Heparin modulates chemokines in human endometrial stromal cells by interaction with tumor necrosis factor α and thrombin

Julia Spratte, M.D.,[a] Magdalena Schönborn, B.S.,[b] Nora Treder, B.S.,[b] Frauke Bornkessel, B.S.,[b] Marek Zygmunt, M.D., Ph.D.,[b] and Herbert Fluhr, M.D., Ph.D.[a]

[a] Department of Obstetrics and Gynecology, University of Heidelberg, Heidelberg; and [b] Department of Obstetrics and Gynecology, University of Greifswald, Greifswald, Germany

Objective: To study the impact of heparins on chemokines in decidualized human endometrial stromal cells (ESCs) in vitro.
Design: In vitro experiment.
Setting: Research laboratory.
Patient(s): Premenopausal women undergoing hysterectomy for benign reasons.
Intervention(s): ESCs were isolated from hysterectomy specimens, decidualized in vitro and incubated with unfractionated heparin and low-molecular-weight heparins (LMWHs) as well as tumor necrosis factor (TNF) α or thrombin with or without heparins.
Main Outcome Measure(s): Chemokines CXCL1, CXCL5, CXCL8, CCL2, and CCL5 were measured with the use of ELISA, and CXCL5, CXCL8, CCL2, and CCL5 were detected with the use of real-time reverse-transcription polymerase chain reaction. Cell viability was determined with the use of a fluorometric assay.
Result(s): TNF-α and thrombin stimulated distinct patterns of chemokines in ESCs. Unfractionated heparin and LMWHs attenuated the TNF-α–mediated induction of CXCL8 and enhanced CXCL5, CCL2, and CCL5. The stimulating effect of thrombin on CXCL8 could be inhibited by heparin, whereas heparin had no impact on thrombin-induced CXCL1 and CCL2. Nuclear factor of transcription κB signaling mediated the effects of TNF-α. The effects of thrombin were mediated via extracellular signal–regulated protein kinases 1/2.
Conclusion(s): Heparins have modulating effects on TNF-α– and thrombin-induced endometrial chemokines, which might have implications in the regulation of endometrial receptivity and early implantation. (Fertil Steril® 2015;103:1363–9. ©2015 by American Society for Reproductive Medicine.)
Key Words: Heparin, TNF-α, thrombin, chemokines, endometrium

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/sprattej-heparin-modulates-endometrial-chemokines/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Unfractionated heparin as well as low-molecular-weight heparins (LMWHs) are polysulfated glycosaminoglycans known to have pleiotropic effects beyond their classic anticoagulant properties (1, 2). The efficacy of heparins for the prophylaxis and treatment of repeated implantation failure and habitual miscarriages has been suggested by several studies (3–6). In addition, the risk of pregnancy complications such as preeclampsia and intrauterine growth restriction can be reduced by the application of heparins in selected patients, even if they are not suffering from thrombophilic disorders (7–10). Being aware that these placenta-mediated diseases during pregnancy are based on disorders during the process of implantation, this initial phase of pregnancy seems to be very interesting for the use of heparin independently from its anticoagulant properties in a prophylactic regime. This hypothesis is further supported by the direct effect of heparins on the decidualization of human endometrial stromal cells (ESCs) (11, 12).

Chemokines are small peptides that are classified into four groups (C, CC,

Received September 26, 2014; revised February 2, 2015; accepted February 17, 2015; published online March 23, 2015.
J.S. has nothing to disclose. M.S. has nothing to disclose. N.T. has nothing to disclose. F.B. has nothing to disclose. M.Z. has nothing to disclose. H.F. has nothing to disclose.
Supported by a grant from the German Research Foundation, Bonn, Germany, to H.F. (grant FL 667/2-1).
Reprint requests: Julia Spratte, M.D., Department of Obstetrics and Gynecology, University of Heidelberg, Im Neuenheimer Feld 440, 69120 Heidelberg, Germany (E-mail: julia.spratte@med.uni-heidelberg.de).

Fertility and Sterility® Vol. 103, No. 5, May 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.02.023

CXC, and CX_3C) according to their amino acid sequences and play an important role in regulating the migration of inflammatory cells (13). They are essentially implicated in endometrial physiology and play a role in proliferation, angiogenesis, decidualization, and menstruation (14). Furthermore, chemokines are present at the fetal-maternal interface and are of special importance for the recruitment and regulation of immune cells in the secretory endometrium and the early decidua (15). Chemokines have also been shown to be directly involved in the coordination of the trophoblast invasion into the maternal decidua and vasculature (14, 15).

Tumor necrosis factor (TNF) α is a proinflammatory T-helper 1 cytokine playing a significant role in the physiology and pathology of the human endometrium and early implantation by regulating angiogenesis, apoptosis, proliferation, differentiation, and leucocyte trafficking (16). We could recently show that unfractionated heparin can interact with the transcription factor nuclear factor of transcription (NF) κB in human ESCs, thereby inhibiting the TNF-α–induced expression of interleukin (IL) 6 and CXCL8 (17).

Thrombin is a serine protease activated at the end of the coagulation cascade converting fibrinogen to fibrin. Thrombin can also activate G-protein–coupled proteinase–activated receptors and the extracellular signal–regulated protein kinases (Erk) pathway (18, 19). It has been shown that women suffering from recurrent miscarriage have increased levels of thrombin-antithrombin complexes, underscoring the pathophysiologic relevance of these molecules (20). Additionally, thrombin seems to be an enhancer of soluble fms-like tyrosine kinase 1 (sFlt-1) expression in decidual cells, thereby promoting preeclampsia (21). In the secretory endometrium, thrombin is known to stimulate the production of chemokines, vascular endothelial growth factor, and matrix metalloproteinases (22–25). Similar effects of thrombin have also been observed in first-trimester as well as term decidual cells (26, 27).

Based on these facts, we investigated whether heparin has an effect on the production of chemokines in human ESCs in vitro. We focused on the two proinflammatory and pathophysiologically relevant molecules TNF-α and thrombin as typical stimulators of chemokines.

MATERIALS AND METHODS

Tissue Collection and Cell Culture

Endometrial tissue samples were obtained from premenopausal women undergoing hysterectomy for benign reasons after written informed consents. The study protocol was approved by the Institutional Ethical Board of the University of Greifswald, Germany. ESCs were isolated, cultured, and characterized as described previously (28). Briefly, minced endometrial tissue was digested by means of incubation with collagenase (Biochrom) and the dispersed endometrial cells were separated with the use of filtration. ESCs were maintained in DMEM/F-12 cell culture medium without phenol red (Gibco/Invitrogen) containing 10% charcoal-stripped fetal bovine serum (Biochrom) and 50 μg/mL gentamycin (Ratiopharm). The purity of ESC cultures was proven by means of standard immunofluorescent staining of vimentin.

Decidualization In Vitro and Experimental Conditions

ESCs were decidualized in vitro by incubating the cells with 1 μmol/L progesterone and 30 nmol/L 17β-estradiol (both from Sigma-Aldrich) for 9 days. Decidualization was proven by measuring a significantly increased secretion of insulin-like growth factor–binding protein 1 and prolactin as described previously (28). Decidualized ESCs were incubated with the following agents: human TNF-α (Biosource), human thrombin (Sigma-Aldrich), unfractionated heparin (Ratiopharm), tinzaparin (Innohep; LEO Pharma), dalteparin (Fragmin-P; Pharmacia), enoxaparin (Clexane; Sanofi-Aventis), reviparin (Clivarin; Abbott), pathenolide (Calbiochem/Merck), PD98059 (Cell Signaling Technology), and antithrombin III, (Kybernin-P; CSL Behring). The different combinations, dosages, and incubation times of the mentioned agents are indicated subsequently in detail in the corresponding figures as well as in the Results section.

Cell Viability Assay

The relative number of viable ESCs under the influence of the indicated agents was measured at the end of the respective experiments with the use of the Celltiter-Blue assay (Promega) following the manufacturer's instructions. Fluorescence was recorded with the use of the Fluostar Optima system (BMG Labtech).

Enzyme-linked Immunosorbent Assays

Cell culture supernates were collected after the respective treatments of the cells and analyzed for the chemokines CXCL1, CXCL5, CXCL8, CCL2, and CCL5 with the use of commercially available Duoset ELISA kits from R&D Systems. The sensitivities of the assays were 31.3 pg/mL (CXCL1), 15.6 pg/mL (CXCL5, CXCL8, and CCL2), and 1 pg/mL (CCL5). There was no significant cross-reactivity or interference. Intra- and interassay variabilities were $<5\%$. All assays were performed according to the manufacturer's instructions. Absorbance values were measured with the use of the Fluostar Optima system and normalized to the relative number of viable ESCs.

Real-time Reverse-transcription Polymerase Chain Reaction

Total ribonucleic acid (RNA) was isolated from ESCs with the use of Peqgoldtrifast (Peqlab) and reverse-transcribed with the use of the High Capacity cDNA Archive Kit (Applied Biosystems). Semiquantitative real-time polymerase chain reaction (PCR) was performed to quantify the mRNA levels of CXCL5, CXCL8, CCL2, and CCL5 in relation to the housekeeping gene β-actin. cDNA samples were amplified with the use of the Power Sybr Green PCR Master Mix (Applied Biosystems) and the respective forward and reverse primers. The primers (Invitrogen) were designed with the use of Primer Express Primer Design Software version 2.0 (Applied Biosystems) with the resulting amplicons having an intron-overlapping sequence. The sequences of the primers used are summarized in Table 1.