Heparin inhibits proliferation of myometrial and leiomyomal smooth muscle cells through the induction of α-smooth muscle actin, calponin h1 and p27

Akiko Horiuchi1, Toshio Nikaido1,3, Zhai Ya-Li1, Kazuko Ito1, Ayaka Orii1 and Shingo Fujii2

1Department of Obstetrics and Gynecology, Shinshu University School of Medicine, 3–1–1 Asahi, Matsumoto 390–8621, and 3Department of Gynecology and Obstetrics, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606–8397, Japan

© European Society of Human Reproduction and Embryology

Molecular Human Reproduction vol.5 no.2 pp. 139–145, 1999

Introduction
Mast cells (MC) are widely distributed in human tissues, including the human uterus. However, the function of mast cells in uterine smooth muscle has not been clearly established. Mast cells possess secretory granules containing such substances as heparin, serotonin, histamine and many cytokines. To help establish the role of mast cells in the human myometrium, the action of heparin was investigated using smooth muscle cells (SMC) from normal myometrium and from leiomyoma. The proliferation of cultured myometrial and leiomyomal SMC was inhibited by heparin treatment. Flow cytometric analysis showed that the population of mast cells in the human myometrium, the action of heparin was investigated using smooth muscle cells (SMC) from normal myometrium and from leiomyoma. The proliferation of cultured myometrial and leiomyomal SMC was inhibited by heparin treatment. Flow cytometric analysis showed that the population in the G1 phase of the cell cycle increased under heparin treatment. Western blotting analysis showed that markers of SMC differentiation such as α-smooth muscle actin (α-SMA), calponin h1 and cyclin-dependent kinase inhibitor p27 were induced by heparin, whereas cell-cycle-related gene products from the G1 phase of the cell cycle, such as cyclin E and cdk2, were not changed. Taken together, these results indicate that heparin inhibits the proliferation of myometrial and leiomyomal SMC through the induction of α-SMA, calponin h1 and p27. We suggest that heparin from mast cells may induce differentiation in uterine SMC and may influence tissue remodelling and reconstruction during physiological and pathophysiological events.

Key words: α-smooth muscle actin/heparin/mast cells/myometrium and leiomyoma/p27

Mast cells are widely distributed in human tissues, including the human uterus. However, the function of mast cells in uterine smooth muscle has not been clearly established. Mast cells possess secretory granules containing such substances as heparin, serotonin, histamine and many cytokines. To help establish the role of mast cells in the human myometrium, the action of heparin was investigated using smooth muscle cells (SMC) from normal myometrium and from leiomyoma. The proliferation of cultured myometrial and leiomyomal SMC was inhibited by heparin treatment. Flow cytometric analysis showed that the population in the G1 phase of the cell cycle increased under heparin treatment. Western blotting analysis showed that markers of SMC differentiation such as α-smooth muscle actin (α-SMA), calponin h1 and cyclin-dependent kinase inhibitor p27 were induced by heparin, whereas cell-cycle-related gene products from the G1 phase of the cell cycle, such as cyclin E and cdk2, were not changed. Taken together, these results indicate that heparin inhibits the proliferation of myometrial and leiomyomal SMC through the induction of α-SMA, calponin h1 and p27. We suggest that heparin from mast cells may induce differentiation in uterine SMC and may influence tissue remodelling and reconstruction during physiological and pathophysiological events.

Molecular Human Reproduction vol.5 no.2 pp. 139–145, 1999

Heparin inhibits proliferation of myometrial and leiomyomal smooth muscle cells through the induction of α-smooth muscle actin, calponin h1 and p27

Akiko Horiuchi1, Toshio Nikaido1,3, Zhai Ya-Li1, Kazuko Ito1, Ayaka Orii1 and Shingo Fujii2

1Department of Obstetrics and Gynecology, Shinshu University School of Medicine, 3–1–1 Asahi, Matsumoto 390–8621, and 3Department of Gynecology and Obstetrics, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606–8397, Japan

© European Society of Human Reproduction and Embryology

Molecular Human Reproduction vol.5 no.2 pp. 139–145, 1999

Introduction
Mast cells (MC) are widely distributed in most human tissues and neoplasms, including those of the uterus (Crow et al., 1994). However, in humans, the role played by uterine MC is not clearly understood. Heparin is released from MC (Lydyard and Grossi, 1993) and is a well-known anticoagulant. Moreover, it has also been shown to decrease vascular smooth muscle cell (SMC) mitotic activity both in vivo and in vitro (Clowes and Karnovsky, 1977; Guyton et al., 1980; Clowes et al., 1988), to prevent the phenotypic modulation of SMC and to induce α-smooth muscle actin (α-SMA) (Desmouliere et al., 1992). These functions have also been found in airway and intestinal smooth muscle (Cochran et al., 1987; Johnson et al., 1995). It has been reported that heparin binds directly to the SMC surface and that it inhibits SMC proliferation (Reilly et al., 1986). In addition, in a study using heparin–sepharose chromatography, it has been found that heparin retained full activity in a medium containing serum depleted of all heparin-binding proteins (Orlandi et al., 1994). In contrast, it has been suggested that heparin may inhibit cellular proliferation by binding endogenous growth factors and cytokines, or by displacing growth factors from their binding sites (Reilly et al., 1988; Bono et al., 1997). Its ability to inhibit proliferation seems to be related to the negative charge on the heparin molecule, as the most highly charged fragments are the most potent inhibitors (Wright et al., 1989). Heparin probably acts in the G1 phase of the cell cycle since it must be present before cells enter the S phase if it is to produce its maximum inhibitory effect on SMC proliferation (Reilly et al., 1989). Furthermore, heparin may suppress the expression of proto-oncogenes such as c-fos and c-myc (Pukac et al., 1992) and it interferes directly with the protein kinase C pathway (Herbert et al., 1996). However, the effect of heparin on human myometrial and leiomyomal SMC remains unknown.

In the present study, in an attempt to gain a better understanding of the role of MC in the human uterus, we analysed the responses of both normal uterine myometrial tissue and leiomyoma tumours to heparin treatment. Analysis of the proliferation of cultured cells was performed by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. In addition, cell cycle analysis was carried out using flow cytometry and Western blotting using antibodies against α-SMA, calponin h1 and the G1 cell-cycle-related gene products, cyclin E, cyclin-dependent kinase 2 (cdk2) and the cdk inhibitor, p27.
Materials and methods

Cell culture

Tissue for cell culture was obtained from seven premenopausal women with symptomatic uterine leiomyoma who were undergoing elective hysterectomy. None were receiving any type of hormonal or drug therapy. The tissues were used after obtaining written consent from the patients. Endometrial dating of the experimental uteri was carried out by the method of Noyes et al. (1950). Of the seven patients, four were in the proliferative phase and three in the secretory phase of the menstrual cycle.

Briefly, tissue was collected in Dulbecco’s modified Eagle’s medium (DMEM; Nissui Pharmaceutical Co, Tokyo, Japan) with 10% fetal bovine serum and 1% antibiotic–antimycotic solution (Gibco Laboratories, Grand Island, NY, USA), then transported directly to the laboratory at room temperature. The tissue was rinsed in medium and minced into fine pieces. Samples were aspirated and placed into tubes containing DMEM and 0.4% collagenase (Wako, Osaka, Japan) in which they were kept for 4 h at 37°C with continuous mixing until a suspension was evident. The cell suspension was separated from the undigested fragments, diluted in an equal volume of calcium-, and magnesium-free Dulbecco’s phosphate-buffered saline [PBS(−); Nissui], then centrifuged at 100 g for 10 min. The supernatant was discarded and the cell pellet was suspended in DMEM with 10% fetal bovine serum and 1% antibiotic–antimycotic solution. A cell solution containing 4×10^4 cells/ml was placed in a 75 ml flask (Becton Dickinson Laboratories, Lincoln Park, NJ, USA). Incubation was carried out at 37°C in 5% CO₂ in air. Cultures were determined to be pure SMC cultures (>98%) by immunostaining for α-SMA (Dako, Glostrup, Denmark), which are markers for SMC (Nowak et al., 1993). Cells used in the experiments were from passages 1 or 2.

Analysis of cell proliferation

Cell proliferation in cultures of myometrium or leiomyoma was assessed by the MTT colorimetric method, which is a convenient way to detect enzymes associated with DNA synthesis in dividing cultures (Mosmann, 1983). Secondary cultured cells from myometrial or leiomyomal tissue were plated at a density of 1×10^5 cells in a 75 ml flask in DMEM with 10% fetal bovine serum and 1% antibiotic–antimycotic solution. The following day, heparin was added at a concentration of 100 IU/ml. After 3 days incubation, the cultured cells were analysed by Western blotting for α-SMA, calponin h1 and G1 cell-cycle-related gene products. Cultured cells from normal myometrium or leiomyoma were lysed in a lysis buffer, consisting of 50 mM Tris–HCl, pH 8.0, 0.25 M NaCl, 0.5% NP-40, 1 mM phenylmethylsulphonyl fluoride (PMSF; Sigma), 1 µg/ml aprotinin (Boehringer Mannheim, Mannheim, Germany), 1 µg/ml leupeptin (Boehringer Mannheim), and 20 µg/ml N-tosyl-l-phenylalanyl chloromethyl ketone (TPCK; Boehringer Mannheim). The lysates were centrifuged at 13 000 g for 20 min at 4°C and the supernatants were stored at −80°C. Extracts equivalent to 30 µg of total protein were separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (10% acrylamide) and transferred onto nitrocellulose membranes (Hybond-TM-C super; Amersham, High Wycombe, UK). The membranes were blocked in TBST (0.2 mM NaCl, 10 mM Tris, pH 7.4, 0.2% Tween-20) containing 5% non-fat dry milk and 0.02% NaN₃ for 1 h. This was followed by incubation first with specific antibodies against α-SMA (Dako), calponin h1 (Sigma) and the cell-cycle-related gene products, cyclin E, cdk2 and p27 (Amersham) in TBST containing 2% milk. Membranes were then incubated with sheep anti-mouse Ig (Amersham) in TBST containing 2% non-fat dry milk. Bound antibody was detected with the aid of an enhanced chemiluminescence (ECL) system (Amersham). The relative expression of α-SMA, calponin h1 and cell-cycle-related gene products on the immunoblotting filters was analysed by densitometric analysis using a Quantity One Scan System (ATTO Co, Tokyo, Japan). The intensity generated under heparin treatment is expressed as a percentage of the corresponding untreated control value.

Statistical analysis

The data are presented as the mean ± SE. The significance of differences was assessed by the Kruskal–Wallis test or by the Mann–Whitney U-test. Differences were considered to be significant when P < 0.05.

Results

Analysis of cell proliferation

After 3 days of heparin treatment at a concentration of 10, 50 or 100 IU/ml, the MTT assay showed that the number of day, heparin was added at a concentration of 100 IU/ml. The cells were incubated in culture flasks in DMEM with 10% fetal bovine serum with or without heparin to allow an analysis of the cell cycle.

After 3 days incubation, each group of cells was collected and washed with PBS(−) three times. The cells were fixed in 70% ethanol and stored at −20°C. To reduce the effects of contact inhibition, control cells were allowed to reach 70% confluency at the time of Fluorescence Activated Cell Sorter (FACS) analysis. Then, the cells were resuspended in a DNA stain solution containing propidium iodide (20 µg/ml; Calbiochem, CA, USA) and RNase (1.8 IU/ml; Sigma, St Louis, MO, USA). The cells were analysed with a FACSScan flow cytometer equipped with an argon laser (488 nm; Becton Dickinson Immunocytometry System, Mountain View, CA, USA). The experiments were repeated three times for each of six cultures. When assessed by the Mann–Whitney U-test, the differences were considered to be significant when P < 0.05.
Inhibition of muscle cell growth by heparin

Figure 1. Effect of heparin on proliferation rate of cells cultured from myometrial and leiomyomal smooth muscle. Absorbance is plotted as a function of heparin concentration. Values are shown as mean ± SE. Significantly different from control values: * P < 0.05 and ** P < 0.01.

cultured cells per 96-well plate was reduced by heparin in a dose-dependent manner (Figure 1). The cell numbers indicated significant inhibition of cell proliferation in myometrial cells at the two highest concentrations: to 70% of control with 50 IU/ml heparin and to 57% of control with 100 IU/ml heparin (P < 0.05). The cell numbers also indicated significant inhibition in leiomyomal cells at the highest concentration: to 70% of control with 100 IU/ml heparin (P < 0.05). There was no significant difference between myometrial cells and leiomyomal cells in terms of the inhibition of cell proliferation. In addition, no significant differences in response to heparin were noted between cells obtained at different phases of the menstrual cycle. Growth curves showed that a significant inhibition of myometrial cell proliferation was first obtained on day 2 (P < 0.01; Figure 2A), whereas a significant inhibition of leiomyomal cell proliferation was not obtained until day 3 (P < 0.05; Figure 2B). The proliferative activity of myometrial SMC tended to be higher than that of leiomyomal SMC under control conditions. However, the growth curves showed no significant difference between myometrial cells and leiomyomal cells with respect to the inhibition of cell proliferation.

Cell cycle analysis

Representative results of the cell cycle analysis performed on cells from myometrial and leiomyomal smooth muscle are shown in Figure 3. A significant increase in the fraction of cells in the G1 phase of the cell cycle (81.7 ± 1.3%; P < 0.05) together with significant decreases in the S and G2/M phases (5.2 ± 3.5 and 12.9 ± 3.9%; P < 0.05) occurred after heparin treatment (by comparison with the corresponding values for cultured myometrial SMC without heparin, which were: G1, 64.8 ± 3.9%; S, 14.1 ± 1.3%; G2/M, 20.4 ± 2.4%). Similar results were obtained for leiomyomal SMC. The values obtained were: in the G1 phase, 81.0 ± 0.9% versus 68.1 ± 2.9% (P < 0.05); in the S phase, 7.5 ± 2.3% versus 13.1 ± 3.1% (P < 0.05); and in the G2/M phase, 11.5 ± 2.2% versus 18.7 ± 2.0% (P < 0.05). The results suggest that heparin produces a block of the G1–S transition or G1 arrest in both myometrial and leiomyomal SMC.

Western blotting analysis

Protein extracts from cells treated for 3 days with 100 IU/ml of heparin were analysed by Western blotting to detect the expression of α-SMA and calponin h1, which are markers of the differentiation of SMC. The results are shown in Figure 4A. The 42 kDa band of α-SMA and the 34 kDa band of calponin h1 were both increased by treatment with heparin in myometrial SMC and in leiomyomal SMC. For myometrial SMC, the ratio between the values obtained for the expression of α-SMA with or without heparin treatment was 1.59 (P < 0.01). For leiomyomal SMC, the values obtained for this ratio were 1.48 (P < 0.01) (Figure 4B). The corresponding ratios for the expression of calponin h1 were: 1.17 in myometrial SMC (P < 0.05), 1.17 in leiomyomal SMC (P < 0.05) (Figure 4B).

To confirm that heparin induced a block of the G1–S transition or G1 arrest in these SMC, we analysed the expression of the G1-phase-related gene products cyclin E, cdk2 and p27 (Figure 5A). Neither the 50 kDa band of cyclin E nor the 33 kDa band of cdk2 was changed by heparin treatment in myometrial or leiomyomal SMC (Figure 5B). In contrast, the 27 kDa band of p27, which is a cdk inhibitor, was increased by treatment with heparin in both myometrial and leiomyomal SMC (Figure 5A). The ratio between the values obtained for the expression of p27 in myometrial SMC with or without heparin treatment was 1.67 (P < 0.05). The values obtained for this ratio in leiomyomal SMC was 1.71 (P < 0.05) (Figure 5B). In contrast, suppression of proliferating cell nuclear
Figure 2. Effect of heparin treatment at 100 IU/ml on the proliferation rate of cells cultured from myometrial and leiomyomal smooth muscle. (A) Effect of heparin treatment at 100 IU/ml on proliferation rate of myometrial smooth muscle cells (SMC). (B) Effect of heparin treatment at 100 IU/ml on proliferation rate of leiomyomal SMC. Values are shown as mean ± SE. Significantly different from control values: *P < 0.05 and **P < 0.01.

Figure 3. Analysis of cell cycle by flow cytometry. Cell cycle analysis was conducted for myometrial and leiomyomal smooth muscle cells (SMC) treated or not treated with heparin at 100 IU/ml. Figure shows representative data.

antigen (PCNA), which is a cofactor of DNA polymerase δ, was observed after heparin treatment, the ratios being 0.59 for myometrial SMC (P < 0.05) and 0.51 for leiomyomal SMC (P < 0.05) (Figure 5B).

Discussion

We have demonstrated that heparin inhibits the proliferation of SMC from the human myometrium and from leiomyoma, both effects occurring in dose-dependent manner. With respect to this inhibitory action, there was no significant difference between myometrial and leiomyomal SMC. However, leiomyomal SMC inhibition began later than that in the normal myometrium. We postulate this lag occurred because the proliferative rate of myometrial SMC tended to be higher than that of leiomyomal SMC under control conditions, or because leiomyomal SMC were in some way more resistant to heparin treatment than myometrial SMC. The results also revealed a marked increase in the fraction of cells in the G1 phase of the cell cycle after heparin treatment. This suggests that the anti-proliferative effect of heparin on SMC from human myometrium and leiomyoma is related either to a block of the G1–S transition or to a G1 arrest.

The transition from G1 to S is controlled by the concerted actions of protein kinases, the activities of which are modulated by families of regulatory proteins in both a positive (cyclins) and a negative [cyclin-dependent kinase inhibitors (CDI)] manner. A family of CDI plays a major role in the cell cycle
Inhibition of muscle cell growth by heparin

Figure 4. Analysis of the expression of α-smooth muscle actin (SMA) and calponin h1. (A) Expression of α-SMA and calponin h1. The 42 kDa band of α-SMA and the 34 kDa band of calponin h1 were increased by heparin treatment in both myometrial and leiomyomal smooth muscle cells (SMC). (B) The relative expression of α-SMA and calponin h1 as estimated by densitometric analysis of the immunoblotting filters. The intensity generated under heparin treatment is expressed as a percentage of the untreated control value. Values are shown as mean ± SE. Significantly different from control values: *P < 0.05 and **P < 0.01.

Figure 5. Analysis of the expression of the G1 phase-related gene products cyclin E, cdk2, proliferating cell nuclear antigen (PCNA) and p27. (A) The 50 kDa band of cyclin E and the 33 kDa band of cdk2 were not changed by heparin treatment in either myometrial or leiomyomal smooth muscle cells (SMC). In contrast, the 27 kDa band of cdk inhibitor p27 was increased by heparin treatment in both myometrial and leiomyomal SMC. (B) The relative expressions of cyclin E, PCNA, cdk2 and p27 were estimated by densitometric analysis of the immunoblotting filters. The intensity generated under heparin treatment is expressed as a percentage of the untreated control values. Values are shown as mean ± SE. Significantly different from control values: *P < 0.05.

machinery (Sherr and Roberts, 1995). In this study, our analysis of Western blotting showed: (i) a suppression of PCNA after heparin treatment that was consistent with the results obtained using the MTT method; and (ii) that heparin increased the expressions of α-SMA, calponin h1 and p27. In contrast, no marked change in the expressions of cyclin E and cdk2 was observed in cells treated with heparin after 3 days incubation. It is possible that the other G1 cell-cycle-related molecules or an increased activity of cyclin-associated kinase may have participated in the block of the G1–S transition or G1 arrest induced by heparin treatment. Since α-SMA and calponin h1 are expressed in differentiated SMC, our data suggest that the anti-proliferative effect of heparin is closely associated with an up-regulation of p27 and an induced differentiation of SMC.
With respect to the effect of heparin on myometrial and leiomyomal SMC, our results suggest that: (i) heparin inhibits SMC proliferation; (ii) heparin increases the expression of α-SMA; and (iii) heparin most likely acts in the G1 phase of the cell cycle. This is consistent with results previously obtained from vascular SMC. Although there are many reports showing heparin inhibition of vascular SMC proliferation (see Introduction), this is the first report showing heparin inhibition of myometrial and leiomyomal SMC. Our results show that the effect of heparin is associated with an up-regulation of p27 and an induced differentiation of SMC. On these grounds, we postulate that heparin may be of therapeutic or preventative value in cases of leiomyoma.

It is becoming increasingly clear that in many instances the cellular phenotypic features of vascular SMC are modulated locally by cytokines and growth factors and by extracellular matrix (ECM) components, rather than being controlled by genetically irreversible steps, as was believed for a long time (Ross, 1993). However, the control of SMC proliferation and cellular phenotypic features in the uterus have not been clearly explained. It has been reported that a variety of hormones, growth factors and mitogens can affect the proliferation of uterine SMC (Fayed et al., 1989; Koutsilieris et al., 1992; van der Ven et al., 1994). Recently, heparin-binding growth factors, which include platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), have been isolated from human leiomyomas and normal myometrium, and it has suggested that the enhanced growth of leiomyomas may be due, in part, to the presence of the large quantities of bFGF that are stored in the ECM of leiomyomas (Mangrulkar et al., 1995). Since heparin may inhibit cellular proliferation by binding endogenous growth factors or by displacing growth factors from their binding sites (Reilly et al., 1988; Bono et al., 1997), heparin may operate by binding heparin-binding bFGF and so inhibit the proliferation of normal myometrial and leiomyomal SMC.

In addition, many of the heparin-binding group of growth factors including bFGF, vascular endothelial growth factor (VEGF), heparin-binding epidermal growth factor (HBEGF), PDGF and transforming growth factor-β (TGF-β) regulate the process of angiogenesis or have other effects on vascular structures. These growth factors or their receptors are differentially regulated in leiomyomas and the endometrium of patients with leiomyoma-related complications (Stewart and Nowak, 1996). This being so, heparin may potentially be of use to regulate angiogenesis in women with leiomyoma or abnormal bleeding.

We have reported that there are many MC among the myometrial SMC (Mori et al., 1997a) and that the SMC of the myometrium produce stem cell factor (SCF) (Mori et al., 1997b), which is a major regulator of human MC (Valent et al., 1992; Sperr et al., 1993) through the receptor for SCF (c-kit) (Zsebo et al., 1990). Our results and those obtained by others, suggest that there is an interaction between uterine SMC and MC. In addition, it has been shown that the number of MC in the rodent uterus decreases around the time of implantation (Hore and Mehrrota, 1988) and that a factor secreted by the human embryo stimulates cytokine release of endometrial MC in the uterus (Cocchiara et al., 1996). These findings suggest that there is a physiologically relevant interaction between MC and the adjacent endometrium. However, we have reported that the endometrium contains a significantly smaller number of MC than the myometrium (Mori et al., 1997a). Comparison of the interaction between the granulated lymphocytes that exist in the functional layer of the endometrium and the endometrial stromal cells during the menstrual cycle (Bhartiya et al., 1996), indicates that the interaction between MC and the endometrial stromal cells in the functional layer may be negligible. However, while the proliferative activity of myometrium and leiomyoma increases in the secretory phase of the menstrual cycle and during pregnancy (Kawaguchi et al., 1989; 1991), the number of MC does not change during the menstrual cycle (Mori et al., 1997a;b, Orii et al., 1998). Thus, the physiological interactions between MC and the adjacent endometrium, and between MCs and SMC proliferation cannot be explained simply as a heparin effect.

In a recent study, the number of MC was found to be lower in usual leiomyomas than in the normal myometrium (Orii et al., 1998). Since differences in cellularity and in the collagen matrix were found to correlate with the number of MC in usual leiomyomas (Orii et al., 1998), changes in the levels of MC products such as heparin may possibly be involved in the maintenance of a differentiated state as well as in phenotypic modulations of pathological phenomena such as inflammation and tumour formation (Singh et al., 1992). These results suggest that an interaction between SMC and MC may influence tissue remodelling and reconstruction during both physiological and pathophysiological events. However, the functional roles of uterine MC and the details of their relationship with the myometrium and uterine smooth muscle tumours have not been fully characterized. Clearly, further research will be needed to establish the functional importance of the effects of heparin on leiomyoma.

In conclusion, we have demonstrated an inhibitory effect of heparin on the proliferation of myometrial and leiomyomal SMC. A marked increase in the fraction of cells in the G1 phase of the cell cycle was observed in both tissues after heparin treatment. This study also showed that heparin induced cytoskeletal muscle proteins, such as α-SMA, calponin h1 and cell cycle inhibitor p27, suggesting that heparin inhibits the proliferation of uterine SMC and may induce differentiation of SMC, and that the interaction between SMC and MC products such as heparin may influence tissue remodelling and reconstruction during pathophysiological events.

Acknowledgements
This work was supported in part by a Grant-in-aid for Scientific Research from the Ministry of Education, Science, Sports and Culture (No.08457438, 09877318, 09671671), Japan.

References

References
Inhibition of muscle cell growth by heparin


Received on July, 20; accepted on October 16, 1998