the effects of CA and SCA on the vascular cell functions as well as the blood coagulation-fibrinolytic system have not been investigated.

We hypothesized that Na-SP and CA with or without chemical modifications may act on the arterial endothelial and smooth muscle cells and exhibit beneficial effects for the prevention of atherosclerosis. In this review, we describe the effects of Na-SP and CA/SCA on the arterial endothelial and smooth muscle cells in culture.

ARTERIAL SMOOTH MUSCLE CELL PROLIFERATION[29,30]

Since arterial smooth muscle cell hyperplasia is the hallmark of atherosclerosis, the effect of Na-SP was investigated using a culture system of these cells. Our experiments demonstrated that Na-SP strongly inhibits the proliferation of the arterial smooth

muscle cells. Although dextran, dextran sulfate, chondroitin sulfate, dermatan sulfate, and hyaluronan did not influence the proliferation, heparin and heparan sulfate significantly inhibited it. However, the inhibitory effect of Na-SP is markedly stronger than that of heparin and heparan sulfate, suggesting that the strong inhibitory effect of Na-SP on the arterial smooth muscle cell proliferation requires a certain sequence of the Na-SP polysaccharide structure. In addition, calcium spirulan (Ca-SP), which is prepared by replacing the sodium ion in the Na-SP molecule by calcium ion, also inhibited the arterial smooth muscle cell proliferation. However, H-SP, which is prepared by removing the sodium ion from the Na-SP molecule, and desulfated SP cannot inhibit the proliferation. These results suggest that the molecular conformation of Na-SP that is maintained by the sodium or calcium ion bound to the sulfate group is also required for the inhibitory effect. Furthermore, the inhibitory effect of Na-SP on the arterial smooth muscle cell proliferation was completely retained even after the molecule was depolymerized to yield a molecule of M_r ~14700. Taken together, Na-SP is a potent inhibitor of the arterial smooth muscle cell proliferation, and this inhibitory effect requires a molecular mass of M_r ~14700 or more, a sulfate group, and a sodium ion.

On the other hand, it was shown that both CA (M_r ~17000; prepared as a homopolymer of *N*-acetylneuraminic acid) and SCA (M_r ~22000; containing 13.4% sulfur) inhibited the proliferation of the arterial smooth muscle cells to a similar extent. The inhibitory effect of CA and SCA was almost equal to that of heparin but weaker than that of Na-SP and Ca-SP. Therefore, it was revealed that CA with or without sulfate groups as well as Na-SP/Ca-SP is a moderate inhibitor of the arterial smooth muscle cell proliferation.

MAINTENANCE OF VASCULAR ENDOTHELIAL CELL MONOLAYERS[31–33)]

The maintenance of the vascular endothelial cell monolayers is important for the prevention of atherosclerosis because the vascular lesion is initiated by the functional damage to these cells. It was revealed that Na-SP/Ca-SP inhibited the repair of the damaged vascular endothelial cell monolayer *via* inhibiting the proliferation without nonspecific cell damage. However, the inhibitory effect of Na-SP on the endothelial cell proliferation is weaker than that on the arterial smooth muscle cells. In addition, desulfation of Na-SP resulted in the loss of the inhibitory effect on the vascular endothelial cell proliferation. It is suggested that similar to the case of arterial smooth muscle cells, the molecular conformation of Na-SP that is maintained by the sodium or calcium ion bound to the sulfate group is also required for the inhibitory effect on the endothelial cell proliferation.

The effects of CA/SCA on the vascular endothelial cells are complicated. First, CA causes nonspecific cell damage to the cells in a monolayer. Second, the injurious effect of CA on the endothelial cell monolayers is reduced depending on the degree of sulfation. Thus, a highly sulfated SCA is nontoxic to the monolayers. Third, CA is nontoxic to the proliferating endothelial cells but markedly inhibits the proliferation of these cells. This inhibitory effect is much stronger than that of Na-SP/Ca-SP. Fourth, sulfation of CA diminishes the inhibitory effect of CA on the endothelial cell proliferation; however, SCA does not inhibit the proliferation. Therefore, CA has two distinct effects on the vascular endothelial cells — an inhibitory effect on their proliferation and an injurious effect on their monolayers. Furthermore, these cell density-dependent effects of CA can be reduced by sulfation. In other words, SCA shows neither the potent inhibitory effect on the proliferation nor the toxic effect on the integrity of vascular endothelial cell monolayer.

FIBRINOLYTIC ACTIVITY AND METABOLISM OF ANTICOAGULANT PROTEOGLYCANS IN VASCULAR ENDOTHELIAL CELLS[34–37)]

Vascular endothelial cells play an important role in the regulation of the blood coagulation-fibrinolytic system. These cells synthesize and secrete fibrinolytic proteins, such as t-PA, urokinase-type plasminogen activator (u-PA),[38)] and plasminogen activator inhibitor type 1 (PAI-1).[39)] Since both t-PA and u-PA convert plasminogen to plasmin that degrades fibrin, the fibrinolytic activity in the circulating blood depends on the balance between t-PA/u-PA and their common inhibitor PAI-1. Because there is a strong interrelationship between atherosclerosis and the blood coagulation-fibrinolytic system, we investigated the effects of Na-SP on the secretion of fibrin-

Table 1. The Effects of Na-SP and CA/SCA on Vascular Endothelial and Smooth Muscle Cells

	Proliferation of vascular smooth muscle cells	Proliferation of endothelial cells	Maintenance of endothelial cell	Release of proteoglycans from endothelial cell monolayers	Fibrinolytic activity of endothelial cells monolayers
Na-SP	↓	↓	↓	↑	↑
H-SP	→	N.D.	N.D.	→	N.D.
Desulfated SP	→	↑	N.D.	→	N.D.
Depolymerized SP	↓	→	N.D.	↑	N.D.
CA	↓	↓	↓	N.D.	N.D.
SCA	↓	→	→	N.D.	N.D.

↑, Stimulation; →, no effect; ↓, inhibition; N.D., not determined.

olytic proteins from cultured human coronary endothelial cells. It was found that Na-SP increases the secretion of u-PA and decreases that of PAI-1, resulting in an enhancement of the activity of both t-PA and u-PA in the liquid phase. The effect of Na-SP on the secretion of fibrinolytic proteins in the endothelial cells is completely different from that in fibroblasts,[29] although the mechanism for the cell-type dependency is unknown.

The endothelial cells synthesize and secrete not only fibrinolytic proteins but also anticoagulant substances such as proteoglycans. The predominant proteoglycans in the cells are the large heparan sulfate proteoglycan perlecan and the small leucine-rich dermatan sulfate proteoglycan biglycan.[40] Both perlecan and biglycan exhibit antithrombin activity *via* the activation of antithrombin III and heparin cofactor II, respectively.[41,42] Na-SP stimulates the release of both perlecan and biglycan with intact core proteins from the endothelial cell monolayers by inhibiting the association of proteoglycans with the cell monolayer. It is suggested that there are two possible mechanisms by which Na-SP elevates the anticoagulant activity in the liquid phase surrounding the vascular endothelial cells — the direct activation of heparin cofactor II in the blood and the indirect activation of antithrombin III and heparin cofactor II by the stimulation of endothelial perlecan and biglycan release.

CONCLUSION

The attempt to apply the biological activities of polysaccharide in preventing atherosclerosis has been insufficient. Modulation of the vascular endothelial and smooth muscle cell functions is one of the effective strategies of preventing atherosclerosis. Our data about the effects of Na-SP and CA/SCA on the vascular endothelial and smooth muscle cells are summarized in Table 1. Many problems exist in clarifying the biological effects of depolymerized Na-SP/SCA on the vascular cells and the application of the polysaccharides as preventive agents of atherosclerosis. Nevertheless, our data suggest that depolymerized Na-SP and SCA may function as the precursors of beneficial agents that prevent atherosclerosis because they do not influence the maintenance of the endothelial cell layer but inhibits the proliferation of the arterial smooth muscle cells. With respect to Na-SP, an activation of the endothelial fibrinolytic system was observed. The effect of depolymerized Na-SP and SCA on the fibrinolytic protein secretion, the metabolism of proteoglycans, and other anticoagulant and fibrinolytic functions of the endothelial cells should be investigated further.

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