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THE EFFECT OF INCREASING HEPARIN DOSES AND OF HEPARINOID ON PLATELET FACTOR 4 (PF4) RELEASE IN NORMAL SUBJECTS (*)

EFFETTO DI AUMENTATE DOSI DI EPARINA E DI UN EPARINOIDE SULLA LIBERAZIONE DI FATTORE PIASTRINICO 4 (PF4) NEI SOGGETTI NORMALI

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The effect of different heparin doses on PF4 release and the ability of a second dose of heparin to mobilize more PF4 was studied in normal subjects. A heparinoid was also administred to 2 normal subjects.
The administred heparin dose seemed to be important in influencing the release of PF4 which correlated well with the prolongation of APTT.
A second bolus of heparin given 2 hours after the first, failed to elicit a second peak of this platelet protein in spite of the fact that APTT showed a marked prolongation.
The platelet concentration also seemed to play an important role in the release of heparin-released PF4 (HR-PF4). The injection of the heparinoid induced a very small release of PF4, well correlated with the level of APTT.

KEY WORDS: PF4, heparin, heparinoid.

Among the variety of substances having a potent anti-heparin activity, platelet specific protein PF4 has been widely investigated in recent years [1 11]. Platelet factor 4 is a protein localized in the platelet alpha granules, and is released during platelet activation. It consists of eight subunits, each with a molecular weight of about 7000 D. Under physiological conditions, they are maintained in solution by a proteo-glycan-carrier [13]. With the introduction of a specific and sensitive radioimmunoassay it has been possible to evaluate the levels of PF4 in a variety of clinical conditions [4 5 6]. Since

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heparin is widely used in the prophylaxis and treatment of thromboembolic disorders, a relationship between heparin and PF4 seems to be of great interest. It has been reported by Dawes [8] that the injection of a bolus of heparin stimulates an immediate release of PF4. On the contrary, the other specific protein (beta-thromboglobulin, βtg) is not affected [8]. This was previously confirmed by us [3].

In a recent study [7] it was also demonstrated that the rise in plasma PF4 coincided with, and appeared to be a response to the increase in plasma heparin concentration rather than to the absolute heparin level. In addition, repeated injections of heparin did not induce further release of PF4 after the first injection. It has also been suggested that the endothelium was the immediate source of the PF4 released by heparin; in fact, the presence of PF4 on the vascular endothelium was demonstrated.

The aim of our study was to evaluate the level of PF4 under different and repeated conditions of heparin administration and also to see if there was a correlation with platelet concentration. In addition, we wished to see if a glucoronyl-glycosamino-glycan-sulphate (3GS) was able to induce PF4 release.

Material and methods

Eleven apparently healthy males aged between 20 and 50 years with a body weight varying from 66 to 79 kg were investigated. One male 37 years of age with a body weight of 79 kg suffering from mild thrombocytosis (platelet count = 660000 μl) was also studied.

Sample collection blood was drawn from an antecubital vein with clear venipuncture. The first 6 ml of blood were used for the determination of the activated partial thromboplastin time (APTT) (4.5 ml) and the platelet count (1.5 ml). Two and a half ml were used for the PF4 evaluation. The blood was collected through a butterfly (21 × 3/4,12" tubing, Abbott, North Chicago, USA) into a precooled syringe and immediately put into a precooled plastic tube containing an anticoagulant mixture of Theophilline 30 mM, EDTA 10% pH 7.4 and PGE_1 1 μg/ml (Edimburgh cocktail) [12].

The tubes were then placed into melting ice for a maximum of 30 min. and then centrifuged at 2500 g for 30 min. at 4°C. Using a plastic tip at 1 cm below the top layer, 0.5 ml of platelet poor plasma (PPP) was carefully aspirated and stored at —20°C for up to three weeks before assaying. Six subjects and the patient with mild thrombocythemia were injected with different concentrations of mucous heparin, namely 150, 312, 625, 1250, 2500, 5000 I.U. of Liquemin[R] Roche (1000 IU ∼ 6.6 mg) respectively.

Two normal subjects were injected with one vial of 3GS (Vessel[R], Alfa Farmaceutic, Italy, containing 180 LU ∼ 18 mg) [14]. Two other subjects were injected with two « boli » of mucous heparin (Liquemin[R], Roche) of 5000 I.U. each, the second bolus being injected two hours after the first. Blood samples were collected before and 5, 15, 30, 60, and 120 minutes after the drug injection. In the two

TABLE I.

Platelet factor four (PF4) and APTT values obtained in patients before and after heparin and heparinoid injection.

Livelli di PF4 e APTT ottenuti nei pazienti prima e dopo la somministrazione di eparina o eparinoidi.

		Time (minutes)						Platelet count/μl
		0	5	15	30	60	120	
Heparin (Liquemin Roche) 150 IU	PF4	3.5 ng/ml	63.1	26.1	7.6	6	4.0	190000
	APTT	46.6 sec.	52.9	55.2	50.7	50.7	45	
312 IU	PF4	6.3	72.3	49.0	8.7	5.5	2.4	146000
	APTT	33.3	65.4	50.7	40.5	38.6	33.3	
625 IU	PF4	4.9	69.7	48.4	19.2	7.2	3.2	255000
	APTT	43	73.9	68.7	60.4	48.9	41.7	
1250 IU	PF4	10.1	90.0	40.0	13.6	11.6	12.5	400000
	APTT	38.4	180.3	81.6	68.1	53.4	39.4	
2500 IU	PF4	7.5	225.0	85	21.0	16.5	5.5	320000
	APTT	33	236	151	111.2	92.4	33.6	
5000 IU	PF4	9.5	232.8	137.7	39.8	13.2	7.0	152000
	APTT	33.5	598	285.5	190.8	92.4	33.6	
5000 IU	PF4	7.8	318.0	191	66.5	31.6	14.7	665000
	APTT	39.6	611	383	202	90.1	44.7	
Glucoronyl-glycosamino-glycan-sulphate (3GS) pat. 1	PF4	5.8	44.5	37.2	16.5	3.7	5.6	144000
	APTT	35.4	51.6	45.9	40.3	37.5	34.8	
pat. 2	PF4	5.4	48.6	21.2	4.1	1.8	1.0	305000
	APTT	31.8	48.6	41.6	37.9	32.5	31.2	

subjects with the second bolus of heparin, two other samples were collected 5 and 30 min. after the second bolus.

The samples that presented high values of PF4 were diluted using 0.01 M Tris buffer with 0.1 M of sodium chloride containing 0.2% of bovine albumin (Abbott Labs, North Chicago, USA) in order to reach a concentration measurable on the standard curve of the RIA assay.

Platelet count was determined from the whole blood using an HPC 52 platelet counter (Hycel) and expressed per µl.

APTT was carried out using an ellagic acid activated thromboplastin (Cephaloplastin, Dade Labs., USA).

Platelet factor 4 was determined using a commercial radioimmunoassay kit supplied by Abbott Labs., North Chicago, USA, and expressed as ng/ml [10].

Results

The results are summarized in table I which contains the levels of PF4, APTT and platelet count observed in the subjects studied.

After the administration of heparin there was an immediate release of PF4 (Table I) followed by a subsequent clearance. The normalization time of the level of PF4 after the drug injection was dependent on the maximum peak level obtained at 5 minutes. The patients who presented a small increase had a normalization time between 15 and 60 minutes. On the contrary, those with the highest levels returned to normal after 60 to 120 minutes. The APTT also showed a prolongation that was in agreement with the level of PF4 with a maximum at the 5th minute following heparin administration. In addition, the two subjects who were injected with the same amount of 5000 I.U. of heparin, although showing the same level of APTT, had a different concentration of PF4 at 5 minutes which correlated with their platelet count. The injection of 3GS (Table I) also showed a slight increase of PF4 in agreement with a moderate increase of APTT. A second bolus of heparin was unable to produce any further release of PF4 although the APTT showed a clear prolongation (Fig. 1).

Discussion

The first intravenous injection of heparin bolus caused an immediate release of platelet factor 4 (HR-PF4) as measured by radioimmunoassay. However, a second peak was not seen after the injection of a second blus at 120 minutes. On the contrary, APTT showed a prolongation as expected even after the second bolus. We did not measure the plasma level of the other specific protein βtg since it has been shown that the anti-heparin activity of βtg is extremely low

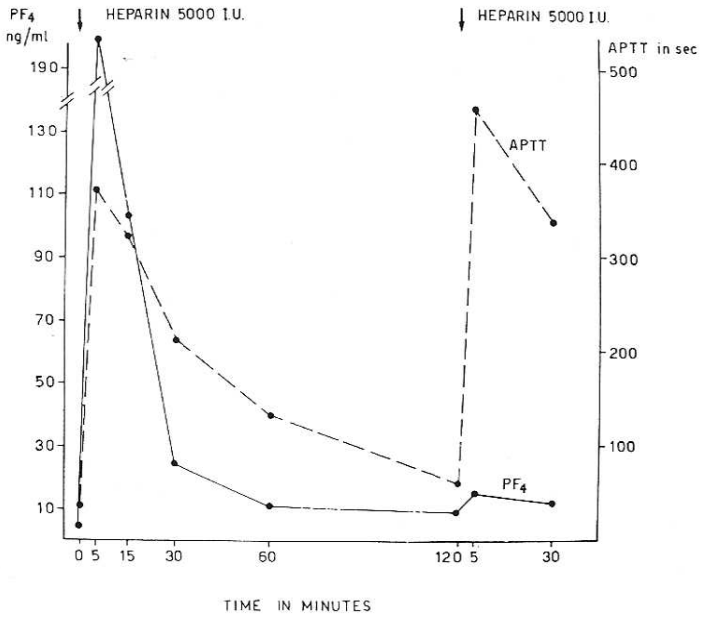

Fig. 1.

Effect of two heparin administrations in a normal subject. The second bolus of heparin fails to elicit a significant PF4 release whereas the effect on APTT is maintained. An identical pattern was observed on the other subject investigated.

Effetto di due somministrazioni di eparina in un soggetto normale. Il secondo bolo di eparina è incapace di indurre una significativa liberazione di PF4, pur essendo immutato l'effetto sul APTT. Un identico modello è osservabile nell'altro soggetto studiato.

and that the plasma level of βtg is not affected by heparin administration [8 15].

These data are in agreement with and confirm the previous experiences by Dowes and us [3 7 8]. In addition, in spite of the fact that different doses of heparin were injected in different subjects with different platelet counts, the administration of heparin determined a small and almost identical HR-PF4 level until 2500 I.U. were used.

Only when we administered a concentration of heparin that probably mobilized all the PF4 from the binding site of the endothelial cells we did notice a great increase in accordance with the platelet count. In fact, the two subjects who were injected with 5000 I.U. had a HR-PF4 level that correlated with their platelet count, even though they presented the same APTT prolongation. This probably reflected the same plasma levels of heparin. We cannot exclude, however, that

other factors such as the presence of other anti-heparin substances or the condition of the vessel wall could influence this release [2].

The injection of 180 lipasemic units (~ 18 mg) of 3GS was able to induce only a minimal increase of HR-PF4 which corresponded to about half the level obtained with the injection of 1250 I.U. (~ 8.2 mg) heparin. This was even less than the level obtained with 150 I.U. of heparin. In addition, the APTT showed only a very small prolongation.

The major fractions of 3GS contain heparan sulphate-like glycosamino-glycans (GAGs), but they also contain considerable amounts of chondroitin-sulphates and dermatan-sulphates as shown by chemical and electrophoretic analysis [14].

These GAGs possess less N-sulphated glucosamines and have higher proportions of the non-sulphated glucuronic acid residues. Since it has been suggested that the conformation of the uronic acid, the location of the sulphate groups and, in particular, the location of the carboxyl group can alter the binding affinity for PF4 [9], this could explain the presence of a smaller peak of PF4 after 3GS administration as compared to that obtained after the bolus of heparin.

RIASSUNTO

Sono stati studiati, in soggetti normali, gli effetti di dosi scalari di eparina sulla « liberazione » del PF4 e la capacità di una seconda dose di mobilizzare ulteriormente il PF4. E' stato inoltre valutato l'effetto di un eparinoide iniettato in due soggetti normali.

La dose di eparina somministrata sembra essere importante nell'influenzare la mobilizzazione di PF4 ed è correlata al prolungamento dell'APTT.

Un secondo bolo, somministrato due ore dopo il primo, non induce un nuovo picco di PF4, malgrado sia presente un notevole allungamento dell'APTT.

Anche il numero delle piastrine sembra giocare un ruolo importante nella liberazione del PF4 sotto stimolo eparinico (HR-PF4).

La somministrazione di un eparinoide libera una piccola quantità di PF4, ben correlata con i bassi livelli di APTT.

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