Angiogenic Response of Placental Villi to Heparin

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OBJECTIVE: To estimate the angiogenic effect of heparin on human umbilical vein endothelial cells cultured in conditioned media from normal and severely preeclamptic human placental villi.

METHODS: Normal first- and second-trimester floating placental villi were explanted in control conditions and increasing concentrations of heparin (unfractionated and low molecular weight heparin) across the clinical prophylactic and therapeutic range (0.025–25 units/mL). At 96 hours, the placenta-conditioned media was tested for angiogenic activity in a human umbilical vein endothelial cell in vitro angiogenesis assay. Total capillary-like tube length and number of branch points were determined from photographs that did not contain information about experimental conditions. The response of placenta-conditioned media from preterm severely preeclamptic pregnant women exposed to low molecular weight heparin also was assessed and compared with both preterm and term control groups.

RESULTS: Unfractionated heparin significantly promoted angiogenesis (0.25 units/mL compared with control: relative branch points 185±32% [mean±standard error of the mean], $P<.05$), whereas low molecular weight heparin had no significant effect. Addition of unfractionated or low molecular weight heparin to first- and second-trimester placenta-conditioned media significantly promoted angiogenesis with the response to low molecular weight heparin more than double that of unfractionated heparin (low molecular weight compared with unfractionated heparin at 2.5 units/mL: relative branch points 930±158% compared with 398±90%, $P<.05$). Placenta-conditioned media from pregnancies with severe preeclampsia arrested angiogenesis in comparison with both preterm and term pregnancies and was not significantly restored by the addition of low molecular weight heparin.

CONCLUSION: Unfractionated and low molecular weight heparin promote in vitro angiogenesis in healthy first- and second-trimester placenta-conditioned media. The nonanticoagulant actions of heparin may be relevant to the prevention of severe preeclampsia.

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Heparins (unfractionated and low molecular weight heparin) are prescribed during pregnancy and the postpartum period to prevent venous or arterial thromboembolism.[1] They inhibit coagulation by facilitating antithrombin III binding to factor IIa (thrombin) and factor Xa through their antithrombin III receptors.[2] Heparins are also used in prophylactic doses in an attempt to prevent recurrent miscarriage or to improve pregnancy outcomes either in the presence or absence of thrombophilia, although recent large trials have demonstrated no benefit.[3–6] The underlying hypothesis is that heparin preserves placental function by preventing villi infarction; however, none of these studies has examined the heparin-exposed placenta after delivery.

Severe preeclampsia is characterized by hypertension resulting from elevated systemic vascular resistance. In general the placentas are small and damaged at delivery[7] and release reduced amounts of placenta-like growth factor and vascular endothelial growth factor, which promote angiogenesis, while

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secreting large amounts of the truncated splice variant of their receptor, soluble FMS-like tyrosine kinase-1.[8] Serum from preeclamptic women is thus intensely antiangiogenic as a result of high levels of soluble FMS-like tyrosine kinase-1.[9] In women with previous severe preeclampsia, prophylactic heparin administration significantly reduces the risk of recurrent disease,[10] suggesting a proangiogenic action of heparin. Interestingly, an increasing body of evidence indicates that heparin exerts nonanticoagulant actions that may be mediated by an effect on angiogenesis. For example, heparins prolong survival in patients with metastatic cancer[11,12] and this action is maintained when the antithrombin-III binding sites are removed or inactivated in truncated nonanticoagulant heparin.[2] We therefore tested the hypothesis that heparin can exert nonanticoagulant actions in the placenta that may be relevant to the prevention of severe preeclampsia using a human umbilical vein endothelial cell in vitro angiogenesis assay.

MATERIALS AND METHODS

Unfractionated (sodium heparin) and low molecular weight heparin (dalteparin sodium) were used in concentrations of 0.025, 0.25, 2.5, and 25 units/mL to span the range of maternal circulating plasma levels found in pregnant women receiving subcutaneous injections of prophylactic (5,000 units/d; equivalent to 0.25 units/mL) or therapeutic (more than 10,000 units/d; equivalent to 2.5 units/mL) doses.[1]

Ethics committee approval for collection of placental tissues was obtained from Mount Sinai Hospital Research Ethics Board (REB #04-0018), and all patients provided written informed consent. First- and second-trimester placental tissues were collected from seven healthy ultrasound-dated viable singleton pregnancies undergoing elective social terminations at a median of 10 weeks (range, 7–18 weeks) of gestation. Placental tissues were also obtained after vaginal or cesarean delivery from seven women with term uncomplicated pregnancies, seven women with spontaneous preterm delivery, and seven women with severe preeclampsia[13] (Table 1).

Placental villous explants were prepared as previously described in detail.[14] Briefly, individual villous clusters were dissected in sterile cold phosphate-buffered saline and explanted in a 24-well culture plate containing serum-free media in the absence or presence of increasing doses of unfractionated or low molecular weight heparin (Fig. 1A). Villous explants were maintained in a humidified incubator at 37°C in 8% O_2/5% CO_2 for 96 hours, at which point media was collected (placenta-conditioned media) and stored at −80°C for further analysis.

Pooled human umbilical vein endothelial cells were maintained in endothelial basal medium with endothelial cell growth medium SingleQuot growth supplements (excluding heparin) at atmospheric 95% O_2/5% CO_2 in a humidified incubator at 37°C. Media was exchanged every 48 hours until confluency, at which point cell monolayers were passaged with 0.25% trypsin-EDTA. All experiments were performed using third-passage cells.

Heparin toxicity was assessed using the CytoTox-ONE Homogeneous Membrane Integrity Assay according to the manufacturer's protocol. Plates were prepared using 50 microliters of serum-free media alone or with increasing concentrations of unfractionated or low molecular weight heparin and 50 microliters (5,000 cells per well) of human umbilical vein endothelial cells. Plates were incubated and toxicity assessed at 24 and 48 hours (excitation wavelength of 560 nm, emission wavelength of 590 nm).

The effect of heparin alone and heparin incorporated into placenta-conditioned media on human umbilical vein endothelial cell proliferation was tested using the CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay according to the manufacturer's protocol. Plates were prepared as described for the toxicity assay (using first- and second-trimester pla-

Table 1. Demographic Details

	Term (n 7)	Preterm (n 7)	Preeclampsia (n 7)
Maternal age (y)	35 (33–37)	30 (18–36)	33 (24–39)
Gravidity	2 (1–4)	2 (1–5)	3 (1–10)
Parity	1 (0–2)	1 (0–1)	1 (0–3)
Systolic blood pressure (mm Hg)	125 (114–137)	117 (100–144)	165 (147–186)
Diastolic blood pressure (mm Hg)	74 (57–93)	76 (68–85)	98 (90–121)
Proteinuria	0	0	+1 or higher
Gestation (wk)	39 1/7 (38 2/7–40 5/7)	34 3/7 (31 4/7–36 3/7)	33 0/7 (30 0/7–37 1/7)
Birth weight (centile)	71% (46–95%)	46% (11–97%)	16% (3–54%)

Data are as median (range).

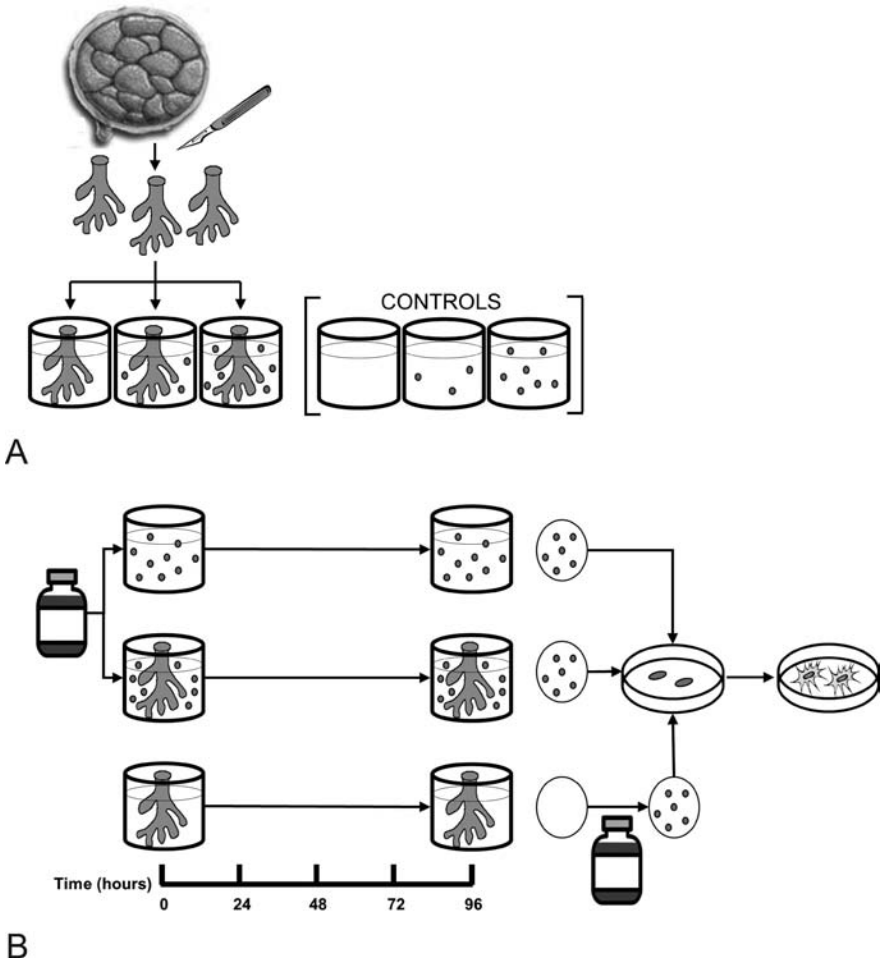

Fig. 1. Schematic of methods. **A.** Small samples of placental villous tissue were dissected into individual villous clusters and then explanted as floating villi into culture media (with or without increasing concentrations of heparin [small circles]). Controls (without placental villi) were set up in a similar fashion. After 96 hours, media was collected for further analysis. **B.** Three experimental conditions included: media (without villous explants) containing increasing concentrations of heparin (upper panel), placenta-conditioned media where heparin was added at time 0 hours (middle panel), placenta-conditioned media in which heparin was added at time 96 hours (lower panel). The addition of heparin either before or after the 96-hour incubation period distinguished a placenta-dependent from a placenta-independent effect of heparin on angiogenesis. Media then was added to the Matrigel angiogenesis assay and tube formation assessed at 20 hours.

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centa-conditioned media as appropriate). Plates were incubated and proliferation determined at 24-hour intervals (absorbance at 490 nm).

Human umbilical vein endothelial cells seeded on Matrigel will invade the underlying fibrin matrix and differentiate to form capillary-like structures; the length and number of tubules provide a quantitative measurement of angiogenesis. Growth-factor reduced Matrigel was thawed on ice at 4°C. Each well of a 96-well plate was coated with 50 microliters of Matrigel and placed at 37°C for 1 hour to solidify. Next, 50 microliters of media was added to each well as follows: 1) media with and without increasing concentrations of unfractionated or low molecular weight heparin; 2) placenta-conditioned media from villous explants after 96 hours incubation in which unfractionated or low molecular weight heparin was added at time 0 hours; 3) placenta-conditioned media from villous explants after 96 hours incubation where unfractionated or low molecular weight heparin (0.25-units/mL dose) was added at time 96 hours (to distinguish a placenta-dependent compared with placenta-independent effect of heparin on endothelial cell angiogenesis) (Fig. 1B). Human umbilical vein endothelial cells were then added to each well (50 microliters at 15,000 cells per well) and the plates incubated at atmospheric 95% O_2/5% CO_2 at 37°C for 20 hours. The entire surface of each well was then immediately photographed at low power (4×) using a Nikon DMRX light microscope. Individual well photographs were blinded to the background experiment (Fig. 2). For each photograph, endothelial tube formation was quantified as follows: 1) the total length of tubes and 2) the number of branch points (three or more tube branches from a single point). For each experiment, tube length and branch point data were similar and therefore only branch point data are provided.

Each type of endothelial cell culture experiment (proliferation, toxicity, and angiogenesis assays) was performed on the same batch of human umbilical vein endothelial cells. Each experiment to produce

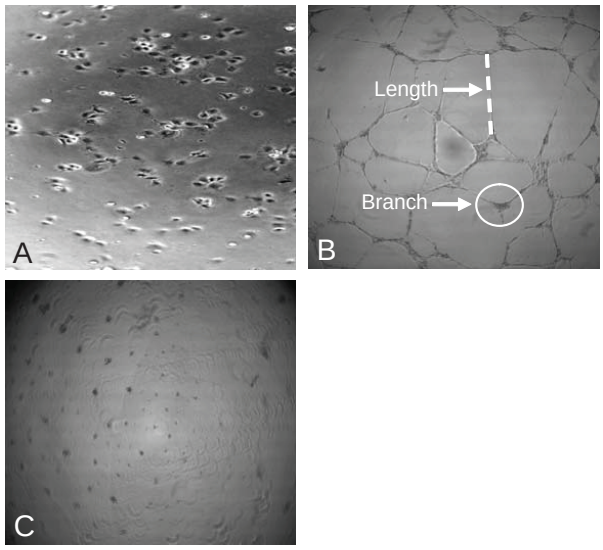

Fig. 2. Human umbilical vein endothelial cell in vitro angiogenesis assay. **A.** Human umbilical vein endothelial cells seeded on Matrigel at time 0 (10× magnification). These cells will invade the underlying fibrin matrix and differentiate to form capillary-like tubular structures, as illustrated in **B. B.** The angiogenic potential of the media is quantified by measuring 1) the total length of tube structures and 2) the number of branch points (defined as three or more tube branches from a single point after 20 hours of exposure) (4× magnification). **C.** Representative image demonstrating an arrest of angiogenesis using media conditioned by placental villi from a severely preeclamptic pregnancy (4× magnification).

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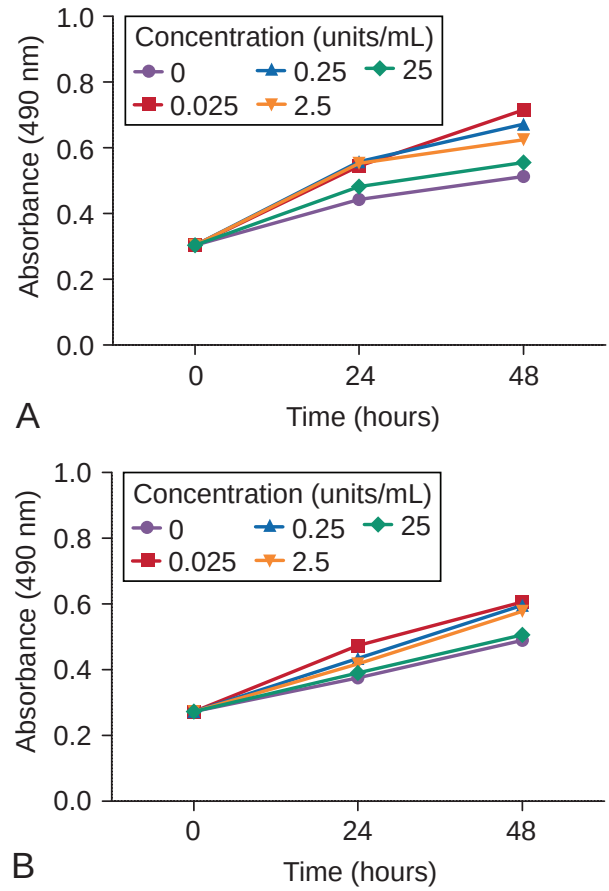

Fig. 3. Human umbilical vein endothelial cell proliferation in the absence and presence of increasing concentrations of heparin. Absorbance at 490 nm increased significantly at 24 and 48 hours, reflecting cell proliferation over time. Increasing concentrations of unfractionated (**A**) or low molecular weight heparin (**B**) had no significant effect on cell proliferation at 24 and 48 hours. Data are shown as mean±standard error (SE) of three separate experiments, each performed in triplicate.

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conditioned media from placentas exposed to incrementally increasing concentrations of heparin was set up in triplicate using individual placentas. Sample size was determined based on preliminary observations in three sets of experiments (not included) and from previously published research.[9,12] Data are presented as mean±standard error. For all experiments, assumptions of normal distribution were not valid as a result of lack of independence of replications and, as a result, nonparametric analyses were performed. Statistical significance of differences in means between groups was determined using the Kruskal-Wallis followed by Dunn's multiple comparison test with a value of $P<.05$ considered significant.

RESULTS

To determine that any observed effect of heparin on human umbilical vein endothelial cell angiogenesis was unrelated to cytotoxic heparin effects, cell survival was assessed through a membrane integrity cell toxicity assay. Endothelial cells were cultured in the absence and presence of increasing concentrations of unfractionated or low molecular weight heparin and cell viability was assessed over a 48-hour period. Increasing unfractionated or low molecular weight heparin exposure up to 25 units/mL did not significantly effect cell viability compared with cells cultured without heparin (data not shown).

To distinguish an effect of heparin on endothelial cell proliferation from cell differentiation, human umbilical vein endothelial cell proliferation was assessed using a cell uptake and bioreduction assay over a 48-hour period. Increasing concentrations of unfractionated or low molecular weight heparin had no significant effect on cell proliferation (Fig. 3). When

endothelial cells were cultured in placenta-conditioned media from healthy first- or second-trimester explants in the absence and presence of unfractionated or low molecular weight heparin, again, no significant difference in cell proliferation was observed (data not shown). Therefore, any differences in tube formation in subsequent experiments are likely to be mediated by a specific effect on human umbilical vein endothelial cell differentiation.

To assess the angiogenic potential of heparins alone, human umbilical vein endothelial cells were grown on Matrigel in the absence and presence of increasing concentrations of heparin (Fig. 4). Increasing concentrations of unfractionated heparin up to 2.5 units/mL significantly promoted angiogenesis illustrated by increases in total branch points (0.25 units/mL compared with control: relative branch points 185±32%; Kruskal-Wallis with Dunn's multiple comparison test, $P<.05$), whereas the highest dose (25 units/mL) showed no effect. By contrast, low molecular weight heparin did not promote angiogenesis, whereas the highest dose (25 units/mL) significantly repressed angiogenesis (25 units/mL compared with control: relative branch points 49±10%).

The addition of increasing concentrations of unfractionated heparin during the 96 hours of conditioning by healthy first- and second-trimester placental explants significantly promoted human umbilical endothelial cell angiogenesis when compared with media cultured in the absence of heparin (2.5 units/mL compared with control: relative branch points 398±90%) (Fig. 5A). This proangiogenic effect was independent of an interaction between unfractionated heparin and the placental villi because the addition of unfractionated heparin (0.25 units/mL) after the 96-hour culture period had the same effect as addition at the start of the culture period (0.25 units/mL at time 0 compared with time 96 hours: relative branch points 224±24% compared with 205±27%).

Similarly to unfractionated heparin, the addition of low molecular weight heparin to the culture media of healthy first- and second-trimester placental explants significantly promoted human umbilical vein endothelial cell angiogenesis compared with media without heparin (2.5 units/mL compared with control: relative branch points 930±158%) (Fig. 5B). The response to low molecular weight heparin was significantly greater than to unfractionated heparin with branch points more than double across the range of heparin concentrations tested (low molecular weight compared with unfractionated heparin at 2.5 units/mL; $P<.05$). However, unlike unfractionated heparin, this proangiogenic effect was dependent on an interaction between low molecular weight heparin and placental villi during the 96-hour culture period because the addition of low molecular weight heparin (0.25 units/mL) at 96 hours did not increase angiogenesis (0.25 units/mL at time 0 compared with 96 hours: relative branch points 569±100% compared with 198±123%).

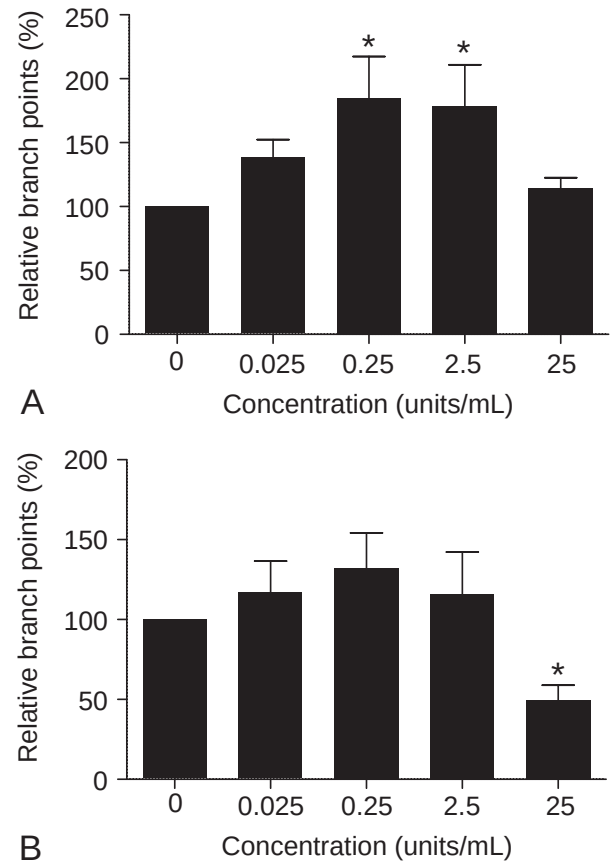

Fig. 4. Effects of unfractionated and low molecular weight heparin on human umbilical vein endothelial cell in vitro angiogenesis. Human umbilical vein endothelial cells were seeded on Matrigel with unconditioned media containing increasing concentrations of either (**A**) unfractionated or (**B**) low molecular weight heparin and photographed after 20 hours. Capillary tube-like formation was assessed by the number of branch points (and the total tube length, data not shown) per well, represented as a percentage of the control (media without heparin). Unfractionated heparin significantly promoted angiogenesis at 0.25 and 2.5 units/mL of concentration. In comparison, similar concentrations of low molecular weight heparin had no effect on angiogenesis, whereas the highest dose (25 units/mL) significantly inhibited angiogenesis. Data are shown as mean±standard error of three different experiments, each performed in triplicate. *Statistical significance compared with control (no heparin) (Kruskal-Wallis followed by Dunn's multiple comparison test, $P<.05$).

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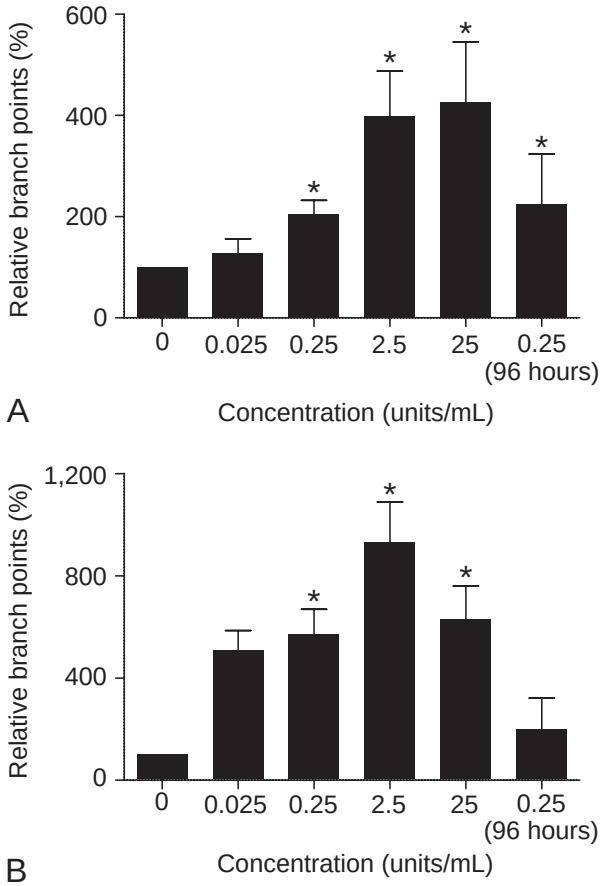

Fig. 5. Effect of unfractionated and low molecular weight heparin on human umbilical vein endothelial cell in vitro angiogenesis in conditioned media from healthy first- and early second-trimester placentas. Both unfractionated (**A**) and low molecular weight heparin (**B**) significantly promoted angiogenesis in placenta-conditioned media at 0.25, 2.5, and 25 units/mL. The unfractionated heparin effect was independent of an interaction with placental villi during the 96-hour culture (no significant difference between 0.25 units/mL added at time 0 compared with time 96 hours), whereas the low molecular weight heparin effect required an interaction with placental villi during the 96-hour culture (significant difference between 0.25 units/mL added at time 0 compared with time 96 hours). Data are represented as a percentage of the control (media without heparin) and shown as mean±standard error of seven different experiments, each performed in triplicate. *Statistical significance compared with control (no heparin) (Kruskal-Wallis followed by Dunn's multiple comparison test, $P<.05$).

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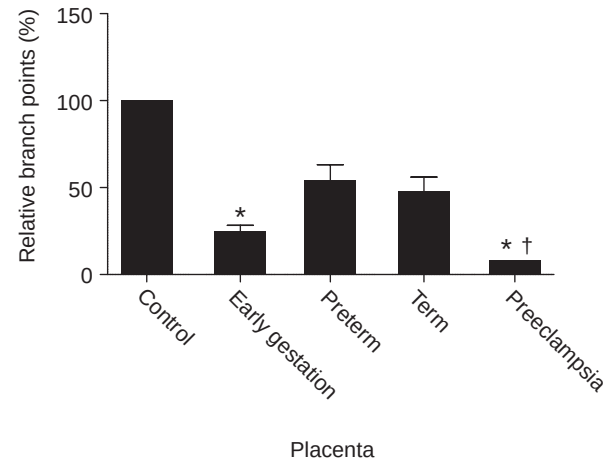

Fig. 6. Effect of gestation and preeclampsia on human umbilical vein endothelial cell in vitro angiogenesis. Placenta-conditioned media from healthy first-trimester and early second-trimester explants (early gestation) significantly repressed angiogenesis in comparison with unconditioned media. Placenta-conditioned media from both preterm and term pregnancies showed attenuated nonsignificant responses compared with unconditioned media. Contrast with placenta-conditioned media from severely preeclamptic pregnancies that mostly arrested angiogenesis. Data are represented as a percentage of the control (unconditioned media) and shown as mean±standard error of seven different experiments, each performed in triplicate. *Statistical significance compared with control group (no heparin); †statistical significance compared with preterm and term control groups (Kruskal-Wallis followed by Dunn's multiple comparison test, $P<.05$).

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Placenta-conditioned media alone (ie, without heparin) from normal first- and early second-trimester explants significantly inhibited angiogenesis in comparison with unconditioned media (first- and second-trimester compared with control: relative branch points 25±4%) (Fig. 6). Placenta-conditioned media from preterm and healthy term deliveries demonstrated nonsignificant attenuations of angiogenesis.

Given the similar but more robust proangiogenic effect of low molecular weight heparin compared with unfractionated heparin, additional experiments were performed using only the former. Placenta-conditioned media from pregnancies with severe preeclampsia arrested angiogenesis in comparison with both preterm and term pregnancies (Fig. 6). Addition of low molecular weight heparin to severely preeclamptic placenta-conditioned media restored a minimal and nonsignificant degree of endothelial tube formation in three of seven cases (Fig. 7). When low molecular weight heparin was incorporated into conditioned media with both preterm and term placentas, similar but nonsignificant additional angiogenic responses were observed (data not shown).

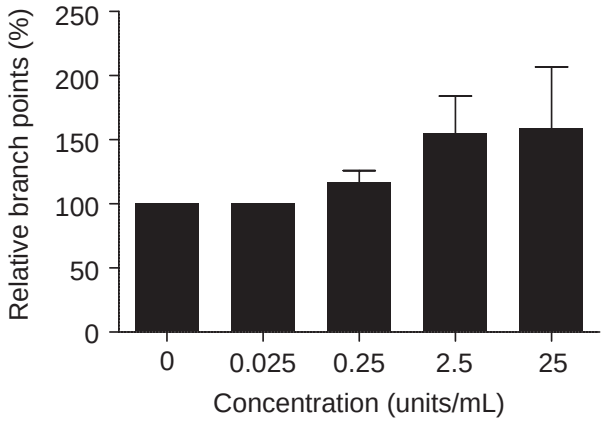

Fig. 7. Effect of low molecular weight heparin on human umbilical vein endothelial cell in vitro angiogenesis in conditioned media from severely preeclamptic placentas. Addition of low molecular weight heparin to placenta-conditioned media from pregnancies with severe preeclampsia promoted a limited (but nonsignificant) degree of angiogenesis in three of seven cases, whereas no angiogenesis was demonstrated in four of seven cases. Data are represented as a percentage of the control (media conditioned by preeclamptic villi without heparin) and shown as mean±standard error of seven different experiments each performed in triplicate. *Statistical significance compared with control group (no heparin) (Kruskal-Wallis followed by Dunn's multiple comparison test, $P<.05$).

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DISCUSSION

In addition to their well-established anticoagulant properties, heparins have broader actions, including anti-inflammatory and antitumor activities.[12,15] Structurally, heparins are similar to heparan sulfates, long proteoglycan molecules attached to cell membranes, and the extracellular matrix. Here both heparan sulfates and heparins regulate angiogenesis by binding angiogenic growth factors and facilitating or inhibiting interaction with their receptors.[16]

Using an established in vitro angiogenesis assay specific to floating chorionic villi from the human placenta,[14] we demonstrated that heparins influence new vessel formation through cellular differentiation because neither heparin species had any effect on endothelial cell viability or proliferation. Unfractionated heparin promoted angiogenesis at levels equivalent to prophylactic and therapeutic dosing. Similar doses of low molecular weight heparin had no effect, whereas high doses inhibited in vitro angiogenesis. Collen et al[17] also showed that endothelial tube formation was facilitated by unfractionated heparin and repressed by low molecular weight heparin when added directly to the fibrin matrix.

These differential effects of unfractionated and low molecular weight heparin on angiogenesis may reflect their varying structures. Unfractionated heparin is a heterogeneous mixture of polysaccharide molecules (200–300 saccharide units) with a mean molecular weight of 12–15 kDa.[1] Unfractionated heparin may be depolymerized producing smaller low molecular weight heparin fragments with chain lengths of 12–18 units and molecular weights between 3–6 kDa.[1] Such changes prolong the half-life and may reduce the risk of heparin-induced thrombocytopenia and osteoporosis making low molecular weight heparin the preferred agent in clinical practice.[1] Naturally occurring heparin sulfates (and longer heparin fragments) bind potent angiogenic factors within tissues such as fibroblast growth factor and vascular endothelial growth factor.[16] Low molecular weight heparin fragments may be too small to facilitate these interactions, which may explain the apparent antiangiogenic influence found at high doses in this study. Khorana et al[18] demonstrated that low molecular weight heparins less than 6 kDa (but not unfractionated heparins or larger low molecular weight heparins) inhibited fibroblast growth factor-stimulated endothelial tube growth on Matrigel. In vivo studies have also shown that heparins inhibit angiogenesis in the rat mesentery and rat cornea in a size-specific manner.[19,20] In clinical studies, patients with cancer receiving low molecular weight heparin survive longer than those allocated to unfractionated heparin and this difference may be related to an antiangiogenic action of the former on tumor cells.[21]

When media was conditioned for 96 hours by first- and early second-trimester placental villi, we observed a near 50% reduction in angiogenic activity measured either by length of tube formation or creation of new branch points. This observation suggests that placental villi release soluble FMS-like tyrosine kinase-1 in excess of the proangiogenic factors placenta-like growth factor and vascular endothelial growth factor. This seems counterintuitive because pregnancy is a proangiogenic state characterized by blood volume expansion, systemic vasodilation, and elevated cardiac output.[22] However, the human placenta comprises both the chorionic villi of the definitive placenta and the decidualized endometrium, the latter synthesizing large amounts of proangiogenic growth factors.[23] Because the decidua is larger than the placenta throughout most of the first trimester and is only perfused by maternal blood at the end of the first trimester,[24] it is likely that the antiangiogenic response of placental villi from the early developing human placenta is effectively counterbalanced by the

biologic actions of the decidua. Because placental size increases relative to the decidua in the second trimester, the decline we observed in this phenomenon in preterm and term placental villi is likely a component of the physiological adaptations of normal pregnancy. Because preeclampsia has its origins in early pregnancy,[25] the inability of placental villi to repress their antiangiogenic action may be a key pathologic mechanism to initiate the disease during the early second trimester. Both unfractionated and low molecular weight heparin, in concentrations encompassing circulating levels for both prophylactic and therapeutic dosing regimens in pregnancy, are able to reverse this phenomenon. The effect was significant but partial for unfractionated heparin and was complete for low molecular weight heparin.

Unfractionated heparin is a cofactor for the maintenance of trophoblast stem cells by fibroblast growth factor 4.[26] We previously demonstrated that heparin with fibroblast growth factor 4 is a potent mitogen to villous cytotrophoblasts in floating first-trimester placental villi.[14] Heparin also promotes proliferation and prevents apoptosis in BeWo cells.[27] One explanation for the observed phenomenon of reversing an antiangiogenic potential is that heparin promotes cytotrophoblast proliferation leading to enhanced syncytial fusion into the syncytiotrophoblast layer that secretes proangiogenic growth factors into the media (representing maternal blood in the intervillous space). Because low molecular weight heparin, but not unfractionated heparin, required an interaction with the placental villi to generate its proangiogenic effect, we speculate that this purified form of heparin is more able to penetrate the syncytiotrophoblast to interact with the underlying villous cytotrophoblasts.

During the course of the study, we demonstrated that maternal serum from severely preeclamptic women is intensely antiangiogenic, as previously observed.[28] Low molecular weight heparin, even in supratherapeutic doses, was only able to restart a minimal degree of angiogenesis in conditioned media from three of seven preeclampsia cases. Because these pathologic placentas have established defects in the villous trophoblast, including depletion of villous cytotrophoblasts and focal areas of syncytiotrophoblast necrosis,[29] they may, in contrast to healthy first- and early second-trimester villi, be unable to respond to heparin at the level of villous cytotrophoblasts that are responsible for constant regeneration of normal syncytiotrophoblast. Therefore, the lack of reversal of an antiangiogenic state by heparin in placental villi from established severe preeclamptic placental villi is not inconsistent with a preventive role of heparin started at the end of the first trimester to women at milder forms of the disease, before these trophoblast defects develop.[10]

Our findings have considerable significance for the use of heparin to prevent serious perinatal complications. First, our data give insight into the mechanism of action of low molecular weight heparin to prevent recurrent severe preeclampsia.[10] Second, they may be relevant to recent trial data indicating that heparin may promote implantation success after in vitro fertilization.[5] Third, because these effects are unlikely to be mediated by the antithrombin III anticoagulant receptors, they may be preserved in nonanticoagulant truncated heparins. If so, the potential to promote better placental function through heparin without anticoagulant risks will have enormous safety implications in obstetrics.

The limitations of our study include our sample size, potential selection bias, and the inherent variability in the underlying placental pathology of placentas from women with severe preeclampsia that may result in coexistent fetal growth restriction. We attempted to overcome these limitations using the resources of our Biobank team that could attend consecutive deliveries for sample collection on a 24-hour basis. In future larger studies, we intend to compare the effects of heparin across the full range of disease, especially milder cases without coexistent fetal growth restriction because they form the bulk of the disease burden of preeclampsia.[10]

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