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Anti-proliferative effects of *O*-acyl-low-molecular-weight heparin derivatives on bovine pulmonary artery smooth muscle cells

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Abstract Heparin (HP) inhibits the growth of several cell types *in vitro* including bovine pulmonary artery (BPA) smooth muscle cells (SMCs). In initial studies we discovered that an *O*-hexanoylated low-molecular-weight (LMW) HP derivative having acyl groups with 6-carbon chain length was more potent inhibitor of BPA-SMCs than the starting HP. We prepared several *O*-acylated LMWHP derivatives having 4-, 6-, 8-, 10-, 12-, and 18- carbon acyl chain lengths to determine the optimal acyl chain length for maximum anti-proliferative properties of BPA-SMCs. The starting LMWHP was prepared from unfractionated HP by sodium periodate treatment followed by sodium borohydride reduction. The tri-*n*-butylammonium salt of this LMWHP was *O*-acylated with butanoic, hexanoic, octanoic, decanoic, dodecanoic, and stearyl anhydrides separately to give respective *O*-acylated LMWHP derivatives. Gradient polyacrylamide gel electrophoresis (PAGE) was used to examine the average molecular weights of those *O*-acylated LMWHP derivatives. NMR analysis indicated the presence of one *O*-acyl group per disaccharide residue. Measurement of the inhibition of BPA-SMCS as a function of *O*-acyl chain length shows two optima, at a carbon chain length of 6 (*O*-hexanoylated LMWHP) and at a carbon chain length 12-18 (*O*-dodecanoyl and *O*-stearyl LMWHPs). A solution competition SPR study was performed to test the ability of different *O*-acylated LMWHP derivatives to inhibit fibroblast growth factor (FGF) 1 and FGF2 binding to surface-immobilized heparin. All the LMWHP derivatives bound to FGF1 and FGF2 but each exhibited slightly different binding affinity.

Keywords Heparin · Low molecular weight heparin · *O*-acylated · Smooth muscle cells · Surface plasmon resonance

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Introduction

In response to a vascular injury, smooth muscle cells (SMCs) change phenotype, multiply, and migrate as part of the remodeling process [1, 2]. Excessive repair, sometimes leads to an excessive remodeling of the vessel and creates even more complications due to the dysregulation of the repair program, this contributes to the pathogenesis of several vascular disorders, such as restenosis, and hypertension [3]. Proliferation and regression of a specialized cell to an unspecialized form of SMCs seems to play the major role in the formation of lesions found in pulmonary arteries of patients with pulmonary artery hypertension (PAH) [4]. From many compounds, HP has been found to be an effective anti-proliferative agent for growth inhibition of SMCs as shown by us and others [5, 6]. Heparin has been found effective in various vascular SMC tissue cultures *in vitro* [7, 8], and in the rat and rabbit injury models *in vivo* [9, 10].

Heparin (HP) (Fig. 1) is a linear sulfated natural polysaccharide having multiple properties namely anti-coagulation, anti-proliferation and antiinflammation [11]. It consists of 1→4 linked uronic acid and α-D-glucosamine,

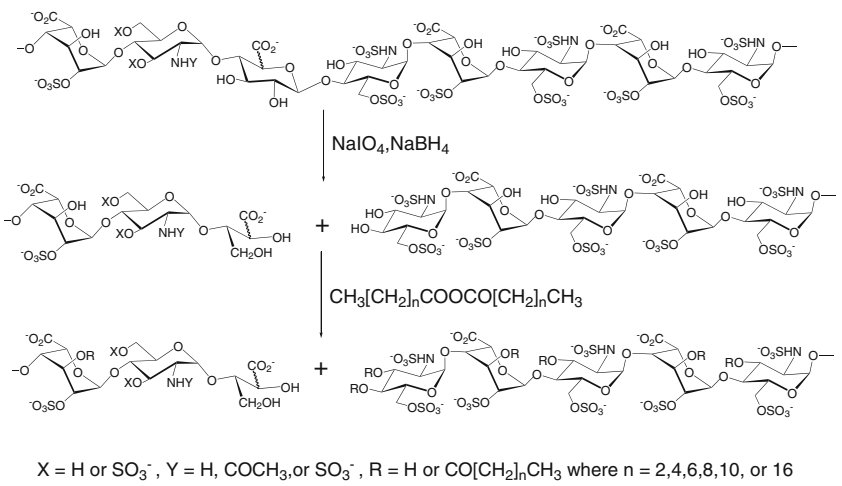

Fig. 1 Synthesis of *O*-acylated LMWHP. The HP chain is cleaved using $NaIO_4$ and $NaBH_4$ at the vicinal diols in the variable disaccharide residues. The resulting LMWHP is acylated with various acyl anhydrides to obtain the *O*-acyl LMWHP derivatives

(GlcN) repeating disaccharide units [1]. The uronic acid usually represents the majority of α-L-iduronic acid and small amount of β-D-glucuronic acid [12] while GlcN can be either *N*-sulfated or *N*-acetylated. HP is a polyelectrolyte having a high negative charge density [13, 14]. In previous studies, we showed that different commercial HP preparations differ in their anti-proliferative properties [15]. HP core protein has no anti-proliferative activity, its molecular size does affect the anti-proliferative properties and its anti-proliferative properties reside in the GAG chain [16]. We further found that in chemically modified HPs, (a) fully *O*-sulfonated HP did not enhance the growth inhibition properties over that of native HP [17], (b) both *N*-sulfo and *N*-acetyl are essential for the growth inhibition properties [18], (c) loss of 6-*O*-sulfo groups in GlcN residues of HP decreases it's growth inhibition properties [19], (d) 2-*O*-sulfo group of L-iduronic acid residues in HP are significantly important for the growth inhibition properties [5], and (f) hexanoylation of HP at 3-*O*-position increased the growth inhibition properties of that parent HP [20].

Matsuda *et al*. [21] found that an alkylated HP obtained by treatment of the HP sodium salt with an oxidizing agent to generate a lactone ring at its terminal end followed by reacting with the alkylamine group, having 4–18 carbons, gave alkylated HP derivatives. These HP preparations were found to reduce the growth rate of SMCs by increase in the alkyl group chain length [22]. Earlier, Barzu *et al*. [23] and Pukac *et al*. [24] also reported that growth inhibition properties of SMCs by *O*-acylated HP derivatives changed with the change in number of carbon atoms present in their acyl chain.

The purpose of this investigation was to assess the optimal carbon chain length of acyl group in LMWHP affording the most potent *O*-acyl LMWHP derivative that could be used clinically for treatment of pulmonary hypertension where pulmonary artery SMCs growth increases.

Experimental

Materials

Unfractionated HP, from porcine intestinal mucosa, was obtained from Celsius Laboratories (Cincinnati, OH) and fetal bovine serum was obtained from BioWhittaker (Walkersville, MD), respectively. Cell culture medium, RPMI-1640 (Mediatech, Washington, DC), contained streptomycin (Lilly, Indianapolis, IN), penicillin (Pfizer, New York, NY), and amphotericin B (GIBCO, Grand Island, NY), which were purchased commercially. Trypsin EDTA and other chemicals used to modify unfractionated HP were purchased from Sigma Aldrich (St. Louis, MO). Fibroblast growth factor 1 (FGF1) and fibroblast growth factor 2 (FGF2) were gifts from Amgen (Thousands Oaks, CA). All chemicals and equipments used in gel electrophoresis were from BioRad Laboratories (Hercules, CA).

Methods

Periodate-oxidation of HP to prepare LMWHP

HP was fragmented by periodate oxidation based on a modification of an earlier procedure (Fig. 1) [25]. Briefly, HP sodium salt (20 g, 1.43 mmol) was dissolved in 175 mL of distilled water. The pH was adjusted to 5.0 using 1 M HCl. $NaIO_4$ (15 g, 0.070 mol), dissolved in 500 mL of water, was added in a single portion with stirring. The pH was readjusted to 5.0 using 1 M HCl and left for 24 h at 4°C in the dark. The solution was dialyzed against 4 vol of water (with one change of water) for 15 h at 4°C. To the approximately 1.5 L of solution obtained after dialysis, 32 mL of 10 M NaOH was added. The solution was stirred at room temperature for 3 h. To prevent the development of colored products, this step was done in the dark. $NaBH_4$ (1 g, 0.026 mol) was added in one portion, and approximately

1.5 L of the solution was stirred for 4 h. The pH was then adjusted to 4.0 using 37% HCl, and the solution was stirred for an additional 15 min. The solution was neutralized to pH 7.0 using 1 M NaOH. NaCl (32.8 g, 0.56 mol) was added, followed by 2.54 L of ethanol. The solution was left for 3 h without stirring, and the precipitate was recovered by centrifugation at 7000 × g for 40 min. The precipitate was recovered by decantation and suspended in 400 mL ethanol. The solution was filtered using a Buchner funnel and the recovered solids were left to dry for 5 h under vacuum yielding 13.6 g of the product. The product was dissolved in 190 mL of water. NaCl (2.8 g, 0.05 mol) was added, and the pH was adjusted to 3.5 using 1 M HCl. The volume was adjusted to 280 mL using water. Absolute ethanol (240 mL) was added with stirring. The solution was stirred for 15 min and then left without stirring for 10 h at room temperature. After decanting, the precipitate was recovered and dissolved in water. The ethanol was removed by rotary evaporation under reduced pressure and the aqueous solution was freeze-dried affording 9.8 g of LMWHP.

Preparation of the tri-*n*-butylammonium salt of LMWHP

The sodium salt of LMWHP was converted to the tri-*n*-butylammonium salt using a modification of previously described conditions [9, 22]. Briefly, LMWHP (10 g) was dissolved in water (50 mL) and passed through a pre-equilibrated column (300 mL) of Dowex 50WX8 (H^+) cation-exchange resin. The pH of the eluent (500 mL) was adjusted to pH 6.0 using tri-*n*-butylamine (25 mL, 0.1 mol). Excess tri-*n*-butylamine was eliminated by concentrating it to 100 mL by rotary evaporation under reduced pressure. The concentrate was diluted with 10 volumes of water and lyophilized to afford the tri-*n*-butylammonium salt (13 g).

Preparation of *O*-acyl derivatives of LMWHP

The tri-*n*-butylammonium salt of LMWHP was separately acylated with butanoic, hexanoic, octanoic, decanoic and stearyl anhydride, using a modification of methods previously described (Fig. 1) [1, 9]. Briefly, the dry tri-*n*-butylammonium salt (12 g), dissolved in dry DMF (110 mL) was cooled to 0°C under an argon atmosphere. 4-Dimethylaminopyridine (0.7 g, 5.7 mmol), either butanoic, hexanoic, octanoic, decanoic, dodecanoic or stearyl anhydride (0.1 mol), and tri-*n*-butylamine (27 mL, 0.1 mol) were successively added in single portions, and the reaction was allowed to proceed under air at room temperature for 24 h. After cooling to 0°C, 5% $NaHCO_3$ in water (230 mL) was gradually added, and the solution was stirred at room temperature for 48 h. Excess $NaHCO_3$ was eliminated by slow, drop wise addition of 1 M HCl (200 mL) until a pH of 4.0 was reached, and then the pH was readjusted to 7.0 with 1 M NaOH (150 mL). Cold denatured (95%) ethanol (5 L, 5 vol) was added with stirring. The sample was allowed to sit overnight at 4°C to create a precipitate. For butanoic, hexanoic, octanoic, decanoic, dodecanoic *O*-acyl LMWHP, the precipitate was recovered by decanting and was dissolved in 0.2 M NaCl (110 mL). The precipitation procedure was repeated by adding absolute ethanol (570 mL). For stearyl *O*-acyl-LMWHP, the precipitate was recovered by decanting, dissolved in water and then adjusted to pH 3.5 by 1 M HCl. Equal volume of diethyl ether was added to extract excess of byproduct, stearic acid. The aqueous portion was collected and the extraction was performed totally three times and NaCl was added to the three combined aqueous portions to make the final concentration of NaCl 0.2 M. Absolute ethanol (four volumes) was added to one volume of the salt solution and the product was allowed to precipitate overnight at 4ºC. The precipitate was recovered by centrifugation 7,000 × g for 40 min, dissolved in water (110 mL), and passed through a column (300 mL) of Dowex 50WX8 (H^+) cation-exchange resin and 600 mL was recovered. The acid was neutralized to pH 7.0 with 10 M NaOH, and the solution was filtered through a 0.22-μm Millipore filter. After lyophilization, *O*-acylated LMWHP derivatives were obtained as an off-white powder.

Gradient polyacrylamide gel electrophoresis

The *O*-acylated LMWHP derivatives were analyzed by PAGE. An equal amount of each sample (5 μL at 2 mg/mL) was combined with 5 μL of 50% (w/v) sucrose in water, and the mixture was loaded into a stacking gel of 5% (total acrylamide) and fractionated with a 22% resolving gel. Electrophoresis was performed at 200 V for 80 min. The gel was stained and fixed with Alcian Blue in 2% (v/v) acetic acid and destained with water. The average molecular weights of *O*-acylated LMWHP derivatives were estimated using a banding ladder of heparin oligosaccharide standards prepared from bovine lung heparin [26].

^{1}H NMR spectroscopy analysis

The *O*-acylated LMWHP derivatives (10 mg) were each dissolved in 0.5 ml deuterium oxide (99.996 atom% purity) and freeze-dried three-times to remove the exchangeable protons. The samples were re-dissolved in 0.5 mL D_2O. Spectra were recorded at 300 K on Varian Unity 500 (500 MHz) FT-NMR spectrometer. The degree of substitution (*O*-acylation) was determined from the ratio of the integrated area of the peaks assigned to the aliphatic methyl protons of the hexanoyl group (0.91 ppm) to the anomeric proton of IdoA2S (5.08 ppm).

Bovine pulmonary artery smooth muscle cells culture assay

BPA-SMC proliferation assays were preformed as previously described [15, 20]. Briefly, isolated BPA-SMCs in passages 4–6 were seeded at 1.5×10^4 cells/well into 6-well tissue culture plates, grown for 2 days, then growth arrested for 48 h by reducing the serum concentration of the medium from 10% to 0.1%. The medium was then changed for experimental samples to contain either standard medium [RPMI-1640 with 10% fetal bovine serum (FBS)], growth arrest media (0.1% FBS) or standard media containing HPs and their chemically modified derivatives. All media contained streptomycin (10 μg/mL), penicillin (100 U/mL), and amphotericin B (1.25 μg/mL). After 4 days, BPA-SMCs present in the cell culture wells were rinsed with Hank's balanced salt solution to remove the remaining cell culture medium. No dead cells (Trypan Blue exclusion) were observed in either the control preparations or in the LMWHP derivative-treated culture media. After detachment of BPA-SMCs with trypsin/EDTA, the cell numbers were determined by direct cell count. The LMWHP derivative was dissolved (1 mg/mL) in distilled sterile water, and 14 μL, 140 μL, or 1.4 mL of this solution was added to the culture medium (13.99, 13.86, and 12.6 mL, respectively). Then 2 mL of the resulting media was added to each well. The *O*-acyl LMWHP derivatives and parent LMWHP samples were completely soluble, as the culture medium was clear (no turbidity) after addition of the LMWHP derivatives. In the present study 1 μg/mL, 10 μg/mL, and 100 μg/mL of LMWHP derivatives were used. The percent growth inhibition was calculated as follows:

Percent Inhibition

$$= \left(1 - \frac{\textit{net growth in the presence of heparin}}{\textit{net growth in control dishes}}\right) \times 100$$

Surface plasma resonance (SPR) analysis

SPR was performed on a BIAcore 3000 instrument (GE Healthcare, Uppsala, Sweden). Buffers were filtered (0.22 μM) and degassed. The biotinylated heparin was prepared by reaction of sulfo-*N*-hydroxysuccinimide long-chain biotin (Piece, Rockford, IL) with free amino groups of unsubstituted glucosamine residues in the polysaccharide chain following a published procedure [27]. Biotinylated heparin was immobilized to streptavidin (SA) chip based on the manufacturer's protocol. In brief, 20 μL solution of the heparin-biotin conjugate (0.1 mg/mL) in HBS-EP buffer (10 mM 4-(2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES), 150 mM sodium chloride, 3 mM ethylenediaminetetraacetic acid (EDTA), 0.005% polysorbate surfactant P20 pH 7.4) was injected over flow cell 2 (FC2) of the SA chip at a flow rate of 10 μL/min. The successful immobilization of heparin was confirmed by the observation of a 100 resonance unit (RU) increase in the sensor chip. The control flow cell (FC1) was prepared by 1 min injection with saturated biotin.

Solution competition SPR study was performed to test the fibroblast growth factor (FGF) 1 and FGF2 binding competition between surface heparin and different *O*-acylated LMWHP derivatives. FGF1 or FGF2 (250 nM) mixed with different of concentrations of *O*-acylated LMWHP derivatives in HBS-EP buffer were injected over heparin chip at a flow rate of 30 μL/min, respectively. At the end of the sample injection, the same buffer was flowed over the sensor surface to facilitate dissociation. After a 2 min dissociation time, the sensor surface was fully regenerated by injecting 30 μL of 2 M NaCl. The response was monitored as a function of time (sensorgram) at 25°C. For each set of competition experiments on SPR, a control experiment (only protein without LMWHP) was performed to make sure the surface was completely regenerated and that the results obtained between runs were comparable.

Statistics

Results are reported as mean ± standard error of the mean. Comparisons among groups were made with a factorial analysis of variance (ANOVA), using the STATVIEW software package (SAS Institute, Cary, NC 27513) for Macintosh computers. If ANOVA were significant, multiple comparisons were made using the Fisher protected least significant difference (PSLD) test. Significance was set as $p<0.01$.

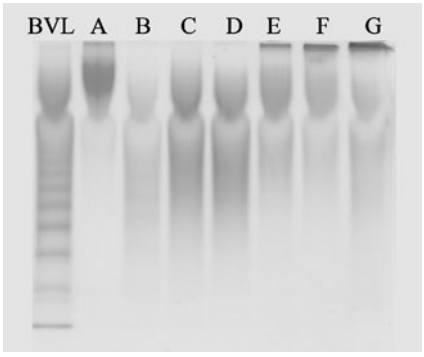

Fig. 2 Polyacrylamide gel electrophoresis (PAGE) analysis of *O*-acylated LMWHP derivatives. Lane BVL: bovine heparin ladder; lane A: unfractionated original heparin with MW_{avg} of 11.8 KD; lane B: butanoylated LMWHP with MW_{avg} of 8.9 KD; lane C: hexanoylated LMWHP with MW_{avg} of 9.0 KD; lane D: octanoylated LMWHP with MW_{avg} of 8.8 KD; lane E: decanoylated LMWHP with MW_{avg} of 8.9 KD; lane F: dodecanoylated LMWHP with MW_{avg} of 9.6 KD; lane G: stearyl LMWHP with MW_{avg} of 9.3 KD

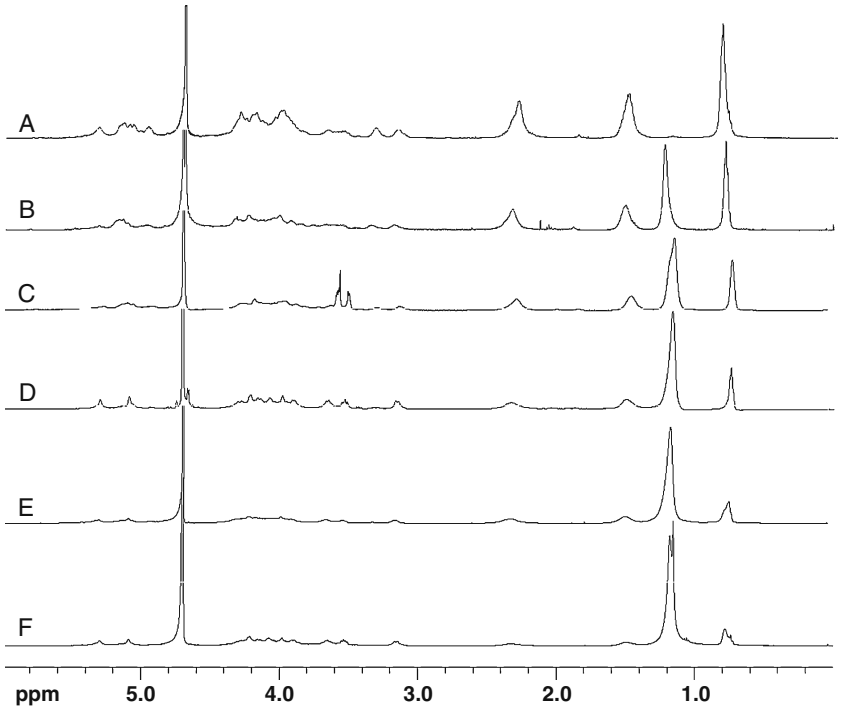

Fig. 3 ^{1}H-NMR of LMWHP derivatives. A: butanoylated LMWHP; B: hexanoylated LMWHP; C: octanoylated LMWHP; D: decanoylated LMWHP; E: dodecanoylated LMWHP; F: stearyl LMWHP

Results and discussion

Unfractionated HP was partially depolymerized by periodate cleavage of the vicinal diol groups of unsulfated glucuronic and iduronic acid residues in the variable domains of the heparin polysaccharides (Figs. 1). The original unfractionated HP had an average molecular weight of 11.8 KD and the resulting LMWHP had an average molecular weight of

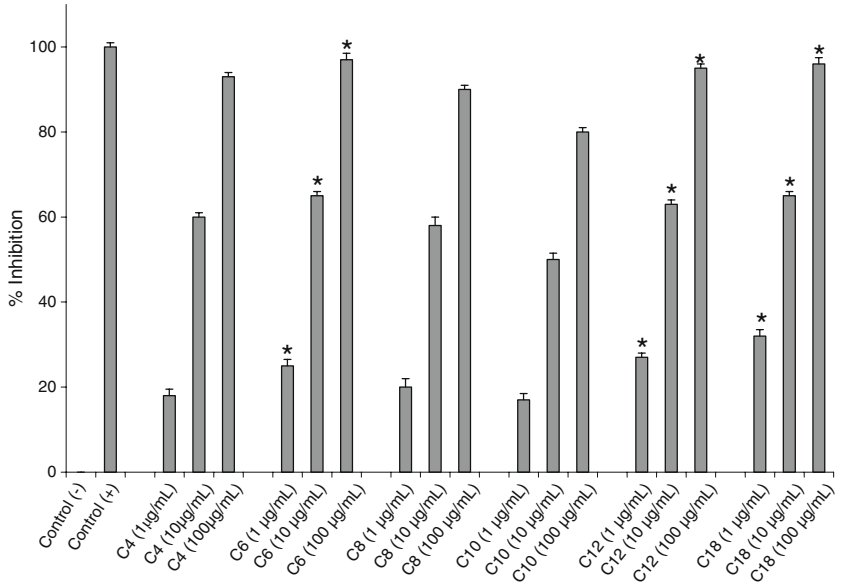

Fig. 4 Inhibition of BPA-SMC proliferation by *O*-acyl LMWHP derivatives. The percent inhibition of BPA-SMCs grown in media containing 10% FBS (without HP) is used as the negative control (−) and is set at zero. The percent inhibition of BPA-SMCs grown in media containing 0.1% FBS (without HP) is used as the positive control (−) and is set at 100%. Six groups of three bars are shown. The three pairs in each group correspond to *O*-acyl LMWHP derivatives at concentrations of 1 μg/mL, 10 μg/mL, and 10 μg/mL, respectively. The six groups correspond to *O*-butanoyl LMWHP (C4), *O*-hexanoyl LMWHP (C6), *O*-octanoyl LMWHP (C8), *O*-decanoyl LMWHP (C10), *O*-dodecanoyl LMWHP (C12), and *O*-stearyl LMWHP (C18), respectively. Significance (*) is shown for C6, C12, and C18 derivatives at all concentrations compared to C4 derivative. The LMPWHP parent without *O*-acyl group substitution gave 50% inhibition at 10 μg/ml [20]. *Error bars* correspond to standard deviations

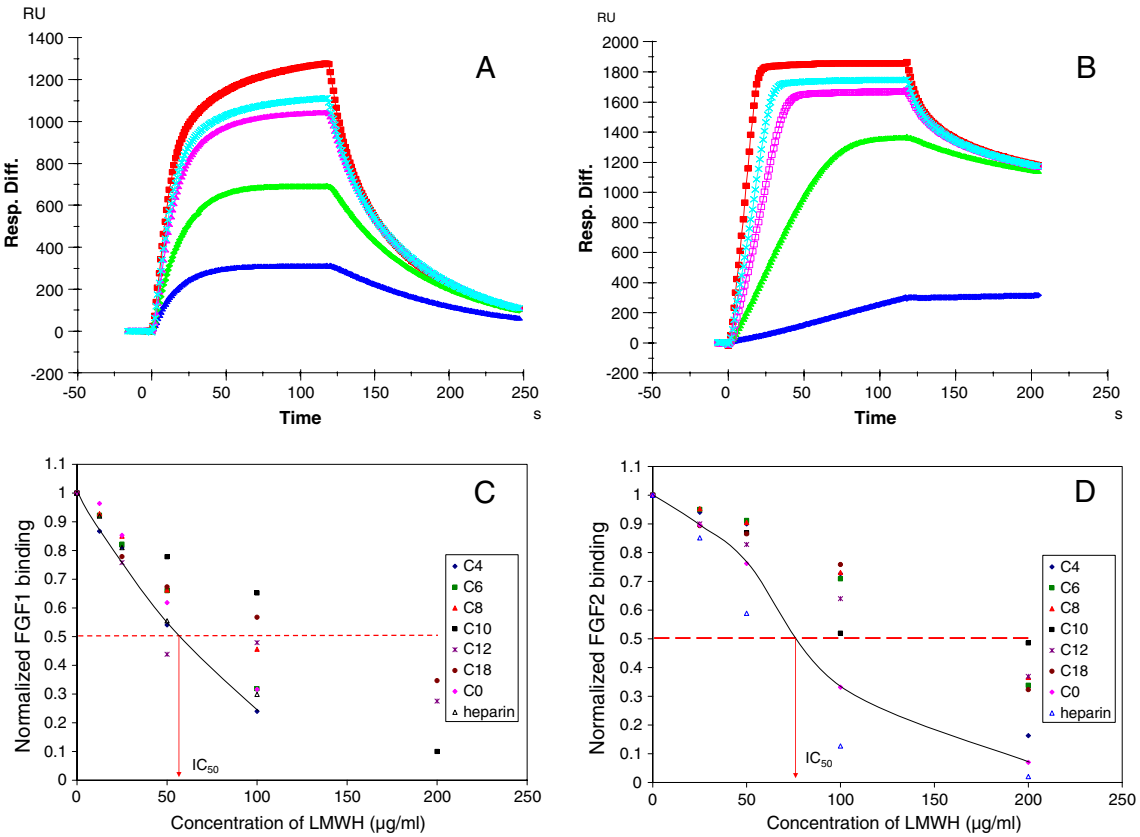

Fig. 5 SPR sensorgrams for IC_{50} measurement of different *O*-acylation of LMWHP (inhibiting FGF1 or FGF2 binding to surface heparin) by solution/surface competition SPR. Panel **a** Competition SPR sensorgrams of FGF1-heparin interaction inhibiting by different concentration of C4. FGF1 concentration was 250 nM, and concentrations of C4 in solution (from top to bottom) were 0, 12.5, 25, 50 and 100 μg/mL, respectively; Panel **b** Competition SPR sensorgrams of FGF2-heparin interaction inhibiting by different concentration of C4. FGF2 concentration was 250nM, and concentrations of C4 in solution (from top to bottom) were 0, 25, 50, 100, and 200 μg/mL, respectively; Panel **c** IC_{50} calculation of different *O*-acylation of LMWHP using FGF1 inhibition data from surface competition SPR. Panel **d** IC_{50} calculation of different *O*-acylation of LMWHP using FGF2 inhibition data from surface competition SPR

8 KD as determined by PAGE analysis. The LMWHP was then acylated with acyl anhydrides of various carbon chain lengths ranging from C4 to C18 and the resulting *O*-acyl LMWHPs were purified. PAGE analyses were consistent with a level of substitution of approximately one *O*-acyl group per disaccharide repeating unit in the *O*-acyl LMWHP derivatives, showing average molecular weights of 8.8–9.6 KD compared to 8 KD for the LMWHP parent (Fig. 2). Careful integration of the ^{1}H-NMR peaks (Fig. 3), assigned to the aliphatic methyl protons of the acyl group (0.91 ppm) to the anomeric proton of IdoA2S (5.08 ppm), confirmed that each *O*-acyl LMWHP had been substituted on the average with one acyl group per disaccharide repeating unit. This consistent level of mono acyl substitution, while somewhat surprising, is consistent with our previous studies on an *O*-hexanoyl LMWHP [20] and suggests that the acylation reaction is limited by the steric demands of the *O*-acyl group.

The LMWHP derivatives were tested for their effect on the growth of BPA-SMCs (Fig. 4) all exhibit inhibitory activity. FBS is required for SMC proliferation. Our assay is designed for full proliferation in the presence of 10% FBS (standard medium, negative control) and no proliferation in the presence of 0.1% FBS (growth arrest medium, positive control). This assay has been validated and is routinely performed in our laboratory using low serum as a positive control [1, 5, 15, 17, 19, 20]. Others [28] have also demonstrated that inhibits SMC proliferation in FBS. The inhibitory effect peaks for butanoylated (C4) LMWHP, and again for *O*-dodecanoylated (C12) LMWHP and *O*-stearyl (C18) LMWHP derivatives at all concentrations (1 μg/ml, 10 μg/ml and 100 μg/ml) measured. The C4, C12 and C18 *O*-acyl LMWHP derivatives exhibit significantly (as indicat-

Table 1 IC_{50} (μg/ml) of different *O*-acylation of LMWHP derivatives inhibiting FGF1 or FGF2 binding to surface heparin measured by solution/surface competition SPR

	C4	C6	C8	C10	C12	C18	C0	heparin
FGF1	55	70	90	130	95	130	65	57
FGF2	140	155	165	160	155	170	75	60

ed by asterisks in Fig. 4) higher anti-proliferative activities than the other LMWHP derivatives and parent LMWHP without an *O*-acyl group.

O-acyl substituted heparin derivatives, such as those described in the current study, may offer therapeutic potential in inhibiting SMC growth. Moreover, others have demonstrated that *O*-acyl heparin derivatives have enhanced biological potency *in vivo* due in part to their increased half-life compared to underivatized heparin [24, 29].

It is well known that the fibroblast growth factor (FGF) signaling plays ubiquitous roles throughout the human life cycle [30]. In the embryo, FGFs are master regulators of mesenchymal-epithelial communication and thus are required for organogenesis and pattern formation [31]. In the adult, FGFs continue to regulate tissue homeostasis but also play important roles in wound healing, tissue repair, and cell proliferation [30]. Extensive studies on the FGF family show that receptor dimerization is a mandatory event in FGF signaling and, in addition to the FGF ligand, requires the presence of the highly sulfated heparin/HS polysaccharide chains of HSPGs [32].

Acidic and basic FGF (FGF1 and FGF2) are the most prominent members of the FGF familiy and are involved in signaling and cell proliferation. It has been reported that the mitogenic actions of fibroblast growth factors (FGFs) are keys to SMC proliferation: reduction in FGF expression inhibits SMC proliferation after intimal injury in humans and laboratory animals [33]. Ghiselli *et al*. (2003) also reported that pharmacologically relevant concentrations of ethanol inhibited the induction of SMC growth by FGF1 and FGF2 [34]. Thus, we tested the ability of LMWHP derivatives to inhibit the binding of FGF1 and FGF2 to surface-immobilized heparin using solution/surface competition SPR. FGF1 or FGF2 (250 nM) mixed with different of concentrations of LMWHP derivatives were injected over the heparin chip. Once the active binding sites on FGF1 molecules were occupied by LMWHP derivatives in the solution, the binding of FGF1 to the surface-immobilized heparin should decrease resulting in a reduction in SPR signal (Fig. 5a). Similar solution/surface competition experiments were conducted with FGF2 (Fig. 5b). The IC_{50} values (concentration of competing analyte resulting in a 50% decrease in response units (RU)) can be calculated from the solution/surface competition SPR. The competition plots (FGF1 or FGF2 binding signal (normalized) *versus* LMWHP derivatives concentration in solution) are shown in Fig. 5c and d. The calculated IC_{50} values for LMWHP derivatives are shown in Table 1 in comparison with non-derivatived LMWHP (C0) and unfractionated heparin. These data show that all the LMWHP derivatives are capable of interfering with FGF1 and FGF2 binding to heparin. In general, the IC_{50} values (Table 1) show that the *O*-acylation of LMWHP reduced the affinity to FGF1 and FGF2 in comparison with non-derivatived LMWHP (C0) and unfractionated heparin. The affinity of LMWHP derivatives to FGF1 decreased with the size from C4 to C10. The affinities of all LMWHP derivatives to FGF2 were comparable to one another. Thus, since all the *O*-acyl LMWHP derivatives show comparable activities towards FGF1 and FGF2, the *O*-acyl chain length providing optimal pharmacokinetics/pharmacodynamics will be selected for future investigation in animal studies.

In conclusion, we have shown that *O*-acylation of LMWHP by either *O*-hexanoyl, *O*-dodecanoyl and *O*-stearyl group enhances its anti-proliferative activity on BPA-SMCs. These derivatives may be effective candidates for treatment of pulmonary hypertension where SMC proliferation plays an important role.

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