Inhibition of Neointimal Proliferation of Rat Carotid Artery by Sulodexide

Sulodexide, a glycosaminoglycan-containing compound, is known to have an antiproliferative effect on vascular smooth-muscle cells, *in vitro*, as well as antithrombotic and fibrinolytic effects. But there are few reports about the effect on neointimal proliferation *in vivo*. In this study, we examined whether Sulodexide was effective in the inhibition of neointimal proliferation after vascular injury. Ten-week-old Sprague-Dawley rats were subjected to vascular injury by endothelial denudation of the common carotid artery by using a balloon catheter. They were then allocated randomly into a control group (saline 2 ml for 3 days, and then 1 ml for 18 days, IM) and a treated group (Sulodexide 10 mg/kg/day for 3 days, and then 4 mg/kg/day for 18 days, IM). Three weeks after vascular injury, we analyzed the neointimal proliferation using morphometry. The neointimal proliferation was significantly reduced in the treated group compared to the control group (Ratio of neointimal area to medial area; 118.39±6.80% in the treated group, 177.25±17.25% in the control group). This result showed that Sulodexide might be effective in reducing the rate of restenosis after balloon angioplasty. ***(JKMS 1997; 12: 210~4)***

Key Words : *Glycosaminoglycan, Angioplasty, Neointimal proliferation, Restenosis,*

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Received : February 19, 1997
Accepted : April 3, 1997

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INTRODUCTION

Despite advances in the clinical use of percutaneous transluminal coronary angioplasty, restenosis presently limits the long-term success of PTCA (1). Restenosis is mediated in part by an uncontrolled proliferation and extracellular matrix synthesis by modified smooth-muscle cells that migrate to the site of the balloon injury (2). Neointimal proliferation of the restenosis lesion is the end point of reparative processes initiated by vascular injury.

Sulodexide is a glycosaminoglycan taken from the intestinal mucosa of mammals, which consists of 80% heparan sulfate and 20% dermatan sulfate. The biologic effects of Sulodexide are dependent on the action of these two glycosaminoglycans, even though the exact mechanism of these two agents on the inhibition of cell proliferation is not well known. Heparan sulfate inhibits thrombus formation by potentiating anti-factor Xa activity of antithrombin III (3, 4). Dermatan sulfate has been known to inactivate the thrombin by binding with heparin cofactor II (5). In comparison with heparin, these two glycosaminoglycans have low anticoagulant activity, but Sulodexide is equally effective in reducing the proliferation of smooth-muscle cells *in vitro* (6).

Our experiments were designed to investigate the effects of Sulodexide on the neointimal proliferation *in vivo*, using endothelial denudation in the rat carotid artery model.

METHODS

Animals and study design

Thirty-eight male Sprague-Dawley rats weighing 280 to 350g (10 weeks of age) were used for this study. To assess the effect of Sulodexide, rats were randomly divided into two groups, control and treated. The rats in the treated group received 5 mg/kg Sulodexide (Alfa-Wassermann Co., Bologna, Italy) intramuscularly immediately prior to balloon denudation, and received the same dose of the drug twice a day for 3 days. Then the dose was reduced to 2 mg/kg, twice a day for 18 days. The rats in the control group received 2 ml of saline intramuscularly immediately prior to balloon injury, and received the same dose of saline twice a day for 3 days. Then they were given 1 ml of saline, twice a day, for 18 days. Three weeks after balloon carotid artery injury, histologic and morphologic analyses of all vessels were performed.

Balloon-injury procedure

The rats were anesthetized with ketamine (100 mg/kg) by intraperitoneal injection. Through a midline neck incision, the distal common carotid artery and the region of the bifurcation were exposed. A 2F Fogarty balloon catheter (Baxter Healthcare Corp., Irvine, CA, U.S.A.) was introduced through the external carotid artery and advanced into the thoracic aorta. The balloon was inflated with saline to distend the common carotid artery and was then pulled back to the external carotid artery. After three repetitions of this procedure, the catheter was removed and the external carotid artery was ligated.

Morphometric analysis

Three weeks after the balloon injury, the rats were sacrificed with an overdose of sodium pentobarbital (75 mg/kg). Via a midline abdominal incision, the distal abdominal aorta was exposed and rinsed with 50 cc saline solution through an 18 G catheter. *In vivo* fixation with 4% formalin at a pressure of 120 mmHg was performed over 5 minutes. The carotid artery was isolated from adherent tissue and kept in 4% formalin for morphometric analysis. Vessels were embedded in paraffin, and the middle one-third of the damaged artery was serially sectioned and morphometric analysis of each arterial segment was performed with a computer-based morphometric system (OPTOMAX V Image Analyzer ; Analytical Measuring System, Pampisford, Cambridge, U.K.). The cross-sectional areas of the external elastic lamina (total area), within the internal elastic lamina (intimal area), and within the lumen (lumen area) were each measured. The degree of myointimal proliferation of the injured carotid artery was expressed as the ratio of the neointimal area over the medial area (intima/media ratio). The mean of four segments exhibiting the greater degree of intima/media ratio was considered as a representative value for comparisons between the two groups.

Statistical analysis

Results were expressed as mean±SEM. Data was analyzed by the use of SPSS/PC software on an IBM PC computer. Statistical comparisons were performed by the use of an independent t-test. A value of $p<.05$ was considered significant.

RESULTS

Histological analysis

Hematoxylin and eosin-stained sections of all segments were examined. As shown in Fig. 1, proliferation of the neointima, consisting of circumferentially-uniform, multi-

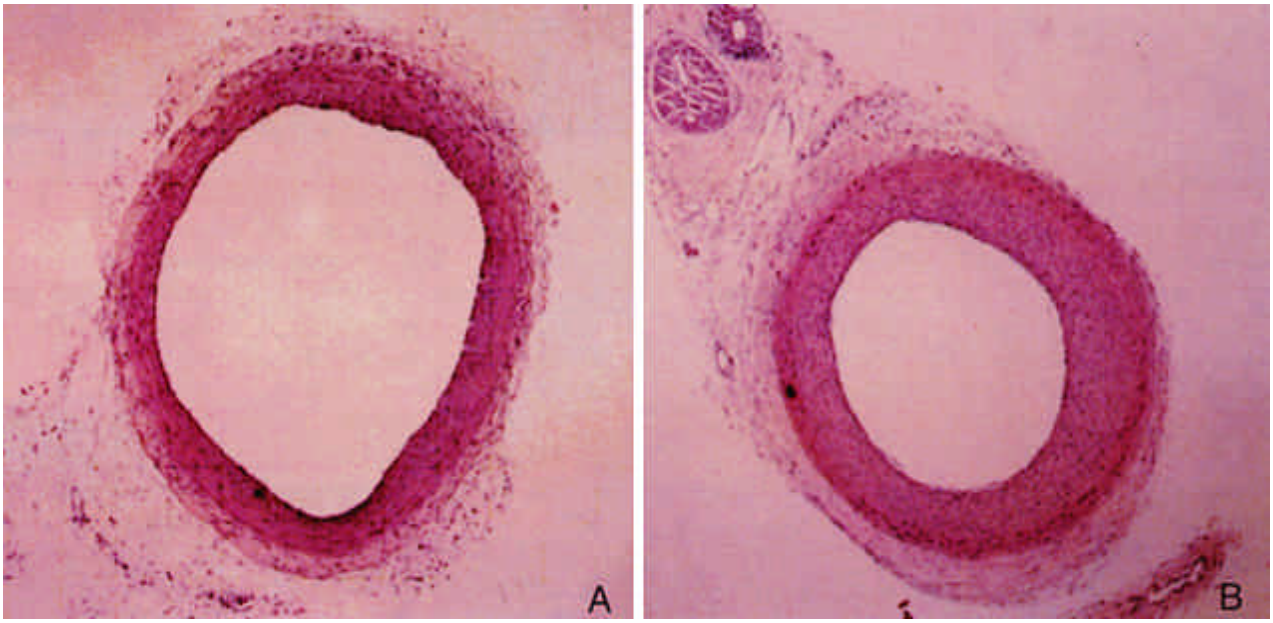

Fig. 1. Photomicrograph of injured common carotid artery 21days after balloon injury showing neointimal proliferation. Morphometric analysis revealed significant reduction of neointimal proliferation in treated group. Hematoxylin-Eosin stain. **A** : Sulodexide-treated group (40x magnification). **B** : Control group (40x magnification).

Table 1. Effect of sulodexide treatment on neointimal proliferation and total occlusion after balloon injury of rat carotid artery

	Sulodexide (n=17)	Control (n=21)	p Value
Total occlusion	1 (5.8%)	5 (23.8%)	NS
Area stenosis (%)	30.01±2.37	41.78±3.79	0.015
Intima/Media ratio (%)	118.39±6.80	177.25±17.25	0.005
Media/Area within external elastic lamina (EEL) (%)	19.94±0.47	20.35±0.76	NS

ple layers of smooth-muscle cells, was observed in the damaged-vessel segments in both the treated and control groups. The degree of thickening was somewhat variable, but in the treated group the degree of neointimal proliferation was less extensive than in the control group. Thrombotic occlusion of injured vessels was noted in both groups (Table 1), but the percentage of total occlusion was higher in the control group than the treated group (23.8% vs. 5.8%, p=NS). These cases were excluded for morphometric analysis.

Morphometric analysis

Morphometric analysis showed that there was no significant change in the medial thickness to the total thickness of injured carotid arteries in both groups. The ratio of the neointima to the medial thickness was significantly less in the treated group than in the control group (118.39±6.80% vs 177.25±17.25%, p=0.005). The percentage of area stenosis (luminal area/area within internal elastic lamina ratio) was also significantly less in the treated group (30.01±2.37% vs 41.78±3.79%, p<=0.015) (Table 1.).

DISCUSSION

This study demonstrates that Sulodexide, which contains the middle-or-low molecular weight glycosaminoglycans-heparan sulfate and dermatan sulfate (Sulodexide, Alfa-Wassermann Co., Bologna, Italy), inhibits neointimal proliferation and reduces the incidence of thrombotic occlusion after a balloon-induced endothelial denudation of a rat carotid artery. Heparan sulfate reduces thrombus formation by inhibiting the activated Factor X through activation of AT III, whereas dermatan sulfate inhibits thrombus formation by enhancing Heparin Cofactor II-dependent inactivation of soluble thrombin (7). But the mechanism underlying the anti-proliferative effects of these heparin-like compounds is still poorly understood. The antiproliferative property of heparan sulfate *in vivo* has been well documented (8, 9), and the structural determinant of growth-inhibitory activity is different from that of anticoagulant activity (10, 11). There is no evidence of an antiproliferative property for dermatan sulfate (12). In some cancer cell lines, dermatan sulfate enhanced the growth rates of the tumorigenic cells (13). In the present study, the mechanism of the antiproliferative effect of heparan sulfate was not investigated. Sulodexide has lipid-lowering effects by acting on lipoprotein lipase as well as antithrombotic and antiproliferative effects (14, 15). Recently, Violaris et al. (16) showed that the presence of angiographically-identifiable thrombus at the time of the angioplasty is associated with higher restenosis. Because of the dual anticoagulant and antiproliferative effects of heparin, it has been extensively studied as an inhibitor of intimal hyperplasia after vascular injury. Heparin inhibits smooth-muscle cell proliferation, *in vitro* and *in vivo*, and decreases intimal thickening in an injured rat carotid artery (17). But trials with heparin in human coronary angioplasty have failed to reduce the restenosis rate (18, 19). There are many possible explanations for this discrepancy, including differences in the route, dose, and duration of heparin administration, differences in the mechanisms of arterial injury and interspecies differences in neointimal proliferation. The procoagulant activity after balloon injury is highest in the first 3~5 days and returns to baseline within two weeks (20). The dose of Sulodexide used in the present study was 10 mg/kg/day for the first 3 days, which is compatible with anti-factor Xa activity of 8,000 U/kg of heparin (21) and then it was reduced to 4 mg/kg/day for the next 18 days.

Sulodexide, which is a mixture of heparan sulfate and dermatan sulfate, has less antithrombotic activity than heparin (22), but a more potent antiplatelet activity than heparin (21). *In vivo*, Sulodexide has a dose-dependent antiaggregation effect on platelet which can already be seen with the dose of 0.5 mg/kg, a dose which does not produce anticoagulant effects. In addition, as one of the nonheparin glycosaminoglycans, Sulodexide is resistant to platelet factor 4 which is a natural neutralizer of heparin (23). In humans, Sulodexide showed a reduction of fibrinogenemia and platelet aggregation without prolongation of partial thromboplastin time and hemorrhagic risk (24). In this study, with a relatively high dose of Sulodexide which was compatible with anti-factor Xa activity of 8,000 U of heparin, significant inhibition of

neointimal hyperplasia was achieved without hemorrhagic complications.

In conclusion, although many clinical-pharmacological trials including anticoagulants, corticosteroids, calcium-channel blockers, ACE inhibitors, and others have failed to show a reduction of restenosis despite their successful results in animal studies (25~28), Sulodexide has demonstrated that it might decrease the rate of restenosis after balloon angioplasty with a proper dosage and delivery method.

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