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Evidence of in vivo platelet activation during heparin anticoagulation in pregnancy for prophlaxis or treatment of thromboembolism

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Summary

Conventional unfractionated heparin substantially enhances spontaneous platelet aggregation in pregnancy in vitro, and may cause platelet activation in healthy volunteers in vivo. It is unknown, however, whether therapeutically administered heparin affects platelet behavior during pregnancy. In a parallel group ex vivo study, 8 third trimester pregnant patients requiring anticoagulation with heparin exhibited a trend to a greater spontaneous platelet aggregation, in comparison to 11 age-matched healthy third trimester pregnant controls. This is consistent with heparin-induced platelet activation in vivo during therapeutic anticoagulation. Peak aggregation in the heparin-treated group was 48 ± 4% compared to 37 ± 5% in the healthy controls, (P = 0.086 ANOVA); and significant time treatment interaction (P = 0.03 ANOVA). There was also a weak positive correlation (r = 0.54) between the peak % spontaneous platelet aggregation and the activated partial thromboplastin time ratio during heparin administration.

Keywords: *In vivo platelet activation, Pregnancy, Unfractionated heparin, Thromboembolism, APTT ratio.*

Résumé

L'heparine conventionel infractione augmente de maniere substantielle l'aggregation spontanee des platelettes sanguine in vitro pendant la grossesse. Ceci pourrait cause l'activation des platelettes chez les volontaire en bonne sante in vivo. Il n'est pas encore connue, cep-endant si l'heparine, pendant la grossesse. Dans un groupe parallele etudie ex-vivo, 8 patients ayant une grossesse du 30 trimestre et necessitant une angicoaligation avec l'hep arine ont exhibe une tendance a une grande aggregation spontanee des platellettes compare a un cas similarire chez 11 femmes et en bonne sante au troisieme trimestre de leur grossesse. Le resultat est conoistant avec l'activation des platelletes in vivo induite par l'heparine pendant la therapy de l'anticoagulation. Le pick de l'aggregation chez les femmes traits a l'heparin avait ete de 48 ± 4% compare a 37, ± 5% chez, les controles bien portant (P = 0,00086 ANOVA); et un temps d'interaction de traitement significantif (P = 0,03 ANOVA). Il y avait aussi une correlation positive faible (r = 0,54) entre le pourcentage de pick d'aggregation spontanee et le temps partiel du ratio de la tromboplastine active, pendant l'administration de l'heparine.

Introduction

Thromboembolism remains a major contributor to maternal morbidity and mortality during pregnancy [1,2]. Human pregnancy is characterised by physiological hypercoagulabiltiy, manifested in part by increased spontaneous platelet aggregation (SPA) in vitro and platelet activation in vivo [3].

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The activation of the coagulation cascade in pregnancy predisposes to thrombotic complications, including fatal pulmonay embolism [1,2]. Unfractioned heparin is the anticoagulant of choice during pregnancy [4], but its use can be associated with thrombocytopenia and paradoxical thrombosis [4,5]. Heparin, in vitro, has been shown to substantially enhance SPA in citrated whole blood from pregnant women [6,7], and may induce platelet activation in vivo in healthy volunteers, following the administration of single intravenous doses [8]. However, the effects of the prophylactic or therapeutic administration of heparin on platelet behaviour during pregnancy have not been described. If heparin administration in pregnancy is associated with further platelet activation in vivo, it may aggravate the problems of thrombocypenia and thromboembolism [5]. Although heparin-induced-thrombocytopenia is a well recognized phenomenon, both within and without pregnancy [5], the mechanism is unknown and platelet aggregation in vivo has been proposed as a possible explanation [9]. It is possible, however, that heparin-induced platelet activation in vivo occurs mostly at a subclinical level during pregnancy. The aim of our preliminary study was to examine the effect of the therapeutic or prophylactic administration of heparin on in vivo platelet behaviour in pregnancy.

Materials and method

A parallel group ex vivo study was undertaken in two groups of pregnant women during the third trimester of their pregnancy. Eight pregnant women aged 26 ± 3 years, with a gestational age of 35 ± 3 weeks were admitted to the Obstetrics and Gynaecology wards of the City Hospital and Queen's Medical Centre, Nottingham. They received subcutaneous unfractionated heparin for the prophylaxis or the treatment of deep venous thrombosis. Each patient consented to undertake the study after informed consent and prior ethical committee review and approval by the Queen's Medical Centre and City Hospital, Nottingham, ethical review committees. Patients receiving medications other than heparin or who were on warfarin therapy were excluded. Eleven healthy pregnant women, matched for age (29 ± 3 years) and gestational age (34 ± 5 weeks), without a personal or family history of thromboembolic disease and who were not receiving any medications consented to serve as the control group. The clinical and demographic characteristics of the patients who received heparin are summarized in Table 1.

Assessment of spontaneous platelet aggregation in citrated whole blood

Blood for platelet aggregation studies was collected into 10 ml tubes containing 3.13% trisodium citrate dehydrate to give a final volume of 9 parts of blood to 1 part of citrate. Platelet aggregation was determined using a single platelet counting technique described by Fox et al.

1932 [10]. After 20 minutes of the incubation of the citrated blood samples at 37 °C, aliquots of 480 ul of the citrated blood samples were transferred into polystyrene tubes and stirred at 1,000 rpm at 37 °C with a magnetic stirring base. Saline water [20 ul] was then added to initiate the aggregation. Platelets counts in subsamples of the whole blood were fixed using a WU and Heak platelet fixative (WU and Hoak, 1974 [11]. They were measured before and after [15, 30,45, 60, 120, 240, and 480s] after the addition of saline. The counts were undertaken using a clay – Adams Ultra-F10 100 platelet counter [10]. In patients on steady state heparin therapy, blood was collected for the assay within 1 hour of heparin dosing and platelet assay undertaken within 30 minutes of collection.

Statistical Evaluation

Comparison of the SPA between the heparin and the control groups was undertaken using 2-way repeated measures analysis of variance (ANOVA). This included as assessment of the main treatment effect and time-treatment interaction. The demographic data of the 2 groups were compared by unpaired t-tests. The relationship between the activated partial prothrombin time (APTT) and the peak SPA was assessed by linear regression analysis. The null hypotheses were rejected at $P<0.05$. "State view 512" package was employed in data analysis.

Results

In the heparin group, 6 patients received heparin for prophylaxis and in 2 patients, it was administered for the treatment of deep venous thrombosis (Table 1).

Table 1: Clinical and demographic data of the third trimester pregnant patients receiving subcuntenous heparin

Initials	Age (years)	Gestatunal age (Weeks)	Study day Heparin dose (1000 i.u. day −1)	Peak % SPA	Study day platelet blood count (109/1)	APTT Ratio	Heparin indication & clinical condition
1. S.D.	24	36	10	68	181	1.50	Prophylaxis (Hbsc)
2. S.R.	20	31	25	29	209	1.16	Ileofemoral DVT
3. H.C.	22	27	40	61	231	1.75	DVT
4. A.P.	27	39	45	60	201	2.19	Prophylaxic (Aortic valve disease)
5. K.G.	31	37	20	66	306	1.20	Prophylaxic (History of Pulm. Embolism
6. K.S.	25	36	30	46	106	1.03	Prophylaxic (DVT history)
7 G.L.	29	39	20	33	410	1.20	Prophylaxis (DVT history)
8. S.J.	27	37	15	20	331	1.13	Prophylaxis Stroke

APTT: Activated partial thromboplastin time. DVT: Deep venous thrombosis.

There was no difference in the peak spontaneous platelet aggregation between the prophylactic (47%) and the therapetuic subgroups (50%) so that the groups were merged for comparison with controls (Table 1). The heparin-administered patients exhibited a trend to a higher SFA in comparison to the healthy control groups *($P=0.086$* ANOVA of 1, 8). There was, however, a statistically significant time-treatment interaction *($P = 0.031$* ANOVA *6, 108)* between the groups. This indicate that while there was no overall treatment difference, the effect of time on heparin-induced SPA was different from its effect on SPA in controls. This implies that the difference in SPA may occur at the fewer later time points. Platelet activation was consistently greater in patients on heparin compared to the healthy control group (see Fig. 1).

Fig. 1

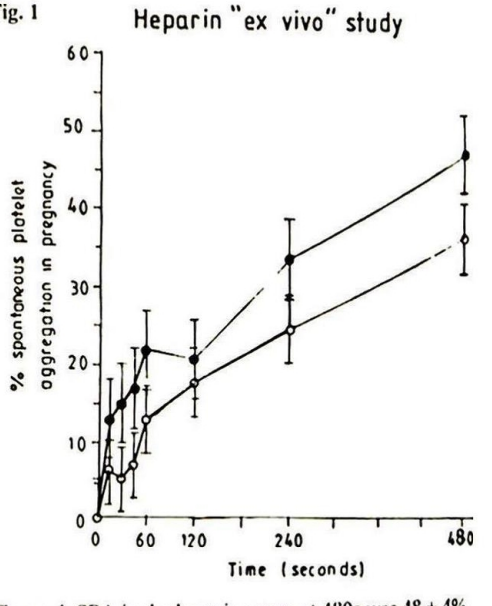

Fig. 1. The time profile of the % spontaneous platelet aggregation, *ex vivo* in citrated whole blood from third trimester pregnant women receiving prophylactic or therapeutic heparin (filled circles, n = 80) and in healthy third trimester pregnant women not receiving heparin (open circles) n = 11. Data are mean ± sem. The overall treatment effect showed a trend to heparin induced platelet activation (P = 0 086 ANOVA) with a significant time treatment – interaction (P = 0.031 ANOVA)

The peak SPA in the heparin group at 480s was 48 ± 4% but 37 ± 5% in the healthy controls. In one patient with Hb Sc genotype receiving heparin for prophylaxis, a pre-heparin aggregation study was possible. Her peak SPA

was substantially increased from 31% before to 68% during heparin administration (Table 1).

There was a trend to a positive correlation between the activated partial thromboplastin time (APTT) ratio and the peak % spontaneous platelet aggregation ($r = 0.54$, $P = 0.082$) in the patients administered heparin. The % peak SPA = 24.9 APTT ratio = 13.1 t = 1.58. The 95% confidence interval for the slope was – 13.7 to 63.4 and for the intercept – 42 to 69.

Discussion

In this preliminary study, we investigated the possibility that the prophylactic or therapeutic use of heparin in third trimester pregnancy is associated with in vivo platelet activation. Our results of a trend to a higher spontaneous platelet aggregation in heparin-treated patients is consistent with the concept that heparin causes in vivo platelet activation during pregnancy. This results have not been previously reported. This proposal is in accord with the findings in healthy volunteers administered single doses of heparin [8] and the well-established pro-aggregatory effects of heparin on platelets in vitro [6,7].

There was no difference in platelet reactivity between the patients receiving heparin prophylactically or for the treatment of deep venous thrombosis, hence the two groups were merged for comparison with controls. This indicates that the higher SPA in heparin-treated patients than in the healthy controls is not accountable by the thrombotic state per se. Heparin-induced thrombocytopenia may occur in up to 5% of patients receiving the drug [9]. In the present study, however, there was no clear evidence of thrombocytopenia, which may be indicative of irreversible aggregation of the platelets in vivo. It is thus plausible that in vivo activation induced by heparin occurs at a subclinical level in majority of the patients. The peak platelet activation appeared to be positively correlated with the APTT ratio ($r = 0.52$), albeit of weak statistical significance. This may suggest that heparin-induced platelet activation in vivo may be heparin concentration dependent. This in vivo platelet activation induced by heparin may increase the risk of thrombosis, which may prove clinically deleterious in a hypercoagulable state such as pregnancy [12].

In conclusion, our preliminary findings indicate a slight degree of in vivo platelet activation during the prophylactic or therapeutic use of heparin in pregnancy. The trend seen in this study probably reflects a type II error and the concentration of measurements in the early phases of SPA. The magnitude of this effect may be dependent on heparin concentration reflected by the APTT ratio during pregnancy. Larger studies are now indicated to evaluate in vivo platelet activation during heparin treatment in pregnancy, and also to compare the effects with those of the newer low molecular weight heparins, which have the potential of inducing less platelet activation [7].

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