Modulation of Actin Isoform Expression in Cultured Arterial Smooth Muscle Cells by Heparin and Culture Conditions

Alexis Desmoulière, Laura Rubbia-Brandt, and Giulio Gabbiani

Heparin inhibits arterial smooth muscle cell (SMC) proliferation in vivo and in vitro; moreover, it reinduces the expression of α-smooth muscle (SM) actin (an accepted marker of SMC differentiation) in SMCs of the intimal thickening that develops after experimentally induced endothelial lesions. We have investigated the effect of heparin on the proliferation and actin isoform expression in cultured rat SMCs. In the presence of 10% fetal calf serum (FCS), heparin-treated primary and passage 5 SMCs showed a decrease of proliferation and an increase of α-SM actin (measured by Western blots or two-dimensional gel electrophoresis) compared with untreated SMCs. When SMCs were cultured in the presence of 10% plasma-derived serum, no proliferation occurred and heparin did not modify α-SM actin expression. This suggests that the action of heparin is related to its antiproliferative activity. SMCs cultured in the presence of 10% FCS plus heparin had the same level of proliferation as SMCs cultured in 5% FCS but had a higher content of α-SM actin. SMCs cultured in 20% rat whole-blood serum had a proliferation similar to that observed in SMCs cultured in 10% FCS but had a higher content of α-SM actin. Moreover, in SMCs cultured in 20% whole-blood serum, heparin inhibited SMC proliferation but did not modify α-SM actin expression. Thus, the action of heparin on α-SM actin expression appears to be partially independent of proliferation and is related to culture conditions. The proportion of α-SM actin mRNA, as measured by Northern blots with an α-SM actin mRNA-specific probe, was increased by heparin compared with cells cultured in 10% FCS; this suggests that heparin acts at the transcriptional or posttranscriptional level. Our results show that heparin acts not only on SMC proliferation but also on SMC differentiation; further investigation along these lines may help in the understanding of the mechanisms of SMC adaptation during normal and pathological conditions. (Arteriosclerosis and Thrombosis 1991;11:244–253)

Inappropriate migration and proliferation of arterial smooth muscle cells (SMCs) are key events in the pathogenesis of atherosclerosis.1,2 SMCs implicated in the atheromatous process show a remodeling of morphological and cytoskeletal features, which consists of a loss of microfilament bundles3 and a switch of actin isoform expression from α-smooth muscle (SM) to β-cytoplasmic predominance.4 Similar features are observed in SMCs of fetal animals (thus suggesting that atheromatous SMCs are dedifferentiated), of intimal thickening after experimental removal of the endothelium, and when SMCs are placed in culture5,6; all these conditions are characterized by SMC proliferation.

Many studies have described the action of mitogenic agents on SMCs of the aortic media and their possible role in the production of the atherosclerotic plaque.1 It is also possible that SMC quiescence is actively maintained in healthy blood vessels by locally produced substances.7–9 Good candidates for this role are proteoglycans and heparin, which inhibit SMC proliferation in vivo10,11 and in vitro.12 Heparan sulfates with antiproliferative activity for SMCs are released from quiescent cultures of aortic endothelium7 and from confluent cultures of SMCs.13 Heparin has also been shown to inhibit SMC modulation from a contractile to a synthetic phenotype,14,15 as well as the switch in actin isoform expression observed in SMCs after a balloon catheter-induced endothelial lesion.9

We have investigated the effect of heparin on actin isoform expression in cultured SMCs and show in this...
article that heparin increases α-SM actin expression in primary and passaged SMCs in the presence of 10% fetal calf serum (FCS) but not in the presence of 10% plasma-derived serum (PDS), suggesting that the action of heparin is related to its antiproliferative activity, as previously suggested by in vivo experiments. Moreover, our results suggest that the action of heparin on α-SM actin expression is partially independent of proliferation and is related to culture conditions.

**Methods**

**Aortic Smooth Muscle Cell Culture and Growth Kinetics**

Six-week-old Wistar rats were used throughout the study. Aortic SMCs were isolated by enzymatic digestion and grown in Dulbecco’s modified Eagle’s medium (DMEM, Gibco AG, Basel, Switzerland) with 10% FCS (Seromed, Biochrom AG, Berlin, F.R.G.). Primary and passage 5 cultures were employed. PDS and whole-blood serum (WBS) were prepared according to Vogel et al. In some experiments, 10^{-5} M serotonin (serotonin creatinine sulfate, Fluka Chemie AG, Buchs, Switzerland), which has a mitogenic effect on SMCs in vitro, was used to induce proliferation in SMC cultures containing 10% PDS. To maintain SMC quiescence without nutritional deprivation and protein breakdown, SMC cultures were kept in a chemically defined serum-free (selenium/insulin/transferrin/[SIT]) medium supplemented with 10^{-8} M insulin (Collaborative Research Inc., Lexington, Mass.), 5 μg/ml transferrin (GIBCO), and 10^{-9} M selenium (Collaborative Research Inc.). Heparin (grade II, Sigma Chemical Co., St. Louis, Mo.) was added 24 hours after plating and generally applied for 7 days.

The cell number was determined by performing hemocytometer cell counts and by measuring total DNA content, divided by the content of DNA per cell. These techniques gave similar results. Briefly, SMC cultures were rinsed in DMEM and trypsinized. After cell counts were completed, SMCs were resuspended in phosphate-buffered saline (PBS, 150 mM NaCl in 10 mM phosphate buffer, pH 7.2) containing 4% aprotinin (Trasylol, Bayer AG, Zürich, Switzerland), 1% leupeptin (Fluka Chemie), 10 mM ethylene glycol-bis(β-aminoethoxy ether)-N,N,N’,N’-tetraacetic acid (EGTA, Sigma), 1 mM phenylmethylsulfonyl fluoride (PMSF, Merck, Darmstadt, F.R.G.), and 7.8% benzamidine (Sigma) and sonicated for measurement of total DNA content. In general, we used the hemocytometer counting results for these calculations. To calculate protein content per cell, total protein content was divided by cell number. In all experimental conditions, the number of seeded cells was 5 × 10^3 cells/cm² (3 × 10^5 cells/100-mm petri dish). After 7 days, cells were counted and results expressed in tables and graphs as follows: no growth = number of seeded cells; 100% growth = number of 10% FCS–treated cells. Other results were calculated as mean percentage ±SEM of 10% FCS–treated cells. The same criteria were used for protein content.

**Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Immunoblotting**

For sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), samples remaining after DNA measurements were mixed with 5× concentrated buffer containing 5% SDS (Bio-Rad Laboratories AG, Glattbrugg, Switzerland), 5% dithiothreitol (DTT, Fluka Chemie), 5 mM PMSF, 5 mM Nεp-tosyl-L-arginine methyl ester (Sigma) in 0.4 M Tris HCl, pH 6.8, and boiled for 3 minutes. Protein content was determined according to Bradford. Forty micrograms protein was electrophoresed on a 5–20% gradient gel and stained with Coomassie Blue. For quantification of total actin, gels were scanned with a computerized laser beam densitometer (Genofit SA, Geneva, Switzerland) as previously described. Actin isoforms were analyzed by two-dimensional electrophoresis using isoelectric focusing in the first dimension. A 10% SDS-PAGE was used for the second dimension, and the gels were again stained with Coomassie Blue. Quantification was performed by densitometric scanning of stained gels, and the relative amounts of actin isoforms were expressed as a percentage of total actin.

Western blotting with anti–α-SM-1, a monoclonal antibody specific for α-SM actin, was performed as previously described. Briefly, 2 μg (primary culture) or 5 μg (secondary culture, passage 5) proteins were electrophoresed on a 5–20% gradient gel. Separated proteins were transferred to nitrocellulose filters, which were incubated with anti–α-SM-1. After three rinses, a second incubation was performed with rabbit anti-mouse immunoglobulin G (IgG) labeled with horseradish peroxidase (Nordic Immunological Laboratories, Tilburg, The Netherlands). Peroxidase activity was revealed with o-dianisidine (Sigma).

In tables and graphs, modifications of α-SM actin expression were evaluated by densitometric scanning (Genofit) of Western blots and expressed as a mean percentage ±SEM of the control condition (10% FCS).

Western blotting with an affinity-purified rabbit anti–chicken gizzard desmin was performed as previously described with minor modifications. Briefly, 40 μg protein was electrophoresed on a 5–20% gradient gel, and immunoblotting was realized as described above, except when a goat anti-rabbit IgG labeled with alkaline phosphatase (Promega Corp., Madison, Wis.) was used for the second step.

**Cell Sorting**

To perform flow cytometric analysis, SMCs were trypsinized and permeabilized with 1% Triton X-100, Sigma. After centrifugation for 5 minutes at 300g, 10⁶ SMCs were resuspended in 1 ml phosphate-buffered saline containing 100 μl 5 M propidium iodide. Fluorescence emission was measured between 595 and 670 nm to exclude the overlapping region of
excitation and emission spectra of unbound propidium iodide. Absolute values for DNA content were obtained by using chicken erythrocytes run simultaneously with the sample. The samples were passed on an EPICS V, Coulter Electronics Inc., Hialeah, Fla., using a Coherent Innova 90 argon-ion laser, Coherent Laser Products Division, Palo Alto, Calif. Data were collected and stored in an MDADS Digital Computer, Coulter Electronics Inc., interfaced with the EPICS V and plotted as linear histograms of DNA content. The relative number of SMCs in the different phases of the cell cycle was estimated according to previous work.6,26

**Immunofluorescence**

Affinity-purified polyclonal rabbit IgGs against SM myosin27 and anti-α-SM-124 were used. Double-immunofluorescence detection of SM myosin and α-SM actin was performed as previously described27 after fixation of cultured SMCs in ethanol for 30 seconds at room temperature. We used fluorescein isothiocyanate–labeled goat anti-rabbit IgG (Nordic Immunological Laboratories) and tetramethylrhodamine isothiocyanate–labeled goat anti-mouse Ig (Nordic Immunological Laboratories). Preparations were observed on a Zeiss Axiophot microscope equipped with epi-illumination, Carl Zeiss Inc., Oberkochen, F.R.G.

**RNA Extraction**

Cells were scraped from petri dishes using a rubber policeman and homogenized in a sterile solution, pH 7.4, containing 4.5 M guanidine isothiocyanate (Fluka Chemie), 50 mM EDTA (Fluka Chemie), 0.1 M β-mercaptoethanol (Fluka Chemie), 25 mM sodium citrate, and 2% N-lauroylsarcosine (Sigma) with a syringe through a 23-gauge needle. RNA was purified by ultracentrifugation through a cushion of 5.7 M CsCl.28 The RNA pellets were resuspended in 10 mM Tris HCl, pH 7.4, 0.5% SDS, and 1 mM EDTA and extracted twice with saturated phenol/chloroform and then once with chloroform/isoamyl alcohol (24:1, vol/vol). The RNA was ethanol precipitated, resuspended in sterile water, and stored at −70°C.

**Northern Blot Hybridization**

Five to 15 μg total RNA was denatured with glyoxal, separated by electrophoresis in a 1% agarose gel, and transferred overnight on a Bio-dry film, Pall Corp., Glen Cove, N.Y. Next day, the membrane was baked for 2 hours at 80°C under vacuum and stained with 0.04% Methylene Blue in 0.5 M sodium acetate to verify correct loading and transfer. Hybridization was performed either with a riboprobe derived from the coding region of the rat α-SM actin mRNA (total actin probe, pRAoαA-C)29 or with a synthetic oligonucleotide derived from the 3’ untranslated region of rat α-SM actin mRNA (M-L. Bochaton, F. Gabbiani, and G. Gabbiani, unpublished observations). The probe pRAoαA-C hybridizes with cytoplasmic and muscular actin mRNAs,29 and the oligonucleotide is specific for α-SM actin mRNA. For the riboprobe,30 labeling was performed according to Melton et al.,30 and Northern blot hybridization was performed as previously described.30 32P labeling of the oligonucleotide and hybridization were performed according to Maniatis et al.31 Filters were exposed to Kodak X-Omat SO-282 film at −70°C between intensifying screens, and developed films were analyzed by means of computerized densitometric scanning.

**Results**

**Proliferation and Protein Content**

Table 1 shows the effect of heparin on the growth of rat aortic SMCs cultured in the presence of 10% FCS. Primary cultures were more sensitive to heparin than passaged SMCs; the growth-inhibitory effect of heparin was clearly dose dependent and leveled off at concentrations higher than 400 μg/ml (data not shown). All subsequent experiments were performed using 200 μg/ml heparin. Table 2 shows the effect of heparin on the growth of passaged SMCs in the presence of FCS, WBS, or PDS. As expected,3 in the presence of 10% PDS and in SIT medium, SMCs did not proliferate. In FCS- and WBS-treated cells, the

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Cell growth</th>
<th>Protein content per cell</th>
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<tbody>
<tr>
<td>10% FCS</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10% FCS+hep</td>
<td>61.4±1.8</td>
<td>132.9±3.9</td>
</tr>
<tr>
<td>5% FCS</td>
<td>62.1±0.9</td>
<td>121.7±4.4</td>
</tr>
<tr>
<td>5% FCS+hep</td>
<td>29.0±0.8</td>
<td>152.7±12.0</td>
</tr>
<tr>
<td>10% WBS</td>
<td>59.7±2.3</td>
<td>176.2±12.2</td>
</tr>
<tr>
<td>10% WBS+hep</td>
<td>23.7±1.1</td>
<td>185.0±12.3</td>
</tr>
<tr>
<td>20% WBS</td>
<td>94.8±4.6</td>
<td>166.0±7.7</td>
</tr>
<tr>
<td>20% WBS+hep</td>
<td>57.4±2.7</td>
<td>165.1±11.3</td>
</tr>
<tr>
<td>10% PDS</td>
<td>None*</td>
<td>213.7±29.4</td>
</tr>
<tr>
<td>10% PDS+hep</td>
<td>None*</td>
<td>249.6±33.8</td>
</tr>
<tr>
<td>10% PDS+serotonin</td>
<td>74.2±3.6</td>
<td>132.1±9.7</td>
</tr>
<tr>
<td>10% PDS+serotonin+hep</td>
<td>26.7±2.3</td>
<td>156.4±11.2</td>
</tr>
<tr>
<td>SIT medium</td>
<td>None*</td>
<td>137.7±3.9</td>
</tr>
</tbody>
</table>

Values are expressed as mean percentage±SEM of control condition (10% fetal calf serum).

*No variation compared with the number of plated smooth muscle cells.

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**TABLE 1. Effect of Heparin on Primary and Passaged Smooth Muscle Cell Growth**

<table>
<thead>
<tr>
<th>Heparin (μg/ml)</th>
<th>Primary</th>
<th>Passaged</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>39.2±4.8</td>
<td>73.0±2.5</td>
</tr>
<tr>
<td>200</td>
<td>33.3±4.5</td>
<td>64.3±6.9</td>
</tr>
<tr>
<td>400</td>
<td>24.5±5.7</td>
<td>56.3±9.6</td>
</tr>
</tbody>
</table>

Values are expressed as mean percentage±SEM of control condition (10% fetal calf serum).
antiproliferative effect of heparin was always obvious. Moreover, in some conditions (i.e., 5% FCS, 10% WBS), cell growth was decreased compared with that observed in 10% FCS. Table 2 also shows the modifications of protein content per cell under the conditions studied. When SMC proliferation was reduced compared with 10% FCS independently of heparin (e.g., in the presence of 10% WBS or 5% FCS) or when SMCs did not proliferate (e.g., in the presence of SIT medium or 10% PDS), protein content per cell was increased. Protein content was also increased in 20% WBS despite the fact that SMC proliferation was similar to that observed in 10% FCS. Heparin-induced cell protein content increased compared with each control only in the presence of FCS. It did not induce polyploidy, since cell numbers in the different conditions determined by counting or by measuring total DNA content divided by content of DNA per cell were not significantly different. Moreover, flow cytometric analysis after DNA staining with propidium iodide showed similar fluorescence histograms in control and heparin-treated SMCs (78.9% of the cells were in the G0-G1 phase of the cell cycle, while 18.8% of the cells were in S-G2), with a negligible proportion of polyploid cells.

Cellular Actin Content

In the presence of 10% FCS, actin represented 18.7±0.4% of cellular proteins in primary cultures and 12.1±0.3% in passage 5 cultures.6 In the presence of 10% WBS or 10% PDS, actin was 15.2±0.2% or 16.5±0.2%, respectively. Heparin did not modify the percentage of actin per total protein.

Immunoblot analysis showed that in the presence of 10% FCS, heparin-treated SMCs contained more α-SM actin when compared with controls (Figures 1A and 1B). Figure 2 shows that the relative increase of α-SM actin content in heparin-treated SMCs was higher in passaged than in primary cultures although heparin exerts a more important antiproliferative effect in primary culture.

Passaged SMCs exposed to 5% FCS had the same degree of proliferation as those exposed to 10% FCS plus heparin, but α-SM actin content was higher in 10% FCS-plus-heparin–treated SMCs (Figures 3 and 5). In 5% FCS, α-SM actin was substantially increased by heparin (Figures 3 and 5) compared with controls.

SMCs cultured in the presence of 10% WBS (Figure 4) contained more α-SM actin when compared with SMCs in 10% FCS. Moreover, when the concentration of WBS was increased to 20%, α-SM actin content per cell protein as well as proliferation were increased compared with 10% WBS (Figures 4 and 5). In WBS-containing medium, heparin inhibited SMC growth but did not modify α-SM actin expression (Figures 4 and 5).

SMCs cultured in the presence of 10% PDS contained more α-SM actin compared with controls (Figure 6); α-SM actin expression was not changed by heparin. When SMCs in the presence of 10% PDS were treated with serotonin, we observed an induction of proliferation and a decrease of α-SM actin. Under these conditions, heparin reduced proliferation and increased α-SM actin content (Figure 6).
The proportion of α-SM actin per total actin was studied by two-dimensional gel electrophoresis (Figures 7a and 7b). Cultured SMCs always showed a β-actin predominance. In control SMCs (Figure 7a), α-, β-, and γ-actin isoforms represented 6.5 ± 0.3%, 67.0 ± 1.3%, and 25.0 ± 1.5%, respectively, of the total actin. The proportions of actin isoforms in heparin-treated SMCs (Figure 7b) changed to 15.8 ± 0.4%, 57.6 ± 1.0%, and 25.1 ± 0.8% for α-, β-, and γ-actin, respectively.

A heparin effect on α-SM actin expression was also observed in human SMC culture (data not shown). We used aortic SMCs from autopsy material (harvested ≤5 hours after death) and uterine artery SMCs after hysterectomy for uterine leiomyomas. Cultures were always obtained by enzymatic digestion. Similar results were observed in bovine aortic SMCs.

Western blotting with a desmin antibody showed that in primary cultures with 10% FCS, desmin expression was not changed by heparin.

Induction of SM myosin, evaluated by immunofluorescence, was not observed in passage 5 heparin-treated SMCs compared with controls with 10% FCS.

Actin Isoform mRNA Expression

Hybridization with the total actin probe pRAaA-C of RNA from cultured SMCs (Figures 8a and 8b) results in two bands. The 2.1-kb band corresponds to cytoplasmic actin mRNAs, and the 1.7-kb band corresponds to α-SM actin mRNA. Densitometric scanning of Northern blots of passaged SMCs in the presence of 10% FCS showed that the percentage of α-SM actin mRNA as a proportion of total actin mRNA passed from 40.5 ± 1.2% in control cells to 61.8 ± 3.3% in heparin-treated cells. Moreover, the
FIGURE 3. Photograph showing effect of heparin on a-smooth muscle (SM) actin expression in passaged smooth muscle cells (SMCs) cultured in the presence of 5% and 10% fetal calf serum (FCS). SMCs were grown in 10% FCS (lanes a, b, e, and f) and 5% FCS (lanes c, d, g, and h) with (lanes b, d, f, and h; 200 μg/ml) or without (lanes a, c, e, and g) heparin. Densitometric scanning of Coomassie Blue-stained gels (lanes a-d) shows that actin percentage per total protein is 12.1±0.3% in 10% FCS and 13.8±0.1% in 5% FCS. Heparin does not significantly modify these percentages. Immunoblotting after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (lanes e-h) shows that heparin increases a-SM actin content of SMCs in the presence of 10% and 5% FCS. By densitometric scanning of Western blots, values expressed as a percentage of 10% FCS value are 315 for 10% FCS+heparin (lane f), 265 for 5% FCS (lane g), and 520 for 5% FCS+heparin (lane h). A→, actin.

FIGURE 4. Photograph showing effect of heparin on a-smooth muscle (SM) actin expression in passaged smooth muscle cells (SMCs) cultured in the presence of fetal calf serum (FCS) and whole-blood serum (WBS). SMCs were grown in 10% FCS (lanes a, b, g, and h), 10% WBS (lanes c, d, i, and j), and 20% WBS (lanes e, f, k, and l) with (lanes b, d, f, j, h, i, and l; 200 μg/ml) or without (lanes a, c, e, g, i, and k) heparin. Densitometric scanning of Coomassie Blue-stained gels (lanes a–f) shows that actin percentage per total protein is 12.1±0.3% in 10% FCS, 15.2±0.2% in 10% WBS, and 16.3±0.3% in 20% WBS. Heparin does not significantly modify these values. Immunoblotting after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (lanes g–l) shows that a-SM actin expression is increased in the presence of FCS but is not modified in the presence of WBS. By densitometric scanning of Western blots, values expressed as percentage of 10% FCS value are 315 for 10% FCS+heparin (lane h), 470 for 10% WBS (lane i), 468 for 10% WBS+heparin (lane j), 538 for 20% WBS (lane k), and 534 for 20% WBS+heparin (lane l). A→, actin.

Discussion

During the development of atherosclerotic lesions in humans or after experimental endoventialization, vascular SMCs respond to factors released from platelets, endothelial cells, or macrophages and migrate from the media into the intima. Proliferating SMCs in vivo assume fetal phenotypic features also observed in cultured SMCs. These changes have been defined morphologically, immunologically, and biologically. The expression of actin isoforms has been shown to be useful in monitoring different phenotypic states of SMCs during pathological and experimental situations or during different culture conditions. In normal arteries, a predominance of the α-SM isofrom is seen; in experimental intimal thickening, human atheromatous plaque, and cultured SMCs, a decrease of the α-SM actin isoform and a predominance of the β-actin isoform develop. This switch in actin isoform expression is more important in actively replicating SMCs compared with quiescent SMCs, suggesting that cell replication is somehow related to this phenomenon. However, if cultured SMCs are treated with γ-interferon, both proliferation and α-SM actin expression are reduced.

Our results are consistent with the assumptions that 1) α-SM actin expression is inversely related to...
SMC proliferation and the action of heparin on α-SM actin expression is related to its antiproliferative activity. In the presence of 10% FCS, heparin inhibited SMC proliferation and increased α-SM actin expression. When SMCs were cultured in the presence of 10% PDS, there was no replication, and α-SM actin level was increased compared with SMCs in 10% FCS but was not modified by heparin. Only when SMCs were induced to replicate by serotonin in the presence of PDS did heparin again inhibit proliferation and increase α-SM actin expression.

However, some of our results indicate that there are exceptions to both the above-mentioned assumptions. In certain conditions (i.e., 10% FCS + heparin, 5% FCS, 10% WBS, and 20% WBS + heparin), the same level of proliferation was observed, but the

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**Figure 5.** Bar graphs of heparin effect (■) on cell growth and α-smooth muscle (SM) actin expression in passaged smooth muscle cells cultured in the presence of fetal calf serum (FCS) and whole-blood serum (WBS). Values are expressed as percentage of control condition (■) (10% FCS). SEMs are not represented in figure but were always lower than 5% of values. Heparin affects proliferation in all conditions but increases α-SM actin expression only in the presence of FCS. In the presence of 10% FCS + heparin, 5% FCS, or 10% WBS, the same proliferative activity is observed, but α-SM actin contents are different. Moreover, increasing the concentration of WBS in the culture medium from 10% to 20% results in significant increases of both proliferation and α-SM actin expression.

**Figure 6.** Photograph showing effect of heparin on α-smooth muscle (SM) actin expression in passaged smooth muscle cells (SMCs) cultured in the presence of fetal calf serum (FCS) or plasma-derived serum (PDS) + serotonin. SMCs were grown in 10% FCS (lanes a, b, g, and h), 10% PDS (lanes c, d, i, and j), and 10% PDS + serotonin (lanes e, f, k, and l) with (lanes b, d, f, h, j, and l; 200 μg/ml) or without (lanes a, c, e, g, i, and k) heparin. Densitometric scanning of Coomassie Blue-stained gels (lanes a–f) shows that actin percentage per total protein is 12.1 ± 0.3% in FCS, 16.5 ± 0.2% in PDS, and 13.6 ± 0.1% in PDS + serotonin. Heparin does not significantly modify these percentages. Immunoblotting after sodium dodecyl sulfate–polyacrylamide gel electrophoresis (lanes g–l) shows that heparin increases α-SM actin content of SMCs only in the presence of 10% FCS or 10% PDS + serotonin. Moreover, α-SM actin content of SMCs cultured in the presence of PDS is increased when compared with α-SM actin content of SMCs cultured in the presence of FCS. By densitometric scanning of Western blots, values expressed as percentage of 10% FCS value are 315 for 10% FCS + heparin (lane h), 370 for 10% PDS (lane i), 375 for 10% PDS + heparin (lane j), 189 for 10% PDS + serotonin (lane k), and 234 for 10% PDS + serotonin + heparin (lane l). A →, actin.
growth of 10% FCS- and 20% WBS-treated SMCs is the same at 7 days, while the percentage of α-SM actin is more important in 20% WBS-treated SMCs. Thus, the assumptions that actin isoform expression is inversely related to SMC proliferation and that the action of heparin on α-SM actin expression is related to its antiproliferative activity are valid when SMCs are cultured in the presence of FCS or of PDS with or without serotonin but are not valid when SMCs are cultured in the presence of WBS. The present results are obtained with whole-sera preparations; further studies are in progress to characterize the components of FCS and WBS responsible for this differential action on α-SM actin expression and on heparin effects.

Generally, an increase in the protein content per cell was observed in heparin-treated SMCs. Using metabolic labeling techniques, Castellot et al. have reported that rates of protein synthesis were not changed by exposure of SMCs to heparin. Heparin has been shown to bind to the SMC surface and to be internalized via high-affinity receptor-mediated endocytosis. It is conceivable that heparin acts on protein turnover by decreasing protein degradation. Heparin interacts with proteins, and some of these interactions may be very specific, for example, between heparin and antithrombin; in most cases, binding is probably relatively nonspecific. Nevertheless, as described for growth factors, proteins may be protected from denaturation and degradation by heparin binding.

We have also observed that the proportion of α-SM actin mRNA was increased in heparin-treated SMCs compared with control cells cultured in 10% FCS, independently of the possible action of heparin on protein turnover. This suggests that heparin influences the expression of α-SM actin mRNA at the transcriptional or posttranscriptional level.

Heparin interacts with several components of the extracellular matrix, and similarly to other proteoglycans, controls cellular differentiation. Proteoglycans have been implicated in the control of locomotory and adhesive activities. Hamati et al. have shown that inhibition of proteoglycan synthesis alters proliferation and cytoskeletal organization in rat aortic SMCs in culture. Cytoskeleton and extracellular matrix relations via proteoglycan interactions could implicate heparin and heparan sulfate as factors controlling SMC differentiation. Cytoskeletal proteins are generally accepted to be good SMC differentiation markers. However, it should be stressed that heparin appears to act rather selectively on α-SM actin expression, since it does not affect desmin and SM myosin expression under our conditions.

In conclusion, it appears that heparin, in addition to its antiproliferative activity, exerts a remarkable action on the expression of an SMC differentiation marker, namely, α-SM actin. This action is partially related to its antiproliferative activity but also depends on other presently unknown factors. Further
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References


KEY WORDS • atherosclerosis • vascular development • cytoskeleton • myosin • desmin
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