The Journal of International Medical Research 1989; **17**: 36–40

Effects of a Defibrotide–Heparin Combination on Some Measures of Haemostasis in Healthy Volunteers

E. M. Pogliani,[1] M. Salvatore,[1] C. Fowst,[1] R. Girardello[2] and C. Marelli[2]

[1]Department of Internal Medicine, Institute of Biomedical Sciences, Hospital S. Gerardo, Monza, Italy; [2]Medical Department, Roussel Maestretti, Milan, Italy

In an open-study design five healthy volunteers were first given 2500 IU sodium heparin intravenously and then, after 72 h, another injection of the same dosage of sodium heparin followed immediately by 400 mg defibrotide intravenously. In two separate experiments, prothrombin time, activated partial prothrombin time, antithrombin III, tissue plasminogen activator, its inhibitor and plasma heparin levels were measured at baseline and after 15 min in one experiment, and at baseline and after 2 h in the other experiment. The most important finding was that an interaction exists between heparin and defibrotide on haemostatic activity: activated partial prothrombin time was increased three-fold in volunteers given the defibrotide–heparin combination compared with volunteers given heparin alone. This statistically and clinically significant effect was evident 15 min after administration of defibrotide–heparin and was still present after 2 h. Possible explanations for this effect are discussed briefly.

KEY WORDS: Sodium heparin; defibrotide; haemostasis; intravenous administration.

INTRODUCTION

A fibrinolytic effect was recently demonstrated in healthy volunteers undergoing treatment with defibrotide at intravenous dosages of 200 mg four times daily and 400 mg twice daily. There was a significant increase in plasma levels of tissue plasminogen activator (TPA) and a reduction of its inhibitor (PAI); this fibrinolytic effect did not decrease on continued administration of defibrotide.[1]

The important role played by haemostatic factors in the cause of vascular thrombo-embolic diseases indicates that an active therapeutic approach, using combinations of medicines capable of interacting at

Received for publication 20 October 1988; accepted 17 November 1988.

Address for correspondence: Professor E. M. Pogliani, Cattedra di Semeiotica Medica, Istituto di Scienze Biomediche, Ospedale S. Gerardo, Via Donizzetti 106, 20052 Monza, Italy.

different levels on the coagulation – fibrinolysis system, may be effective. It may, therefore, be that defibrotide could be used clinically in the future in association with heparin, orally administered anticoagulants, platelet anti-aggregants and thrombolytic drugs. The effect of any interactions by defibrotide with other medicines on various haemostatic measures must, therefore, be determined in order to highlight any undesired effects (haemorragic tendency) which could emerge from such combination therapy. The present study was designed, with this in mind, to study the interaction between intravenously administered heparin and defibrotide in healthy volunteers.

SUBJECTS AND METHODS

Subjects

Five healthy volunteers (three males and two females), aged 27 – 35 years, were enrolled into this open study. All participants were non-smokers and were not undergoing any type of drug treatment. They were asked not to change their dietary habits or physical activity for the whole observation period.

All laboratory measures on their blood were within normal limits.

Treatments

The five subjects were first given 2500 IU sodium heparin by single intravenous injection and then, after 72 h they were given another injection of sodium heparin at the same dosage followed immediately by bolus intravenous injection of 400 mg defibrotide (Noravid®).

Evaluations

Two experiments were conducted. In the first experiment blood samples were collected before (baseline) and 15 min after completion of administration of heparin or heparin plus defibrotide. These were used to measure prothrombin time (PT, coagulation method), activated partial thromboplastin time (aPTT, coagulometric method), antithrombin III (AIII, amidolytic method, S-2238 Ortho Diagnostic Systems, Milan), TPA and PAI (amidolytic method, S-2251 Ortho Diagnostic Systems, Milan) and plasma heparin levels (amidolytic method, S-2222 Ortho Diagnostic Systems, Milan).[2-4]

In a second experiment on the same volunteers, started after 7 days of washout, the same measures were carried out on blood taken at baseline and 2 h after completion of administration of heparin or heparin plus defibrotide.

Statistical evaluation

Results were expressed as means ±SD. Differences between groups were evaluated by analysis of variance (Tuckey test for multiple comparisons). The limit of significance was $P = 0.05$.

RESULTS

Baseline measurements

No clinically significant difference between the baseline measures for the two experiments (heparin alone or in combination with defibrotide) was evident (Tables 1 and 2).

Intravenous injection of heparin

At 15 min after administration. At 15 min after intravenous administration of heparin, no significant clinical change in PT was noted (16.6 s versus baseline value of 13.5 s; $P < 0.01$). The aPTT was slightly prolonged compared with baseline (33.9 s versus baseline of 25.6 s; not statistically significant) and the effect of heparin on AIII was of little clinical importance (96.8% versus baseline of 83.8%; not statistically significant). The effect on plasma heparin concentration, however, was pronounced (0.516 IU/ml versus baseline of 0.002 IU/ml; $P < 0.01$). There was a slight

Noravid® is the registered trademark of Roussel Maestretti, Milan, Italy.

Table 1
The effect of treatment with 2500 IU heparin alone or in combination with 400 mg defibrotide on various measures of haemostasis (means ± SD) in healthy volunteers at baseline and 15 min after intravenous administration

	Heparin		Defibrotide – heparin	
	Baseline	After 15 min	Baseline	After 15 min
PT(s)	13.5±1.1	16.6±1.0**	13.5±0.9	18.0±2.0**°°
aPTT(s)	25.6±2.1	33.9±2.2	25.4±2.0	71.0±13.5**°°
AIII (%)	83.8±13.1	96.8±15.8	87.4±9.3	104.4±10.9*
Plasma heparin (IU/ml)	0.002±0.001	0.516±0.151**	0.002±0.001	0.596±0.243**
TPA (IU/ml)	9.7±1.1	7.9±1.2	9.1±0.8	5.2±2.7**°°
PAI (IU/ml)	27.4±7.0	34.4±3.1**	27.6±3.9	32.2±4.8**

Statistical analysis by Tuckey's test for multiple comparisons (*P <0.05 vs baseline; ** P <0.001 vs baseline; °°P <0.01 vs heparin alone).
PT, Prothrombin time; aPTT, activated partial thromboplastin time; AIII, antithrombin III; TPA, tissue plasminogen activator; PAI, plasminogen activator inhibitor.

Table 2
The effect of treatment with 2500IU heparin alone or in combination with 400mg defibrotide on various measures of haemostasis (mean±SD) in healthy volunteers at baseline and 2h after intravenous administration

	Heparin		Defibrotide – heparin	
	Baseline	After 2h	Baseline	After 2h
PT(s)	15.8±1.0	16.6±0.9**	13.0±0.3	13.2±0.4°°
aPTT(s)	25.8±0.6	44.6±2.5**	26.5±0.5	69.0±2.6**°°
AIII (%)	100.0±0.0	99.0±1.4	90.0±10.6	93.6±6.0
Plasma heparin (IU/ml)	0.001±0.035	0.585±0.035**	0.002±0.015	0.510±0.056**
TPA (IU/ml)	6.2±0.9	5.5±0.7	6.5±2.5	7.8±1.6
PAI (IU/ml)	27.0±1.4	25.5±0.7	27.8±1.3	26.8±1.3

Statistical analysis by Tuckey's test for multiple comparisons (**P<0.001 vs baseline; °°P<0.01 vs heparin alone).
PT, Prothrombin time; aPTT, activated partial thromboplastin time; AIII, antithrombin III; TPA, tissue plasminogen activator; PAI, plasminogen activator inhibitor.

reduction in TPA after heparin administration (7.9 IU/ml versus baseline of 9.7 IU/ml; not statistically significant) and a statistically significant increase in PAI (34.4 IU/ml versus baseline of 27.4 IU/ml; P<0.01) (Table 1).

At 2 h after administration. The plasma heparin concentration 2 h after injection of heparin was significantly increased (0.585 IU/ml versus baseline of 0.001 IU/ml; P <0.01) and aPTT was also significantly increased (44.6 s versus baseline of 25.8 s; P <0.01). None of the other measures showed any clinically significant change from baseline (Table 2).

Intravenous injection of defibrotide – heparin

At 15 min after administration. There was a modest (clinically non-significant) increase in PT (18.0 s versus baseline of 13.5 s; $P<0.01$) 15 min after intravenous injection of defibrotide – heparin. As occurred when heparin was administered alone, there was also a significant effect on plasma heparin concentration (0.596 IU/ml versus baseline of 0.002 IU/ml; $P<0.01$). The level of AIII also showed a slight increase (104.4% versus baseline of 87.4%; $P<0.05$), but remained within normal limits. The aPTT level increased almost three-fold from its baseline value and was significantly greater than the corresponding value 15 min after injection of heparin alone [71.0 s (defibrotide – heparin) versus 33.9 s (heparin); $P<0.01$]. The TPA level was reduced after administration of the combination (5.2 IU/ml versus baseline of 9.1 IU/ml; $P<0.01$) and PAI was increased (32.2 IU/ml versus baseline of 27.6 IU/ml; $P<0.01$). For both TPA and PAI the changes from baseline were greater following administration of the combination than they were after administration of heparin alone (Table 1).

At 2 h after administration. Measures taken 2 h after administration of defibrotide – heparin combination showed that there was no clinically significant change in PT levels compared with baseline (13.2 s versus baseline of 13.0 s; not statistically significant) and, similarly, there was no clinically significant change in AIII (93.6% versus baseline of 90.0%; not statistically significant). The plasma heparin level, 2 h after administration of the defibrotide – heparin combination, was 0.510 IU/ml (baseline, 0.002 IU/ml; $P<0.01$), which was little different from that after administration of heparin alone (0.585 IU/ml). The level of aPTT (69.0 s) remained prolonged ($P<0.01$) compared with baseline (26.5 s), and was similar to that measured after 15 min (71.0 s). The TPA level was increased (7.8 IU/ml versus baseline of 6.5 IU/ml; not statistically significant) and PAI was moderately reduced (26.8 IU/ml versus baseline of 27.8 IU/ml; not statistically significant) (Table 2).

DISCUSSION

This study shows that there is an interaction between heparin and defibrotide on haemostatic activity. There are many reports in the literature stating that defibrotide does not produce significant changes in blood clotting parameters although shows fibrinolytic activity mediated by an increase of TPA and a reduction of PAI.[1, 5, 6]

In the present study, the combination of defibrotide (400 mg) and sodium heparin (2500 IU) both administered via intravenous injection, caused aPTT to increase to a greater extent than with heparin administered on its own. This effect was statistically and clinically significant. It appeared to be complete within 15 min after administration of the combination therapy and persisted for at least 2 h.

The other measures of blood coagulation were not regarded as undergoing any clinically significant changes. The effects on TPA and PAI levels showed that heparin tends to impede the fibrinolytic system. Numerous studies have previously reported a relationship between heparins and this system,[7–10] although there is still disagreement about the nature of the fibrinolytic activity of heparin. These differences may be explained by the different types of heparin used in these studies and the different tests used to determine fibrinolytic activity.

The present study indicated that heparin is able to impede the fibrinolytic activity of defibrotide, at least within 30 min of administration. This is probably achieved by interaction with the endothelial receptors responsible for the activation of vascular fibrinolysis. This effect tended to diminish 2 h after administration when, presumably, the fibrinolytic activity of defibrotide started to become more pronounced. The mechanism of interaction between defibrotide and heparin on aPTT is unclear. The present study, although preliminary, indicates that defibrotide does not affect the molecular structure of heparin in a way which increases the anticoagulant 'availability' of heparin because the plasma heparin concentrations after administration of heparin alone or in combination with defibrotide are similar. It may be that heparin and defibrotide interact with the endothelial cell wall of the blood vessel. Indeed, defibrotide could strip the endothelial cell binding site of 'circulatory' heparin thereby increasing the already considerable anticoagulant activity of heparin. Studies are intended to be undertaken to determine the nature of the interaction between heparin, defibrotide, and the endothelial cell wall using fluorescein-labelled heparin and endothelial cells cultured *in vitro* in the presence and absence of defibrotide.

In conclusion, the results of this preliminary study indicate that, in cases requiring therapy with heparin and defibrotide, caution should be exercised and close monitoring undertaken to avoid unwanted phenomena, such as haemorrhage, in high risk patients.

REFERENCES

1. Pogliani EM, Salvatore M, Fowst C, *et al*: Studio di bioequivalenza di due schemi posologici di defibrotide su parametri della fibrinolisi in soggetti volontari sani. *Farm Ter* 1987; **2**: 1–5.
2. Abildgaard U, Lie M, Odegard OR: Antithrombin (heparin cofactor) assay with new chromogenic substrate. *Thromb Res* 1977; **11**: 549–553.
3. Ramby M, Norrman B, Wallen P: A new sensitive assay for tissue plasminogen activator. *Thromb Res* 1982; **27**: 743–749.
4. Teien AM, Lie M, Abildgaard U: Assay of heparin in plasma using a chromogenic substrate. *Thromb Res* 1976; **8**: 413–416.
5. Coccheri S, De Rosa V, Dettori AG, *et al*: Effect on fibrinolysis of a new antithrombotic agent: fraction P (defibrotide). A multicentre trial. *Int J Clin Pharmacol Res* 1982; **2**: 227–245.
6. Kumar A, Fareed J, Wehrmacher WH, *et al*: Endothelial function, modulation and control of vascular and thrombotic disorders: experimental results with a polideoxyribonucleotide agent, defibrotide. *Blood* 1986; **68** (suppl 1): 356.
7. Doutremepuich C, Gestreau JI, Kuttler MC, *et al*: Fibrinolytic activity of heparin and low molecular weight heparin fractions. *Progress in Fibrinolysis,* 7th edn (Davidson JF, Donati MB, Coccheri S, eds). London: Churchill Livingstone, pp 303–306.
8. Vairel EG, Bouty-Boye, Toulemonde F, *et al*: Heparin and a low molecular weight fraction enhances thrombolysis and by this pathway exercises a protective effect against thrombosis. *Thromb Res* 1983; **30**: 219–224.
9. Vinazzer H, Stemberger A, Maas S, *et al*: Influence of heparin, of different heparin fractions and of a low molecular weight heparin-like substance on the mechanism of fibrinolysis. *Thromb Res* 1982; **27**: 341–352.
10. Fareed J, Walenga JM, Hoppensteadt DA, *et al*: Studies on the profibrinolytic actions of heparin and its fraction. *Semin Thromb Haemostasis* 1985; **11**: 199–207.