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Pilot Study

PILOT STUDY

The effect of heparins on plasma concentration of heparin-binding protein: a pilot study

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Abstract

Background: Neutrophil-derived heparin-binding protein (HBP) plays a role in the pathophysiology of impaired endothelial dysfunction during inflammation. HBP has been suggested as a predictor of organ dysfunction and disease progression in sepsis. We investigated the effects of heparins on plasma concentrations of HBP in patients undergoing surgery.
Methods: We studied three groups of patients receiving heparins during or after surgery. The vascular surgery group received 3000–7500 U, whereas the cardiac surgery group received 27 500–40 000 U. After major general surgery, the third group received 5000 U of low-molecular-weight heparin (LMWH) subcutaneously. Serial plasma HBP concentrations were measured after these treatments with two different methods: Axis-Shield ELISA and Joinstar FIC-Q100. In addition, plasma myeloperoxidase and syndecan-1 were measured in the cardiac surgery group.
Results: During vascular surgery, heparin induced a six-fold increase in HBP within 2 min, from 3.6 (2.4–5.4) to 21.4 (9.0–35.4) ng ml^{-1} ($P<0.001$). During cardiac surgery, the higher dose of heparin elevated HBP concentrations from 5.3 (2.7–6.1) to 48.7 (38.4–70.1) ng ml^{-1} ($P<0.0001$) within 3 min. Patients receiving LMWH showed an increase from a baseline of 5.7 (3.7–12.1) ng ml^{-1} to a peak HBP concentration of 14.8 (9.5–18.1) ng ml^{-1} ($P<0.0001$) after 3 h. Plasma concentrations of myeloperoxidase, but not syndecan-1, also responded with a rapid increase after heparin. There was a strong correlation between the two methods for HBP analysis ($r=0.94$).
Conclusions: Plasma concentrations of HBP increased rapidly and dose-dependently after heparin administration. Subcutaneous administration of LMWH increases plasma HBP, but to a lesser degree.
Clinical trial registration: ClinicalTrials.gov identifier: NCT04146493.

Keywords: heparin; heparin-binding protein; low-molecular-weight heparin; myeloperoxidase; pilot study; surgical procedure; syndecan-1

Heparin-binding protein (HBP), also named Azurocidin or CAP37, is stored in secretory and primary granules in polymorphonuclear leucocytes. This protein is released as a result of inflammation and can be readily detected in conditions such as sepsis, shock, and trauma.[1] Previous studies have indicated that HBP could have an important role in endothelial plasma leakage and is released upon neutrophil adhesion and extravasation.[2,3] HBP binds to glycosaminoglycans in the endothelial glycocalyx[3,4]; this binding is likely mediated through electrostatic forces and leads to cytoskeletal rearrangement and intercellular gap formation in endothelial cell monolayers.[2–5]

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Plasma HBP has been promoted as a promising early biomarker of sepsis.[6–8] A wide range of infections cause release of HBP,[7 8] and streptococcal M-protein is a well-known generator of HBP release.[9] Increased concentrations of HBP at ICU admission have been strongly associated with respiratory and circulatory failure.[10] Higher plasma HBP concentrations are shown in patients with sepsis and organ failure than in patients with a non-septic illness or mild sepsis. Furthermore, plasma concentrations >15 ng ml^{-1} at ICU admission are associated with increased mortality.[8,11]

However, studies have also shown elevated concentrations of HBP in general ICU patients with or without infection[12] and in patients with shock caused by cardiac arrest, cardiac surgery, or trauma.[13–16] This indicates that HBP may play an important role in the pathophysiology of these conditions and may be a sign of general inflammation, not limited to infections. The molecular mechanism, biological role, and cellular signalling pathways of HBP and the fate of HBP after its secretion remain largely unknown.

Numerous studies have elucidated anti-inflammatory effects on heparin which have mainly been attributed to interactions with histones, complements, selectins, and chemokines,[17,18] but the use of heparin to ameliorate inflammation in the clinical setting is limited because of its anticoagulant effects. Novel heparinoids with significantly less anticoagulant activity while retaining their anti-inflammatory and anti-adhesive effects have been developed.[19] One such drug, sevuparin, has been shown to hinder streptococcus-induced vascular leak in mice[20] and has been tested in clinical trials in sickle-cell disease and malaria.[21,22]

HBP owes its name to its strong heparin-binding capacity and interestingly, heparins are structurally similar to glycosaminoglycans with a strong negatively charged part of the molecule.[23–25] However, the effects of heparins on plasma concentrations of HBP in the clinical setting are poorly studied. A recent study showed that administration of heparin during cardiac surgery increased plasma concentrations of HBP[15] without any apparent biological effects. Moreover, experimental studies also showed that heparins bind to several other proteins including myeloperoxidase (MPO).[20,26–28]

In this study, we hypothesised that heparins may affect plasma concentrations of HBP. We investigated the effects of various doses of unfractionated heparin and low-molecular-weight heparin (LMWH) in patients undergoing surgery. We also measured MPO and syndecan-1, the latter as a marker of glycocalyx integrity. In addition, we compared a novel point-of-care method for HBP analysis with the commonly used commercial ELISA.

Methods

Patient recruitment

This is an observational pilot study in three separate groups of patients in which we wanted to study the effect of different doses of unfractionated heparins and LMWH. The study was approved by the regional ethical review board in Stockholm (no 2018/520-31/1). Informed and written consent was obtained from the patients. For inclusion in all groups, the participants were over 18 yr old and able to understand the study information and protocol. Patients were included when sufficient resources were available.

In the low-dose heparin group, patients were included if they were to undergo routine vascular surgery and were expected to receive a single dose of unfractionated heparin during the early part of surgery. In the high-dose heparin group, patients were included if they were to undergo cardiac surgery where the extracorporeal circulation (ECC) time was expected to be <120 min. In the LMWH group, patients were included if they were to undergo major general surgery with anticipated prolonged postoperative care. This was done as the frequent sampling of blood would be convenient through an indwelling arterial line. These patients were expected to receive postoperative dalteparin during the first few hours after surgery. Preoperative patient characteristics are shown in Table 1.

Procedures

Low-dose heparin

Patients received a single i.v. dose of unfractionated heparin (3000–7500 U) early during vascular surgery, before opening the artery of interest. Plasma concentrations of HBP were measured immediately before heparin and repeatedly at 2, 5, 10, and 60 min after the given dose. Two patients were given an extra dose of heparin before the 60-min sample and these measurements were therefore excluded. The patients underwent the following surgical procedures: carotid endarterectomy (n=4), endovascular aortic repair (n=3), femoro-popliteal bypass (n=2), and popliteal aneurysm surgery (n=1).

High-dose heparin

Patients undergoing open or minimally invasive heart surgery with ECC underwent the following surgical procedures: aortic valve replacement (n=5), coronary artery bypass graft (CABG) surgery (n=2), minimally invasive mitral valve repair (n=1), minimally invasive mitral valve repair and CryoMaze ablation (n=1), and aortic valve replacement and CABG (n=1). We measured HBP before surgery (immediately when an arterial line was in place). The next sample was drawn during surgery, (immediately before high dose of unfractionated heparin was given) and 3, 5, and 10 min after heparin was given, but before the start of ECC. We also measured HBP after 20 and 40 min of ECC. The ECC system was primed with 7000 or 7500 U of heparin which was not included in the initial dose of heparin. While on ECC, patients were given extra doses of heparin according to the local practice with the help of the HeProCalc perfusion system algorithm.[29] The HBP concentrations were also measured just before the patients were given protamine sulfate, and then 3 and 15 min after the whole protamine dose was given. The protamine was given over a median time of 16 min. The last sample was taken 3 h after ECC. Samples for measurement of MPO and syndecan-1 were taken before heparin, 3 min after heparin, after 20 and 40 min of ECC, and immediately before and 15 min after protamine.

Low-molecular-weight heparin

Patients who had undergone major general surgery and received 5000 U of dalteparin after surgery subcutaneously as postoperative thromboprophylaxis were included. We measured HBP before dalteparin administration and 30, 60, 120, 180, and 300 min after. These patients had undergone the following operations: laparotomy because of colon/rectal cancer (n=9), laparotomy because of ovarian cancer (n=5), robotic-assisted radical cystoprostatectomy (n=3), laparotomy because of sarcoma in the pelvic area (n=1), and robotic-assisted abdominoperineal resection (n=1).

Table 1 Preoperative and perioperative patient characteristics. Data are displayed as median (inter-quartile range) for continuous variables and numbers for categorical variables. ACT, activated clotting time; CRP, C-reactive protein; ECC, extracorporeal circulation; GFR, glomerular filtration rate; GI, gastrointestinal; HBP, heparin-binding protein; INR, international normalised ratio; LMWH, low-molecular-weight heparin; NYHA, New York Heart Association; TIA, transient ischaemic attack; WBC, white blood cell count.

	Low-dose heparin vascular surgery (n=10)	High-dose heparin cardiac surgery (n=10)	Low-molecular-weight heparin major abdominal surgery (n=19)
Age (yr)	74 (60–78)	64 (58–67)	64 (61–77)
Male sex	6	9	6
Creatinine (μmol L^{-1})	91.5 (68.0–129.3)	83.0 (77.3–97.0)	73.0 (57.0–87.0)
GFR (ml min^{-1} 1.7 m^{-2})	63 (43.8–83.0)	74.5 (62.3–78.5)	64.0 (55.0–85.0)
INR	1.1 (1.0–1.1)	1.0 (1.0–1.0)	1.0 (1.0–1.1)
WBC ($n\times10^9$ L^{-1})	6.8 (5.3–8.3)	7.9 (5.9–9.1)	7.9 (6.2–10.4)
CRP (mg L^{-1})	5.6 (3.0–12.1)	1.0 (1.0–3.3)	4.0 (1.0–12.8)
Anaesthesia time (min)	279 (212–317)	282 (239–342)	395 (268–532)
Surgery time (min)	173 (127–243)	170 (134–203)	313 (175–409)
Operation start − to given heparin (min)	35 (29–79)	14 (7–16)	-
Operation end to given LMWH (min)	–	–	290 (236–457)
General anaesthesia	6	10	19
Steroids during surgery	6	8	18
Total bleeding (ml)	50 (0–125)	525 (319–750)	700 (200–900)
ECC time (min)	–	103 (78–117)	–
Aortic cross-clamp time (min)	–	67.5 (53.5–82.5)	–
Time ECC start to blood sampling before protamine (min)	–	93.5 (68.5–111.0)	–
Heparin start dose (U)	–	35 000 (27 500–40 000)	–
Total heparin dose (U)	–	50 000 (35 000–63 125)	–
Protamine (mg)	–	225 (188–300)	–
Protamine administration (min)	–	16 (13.5–18.75)	–
Baseline ACT	–	142 (125–146)	–
ACT 3 min after heparin	–	619 (530–666)	–
ACT 3 min after protamine	–	116 (115–119)	–

Blood sampling and biomarker analysis

All blood samples were drawn from an indwelling arterial line, except two samples that were drawn through an indwelling central vein in one patient in the high-dose heparin group who had a temporary arterial line problem. Samples were centrifuged within 20 min at 2000 *g* for 10 min and plasma stored at −80°C.

HBP was analysed in duplicate using the Axis-Shield HBP microtitre plate enzyme-linked immunosorbent assay (ELISA; Axis-Shield Diagnostics, Dundee, UK). The ELISA has been tested by the manufacturer in the presence of heparin concentrations up to 16.3 U ml^{-1} without interfering with the HBP measurements.

Additionally, HBP was assayed in duplicates by a dry fluorescence immunoassay analyser (Joinstar FIC-Q100; Joinstar Biomedical Technology, Hangzhou, ZJ, China) according to the manufacturer's recommendations. This method is a new point-of-care device with the lower detection limit of 5.9 ng mL^{-1} and higher detection limit of 300 ng ml^{-1} for HBP. This method has been tested by the manufacturer in the presence of heparin concentrations up to 16.3 U ml^{-1} without interfering with the HBP measurements.

MPO and syndecan-1 were analysed in duplicate using the Human MPO Instant ELISA Kit Invitrogen and the Human Syndecan 1 (SDC1) ELISA Kit, Invitrogen, respectively.

Heparin interference with HBP measurements

To investigate any interference of heparin in the measurement of HBP, blood samples were drawn from three individuals at baseline in the LMWH group. Both whole blood and plasma were treated *in vitro* with increasing concentrations of heparin (0–125 U ml^{-1}).

Statistics

Power analysis was not performed before including patients as there were no previous data on the effect of heparins on HBP. We started with 10 patients in each group and data were analysed. In the LMWH group, we noticed a trend and decided to add 10 more patients. Data were not considered normally distributed. The Friedman test with Dunn's multiple comparisons test was used for multiple comparison of HBP changes over time in response to the heparins. Comparison between Axis-Shield ELISA and Joinstar FIC-Q100 HBP values was performed with Spearman correlation and Bland–Altman analysis. Samples with Joinstar FIC-Q100 values of <5.9 and >300 ng ml^{-1} were not compared with Axis-Shield ELISA because of these minimum/maximum limits in the former analysis. A P-value <0.05 was considered statistically significant. Statistical analyses were performed using Prism 9 (Graphpad Inc., San Diego, CA, USA). Data are presented as median and inter-quartile range and depicted as box plots.

Results

Patient characteristics

Flowcharts of the studied patients are shown in Fig 1. In the low- and high-dose heparin groups, all recruited patients fulfilled the study protocol. In the LMWH group, 28 patients were recruited but 19 fulfilled the protocol. Eighteen patients had received 5000 U of dalteparin the night before their surgery, approximately 24 h before entering the protocol. One patient received 15 000 U approximately 36 h before the blood sampling. None of the patients in the low- and high-dose heparin groups received LMWH the day before surgery. Perioperative patient characteristics are shown in Table 1.

Low-dose heparin group

In the vascular surgery patients receiving 3000–7500 U of heparin, the HBP concentration increased from 3.6 (2.4–5.4) ng ml^{-1} immediately before heparin to 21.4 (9.0–35.4) ng ml^{-1} 2 min after administration (Fig 2a). Maximum concentrations of HBP were seen after 10 min, 33.4 (15.3–41.5) ng ml^{-1} and these were sustained over the 60-min observation period (Fig 2a, Table 2).

High-dose heparin group

The baseline HBP concentration in the cardiac surgery patients was 3.2 (1.9–4.7) ng ml^{-1}. After thoracotomy and cannulation of the heart, the concentration increased to 5.3 (2.7–6.1) ng ml^{-1}. After a median dose of 35 000 (27 500–40 000) U of heparin, the HBP concentration increased within 3 min to 49 (38–70) ng ml^{-1} (Fig 2b, Table 2). The concentration did not change significantly in the period before EEC. The median time from the 10-min heparin blood sampling until the start of ECC was 8.5 min. During ECC, the plasma HBP concentration gradually increased to a peak of 248 (190–374) ng ml^{-1} just before the initial dose of protamine was given, when the patients had just been taken off the ECC. During ECC, seven patients received extra heparin according to a local protocol based on the patient's age, sex, height, weight, and measurements of activated clotting time[29]: five patients needed heparin repeatedly (Supplementary Table S1). However, the increase in HBP concentration during ECC did not differ between these seven patients and the three patients not receiving extra heparin (Δ211 (136–421) *vs* Δ232 (195–255) ng ml^{-1}, respectively). The high concentrations of HBP decreased significantly 3 min after protamine administration but remained significantly higher than those at baseline even 3 h after the end of ECC (63 ng ml^{-1}, 47–78). There was no correlation between ECC time and HBP concentration (data not shown).

Myeloperoxidase

Similar to HBP, plasma MPO concentration increased rapidly after administration of heparin, from 27.8 (26.6–30.2) to 146.0 (124.1–221.6) ng ml^{-1} (Fig 2d), increasing further during ECC to a maximum of 280.1 (152.4–341.7) ng ml^{-1}. A rapid decrease in plasma MPO concentration was observed after administration of protamine, but this decline was smaller compared with the effect on HBP.

Syndecan-1

The concentration of syndecan-1 at baseline was 3.3 (2–4) ng ml^{-1} and it increased slowly during surgery to reach a maximum of 6.2 (3.8–8.0) ng ml^{-1} at 15 min after protamine administration. One patient was an outlier with concentrations

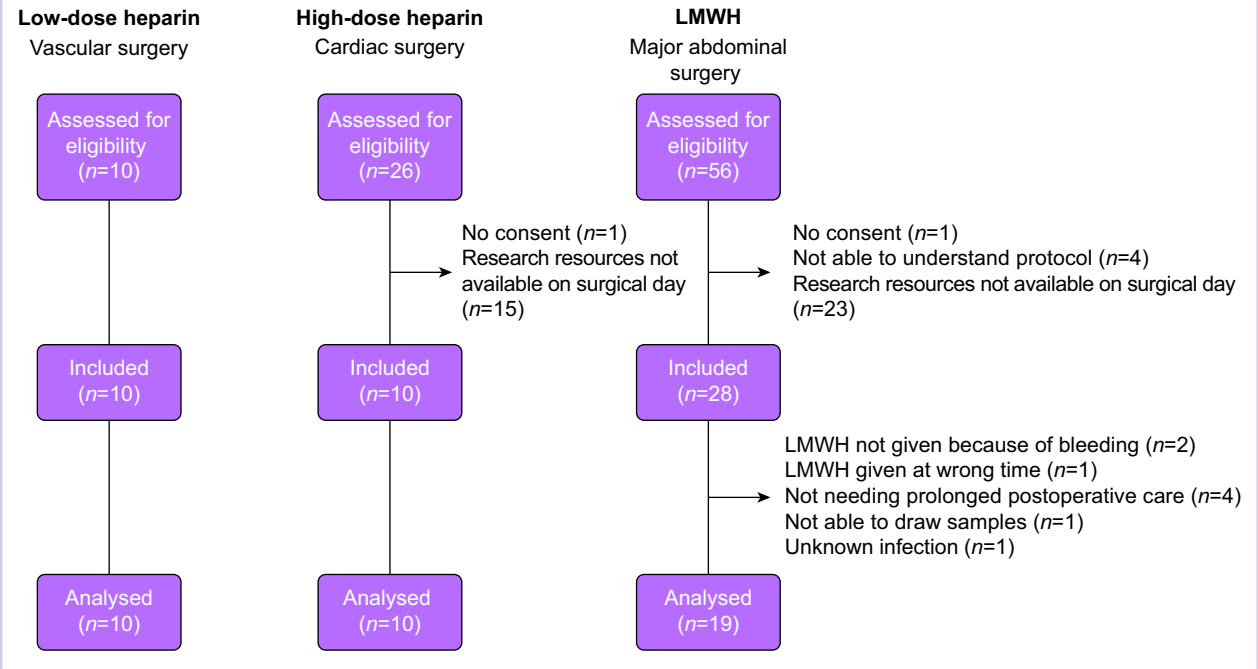

Fig 1. Enrolment of patients. LMWH, low-molecular-weight heparin.

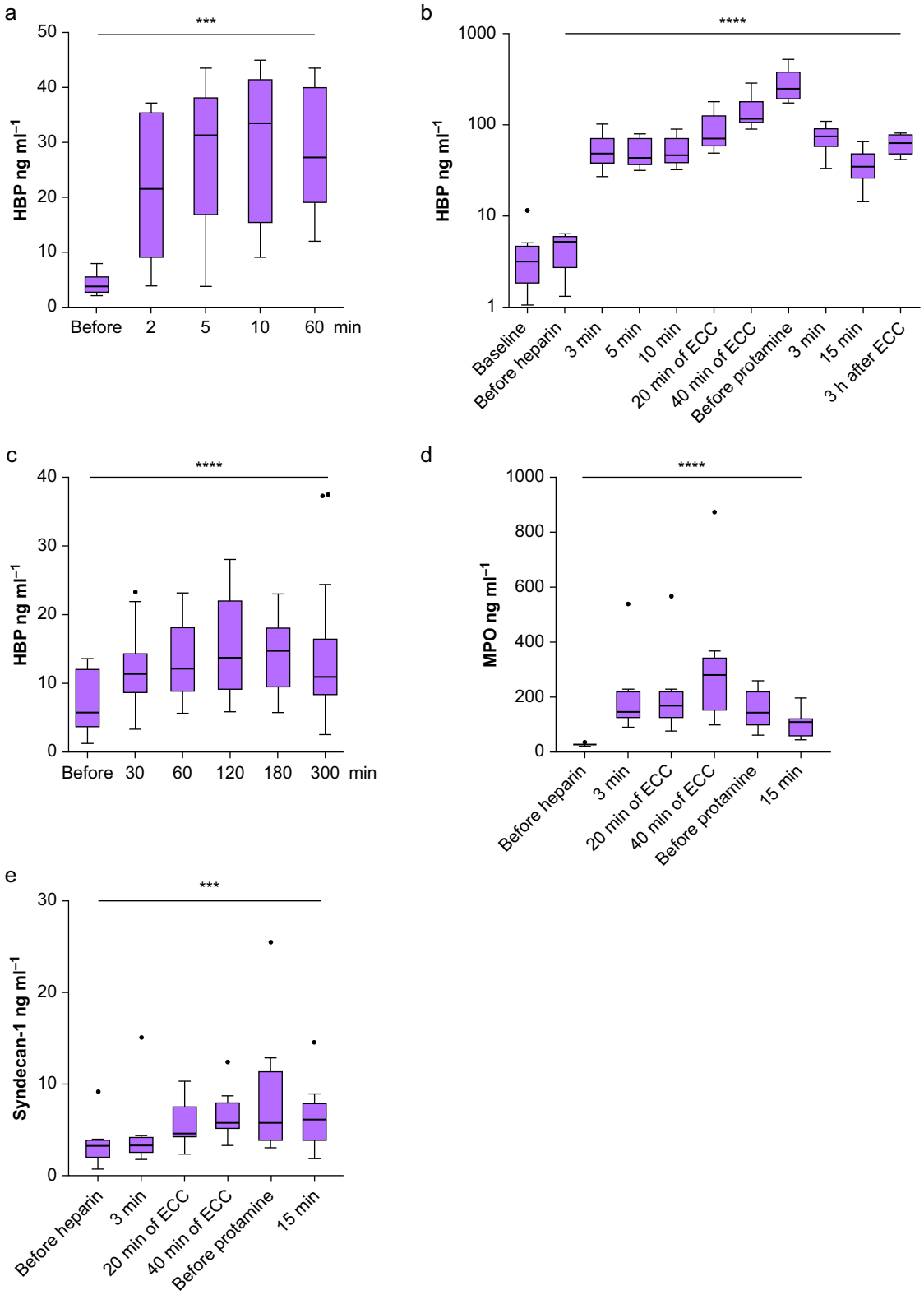

Fig 2. Plasma concentrations of heparin-binding protein (HBP), myeloperoxidase (MPO), and syndecan-1. Data displayed as Tukey box and whisker plots with the median indicated by the horizontal line. (a) Low-dose heparin during vascular surgery (n=8–10). (b) High-dose heparin during cardiac surgery (n=9–10). (c) Low-molecular-weight heparin after major abdominal surgery (n=19). (d) MPO (n=9–10). (e) Syndecan-1 (n=8–10). Friedman non-parametric repeated measures analysis of variance. ***$P<0.001$ and ****$P<0.0001$. ECC, extracorporeal circulation.

Table 2 Plasma concentrations of heparin-binding protein (ng ml^{-1}) in the patient groups. Data are displayed as median (inter-quartile range). In low-dose heparin group: n=8 at time point 60 min after heparin. In high-dose heparin group: n=9 at time point 3 min after heparin. ECC, extra corporeal circulation; LMWH, low-molecular-weight heparin.

Time points	Low-dose heparin (n=10)	High-dose heparin (n=10)	LMWH (n=19)
Before heparin	3.6 (2.4–5.4)		
2 min after heparin	21.4 (9.0–35.4)		
5 min after heparin	31.1 (16.7–37.6)		
10 min after heparin	33.4 (15.3–41.5)		
60 min after heparin	27.2 (19.0–40.9)		
Preoperative		3.2 (1.9–4.7)	
Before heparin		5.3 (2.7–6.1)	
3 min after heparin		48.7 (38.4–70.1)	
5 min after heparin		43.4 (36.6–71.3)	
10 min after heparin		46.9 (38.6–69.6)	
20 min of ECC		71.4 (57.9–127.2)	
40 min of ECC		116.5 (106.2–179.6)	
Before protamine		248.2 (189.9–373.7)	
3 min after protamine		75.1 (58.2–91.2)	
15 min after protamine		34.4 (25.9–48.2)	
3 h after ECC		62.9 (47.4–78.3)	
Before LMWH			5.7 (3.7–12.1)
30 min after LMWH			11.4 (8.7–14.4)
60 min after LMWH			12.1 (8.9–18.2)
120 min after LMWH			13.8 (9.2–22.1)
180 min after LMWH			14.8 (9.5–18.1)
Before heparin			11.0 (8.4–16.5)

of 271–277 ng ml^{-1}. Syndecan-1 concentration was not affected either by heparin or protamine administration (Fig 2e).

Low-molecular-weight heparin group

In the postoperative period after abdominal surgery, all patients were given 5000 U of dalteparin subcutaneously which led to a gradual increase in plasma HBP concentration. The temporal profile was different compared with i.v. heparin with a maximum concentration of 14.8 (9.5–18.1) ng ml^{-1} occurring 180 min after heparin administration (Fig 2c, Table 2).

Point-of-care measurements of HBP

In all patient groups, a parallel analysis of HBP was performed comparing the point-of-care analyser Joinstar FIC-Q100 with the Axis-Shield ELISA method. There was a strong correlation between the two methods (r=0.94, P<0.0001, Fig 3d), but the Joinstar FIC-Q100 values were often slightly higher than the values measured by the Axis-Shield ELISA (Fig. 3a–c). Bland–Altman plots of the patient groups showed a bias between +4.6–8.9 ng ml^{-1} and there was a significant proportional bias in the low-dose heparin and LMWH groups (Supplementary Fig. S1).

Interference

To investigate if heparin would interfere with the Axis Shield-ELISA or Joinstar FIC-Q100, we spiked blood and plasma from three individuals *ex vivo* with increasing concentrations of heparin. Measurements of HBP with the Axis-Shield ELISA were not significantly affected by heparin in blood or plasma (Supplementary Tables S2 and S3). The values obtained with Joinstar FIC-Q100 after spiking of blood were also not significantly affected by heparin. A significant proportion of values in plasma were found to be below the detection limit of the Joinstar FIC-Q100 method, rendering analysis unfeasible. HBP concentrations were significantly higher in blood than in plasma, regardless of additional heparin (P<0.001).

Discussion

In this study, we show that heparins dose-dependently increase plasma concentrations of HBP and that these effects were seen within a few minutes of administration. Conversely, HBP concentrations rapidly decreased after protamine administration but remained above baseline. EEC seems to further increase HBP concentration. Heparin administration also increased plasma concentrations of the neutrophil granule protein MPO.

As indicated by the name, HBP can bind to heparins, as demonstrated in numerous *in vitro* studies.[4,23,24] However, the effect of heparins on plasma concentrations in humans has not been extensively studied. We suggest that the rapid increase in plasma HBP observed in our study upon heparin administration is primarily attributed to the binding and detachment of HBP from endothelial glycosaminoglycans. Subsequently, the heparin–HBP complexes become evident in plasma. However, there may also be a contribution from HBP attached to or released from neutrophils. In a normal homeostatic environment, HBP bound to glycosaminoglycans most likely has a minor contribution to HBP plasma concentrations.[2] In the present study, we did not investigate whether HBP bound to heparin is still biologically active, but animal and *in vitro* studies suggest that HBP is inactivated when bound to heparin.[3,5,20,30] This is further supported by the fact that the very high HBP concentrations observed in the cardiac surgery patients did not seem to induce clinically relevant endothelial dysfunction or oedema formation, which are known effects of high HBP concentrations in other situations.[7,8,31]

The rapid dynamics of HBP in relation to i.v. heparin are interesting. The vascular surgery patients were given a single dose of heparin (3000–7500 U) and within 2 min we found a

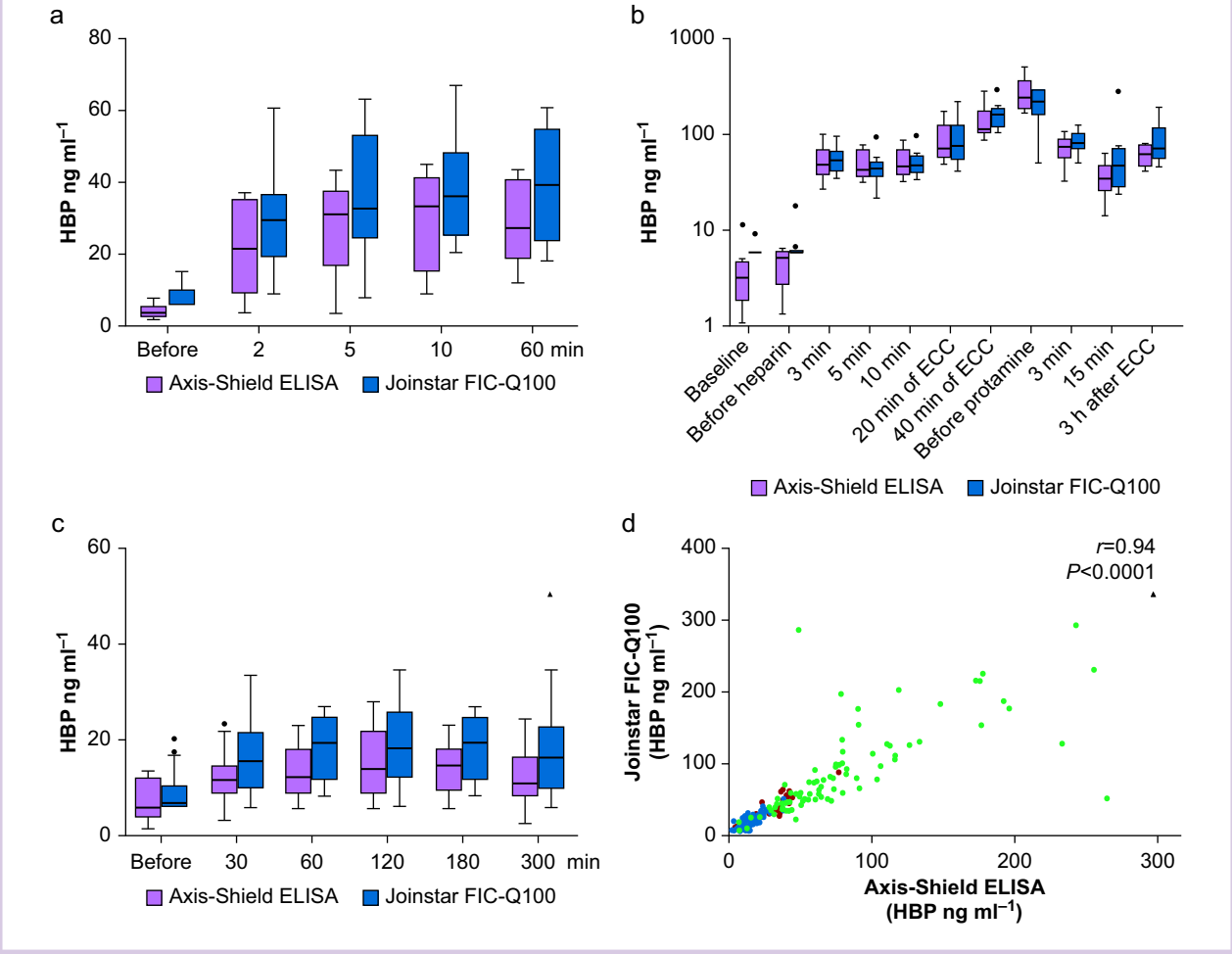

Fig 3. Comparison of Axis-Shield ELISA and Joinstar FIC-Q100 point-of-care plasma concentrations of heparin-binding protein values. (a) Low-dose heparin during vascular surgery. (b) High-dose heparin during cardiac surgery. (c) Low-molecular-weight heparin after major abdominal surgery. (d) Correlation between heparin-binding protein levels measured by Axis Shield-ELISA and Joinstar FIC-Q100 point-of-care methods. Data displayed as Tukey box and whisker plots. Red colour denotes the low-dose heparin group, green colour the denotes high-dose heparin group, and blue colour denotes the low-molecular-weight heparin group. ECC, extracorporeal circulation.

six-fold increase in HBP concentration. Most of the baseline measurements were taken 1 min prior to heparin and therefore it is unlikely that the surgery *per se* would have affected the HBP concentration. Similarly, a higher bolus dose of heparin in the cardiac surgery patients resulted in nine-fold increase in HBP within 3 min. Moreover, the concentration was lowered more than three-fold within only 3 min of protamine administration. Remarkably, this indicates an exceptionally rapid effect of heparin on HBP dynamics. The positively charged binding site of HBP binds with electrostatic forces to the negatively charged sites of glycosaminoglycans and heparin.[2] The administration of highly negatively charged heparin competes with the binding of HBP to the negatively charged glycosaminoglycans rendering HBP–heparin complex measurable in blood. Similarly, electrostatic forces are the cause of heparin–protamine binding capacity resulting in release of HBP from heparin.[32]

We found three other studies where plasma HBP concentrations were measured during cardiac surgery where heparin anticoagulation was used.[15,33,34] These studies have shown high HBP values with as much as a 39-fold increase.[33] The effects and timing of heparin administration were not considered in two of these studies.[33,34] However, a recently published study[15] shows a direct effect of heparin on HBP concentration which is confirmed by our results. In this study, the HBP concentration decreased in a similar manner when the heparin effects were neutralised with protamine. Furthermore, in elegant additional *in vitro* experiments, Sterner and colleagues show that heparin does not induce HBP release from neutrophils but extracts HBP from the endothelial cell surface.[15]

Elevated plasma concentrations of HBP have been associated with trauma[16] but there are, to our knowledge, no published studies on HBP concentration in patients undergoing noncardiac surgery. However, in one study only a minor increase in plasma HBP concentration, from 7.1 to 11.0 ng ml^{-1}, was observed after lung surgery with thoracotomy.[15] In the present study, opening the thorax and cannulating the aorta/

heart did not lead to a relevant increase in HBP; 5.3 ng ml^{-1} compared with 3.2 ng ml^{-1} at baseline before anaesthesia. In addition, the MPO concentration did not increase during the same period, indicating no substantial neutrophil activation. Moreover, the patients in the LMWH group that had undergone major abdominal surgery did not have high postoperative HBP concentrations (5.7 ng ml^{-1}). Together, these findings suggest that surgery *per se* does not lead to a significant increase in HBP.

The exposure of blood to the ECC circuit results in a systemic inflammatory response with widespread activation of neutrophils.[35–37] Therefore, we also wanted to measure MPO in the high-dose heparin group, as an additional neutrophil-derived protein only found in the primary granules. We observed an additional increase in HBP and in MPO during the ECC period. However, the plasma profiles of HBP and MPO seemed to differ during EEC, where MPO increased slower than HBP upon ECC and started to decrease earlier when ECC circuit flows normally are lowered before protamine administration. As several patients received extra heparin during this period, it is somewhat difficult to differentiate whether HBP increased as a result of the extra heparin given or because of widespread activation of neutrophils. However, as this increase seemed similar between patients receiving and not receiving additional heparin, it is likely that ECC itself was the cause of the increase in HBP. The fact that MPO from primary granules was also increased and seemed to display a different pattern, supports the latter.

Protamine is a cationic molecule that binds with anionic heparin to form a stable complex to reverse heparinisation. Protamine could therefore compete with HBP for heparin binding, resulting in displacement of HBP from heparin, rendering HBP free and able to bind to glycosaminoglycans on the endothelium. In contrast to the rapid increase in HBP after heparin, HBP gradually decreased after protamine administration even if the anticoagulant effect was more or less instantly abolished. We cannot explain this delay in HBP disappearance from plasma as both the kinetics of HBP binding back to the glycocalyx and metabolism of HBP are unknown. It is interesting that the HBP concentration increased again to 63 ng ml^{-1} 3 h after ECC. A possible explanation is rebound effects of heparin as the half-time of protamine is only a few minutes[38] compared with a half-time of 1–2 h for heparin. There is also the possibility that disturbances in glycocalyx affinity for HBP plays a role, hindering free HBP from binding again to the glycocalyx during the first hours after surgery. This is partly supported by the increase in syndecan-1, indicating glycocalyx degradation. However, such high concentrations of HBP have been coupled with endothelial dysfunction and oedema formation which was not evident in our patients.

We also observed that subcutaneous LMWH in a thromboprophylaxis dose affects HBP concentration, albeit to a smaller degree compared with i.v. heparin. To our knowledge, there are no clinical studies published on LMWH in relation to HBP concentration. HBP has been suggested as a useful biomarker for predicting outcome in patients with severe infections[5,6,8] and in the ICU setting, high plasma HBP has been associated with severity of disease and increased risk of death.[5,11] With present findings in mind, clinical studies are needed in septic patients receiving LMWH, as they may have higher baseline concentrations of HBP which may lead to an augmented effect of LMWH on plasma HBP in this group of patients. This may interfere with the use of HBP as a prognostic biomarker in sepsis.

We found that the Joinstar FIC-Q100 point-of-care HBP measurements were comparable with those with Axis-Shield ELISA. The correlation between these HBP methods was good, but the Joinstar FIC-Q100 measurements were constantly higher. There was a proportional bias with increasing concentrations of HBP. However, we believe that Joinstar FIC-Q100 is clinically acceptable when used to follow bedside trends, such as in patients with infections or sepsis.

HBP and MPO have been suggested to be involved in promoting endothelial dysfunction and leakage leading to oedema formation.[2,3,27] It can be speculated that the anti-inflammatory effects of heparins arise partly from binding to, and neutralisation of, these and other proteins. Novel heparinoids with low anticoagulant activity are being developed as anti-inflammatory agents.[19] One such heparinoid is sevuparin which inhibits neutrophil-induced endothelial cell activation, counteracts endothelial cell gap formations, and has been tested in clinical situations including sickle cell disease and malaria.[20–22] Whether these heparinoids have the same effects on HBP plasma dynamics as heparin is still unknown, but if so, one could speculate that HBP, especially with a point-of-care method, could be used as a marker to monitor effects of heparinoids. In addition, it would also be interesting to measure the effects of LMWH in patients known to have elevated HBP concentrations, such as in sepsis, as one may speculate that heparin may have an even greater effect in these patients. If so, this may influence the value of HBP as a predictive biomarker in sepsis.

In conclusion, plasma concentrations of HBP are rapidly and dose-dependently increased by heparin. Furthermore, subcutaneous administration of LMWH increases plasma HBP, but to a lesser degree. During cardiac surgery, protamine significantly lowers plasma concentration of HBP. Similar effects of heparin are seen on MPO. The observed effects support the view of heparins as anti-inflammatory agents, by binding and inactivation of proteins involved in vascular inflammation.

Author's contributions

Conceptualisation: HH, EW, AO
Study design: HH, EW, AO
Recruitment of participants: HH
Laboratory measurements: AE
Data collection/analysis: HH, EW, AO, LL
Manuscript preparation and approval: all authors

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bjao.2023.100256.

Declarations of interest

EW and LL are scientific advisors to Modus Therapeutics. The other authors declare that they have no conflicts of interest.

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