Heparin's effect on primary hemostasis detected by PFA-200

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Heparin is widely used in clinical practice; its effect on secondary hemostasis is well known, but its effects on primary hemostasis are controversial. The Innovance® Platelet Function Analyzer-200™ (PFA-200) performs a group of tests that evaluate the primary hemostasis of whole blood. It is frequently used in critically ill patients, but the effect of heparin on its results expressed as closure time is controversial. The purpose of this study was to describe whether different types and doses of heparin may influence closure time results on PFA-200 devices. For this study, 30 patients were recruited and divided into three groups based on the type and dose of heparin being administered. The first group included ten patients who were treated with a high dose of unfractionated heparin (concentration from 3 to 4 mg/kg) before commencement of cardiopulmonary bypass during cardiac surgery. The second group consisted of ten patients from both vascular and thoracic surgery who were administered a low dose of unfractionated heparin (concentration 1 mg/kg). The remaining ten patients from intensive care units received a prophylactic dose of low-molecular-weight heparin monitored by anti-Xa. We compared closure times on PFA-200 devices with collagen/ADP and collagen/epinephrine cartridges before and after anticoagulant administration. The results showed that only a high dose of unfractionated heparin prolonged the collagen/ADP closure time. The other groups failed to show any difference. We consider this finding to be important for clinicians using extracorporeal systems to differentiate primary hemostasis pathology caused by heparin or by extracorporeal devices themselves.

Key words: heparin, platelets, coagulation, PFA-200.

Účinek heparinu na primární hemostázu měřený pomocí PFA-200

Heparin je v klinické praxi široce používán; jeho účinek na sekundární hemostázu je dobře známý, ale jeho účinky na primární hemostázu jsou kontroverzní. Analyzátor funkcí destiček Innovance®-200 ™ (PFA-200) provádí skupinu testů, které hodnotí primární hemostázu plné krve. Často se používá u kriticky nemocných pacientů, ale účinek heparinu na jeho výsledky vyjádřené jako čas srážení („closure time") je kontroverzní. Účelem této studie bylo popsat, zda různé typy a dávky heparinu mohou ovlivnit výsledky PFA-200. V této studii bylo hodnoceno 30 pacientů, kteří byli rozděleni do 3 skupin podle typu a dávky podávaného heparinu. První skupina zahrnovala 10 pacientů, kteří byli léčeni vysokou dávkou nefrakcionovaného heparinu (3 až 4 mg/kg) před zahájením kardiopulmonálního bypassu během operace srdce. Druhá skupina byla tvořena 10 pacienty z cévní i hrudní chirurgie, kterým byla podávána nízká dávka nefrakcionovaného heparinu (1 mg/kg). Posledních 10 pacientů z jednotky intenzivní péče dostalo profylaktickou dávku nízkomolekulárního heparinu monitorovaného pomocí anti-Xa. Porovnávali jsme čas srážení na zařízení PFA-200 a to testy kolagen/ADP a kolagen/epinefrin před a po podání antikoagulancií. Výsledky ukázaly, že pouze vysoká dávka nefrakcionovaného heparinu prodloužila čas srážení u testu kolagen/ADP. Ostatní skupiny nevykazovaly žádný rozdíl. Myslíme si, že toto zjištění je důležité pro kliniky používající mimotělní systémy k rozlišení patologie primární hemostázy způsobené heparinem od patologie způsobené samotnými mimotělními zařízeními.

Klíčová slova: heparin, krevní destičky, koagulace, PFA-200.

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Introduction

Heparin is widely used in clinical practice. Its thrombin inhibition effect through antithrombin is used to prevent thrombosis development and to treat thromboembolic events, especially in critically ill patients. It is also used in patients undergoing extracorporeal membrane oxygenation (ECMO) to prevent circuit clotting, and its use is associated with various bleeding and thrombotic complications [1]. Heparin's effect on secondary hemostasis is well known, but data regarding its effect on primary hemostasis are minimal and controversial. The Innovance® PFA-200™ system is part of a group of tests used to assess primary hemostasis of whole blood. The device evaluates the adhesion of platelets through vWF to the endothelium (collagen) and aggregation upon high shear-stress conditions. The PFA-200 is an updated version of the PFA-100 which has the same mechanical principle, but a new design (a bigger device with touchpad screen and scanner for patient data) [2]. The PFA-100 was introduced in order to provide a simple, rapid assessment of high shear-dependent platelet function using a small amount of citrated blood [3]. Blood samples are aspirated at high shear-stress rates through a capillary in the instrument cartridge and encounter a membrane coated with collagen and epinephrine (PFA collagen/EPI test) or collagen and ADP (PFA collagen/ADP test). The PFA collagen/EPI test is generally used to assess the effect of acetylsalicylic acid on platelets and the PFA collagen/ADP test is used to assess the effect of ADP blockers on platelets. However, both PFA-100 and 200 tests have various clinical applications; it is a useful tool in diagnosing mild/severe von Willebrand diseases, Glanzmann thrombasthenia, Bernard-Soulier syndrome, primary hemostasis pathology during hepatic/renal disorders, and effect of nonsteroidal antirheumatic drugs. It can also be used to monitor mild von Willebrand diseases treated with desmopressin [4], and it may help in the assessment of surgical bleeding risk, especially in cardiac surgery patients undergoing extracorporeal circulation [2, 5]. Both PFA-100/200 test results may be negatively influenced by hematocrit, platelet count, and plasma von Willebrand Factor (VWF) levels [3]. The effect of heparin type and its dose on PFA-100/200 results is not clear. Kottke-Marchant et al. [6] analyzed the effects of unfractionated heparin in vivo (dose of heparin 70–100 units/kg = 0.7–1 mg/kg) using the PFA-100 in patients undergoing coronary angioplasty and showed no changes in the results, while Slaughter et al. in vivo (dose of heparin 300 units/kg = 3 mg/kg) [7] and Williams et al. in vitro (dose of heparin calculated for a 70-kg patient as 60 units/kg = 0.6 mg/kg) [8] found a prolongation in the collagen ADP/closure time after the administration of unfractionated heparin. A study investigating the effect of low-molecular-weight heparin (LMWH) on PFA-100/200 is missing in the literature. Therefore, we decided to perform a study evaluating the effect of different doses of unfractionated heparin (UFH) and low-molecular-weight heparin on PFA-200 results. The purpose of the study was to describe whether different types and doses of heparin may influence the closure time results of PFA-200.

Methods and materials

This study was approved by the Local Ethics Committee of the Motol University Hospital in Prague with the reference number EK-82/20. Informed consent was obtained from all the patients enrolled in the study who were all older than 18 years. Thirty patients were prospectively recruited in the study and a power analysis was not performed, as this was designated as a pilot study. The first group was made up of patients undergoing cardiac surgery (70% coronary bypass, 20% aortic valve replacement, 10% mitral valve replacement) who were administered a high dose of heparin of 3 to 4 mg/kg before the commencement of cardiopulmonary bypass (CPB). The second group consisted of patients undergoing vascular surgery of carotid or aorto-femoral bypasses or those undergoing lung transplantation on ECMO who were administered a low dose of heparin of 1 mg of heparin/kg. The third group was made up of patients with sepsis (with no vasopressors to ensure physiologic absorption of LMWH from subcutaneous tissue) in the intensive care unit (ICU) who were receiving LMWH-enoxaparin subcutaneously (s.c.) every 12 hours as standard prophylaxis of thrombosis. Only those who reached an anti-Xa level of 0.4–0.5 IU/mL were included in the study, thus eliminating possible bias caused by potential variability of efficacy of LMWH in septic patients. Therefore, the Sequential Organ Failure Assessment (SOFA) score or renal function assessment were not necessary in this study. Each group consisted of ten patients.

Each blood sample in all groups was obtained from an arterial catheter (after discarding the first volume of 5 mL of blood) and a volume of 5 mL was collected in a 3.2% sodium citrate VACUETTE® and evaluated by PFA tests after 15–20 minutes of incubation at room temperature. Clotting time (CT) of both collagen/ADP and collagen/epinephrine PFA-200 tests was analyzed in all patients.

Since blood samples of patients treated with clopidogrel did not clot during PFA analysis before and after heparin administration ("no closure" results on both collagen/ADP and collagen/EPI cartridges), they were excluded from the study.

Statistics

A Shapiro-Wilk test of normality was performed to study the data distribution. Since it did not show a normal distribution, the closure times were analyzed in a logarithmic scale: a paired t-test and a Wilcoxon signed-rank test were then performed in all the groups to compare the CT measured with the PFA-200 before and after the administration of heparin for both collagen/ADP and collagen/epinephrine cartridges. The ANOVA test with post-hoc analysis (using Bonferroni correction) was used to compare initial CTs in all groups. The results were considered significant if the P-value < 0.05.

Results

High-dose UFH group

Group 1 (a high dose of UFH) was represented by ten patients (eight males and two females, with an average age of 65 ± 10.08; the average body mass index (BMI) was 29.84 ± 7.12, activated partial thromboplastin time ratio (APTT-r) was 1 ± 0.2, prothrombin time ratio (PT-r) was 1 ± 0.2, platelet count (Plt) was 212 ± 54, and hematocrit (Hct) was 0.40 ± 0.05) with an average dose of UFH of 345 mg ± 111.68. Eight patients were

on 100 mg of acetylsalicylic acid. Blood samples were obtained just before heparin administration and then 5 minutes after administration along with controlled activated clotting time (ACT), which is part of standard procedure. Eight patients were on a long-term dose of 100 mg of acetylsalicylic acid.

Low-dose UFH group

Group 2 (a low dose of UFH) was represented by ten patients (nine males and one female, with an average age of 65.20 ± 9.08 years; the average BMI was 29.67 ± 3.12, APTT-r was 1 ± 0.2, PT-r was 1 ± 0.2, Plt was 207 ± 52, and Hct was 0.36 ± 0.07) with an average dose of UFH of 83.33 mg ± 34.37. Half of the patients underwent lung transplantation surgery while the other half underwent vascular surgery (femoral-popliteal bypass, aortobiiliac bypass, carotid artery surgery, abdominal aorta replacement). Two patients were on a long-term dose of 100 mg of acetylsalicylic acid. Blood samples were obtained just before heparin administration and then 5 minutes after administration along with controlled ACT, which is part of standard procedure.

Low-Molecular-Weight Heparin Group

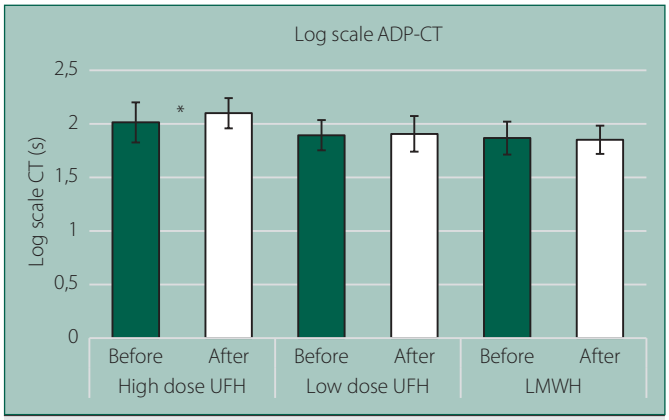
Group 3 (LMWH) was represented by ten patients hospitalized in the ICU (six males and four females, with an average age of 69.1 ± 12.98 years; the average BMI was 28.78 ± 3.63, APTT-r was 1 ± 0.2, PT-r was 1 ± 0.2, Plt was 238 ± 122, and Hct was 0.33 ± 0.04) with an average dose of LMWH of 55 mg ± 20.14 and with anti-Xa level of 0.45 ± 0.07 IU/mL. No patient was on any antiplatelet drug. Blood samples were obtained before the administration of LMWH s.c. and then four hours later simultaneously with anti-Xa control.

The high-dose UFH group with an ACT of 503 ± 178 seconds showed a significant prolongation in ADP-CT after the administration of heparin with a P-value < 0.05 in both the paired t-test and Wilcoxon signed-rank test (Table 1, Graph 1). The collagen/EPI cartridge showed a longer CT after the administration of heparin, but with no statistical significance (Table 1, Graph 2). The low-dose UFH group with an ACT of 240 ± 50 seconds and the LMWH group with an anti-Xa level of 0.45 ± 0.07 IU/mL showed no relevant changes in collagen/ADP or collagen/EPI cartridges (Table 1, Graphs 1 and 2). After analysis of initial CT values among all groups, a borderline significant difference was found between the high-dose UFH and LMWH groups in the collagen/ADP group with p = 0.049. In the collagen/EPI group, there was a significant difference between the high-dose UFH and low-dose UFH groups with p = 0.002 and between the high-dose UFH and LMWH groups with p = 0.009.

Discussion

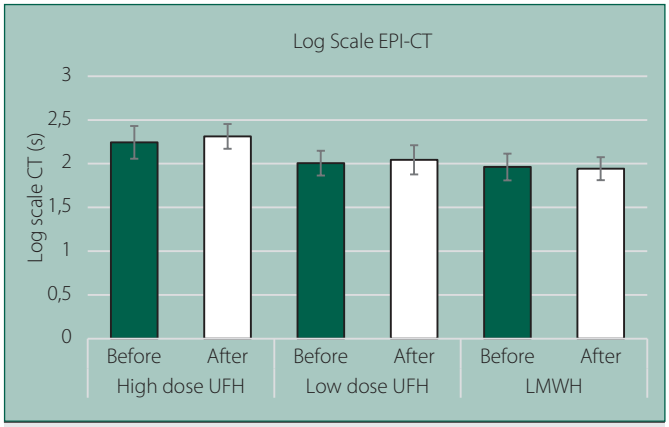
This study suggests that there is a relationship between heparin and primary hemostasis that can be evaluated with the PFA-200. This interaction seems to be dose-dependent and only a high dose of UFH was able to prolong collagen/ADP tests statistically significantly. Low doses

Graph 1. *The PFA-200 closure times of COL/ADP tests in logarithmic scale (seconds). Heparin effect on CT (closure time) in different heparin groups*



*UFH – unfractionated heparin; LMWH – low-molecular-weight heparin; data represented as mean ± SD * represents significant difference (p < 0.05)*

Graph 2. *The PFA-200 closure times of COL/EPI tests in logarithmic scale (seconds). Heparin effect on CT (closure time) in different heparin groups*



UFH – unfractionated heparin; LMWH – low-molecular-weight heparin; data represented as mean ± SD; - there was no significant effect of heparin in any of the groups investigated (p > 0.05)

Table 1. *Log Scale of PFA-200 closure times (seconds)*

Patient group	PFA-200 test	Closure time			
		No Heparin	Heparin	P-value paired T	P-value Wilcoxon
High-dose UFH	ADP	2.01 ± 0.11	2.10 ± 0.15	0.012	0.015
	EPI	2.24 ± 0.19	2.31 ± 0.14	0.296	0.374
Low-dose UFH	ADP	1.89 ± 0.16	1.91 ± 0.17	0.179	0.822
	EPI	2.01 ± 0.14	2.04 ± 0.17	0.493	0.552
LMWH	ADP	1.87 ± 0.09	1.85 ± 0.11	0.701	0.260
	EPI	1.96 ± 0.15	1.94 ± 0.13	0.584	0.445

UFH – unfractionated heparin; LMWH - low-molecular-weight heparin; ADP – collagen/ADP test; EPI – collagen/epinephrine test

of UFH or LMWH do not have a negative effect on primary hemostasis evaluated by PFA-200 tests.

Sobel et al. demonstrated that heparin led to an inhibition of von Willebrand Factor-platelet binding [9]. This finding may explain the mechanism of CT prolongation in the high-dose UFH group. They studied the blood collected from 12 patients after the administration of intravenous porcine heparin before the commencement of CPB during coronary artery bypass surgery. The results were obtained by measuring ristocetin-induced agglutination of normal formaldehyde-fixed platelets which were suspended in the patients' plasma. The authors also used bovine vWF because of its capacity to bind spontaneously to platelet glycoprotein Ib without the presence of ristocetin as a cofactor, and arrived at the same results. These findings suggest that vWF inhibition was caused by heparin excluding ristocetin as a possible cause, concluding that heparin may interfere with vWF-glycoprotein Ib binding in a dose-dependent manner (a concentration of 80–140 U/mL of heparin in plasma completely suppressed agglutination, while 1–5 U/mL produced 10–15% inhibition). Moreover, Kroner and Frey studied heparin and vWF using heparin-Sepharose CL-4B; the substitution of lysine residues 642–645 in the vWF A1 domain determined a reduction in the interaction [10]. The authors suggest that this may indicate a possible heparin-binding domain in vWF causing the inhibition of vWF-dependent platelet hemostatic function. This might explain the prolongation of CT in collagen/ADP tests performed in our study in the group that received a high dose of UFH. The prolongation of collagen/ADP closure time related to a high dose of UFH has also been previously observed by Williams when comparing in vitro effects on CTs evaluated by PFA-100's in healthy individuals by adding UFH directly in sample tubes [8]. Slaughter et al. also demonstrated a prolongation in the collagen/ADP CT related to heparin administration in patients undergoing cardiac surgery by using a high dose of the anticoagulant (ACT required to be maintained greater than 480 s, by administering 300 U/kg bolus of porcine heparin and adding 5000 U if needed). The authors also detected a prolongation of closure time after the commencement of CPB. The administration of protamine sulfate reduced the closure time, but with no statistical significance; these findings may suggest that CPB itself contributed to prolongation [7]. Griffin et al. studied the interaction between heparin and platelets by analyzing platelet function under high shear stress. They added 4 and 20 µg/mL of heparin in blood samples of 12 healthy volunteers and then analyzed them with a Clot Signature Analyzer. The authors suggest that high-dose heparin is able to inhibit both secondary thrombin activity and platelet functional alteration causing an inhibition of platelet function [11].

On the contrary, different authors suggest that heparin has a pro-aggregating effect on platelets. UFH has a greater lipolytic activity than low-molecular-weight heparinoid [12]. This may cause higher levels of non-esterified fatty acids determining the inhibition of the ADPase enzyme. This way, ADP is not converted to adenosine, which normally inhibits platelet aggregation and promotes vasodilation [13]. In addition, higher levels of fatty acids may reduce prostacyclin antiaggregating activity [14]. Therefore, the pro-aggregating effect determines a fall in platelet count, which may turn the blood sample to a refractory form that could explain the longer CT [15].

Interestingly, we discovered that collagen/EPI did not significantly prolong CT after the administration of a high dose of UFH. Williams found the same results in healthy patients after verifying the absence of aspirin in blood samples [8]. Thus, these data suggest that collagen/EPI is not influenced by heparin, excluding antiplatelet therapy as a possible cause of a lack of interaction.

On the other hand, a high dose of UFH influences collagen/ADP CT and this is not the case for low dose UFH regimen. Therefore, any PFA-200 CT alteration found in patients treated with a low dose of UFH (1 mg/kg) or LMWH seems not to be related to the anticoagulant. The results of this study support our recently published data where the PFA-200 test was used to detect ECMO-related pathology of primary hemostasis. We found that in patients on short-term ECMO (perioperative use during lung transplantation) or long-term ECMO (indicated because of cardiac or respiratory failure) there was significant pathology-prolongation of CT of both PFA-200 tests (collagen/ADP and collagen/epinephrine). In that study during perioperative short-term ECMO support, a low dose of UFH (1 mg/kg) was used and, for long-term ECMO support, only a prophylactic dose of LMWH was used with an anti-Xa level of 0.4–0.6 IU/mL (no UFH was used to prevent clotting of the ECMO device) [16]. The finding of that study that ECMO produces primary hemostasis pathology detected by PFA-200 is supported by our results which failed to show a negative effect of low-dose UFH or prophylactic dose of LMWH on PFA-200 tests.

Study limitations

A limitation of the study is that we were not able to enroll only patients without antiplatelet drugs. In practice, most of the patients undergoing CPB are on some antiplatelet drug. Therefore, collagen/epinephrine cartridges in the high-dose UFH group showed a longer CT before the administration of the anticoagulant. These findings are likely related to the preoperative chronic intake of 100 mg of acetylsalicylic acid. There was also a similar prolongation of CT in collagen/ADP cartridges in the high-dose UFH group although the cause of this phenomenon is not clear.

Another limitation of this study might be evaluating only a prophylactic dose of LMWH on PFA-200. However, there are not enough of patients on a therapeutic dose of LMWH (with an anti-Xa level of 0.6–1.2 IU/mL) in the perioperative or postoperative periods.

Conclusion

In our study, a high-dose UFH regimen significantly prolonged the closure time of collagen/ADP tests, which is not the case for collagen/EPI tests. On the other hand, a low-dose UFH regimen and the use of LMWH did not seem to negatively influence both collagen/EPI and collagen/ADP tests.

This finding is important for clinicians using extracorporeal systems to differentiate primary hemostasis pathology caused by heparin or by the extracorporeal device itself.

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