Effect of low-dose heparin treatment on fibrinolysis in patients with previous myocardial infarction

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Effect of Low-Dose Heparin Treatment on Fibrinolysis in Patients with Previous Myocardial Infarction

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Key Words
Unfractionated heparin
Fibrinolysis
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Plasminogen activator inhibitor 1
Euglobulin lysis time
Ischemic heart disease

Abstract

The present study was designed to investigate whether medium-term, low-dose heparin treatment is able to affect the fibrinolytic system. In a randomized cross-over study 10 asymptomatic patients with previous (1–6 years) myocardial infarction underwent two sequential 15-day treatments, respectively, on heparin and on placebo (saline solution), preceded and separated by 10-day wash-out periods. Heparin (as calcium heparin, 12,500 IU in 0.5 ml) and saline (0.5 ml) were subcutaneously administered once a day at 8 a.m. Blood samples for fibrinolysis studies were withdrawn on the first and 15th day of each period immediately before and 4 h after heparin or saline administration before and after 10 min venous occlusion (VO) respectively. Four hours after the first heparin administration tissue plasminogen activator antigen (t-PA ag) levels significantly increased with respect to saline administration ($p < 0.01$ and $p < 0.05$, respectively). After 15-day heparin treatment a decrease in euglobulin lysis time ($p < 0.05$) and an increase in t-PA activity (act) ($p < 0.05$) and in t-PA ag ($p < 0.01$) in comparison with placebo were observed before VO. No statistically significant changes in plasminogen activator inhibitor-1 (PAI-1) levels were found. The variations of fibrinolytic system activity induced by heparin treatment were more marked when evaluated after VO. These results indicate that medium-term low-dose heparin treatment increases t-PA ag formation and/or release with consequent t-PA act increase.

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Introduction

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Material and Methods

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Introduction

Over the last years several in vitro and in vivo studies have suggested that the antithrombotic activity of heparin may stem not only from the potentiation of the inhibitory activity of antithrombin III [1], but also from interactions with the fibrinolytic system [2–5]. However, the results have been obtained with different preparations of heparin, and heparin was administered via differing routes to normal volunteers or patients and with regimens varying from single doses to repeated daily doses [6]. In in vivo studies performed in acute clinical conditions (such as deep vein thrombosis or in relation to surgical procedures) a heparin-induced increase in fibrinolytic activity has been observed and found to be associated with an increase in plasma concentrations of tissue plasminogen activator (t-PA) [7–10] and/or a decrease in plasminogen activator inhibitor 1 (PAI-1) levels [11]. However, these studies were not suitable to investigate the effect of heparin on the fibrinolytic system, because they were often performed in patients with acute conditions in whom sudden changes in haemostatic factors may arise. Moreover, most studies were lacking control groups which are necessary due to the circadian rhythm of the fibrinolytic system [12]. In order to investigate if administration of low-dose heparin is able to enhance fibrinolytic activity, we performed a randomized cross-over study in patients with previous myocardial infarction.

Material and Methods

Patients Investigated

Ten patients (9 males and 1 female) with a mean age of 61 years (range 44–69 years) were investigated after they had given their informed consent. Patients had suffered from a previous myocardial infarction (1–6 years before the study) and were needing no medical therapy. None of them had alterations of plasma lipids, hypertension, obesity, diabetes, liver diseases or malignancies nor had taken any drug for at least 15 days before entering the study. Twenty-five control subjects of equivalent age were used as reference population (table 1).

Study Design

The study was performed according to a randomized cross-over design. Heparin (as calcium-heparin, Italfarmaco, Milan, Italy; 12,500 IU s.c.) or saline solution as placebo were administered once a day at 8 a.m. for two sequential 15-day periods, which were preceded and separated by wash-out periods of 10 days. The first day of each treatment period, after overnight fasting, venous blood was withdrawn before and 4 h after the first administration of heparin or saline. At the end of each treatment period, blood was withdrawn at 8 a.m. on the morning of the fifteenth day (24 h after the previous heparin or saline administration and immediately before the last administration), and 4 h after the last dose.

Blood samples for investigating fibrinolytic activity were obtained in baseline conditions (after a 30-min period of rest in the supine position) and after 10 min of venous occlusion (VO) of the arm performed with a pressure intermediate between the patient's systolic and diastolic arterial pressures.

Venous blood was obtained with rapid flow from antecubital vein using a 19-gauge needle by the two-syringe technique. After discarding the first 3–4 ml, 4.5 ml of blood were transferred into an ice-cold polypropylene tube containing 0.11 mol/l sodium citrate (0.5 ml). Immediately after mixing, one part of anticoagulated blood was added to an equal volume of acetate buffer (0.2 mol/l, pH 3.9) for the determination of t-PA activity (t-PA act). Blood was centrifuged at 1,200 *g* for 20 min at 4 °C. Platelet-poor plasma was separated in small aliquots; 50 µl of 1 mol/l HCl was added to 0.75 ml of acetate plasma and all treated plasma samples were stored at –70 °C.

Fibrinolysis Studies

Euglobulin lysis time (ELT) was performed according to Chakrabarti et al. [13]; t-PA act was measured by end point amidolytic method [14] (Coa-Set t-PA/PAI, Kabi Vitrum, Molndal, Sweden) and expressed in IU/ml. t-PA antigen (t-PA ag) plasma concentration was determined by an Elisa which measures both complexed and free t-PA [15] (Imubind-5 t-PA, American Diagnostica, New York, N.Y., USA). t-PA inhibitory activity (PAI-1 act) was measured with end point amidolytic method [16] (COA-Set PAI, Kabi Vitrum) and

Table 1. Fibrinolytic parameters before and after saline and heparin treatment

		Laboratory control values	1st day		15th day	
			0	4 h	0	4 h
Saline treatment						
ELT, min	before VO	385 ± 172	429 ± 168	376 ± 151	464 ± 205	374 ± 185
	after VO	130 ± 63	165 ± 118	103 ± 70	167 ± 155	73 ± 42
t-PA act, IU/ml	before VO	0.52 ± 0.34	0.72 ± 0.25	0.81 ± 0.24	0.68 ± 0.30	0.84 ± 0.26
	after VO	5.30 ± 3.48	4.75 ± 1.28	5.75 ± 2.55	4.57 ± 1.17	5.71 ± 2.83
t-PA ag, ng/ml	before VO	5.5 ± 2.2	5.2 ± 1.9	5.5 ± 2.2	5.4 ± 2.3	5.7 ± 2.2
	after VO	15.5 ± 5.0	14.2 ± 5.5	17.4 ± 6.8	14.1 ± 5.9	18.8 ± 6.8
PAI-1 act, AU/ml	before VO	10.4 ± 1.7	16.2 ± 3.1	14.8 ± 3.9	15.5 ± 2.5	14.7 ± 3.7
PIA-1 ag, ng/ml	before VO	9.6 ± 4.0	11.5 ± 4.9	8.3 ± 4.5	11.7 ± 8.1	8.5 ± 6.3
Heparin treatment						
ELT, min	before VO	385 ± 172	412 ± 143	365 ± 149	322 ± 171*	215 ± 83*
	after VO	130 ± 63	180 ± 107	77 ± 45	72 ± 61*	29 ± 8*
t-PA act, IU/ml	before VO	0.52 ± 0.34	0.68 ± 0.28	0.95 ± 0.49	1.18 ± 0.73*	1.19 ± 0.37
	after VO	5.30 ± 3.48	4.71 ± 1.01	7.05 ± 1.96*	6.49 ± 2.18	8.37 ± 3.17*
t-PA ag, ng/ml	before VO	5.5 ± 2.2	5.5 ± 2.1	7.1 ± 2.2**	9.8 ± 3.2**	14.8 ± 7.4**
	after VO	15.5 ± 5.0	14.1 ± 5.4	21.4 ± 6.0*	24.2 ± 7.6**	26.1 ± 7.9*
PAI-1 act, AU/ml	before VO	10.4 ± 1.7	15.9 ± 4.5	14.3 ± 3.2	14.1 ± 3.5	13.8 ± 4.9
PAI-1 ag, ng/ml	before VO	9.6 ± 4.0	11.0 ± 6.9	8.9 ± 6.3	11.5 ± 5.5	8.9 ± 3.4

0 = Baseline.
* $p < 0.05$, ** $p < 0.01$, vs. saline treatment.

expressed in arbitrary units (AU) per ml (1 AU was defined as the amount neutralizing 1 IU of t-PA in 10 min). Plasma concentration of PAI-1 (PAI-1 ag) was measured by an Elisa sensitive to free but not complexed PAI-1 [17] (Imubind PAI-1, American Diagnostica). PAI-1 act and PAI-1 ag were measured only before VO. Normal values of fibrinolytic parameters were obtained in our laboratory from control subjects of equivalent age (62 ± 7 years) to that of patients.

Statistical Analysis

Results are reported as means ± SD (x ± SD). Statistical analysis of the results was performed with Anova and Student's t test for paired data. The level of significance was 0.05.

Results

Pretreatment values of ELT, t-PA act, t-PA ag and PAI-1 ag in patients were not significantly different in comparison with age-matched control subjects. On the contrary PAI-1 act values were slightly, but significantly higher in patients ($p < 0.01$, table 1). In all patients a marked activation of fibrinolytic system was induced by VO (table 1).

Table 1 shows the values of fibrinolytic parameters observed at different times before and after either saline or heparin treatment. No differences were found between values measured before and after saline administration ($F < 1.5$ for all parameters). After the first heparin administration ELT was slightly shorter than after saline t
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Discussion

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shorter than after saline treatment both before and after VO, but the difference was not statistically significant. After 15 days of heparin treatment, ELT was found to be significantly reduced in comparison to saline, both before and after VO ($p < 0.05$), in samples withdrawn 24 h after the previous heparin administration. Moreover, 4 h after the last heparin administration (as after the first heparin injection), ELT further shortened and was significantly shorter than 4 h after the last saline administration both before and after VO ($p < 0.05$).

After the first heparin administration t-PA act, measured after VO, significantly increased ($p < 0.01$) with respect to saline administration. After 15-day heparin treatment t-PA act significantly increased in comparison to saline both before ($p < 0.05$) and after VO ($p < 0.01$). Significant differences were also observed between t-PA act values obtained 4 h following the last heparin administration and those obtained after the last saline injection, both before and after VO ($p < 0.01$).

A significant increase in t-PA ag was observed 4 h after the first heparin administration with respect to saline treatment, both before and after VO ($p < 0.01$ and $p < 0.05$). At the end of chronic heparin administration plasma t-PA ag significantly increased with respect to saline both before and after VO ($p < 0.01$). Moreover, t-PA ag levels were higher 4 h after the last heparin injection than 4 h after saline administration, both before and after VO ($p < 0.01$). In contrast to changes of t-PA, both PAI-1 act and PAI-1 ag did not show significant variation after heparin treatment (table 1).

Discussion

This randomized cross-over study shows that both a single administration of subcutaneous unfractionated heparin (12,500 IU) and a 15-day treatment with low-dose heparin are able to induce significant changes in fibrinolytic activity in patients with previous myocardial infarction. The dose of heparin chosen was that employed in a clinical trial which demonstrated that heparin is able to reduce reinfarction in patients with recent myocardial infarction [18]. Changes in fibrinolysis have been measured 4 h after a single dose of heparin (acute administration) and 24 h after the last dose, at the end of a daily 15-day treatment (chronic administration). These two aspects need to be considered separately.

Effects of Acute Heparin Administration

Our data indicate a slight but significant increase in t-PA act and t-PA ag with no changes in PAI-1 after a single administration. Diurnal fluctuations of fibrinolysis measurements cannot account for the observed variations because comparisons were performed with data obtained after placebo treatment at the same hour of the day. The contemporary increase in t-PA-act and the absence of a reduction in free PAI-1 levels rule out significant changes in t-PA/PAI-1 complexes and seem to indicate an actual increase in t-PA plasma levels after a single heparin administration.

Effect of Chronic Heparin Treatment

In this study a profibrinolytic effect of heparin was observed in blood samples obtained 24 h after the last heparin administration when heparin is no longer detectable in plasma. These data are consistent with those of several investigations carried out in acute patients indicating increased levels of t-PA ag after treatments of more than 3 days with unfractionated heparin [2, 7–9]. In contrast, significant increases in t-PA ag, t-PA/PAI complexes and PAI-1 with no changes in ELT were reported by Vergnes et al. [19] in elderly patients without thrombotic events after a

more prolonged (30 and 60 days) prophylactic treatment with heparin. These different results cannot be attributed to differences in methodology, but rather are due to differences in patients investigated. Actually, we studied patients who were on average more than 20 years younger, with previous myocardial infarction and with higher PAI-1 act levels than patients studied by Vergnes et al.

Possible Mechanisms of t-PA Increase after Heparin Treatment

Heparin is able to affect the production of fibrinolytic components by cultured cells. In fact, it was reported to increase intracellular t-PA levels in rat epididymal fat pad capillary endothelial cells [20] and in human umbilical vein endothelial cells [21]. On the other hand, Konkle and Ginsburg [22] noted an increase in t-PA secretion by human umbilical vein endothelial cells not mirrored by a comparable increase in mRNA and this discrepancy was explained by hypothesizing a decrease in t-PA ag 'crypticity'. Thus, a heparin-induced increase in t-PA synthesis is not proved so far. However, extrapolation of findings obtained from cell cultures to clinical studies can be hazardous. So, conclusive data on the mechanism(s) underlying t-PA increase after heparin treatment are still lacking.

In conclusion this study indicates that low-dose heparin administration is associated with increased plasma levels of t-PA in patients with previous myocardial infarction who frequently show an impaired fibrinolytic activity [23] and high levels of PAI-1 [24]. This effect of heparin may contribute to the antithrombotic activity of low-dose heparin treatment in patients with ischemic heart disease.

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