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Effect of low-dose heparin on fibrinogen levels in patients with chronic ischemic heart disease

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Abstract Several prospective studies have demonstrated that high plasma fibrinogen levels are associated with an increased risk of ischemic heart disease. Since in most patients an increased thrombin generation has been reported, we investigated whether the control of thrombin generation could affect plasma fibrinogen levels. Forty male outpatients (20 asymptomatic with previous myocardial infarction and 20 with stable effort angina) were enrolled in a randomized medium-term (6 months) cross-over study. Clottable fibrinogen, according to Clauss, prothrombin fragment 1+2, thrombin-antithrombin complex, and fibrinopeptide A were evaluated in relation to treatment with low-dose heparin. After a 15-day wash-out period, during which patients had been treated only with nitrates if needed, patients were allocated to two sequential periods of treatment with standard heparin (12,500 U, subcutaneously daily) plus antianginal treatment or antianginal treatment alone, separated by a second 15-day wash-out period. At the end of the treatment period with low-dose heparin significant decreases in the plasma fibrinogen (2.5±0.6 g/l vs. 3.3±0.5 g/l, $P<0.001$), prothrombin fragment 1+2 (1.4±0.5 nmol/l vs. 1.9±0.7 nmol/l, $P<0.001$), thrombin-antithrombin (4.5±2.4 ng/ml vs. 9.7±3.6 ng/ml, $P<0.001$), and fibrinopeptide A (2.1±1.1 ng/ml vs. 3.5±2.1 ng/ml, $P<0.001$) were observed compared with the period without heparin. The present results indicate that low-dose heparin can effectively control the increased abnormal thrombin generation and elevated fibrinogen levels in patients with ischemic heart disease, possibly decreasing the risk of cardiovascular death.

Key words Heparin · Fibrinogen · Prothrombin fragment 1+2 · Thrombin-antithrombin complex · Fibrinopeptide A

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Introduction

Several large epidemiological prospective studies have recently indicated a strong association between high fibrinogen levels and mortality from cardiovascular disease [1, 2]. In particular, multivariate analysis indicated a significant independent contribution of fibrinogen level to the risk of ischemic heart disease (IHD). Recently, the levels of fibrinogen and of other hemostatic factors have been reported to be independent predictors of subsequent acute coronary syndromes in patients with angina pectoris [3, 4]. Several explanations have been proposed to link high plasma fibrinogen levels with enhanced susceptibility to thrombosis [1, 5]. It is now largely accepted that atherosclerosis is a chronic inflammatory condition due to monocyte-macrophage infiltration and vascular smooth muscle cell proliferation [6]. Activated monocytes in the plaque express tissue factor [7] and receptors for factor X [8] and fibrinogen [9]. Thus, increased fibrinogen levels might be related to enhanced clotting activation [10]. The aim of this study was to verify if plasma fibrinogen levels could be influenced by the control of thrombin generation in patients with IHD. To this purpose plasma concentrations of fibrinogen, prothrombin fragment 1+2 (F1+2), a marker of factor Xa action on prothrombin, thrombin-antithrombin complex (TAT), a marker of thrombin formation, and fibrinopeptide A (FPA), a marker of thrombin activity, were evaluated in IHD patients before and after low-dose heparin treatment or placebo.

Materials and methods

Patients

Forty male outpatients with chronic IHD were included in the study (mean age 57±8 years, range 45–66 years); 20 were asymptomatic patients who had suffered from previous myocardial infarction (1–6 years before the study) and 20 patients had stable effort angina (11 had a history of previous myocardial infarction). Angina was diagnosed on the basis of typical chest pain, ECG, and treadmill stress

Table 1 Plasma levels of fibrinogen (FBG), prothrombin fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT), and fibrinopeptide A (FPA) of the ischemic heart disease (IHD) patients before and after the two treatments and of control subjects

	IHD patients				Controls
	Placebo treatment		Heparin treatment		
	Baseline	6 months	Baseline	6 months	
FBG (g/l)	3.2±0.5** (2.4–3.9)	3.3±0.5** (2.2–4.3)	3.3±0.5** (2.5–4.1)	2.5±0.6* (1.4–3.9)	2.6±0.5 (0.8–3.8)
F1+2 (nmol/l)	2.0±0.7** (0.5–4.5)	1.9±0.7** (0.6–3.0)	2.1±0.7** (0.7–4.0)	1.4±0.5*** (0.5–2.2)	1.0±0.3 (0.4–1.8)
TAT (ng/ml)	9.7±3.9** (3.9–21.2)	9.7±3.6** (3.9–18.0)	9.8±3.2** (4.7–19.0)	4.5±2.4*** (1.2–10.0)	2.4±1.1 (1.0–5.6)
FPA (ng/ml)	3.7±2.2** (0.6–9.2)	3.5±2.1** (0.6–7.7)	3.7±2.3** (0.8–10.2)	2.1±1.1*** (0.4–5.0)	1.5±0.5 (0.6–2.5)

* $P<0.001$ vs. placebo treatment; ** $P<0.001$ vs. control subjects

test. No patient had evidence of acute or chronic inflammatory disease or neoplasia, which can cause an increase in plasma fibrinogen. Fourteen patients were non-smokers, 16 were smokers, and 10 former smokers who had stopped smoking more than 1 year previously, so that the effect of giving up smoking on fibrinogen levels could not have influenced the results of the study. Patients had received only nitrates for at least 2 weeks before blood sampling. Moreover, they had not taken any drugs able to interfere with platelet function or blood clotting for at least 3 weeks.

A control group composed of 40 clinically healthy subjects (age 55±7 years, range 47–66 years) with a normal lifestyle and no limitations on physical activity was investigated. The two groups were not different for mean age, body weight, percentage of smokers, blood arterial pressure, and plasma lipid profile. The study was carried out according to the principles of the Helsinki declaration. All subjects gave their informed consent for the use of part of their blood for research purposes. Patients also gave their consent to receive drug or placebo treatment.

Study design

The study was performed according to a single-blind randomized cross-over design. Unfractionated heparin (as calcium-heparin, Calciparina, Italfarmaco, Milan, Italy, 12,500 IU) or 0.9% saline solution as placebo were administered once a day at 8 a.m. for two sequential 6-month periods, which were preceded and separated by wash-out periods of 15 days. Patients were instructed to inject the heparin alternatively in the abdominal wall and in the buttock subcutaneous tissue. Placebo was injected intramuscularly in the buttock.

Blood sampling

On the first and last day of each treatment period venous blood was withdrawn in the morning, after overnight fasting, before the administration of heparin or saline solution. Blood was withdrawn carefully from a trauma-free venipuncture with a rapid flow of blood without venous stasis, using a 19-gauge needle by the two-syringe technique. After discarding the first 3–4 ml, 9 ml of blood was collected into polypropylene syringes containing 1 ml of anticoagulant solution for FPA assay purchased from Boehringer Mannheim Italia (Milan, Italy). Within 30 min of blood collection, tubes were centrifuged at 1,000×*g* for 30 min at 4 °C to obtain platelet-poor plasma. The removal of fibrinogen from plasma for FPA assay was carried out by two precipitations with bentonite, and the precipitated plasma was stored at –80 °C prior to assay. Citrated blood samples for F1+2, TAT, and fibrinogen assay (0.129 mol/l trisodium citrate, 1/10 v/v) were centrifuged at 1,500×*g* for 20 min at 4 °C and plasma was stored at –80 °C prior to assay.

Fibrinogen, F1+2, TAT, and FPA assays

Plasma fibrinogen levels were evaluated as clottable fibrinogen according to Clauss [11] using reagents from Baxter (Milan, Italy) with an autoanalyzer (MLA Electra 1000, MLA, Pleasantville, N.Y., USA). Plasma F1+2 and TAT were measured by ELISA [12, 13] using commercial kits (Behring Institute, Scoppito, Italy). Plasma FPA was measured by ELISA according to Amiral et al. [14] using a commercial kit (Boehringer Mannheim Italia). Fibrinogen, F1+2, TAT, and FPA values of 40 control subjects were 2.6±0.5 g/l, 1.0±0.3 nmol/l, 2.4±1.1 ng/ml, and 1.5±0.5 ng/ml respectively.

Statistical analysis

Results are reported as mean, standard deviation (SD), and range. Statistical analysis of the results was performed with analysis of variance and Student's *t*-test for paired and unpaired data. The level of significance was 0.05. For different parameters mean±2 SD of the control group was considered as normal range.

Results

Table 1 summarizes the fibrinogen, F1+2, TAT, and FPA levels of control subjects and patients during the two treatments.

Plasma fibrinogen levels

In IHD patients mean fibrinogen levels were higher than in the control group when patients were not on heparin ($P<0.001$). Fibrinogen significantly decreased ($P<0.001$) after the addition of low-dose heparin to the usual antianginal treatment (Table 1). The decrease was found in 36 of 40 patients (90%). The mean fibrinogen decrease was 17%, 31 with of 40 patients having plasma levels under 3 g/l.

Plasma F1+2 levels

Baseline plasma F1+2 levels were significantly higher ($P<0.001$) in IHD patients than in control subjects. No variations were found during the treatment with antianginal

drugs alone. F1+2 levels obtained before heparin treatment were not different from those found before the period on placebo. At the end of low-dose heparin treatment, the mean F1+2 plasma concentration was significantly lower than at the end of the period on placebo ($P<0.001$, Table 1). A decrease was found in a large (72.5%) percentage of patients (29 of 40); in particular, after low-dose heparin 69% of these 29 patients had F1+2 values within the normal range.

Plasma TAT levels

The pattern of plasma TAT levels was similar to that reported for F1+2 (Table 1). A decrease was found in all patients after heparin treatment compared with placebo, and in 22 of these 40 patients TAT levels were within the normal range.

Plasma FPA levels

The pattern of plasma FPA levels was similar to that reported for F1+2 (Table 1). A decrease was found in 95% of patients after heparin treatment compared with placebo, and in 23 of these 38 patients FPA levels were within the normal range.

Discussion

This study indicates that a 6-month treatment with low-dose heparin is able to significantly reduce plasma fibrinogen levels in IHD patients. The decrease in plasma fibrinogen is associated with a decrease in thrombin formation and activity, as indicated by decreased F1+2, TAT, and FPA plasma levels. Plasma fibrinogen levels are strongly correlated with thrombin activation [10] and the present study lends support to this by demonstrating that low-dose heparin treatment, known to reduce clotting activation [15], is also able to reduce fibrinogen levels.

Explanations other than heparin treatment for the reduction in fibrinogen levels are unlikely. In this cross-over study we instituted a short wash-out period. However, even if the heparin effect carried over into the placebo period, this would only have resulted in a beneficial effect at the beginning of the placebo period in the patients assigned to the heparin-placebo sequence. There may also be some concerns about the different sites of heparin and placebo injection. However, the procedure employed, although involving two different routes of administration ensured the patients were not aware which of the two medications was the active one. Indeed, our data are so clear-cut that they are unlikely to be affected by bias. One mechanism by which low-dose heparin may affect fibrinogen levels is a decrease of clotting activation. Interestingly, increased levels of F1+2 [16] and fibrin/fibrinogen split products [17] have been reported to modulate fibrinogen synthesis by the liver. Thus, the reduction of these products of clotting activation (and subsequent fibrin formation and lysis) would mediate the decrease in fibrinogen levels observed in this study.

The activity of heparin, unrelated to the presence of detectable heparin levels in circulating blood, is distinct from the anticoagulant activity which needs elevated plasma concentrations [18]. The administration of low-dose heparin represents a treatment able to remarkably enhance the antithrombotic properties of the vessel wall. Both in experimental animals [19] and in humans [20, 21] heparin can bind to arterial endothelium in vivo by saturable and reversible receptor binding. In humans the binding occurred at very low plasma heparin concentrations (0.05–0.1 U/ml) which do not affect the activated partial thromboplastin time. On the basis of the available data, the decrease in clotting activation by low-dose heparin could be attributable to an increased ability to inactivate thrombin and factor Xa by antithrombin III and heparin cofactor II bound to endothelium to which heparin specifically binds.

Another mechanism which may explain the results of this study is the binding of heparin to monocytes [22, 23]. In vitro inhibition by heparin of monocyte tissue factor and plasminogen activator inhibitor type 2 production and gene expression has been demonstrated [24]. Therefore, heparin may inhibit the increased monocyte tissue factor expression observed in IHD patients [25]. It is noteworthy that the pro-inflammatory cytokines interleukin-1 (IL-1β), tumor necrosis factor-α, (TNF-α), and especially IL-6 are able to induce fibrinogen synthesis by hepatocytes [26, 27]. Recently, the ability of heparin to inhibit both monocyte cytokine (IL-1β, TNF-α, IL-6) production [28] and mRNA expression [29] has been reported. These data suggest that heparin may reduce hepatic fibrinogen synthesis also via the inhibition of macrophage-produced inflammatory mediators [30]. Thus, heparin may decrease clotting activation and fibrinogen turnover by at least two mechanisms (antithrombin and antiinflammatory).

In conclusion, 6-month low-dose heparin treatment is associated with a decrease in thrombin generation and activity and decreased levels of fibrinogen. Irrespective of its mechanism, a heparin-induced fibrinogen decrease in plasma may reduce the risk of cardiovascular death. Interestingly, low-dose heparin treatment is associated both with a reduction in ischemic episodes in unstable angina [15] and decreased mortality and reinfarction in patients with recent myocardial infarction [31].

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