Sulfated Polysaccharides Modulate Effects of Acidic and Basic Fibroblast Growth Factors on Repair of Injured Confluent Human Vascular Endothelium

Claude Klein-Soyer, Alain Beretz, Jean-Pierre Cazenave, Elisabeth Wittendorp-Rechenmann, Jean-Luc Vonesch, Roger V. Rechenmann, Francis Driot, and Jean-Pierre Mafrand

Semi-automatic analysis of the repair process of a circular mechanical lesion of confluent human vascular endothelial cells in vitro was used to evaluate the contributions of cell migration and cell proliferation. Standard heparin added to culture medium that contained 30% human serum induced an inhibition of cell migration at the lesion margin during the first day after injury. Several sulfated polysaccharides were tested in the presence of 5% human serum. Standard heparin, low molecular weight heparin, or pentosan polysulfate markedly reduced the rate of lesion regeneration. Cell proliferation, measured by $^3$H-thymidine incorporation at the lesion margin, and cell migration were both decreased. In contrast, the combination of acidic fibroblast growth factor with a sulfated polysaccharide accelerated the repair process. Basic fibroblast growth factor combined with a sulfated polysaccharide gave a regeneration rate similar to that of the control; however, at 4 days after injury, the residual lesion was the same when basic fibroblast growth factor was used alone or when it was combined with sulfated polysaccharides. Acidic fibroblast growth factor totally reversed the effects of sulfated polysaccharides on the repair process by enhancing endothelial cell proliferation and allowing endothelial cell migration.

(Arteriosclerosis 9:147–153, March/April 1989)

The effects of vascular injury are of major importance in the development of atherosclerosis.1-3 After a denuding injury, the regrowth of endothelium is a function of the size of the initial lesion. Small wounds are closed by migration of adjacent endothelial cells (EC) without cell replication. In larger denuded areas, the repair process is a combination of cell migration and cell proliferation.4 Standard heparin (st-heparin) has been shown to have a protective effect on EC by binding and inactivating biological substances such as histamine, serotonin, bradykinin, and toxins that may injure EC and by restoring the negative charge of EC after injury.5 Furthermore, heparin stimulates the migration of capillary EC but inhibits the migration of EC from large vessels in vitro.6 The addition of st-heparin to endothelial cell growth factor (ECGF) may greatly improve the growth of sparsely seeded umbilical cord EC.6 Less is known about the influence on maintenance and tissue repair of fibroblast growth factors (FGFs), which present a high affinity for heparin.6 We have recently described an in vitro model of mechanical injury of confluent human EC. In this system, EC are selectively detached with cellulose polycarbonate paper from the underlying matrix, and it is possible to quantify the effects of growth factors and pharmacologic agents during the repair process.10 Our maintenance of an extracellular matrix in the injury area could be significant, because it has been reported that FGFs are deposited by EC in subendothelial extracellular matrices.11-12 Computerized image analysis coupled to autoradiography has been developed and adapted to this injury model.13-14 We have now analyzed the modulation of the regeneration of injured confluent EC, which involves cell migration and proliferation by using several sulfated polysaccharides (SP): st-heparin, low molecular weight heparin (lo-heparin), or pentosan polysulfate (PPS) alone or in combination with purified acidic FGF (aFGF) or basic FGF (bFGF).

Methods

Reagents

St-heparin ([173 U/mg] with an average molecular weight [Mr] of 15 000 and a degree of sulfation [%S] of 10.9) and lo-heparin Cy216, (with an average Mr of 4500, and %S of 8.5) were a gift from the Institut Choay, Paris, France. PPS ([Hemoclar]) with an average Mr of 4700 and %S of 16.6) was from Clin-Midy, Montpellier, France.

Endothelial Cell Culture Conditions

Human umbilical EC were obtained and cultured as described previously.15 Experiments were done in primary...
culture or (in most cases) by using EC cryopreserved in liquid nitrogen.15 16 EC/cm² were seeded in 35 mm (diameter) Petri dishes (ref 2500 HA-20, Coming Glass Works, Coming, NY), which were precoated with a human plasma fraction enriched in fibronectin (50%) by cryoprecipitation.15 The culture medium was M199/RPMI 1640 (vol/vol) containing 10 mM HEPES, 2 mM L-glutamine, antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin), fungizone (2.5 μg/ml) all from Gibco, Paisley, Scotland, UK, and 30% pooled hepatitis B virus and HIV-free heat-inactivated human serum (HS) from the Centre Régional de Transfusion Sanguine, Strasbourg, France.

**Human Acidic and Basic Fibroblast Growth Factors**

The aFGF was purified from human brains obtained 24 to 48 hours after death and stored at ~80°C until used. The extraction and purification were based on previously described procedures17 with some modifications. Briefly, one brain was homogenized and extracted in ice-cold 0.15 M ammonium sulfate (pH 4.5). The extracted material was partially purified by ammonium sulfate precipitation, cation exchange, and heparin-Sepharose (Pharmacia, Uppsala, Sweden) affinity chromatography.18 The bFGF was isolated from human placenta by a combination of salt precipitation, cation exchange chromatography, and heparin-Sepharose chromatography as described previously.18 The mitogenic factor eluted from the affinity column was diluted to a final NaCl concentration of 0.5 M and was reloaded onto a second heparin-Sepharose column (10.0×3.0 cm); then this was washed with 1.0 M NaCl and eluted with a linear gradient from 1.0 M to 2.0 M NaCl for 2 hours at 0.6 ml/minute. The aFGF and bFGF were eluted at 1.0 M and 1.6 M NaCl, respectively. The aFGF and bFGF displayed one band in sodium dodecyl sulfate-polyacrylamide gel electrophoresis of M₉ 16.5 and 17.4, respectively. Both FGFs exhibited one major peak in reverse-phase, high-performance liquid chromatography. After integration of the chromatograms, their purities were estimated to be greater than 95%. The mitogenic activities of both aFGF and bFGF were determined by use of cultured bovine aortic endothelial cells (BAEC) as described.20 The concentrations required to obtain an optimal proliferative response of BAEC were 1 ng/ml for bFGF and 40 ng/ml for aFGF (data not shown). These results are in agreement with already published data.20 Under the same conditions, the concentrations required to obtain optimal proliferation of human EC were 5 ng/ml for bFGF and 50 ng/ml for aFGF. The active fractions were stored at ~80°C until used.

**Mechanical Injury of Confluent Endothelial Cells and Repair Process**

An in vitro model which mechanically denudes confluent human EC has been described elsewhere.19 Briefly, a calibrated circular lesion of 6 mm diameter was made at post-confluence in the cell monolayer center with a disk of cellulose polycarbonate paper (Sepraphore III Gelman, Ann Arbor, MI). EC were selectively detached from the underlying extracellular matrix in the area of disk application. The repair process was quantitatively estimated by planimetry, weighing, or directly projecting the culture dish with a photographic enlarger on a digitizer tablet and then measuring the denudation area with a pen stylus.20 The time course of regeneration was followed for 4 days after injury.

**Experimental Protocol**

The lesions were established at time zero, one lesion per culture dish. Drugs and FGFs were added daily to the culture medium, and the medium was changed at day 2. SP solubilized in M199/RPMI 1640 were added at a final concentration of 50 or 100 μg/ml. The aFGF and bFGF diluted in M199/RPMI 1640 containing 0.5% crystallized bovine serum albumin (Schwartz/Mann, Orangeburg, NY) were added at concentrations that promoted optimal growth of sparse EC cultures (50 ng/ml and 5 ng/ml, respectively). Depending on the experiment, the culture medium contained either 30% or 5% pooled HS. After injury, representative samples were removed every 24 hours until day 4 and were fixed as follows: before fixation, the samples were pulsed for 18 hours with 9.25 kBq/ml ³H-thymidine (³H-Tdr, 37 MBq/ml, 7.4 GBq/mmol, Amersham, Les Ulis, France). Then the culture dishes were rinsed twice with phosphate-buffered saline (PBS) and twice with fixative (2% paraformaldehyde, 0.1% glutaraldehyde in PBS) and were allowed to stay in the fixative for 1 hour at 4°C. After fixation, the dishes were rinsed twice with 70% ethanol and were rinsed extensively (4 to 6 hours) in tap water. After the final rinsing with distilled water, the dishes were allowed to dry until processed for autoradiography.

**Measurement of Antithrombin Activity of Culture Medium and Serum**

No antithrombin activity of culture medium alone or supplemented with serum was detected when residual, active st-heparin was measured with a fluorogenic substrate sensitive to thrombin (Protopath, AHS, Dade, Miami, FL). Furthermore, platelet factor 4 (PF4) was measured by an enzyme-linked immunosorbent assay technique (Asserachrom PF4, Diagnostica Stago, Asnières, France) in the serum batch used for culture and was 6.9 μg/ml. The maximal concentration of PF4 in medium with 30% serum is 2.1 μg/ml; thus the concentrations of st-heparin used in this work were excessive. For example, 1 μg/ml of st-heparin is inhibited by 25 μg/ml PF4.7

**Autoradiography and Image Analysis**

A high efficiency autoradiographic method21 was specifically adapted to cultured EC. A uniform 15 μm thick layer of liquid emulsion (NTB2, Eastman Kodak Company, Rochester, NY) was poured directly onto the EC layer with a micropipette. After a 24-hour exposure, the dishes were submitted to an activated development procedure22 and were stained with May-Grünwald Giemsa. A computer-assisted optoelectronic image analyzing assembly and software, which combines visual counting with automated data acquisition and treatment, was developed.14 Thus, the cell density, the labeling index, and the lesion repair were simultaneously monitored.15
### Table 1. Influence of Standard Heparin on Regeneration of Damaged Endothelium in Presence of 30% Human Serum

| Time (hrs) | No addition | Standard heparin (50 µg/ml) |
|-----------|-------------|-----------------------------|
| T50       | 48.0        | 50                          |
| Residual lesion | 6.8±3.9 | 8.6±2.8                     |

The growth curves were obtained at 24, 48, and 72 hours with two samples per time point. The T50 is the mean of determinations obtained from two different growth curves. The slopes of the regression curves were compared using Student’s t-test and were not significantly different (p>0.05). The size of the residual lesions (mean±SD, n=4) were compared using Student’s t test and were not significantly different (p>0.05).

### Table 2. Influence of Standard Heparin on Regeneration of Damaged Endothelium in Culture Medium Containing 30% Human Serum

| Time (hrs) | Control | St-heparin (50 µg/ml) | Control | St-heparin (50 µg/ml) |
|-----------|---------|-----------------------|---------|-----------------------|
| 24        | 44.0±5.2 | 38.8±3.6*             | 45.5±3.3 | 54.4±3.9†             |
| 48        | 23.0±5.7 | 27.4±4.0              | 70.2±6.5 | 58.5±7.1              |
| 72        | 22.2±3.2 | 22.0±2.8              | 60.7±7.2 | 61.4±7.0              |
| 96        | 21.3±3.1 | 32.1±5.0              | 38.5±6.6 | 37.7±5.6              |

The cell density (expressed as the percentage of the cell density in confluent areas) and the labeling index were determined at the lesion margin by image analysis. The results are the means±95% confidence intervals (n=4).

*p<0.01, †p<0.001, St-heparin-standard heparin.

### Statistical Analysis

The effects on the repair process of SP and FGFS, alone or in combination, were compared by analysis of variance followed by a Newman-Keuls test. We used the statistical software, STAT-ITCF (ITCF, Bolgenville, France). Comparisons between the labeling indexes were carried out on the basis of a χ² test. A so-called "bootstrap method" was used to estimate the 95% confidence intervals in the case of cell density ratios between the edge of the lesion and the confluent areas. Comparisons of mean cell density ratios were made with the Mann-Whitney test.

### Results

#### Effect of Standard Heparin on Repair Process of Injured Confluent Human Endothelium in Presence of 30% Human Serum

The regeneration process of confluent human EC in the presence of culture medium containing 30% HS with or without supplemental (50 µg/ml) st-heparin was studied. The presence of st-heparin in the culture medium did not significantly modify the time necessary to repair 50% of the lesion (T50) (Table 1). Furthermore, the residual lesions that were measured 96 hours after injury were not different. These results indicate that the global process of regeneration was not modified by high concentrations of st-heparin in the culture medium. The cell density at the lesion margin 24 hours after injury (monitored by Image analysis and expressed as the percentage of cell density of confluent areas distant from the lesion) was lower (0.07<p<0.06) in the presence of st-heparin (38.8%±3.6% vs. 44.0%±5.2% for the control) (Table 2). This lowering of cell density could be due either to inhibition of cell migration or to inhibition of cell proliferation. However, the labeling index in heparin-treated EC was significantly higher (54.4%±3.6% vs. 45.5%±3.3%) (p<0.01). This increase of the labeling index at the lesion margin should signify that the cells that participate directly in the repair process are essentially in the proliferative state. As the recovered area was the same with or without st-heparin, we can assume that the contribution of cell migration to the repair process is lower in the presence of st-heparin, that is, that st-heparin inhibits cell migration into the denuded area. This effect was no longer visible after 24 hours; cell density and labeling index were similar in treated and control EC.

#### Modulation of Repair Process by Sulfated Polysaccharides, Alone or with Acidic or Basic FGF

The effects of SP, alone or in combination with aFGF or bFGF, were studied during the repair process of a denuding injury of confluent endothelium. The growth of EC in medium containing 30% HS was optimal and not sensitive to additional mitogenic stimulation. Thus, in further experiments, the serum concentration was reduced to 5% to increase the sensitivity of the proliferative response to the addition of substances that either stimulate or inhibit cell growth. The T50 and the residual lesion 96 hours after injury were analyzed. The addition of aFGF or bFGF to the culture medium (at concentrations supporting optimal growth of sparsely seeded EC) did not significantly modify (p>0.05) the T50 as compared to the controls (Table 3). When st-heparin or PPS alone was added during the repair process, the T50 was significantly increased, indicating a reduction in speed of regeneration (p<0.05). The combination of bFGF with st-heparin or PPS only slightly reduced the T50 obtained in the presence of SP alone. In the presence of aFGF plus a SP, the T50 value was significantly reduced (p<0.05) as compared to that obtained with aFGF alone. In the presence of SP, the size of residual lesions after 96 hours was significantly larger (p<0.05) than for the control or when aFGF or bFGF alone was added to the culture medium (Table 4). When aFGF was combined with SP, a significant reduction (p<0.05) in size of the residual lesion was observed. This was not the case when bFGF was added to SP. The residual lesion was even larger than the residual lesion of the control, of bFGF alone, of bFGF/st-heparin, or of bFGF/PPS, and the lesion was identical to those obtained with SP alone.

#### Analysis of Evolution of Labeling Index at Lesion Margin as a Function of Time

We examined the labeling index and cell density at the lesion margin, which is the strategic place for the events leading to regeneration of the injury. The labeling index
increased in all cases as a function of time and reached a maximum at 72 hours after injury (Figure 1). Between 72 and 96 hours, the labeling index diminished considerably in all samples, this effect was less marked when the associations between aFGF and st-heparin or aFGF and PPS were considered. In the presence of SP, the labeling index was lower than under other conditions during the 48 hours after injury (40% vs. 55%) (p<0.05). At 72 hours, with lo-heparin only, the labeling index was identical to the control (75% of labeled cells at the lesion margin). At 96 hours after injury, the percentage of labeled cells at the lesion margin was diminished when the repair process occurred in the presence of st-heparin (and to a lesser extent with lo-heparin or PPS) as compared to the control (28.3%±0.42% and 55.8%±7.0%, respectively, p<0.05). The addition of aFGF to st-heparin considerably increased the labeling index in the same conditions (70.7%±5.0% vs. 28.3%±0.42%, p<0.05). This effect was less pronounced when aFGF was combined with lo-heparin or PPS. In contrast, the addition of bFGF to st-heparin during the repair process did not modify the low labeling index observed at the lesion margin 96 hours after injury when the regeneration occurred in the presence of st-heparin alone.

### Analysis of Cell Density at a Distance from Lesion, 96 Hours After Injury when aFGF, bFGF, and Sulfated Polysaccharides Alone or in Combination Were Added to Culture Medium

During the repair process, the labeling index was high at the leading edge of the lesion margin, and cell density was considerably diminished. The labeling index progressively diminished, and the cell density increased moving away from the lesion margin. At a distance of 2 mm from the leading edge, the labeling index returned to background value, and the cell density reached a value similar to that in undisturbed areas. The cell densities in various confluent areas at a distance from the lesion margin were compared (Table 5). No major difference in cell density between the different samples was observed except when aFGF was associated with SP. Here, cell density was higher than in samples containing SP alone. These results demonstrate the high proliferative activity observed when aFGF was combined with SP.

### Table 3. Effects on Repair Process of Various Additions, Alone or in Combination

| Addition to medium* | Control (no addition) | Growth factor alone | SP alone | Growth factor + SP |
|---------------------|-----------------------|---------------------|----------|-------------------|
| aFGF/st-heparin     | 55.3±0.8              | 53.2±0.8            | 58.3±0.8 | 41.0±2.4          |
| aFGF/lo-heparin     | 53.8±0.6              | 50.7±0.8            | 61.8±2.1 | 44.7±0.1          |
| aFGF/PPS            | 57.9±3.8              | 54.1±1.4            | 64.9±0.66| 41.6±0.5          |
| bFGF/st-heparin     | 55.4±1.1              | 51.9±2.3            | 64.8±2.8 | 57.1±0.2          |
| bFGF/lo-heparin     | 50.9±0.5              | 52.7±5.2            | 52.5±1.3 | 56.4±3.1          |
| bFGF/PPS            | 53.1±0.7              | 55.4±2.8            | 64.2±1.7 | 58.1±0.4          |

*All additions to medium containing 5% human serum.
†p<0.05.
‡Significance different from T90 of control (p<0.05).

### Table 4. Effects on Repair Process of Various Additions, Alone or in Combination

| Residual lesion† | Control (no addition) | Growth factor alone | SP alone | Growth factor + SP |
|------------------|-----------------------|---------------------|----------|-------------------|
| aFGF/st-heparin  | 21.4±3.1              | 16.5±0.2            | 32.7±0.6 | 3.1±0.4           |
| aFGF/lo-heparin  | 22.2±3.8              | 14.6±2.6            | 27.2±2.4 | 5.7±0.8           |
| aFGF/PPS         | 21.6±0.9              | 14.5±2.7            | 27.2±1.1 | 1.9±0.2           |
| bFGF/st-heparin  | 15.8±5.6              | 19.9±0.5            | 31.0±0.2 | 28.9±0.6          |
| bFGF/lo-heparin  | 18.3±3.2              | 11.1±3              | 26.9±1.1 | 18.9±1            |
| bFGF/PPS         | 11.4±0.3              | 21.1±0.6            | 23.5±2.9 | 28.9±3.6          |

*All additions to medium containing 5% human serum.
†Percent of initial surface 96 hours after injury.
‡Residual lesion significantly different from the residual lesion of control (p<0.05).

The initial diameter of the lesion was 6 mm. Acidic and basic fibroblastic growth factors (aFGF, 50 ng/ml; bFGF, 5 ng/ml) and sulfated polysaccharides (SP): st-heparin, lo-heparin, and PPS (100 µg/ml) were added as described in the Methods section. The T90 is the mean of determinations obtained from two different growth curves including three time points (24, 48, and 72 hours). The T90 values from each experiment were compared by analysis of variance followed by the Newman-Keuls test. Each value is the mean of two values±SD.

St-heparin=standard heparin, lo-heparin=low molecular weight heparin, PPS=pentosan polysulfate.
SULFATED POLYSACCHARIDES, FGFS, AND ENDOTHELIAL REPAIR  Klein-Soyer et al.  151

Table 5. Effects of Various Additions, Alone or in Combination, on Cell Density of Confluent Monolayer Distant from Lesion

| Addition to medium* | No addition | St-heparin (100 μg/ml) | Lo-heparin (100 μg/ml) | PPS |
|--------------------|-------------|------------------------|------------------------|-----|
| None               | 58.0±5.5    | 55.9±8.0               | 57.1±10.9              | 72.6±12.6 |
| aFGF (50 ng/ml)    | 63.8±5.1    | 91.5±9.5†              | 74.4±10.5†             | 94.9±7.9†  |
| bFGF (5 ng/ml)     | 47.9±5.2    | 54.7±5.4               |                        |     |

*All additions to medium containing 5% human serum.
The values are means±95% confidence interval; n=4 to 8.
†Cell density significantly different from control (p<0.05).
Abbreviations are explained in the legend for Table 3.

Figure 1. Labeling index at the lesion margin as a function of time. Acidic fibroblast growth factor (aFGF, 50 ng/ml) and basic fibroblast growth factors (bFGF, 5 ng/ml) and the polysaccharides, standard heparin (st-hep), low molecular weight heparin (lo-hep), and pentosan polysulfate (PPS) at 100 μg/ml were added as described in the Methods section. During repair, the cells were labeled with [3H]thymidine (0.35 kBq/ml) for 18 hours. After fixation and autoradiography, the labeling index was determined at the leading edge of the lesion at various times. The results are the means±95% confidence levels. n=4 to 8.† These were significantly different from the control (p<0.05).

Discussion

The contributions of cell migration and cell proliferation to the repair of an in vitro denuding lesion of EC were determined.14 The proliferative state of individual cells was followed by autoradiography. A decrease in cell density at the lesion margin could be due to migration or to detachment of EC. However, because the cell density at the lesion margin was expressed as a percentage of the density in confluent areas at a distance from the lesion, an eventual cell detachment would not greatly influence the results. The presence of high concentrations of st-heparin in culture medium containing 30% HS did not prevent completion of the regeneration process. The time necessary to repair 50% of the lesion and the lesion remaining 96 hours after injury were similar in samples containing st-heparin and in controls. However, the cell density in regenerated areas in st-heparin–treated EC was lower 24 hours after injury, and the labeling index was significantly higher. These results suggest that st-heparin selectively inhibited EC migration during the repair process. The inhibitory effect of st-heparin on EC migration observed here is in agreement with previous results,7 in which maximal inhibition (91%) of BAEC migration was obtained at a concentration of 1 μg/ml st-heparin in serum-free medium. In the present experiments, we observed an effect of st-heparin only during the 24 hours after injury. Thereafter, the high concentration (30%) of serum used was probably sufficient to overcome the effect of st-heparin. The addition of FGF to the culture medium of sparsely seeded EC has been shown to greatly improve their growth performance.25,26 Furthermore, the combination of st-heparin and ECGF, which is related to the acidic FGFs,5 has made possible long serial cultivation and the cloning of human EC.6 Although aFGF and bFGF can both be purified by heparin affinity chromatography, st-heparin has little effect on the activity of bFGF.6 Until recently, the exact role of FGFs in wound healing or in the
regeneration of endothelium was not well established.\textsuperscript{11,26} It has been shown that topical application of aFGF and st-heparin accelerated dermal wound closure in mice.\textsuperscript{27} We previously reported that a human brain extract containing growth factor activity, which was added to culture medium with 30\% HS, significantly accelerated the regeneration of mechanically damaged EC in culture.\textsuperscript{10} Here we have shown that the regeneration of denuded confluent EC in the presence of low concentrations of HS (5\%) can be modulated in two ways: 1) SP such as st-heparin, lo-heparin, or PPS considerably slowed down the repair process, as indicated by the longer $T_{50}$ (Table 3). This could be partly due to a reduction of the proliferation rate, which was reflected in a lower labeling index at the lesion margin. At the end of the repair process (96 hours), the labeling index at the leading edge of the lesion returned to basal values (Figure 1); at this stage, it can be assumed that cell migration contributes most to the repair process. This has been shown for the recovery of small EC lesions.\textsuperscript{4} SP slowed down the repair at this late stage, as the residual lesions were significantly larger after injury (Table 4). Thus, we suggest that SP inhibit not only EC proliferation, but also EC migration. 2) In contrast, the combination of aFGF with SP completely abolished their inhibitory effects on the repair process and even led to an increase in the speed of regeneration as compared to the controls. Both proliferation and migration of EC could be affected; the residual lesions were considerably reduced, the labeling index at the margin of the lesion increased, and the cell density in undisturbed areas 96 hours after injury was significantly greater.

It has been reported that SP inhibit $^3$H-TdR incorporation at low serum concentrations (<10\%) in proliferating cells.\textsuperscript{28} The affinity of st-heparin for EC is a function of the molecular weight and the negative charge density of the sulfate groups of the molecule. It has also been shown that in the case of the lo-heparin, CY216 (Mr 1800 to 8000), the heavy chains are bound to and internalized in priority by EC.\textsuperscript{29,30} The smaller effector of lo-heparin alone or with aFGF that we observed can be explained, in part, by a reduction of high molecular weight spacers and a reduced degree of sulfatation, 8.5\% versus 10.9\%, for st-heparin. PPS and st-heparin behaved in a similar way in the repair process. PPS has a molecular weight similar to lo-heparin, but its charge density is considerably higher, with a degree of sulfatation of 16.6\%. This shows that both molecular weight and charge density of SP play a role in modulation of the repair of EC lesions.

More recently, it has been reported that heparin in the presence of bFGF suppressed the growth of human EC cultivated in the presence of fetal bovine serum.\textsuperscript{31} In the present experiments, bFGF partially reversed the inhibitory effects of SP on the repair process. The residual lesion 96 hours after injury was small enough that cell migration could be assumed to be the main mechanism of repair, as reported previously for small linear wounds.\textsuperscript{4} The residual lesion in the presence of st-heparin or PPS was similar whether bFGF was present or not, suggesting that bFGF was less efficient in influencing the process of EC migration.

\section*{Acknowledgments}

The authors are grateful to Michel Roos, Bernard Senger, and Jean-Claude Turriot for their statistical evaluations, to Violette Koziel-Vigneron for excellent technical assistance, to Juliette Mulvihill for reviewing the English, and to Marie-Odile Bernhard and Marinette Voyat for skilful manuscript typing.

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Index Terms: sulfated polysaccharide • heparin • pentose polysulfate • fibroblast growth factor • endothelium • injury • repair process • image analysis • autoradiography