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Postoperative Changes in the Plasmatic Levels of Tissue-Type Plasminogen Activator and Its Fast-Acting Inhibitor – Relationship to Deep Vein Thrombosis and Influence of Prophylaxis

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Key words

Postoperative deep vein thrombosis – Tissue plasminogen activator – Plasminogen activator inhibitor

Summary

Fibrinogen, euglobulin lysis time (ELT), tissue-type plasminogen activator (t-PA) antigen, plasminogen activator inhibitor activity (PA-inhibitor) and α_2-antiplasmin (α_2-AP) were measured pre- and postoperatively in 60 patients undergoing total hip replacement. Reduced fibrinolytic activity as assessed by the prolongation of euglobulin lysis time, decrease of t-PA and increase of PA-inhibitor and α_2-AP could be demonstrated. These changes did not correlate with the postoperative deep vein thrombosis (DVT) diagnosed with the ^{125}I-fibrinogen test. However, preoperative PA-inhibitor activity was significantly higher in patients with postoperative DVT ($p < 0.01$). The prophylactic treatment with aspirin (20 patients) and with heparin plus dihydroergotamine (20 patients) induced significant changes in some of those parameters.

This study shows that the decrease of t-PA and the increase of PA-inhibitor may contribute to the reduced postoperative fibrinolytic activity after total hip replacement. PA-inhibitor level might be a useful marker in evaluating the risk of developing DVT in patients undergoing total hip replacement.

Introduction

Impairment of the fibrinolytic system has been found in association with postoperative venous thrombosis (1). A reduction in blood fibrinolytic activity after major surgery is generally accepted (2, 3, 4). Whether defective fibrinolysis is due to a decrease in plasminogen activator or enhancement of inhibitor levels has not been established yet (5, 6, 7). Recently, the existence of a fast-acting inhibitor of plasminogen activator (PA-inhibitor), which may play an important role in the regulation of fibrinolysis, has been demonstrated in human plasma (8, 9, 10). Increased levels in this PA-inhibitor have been observed in clinical and experimental conditions related to thrombotic phenomena (11, 12).

We studied the fibrinolytic activity assessed by the determination of tissue-type plasminogen activator (t-PA) and its fast-acting inhibitor pre- and postoperatively in patients undergoing total hip replacement to further correlate these parameters with the development of postoperative deep vein thrombosis (DVT). The influence of prophylaxis with aspirin or a combination of heparin plus dihydroergotamine (heparin-DHE) on those related changes is also evaluated.

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Materials and Methods

Patients

Sixty patients subjected to total hip replacement were randomized into three groups of 20 patients each:

- Control group. No specific prophylaxis for DVT was given. The mean age was 62 years and the sex ratio M/F was 13/7.
- Aspirin group. They received aspirin 0.5 g twice daily starting preoperatively until the seventh postoperative day. The mean age was 66 years and the sex ratio M/F was 11/9.
- Heparin-DHE group. They received a combination of 5000 IU of sodium heparin (Roche®) plus 0.5 mg dihydroergotamine (Sandoz®) subcutaneously, starting preoperatively and repeated every 12 hr for seven days. The mean age was 58 years and the sex ratio M/F was 11/9.

All patients were screened for DVT by the ^{125}I-fibrinogen uptake test as described (13).

Plasma samples were taken preoperatively and on the first, third and seventh postoperative days in siliconized vacutainer tubes (Terumo®, Leuven, Belgium) containing 1/10 volume of 0.13 mmol/l sodium citrate.

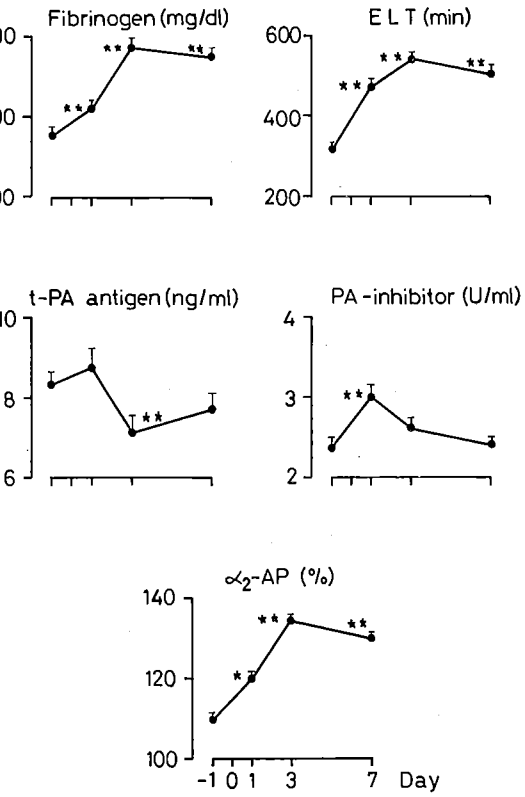
Reagents

Fibrinogen was purified according to Blombäck et al. (14). Plasminogen was prepared by affinity chromatography as described (15). Fibrinogen fragment was obtained by digestion of fibrinogen with CNBr as described (16).

Two-chain human melanoma cell t-PA was kindly provided by Dr. Collen (Leuven, Belgium). The preparation used in the present study had a specific activity of 100000 U/mg as compared to the International Urokinase Standard. Chromogenic substrate S-2251 was obtained from Kabi Diagnostica (Sweden).

Methods

Fibrinogen was determined by Clauss method (17). Euglobulin lysis time (ELT) was performed according to von Kaulla et al. (18). α_2-Antiplasmin (α_2-AP) was determined by chromogenic substrates as described (19). t-PA antigen was measured by a two-site immunoradiometric assay (IRMA) as described (20). The fast-acting inhibitor of plasminogen activator (PA-inhibitor) was determined by adding 2 U of t-PA to plasma diluted four-fold or more with 0.02 M tris HCl, 0.1 M NaCl, 0.01% Triton X-100 pH 8.8 (Tris buffer) and incubated for 1 min a 37° C. The samples (100 µl) were acidified with 100 µl of 0.16 M HCl and incubated 10 min at room temperature to efficiently destroy plasmin inhibitors. The pH was adjusted by the addition of 100 µl of 0.16 M NaOH and 700 µl of Tris buffer. In a microtitre plate (Linbro®, Flow Laboratories) were added 100 µl of the test solution and 100 µl of a mixture containing 0.5 µM human plasminogen, 0.6 mM S-2251 and 0.09 g/l CNBr fibrinogen digest. The plate was incubated at 37° C and the change in absorbance at 405 nM was measured with a titertek multiskan spectrophotometer (Flow Laboratories, Irvine, Scotland). Inhibitor activity was expressed in units of plasminogen activator inhibited per ml.



g. 1 Pre- and postoperative levels of fibrinogen, ELT, t-PA antigen, A-inhibitor and α_2-AP in 60 patients undergoing total hip replacement. ean ± SEM is reported. *p <0,03; **p <0,001

Statistical Methods

Student's t-test was used to compare groups. Student's t-test for paired observations was used to compare the values of different days within each group. The data were processed on a IBM 4341 computer and a BMDP program was used to perform the statistical analysis (21).

Results

Incidence of DVT

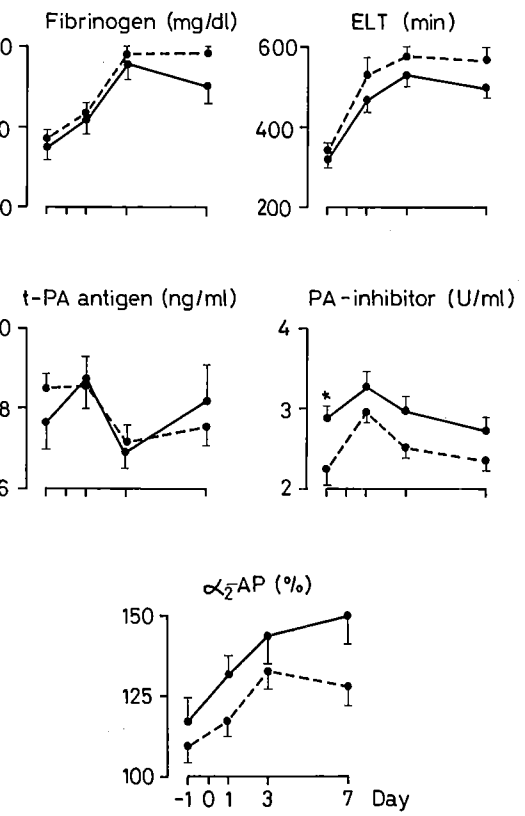
Among the 60 patients, 8 in control group (40%), 1 in aspirin group (5%) and 4 in heparin-DHE group (20%) developed DVT according to the ^{125}I-fibrinogen test. The difference was statistically significant for aspirin group as compared to control group ($p < 0.01$). Only one patient in the control group showed signs and symptoms of pulmonary embolism.

Postoperative Changes

The pre- and postoperative levels in the fibrinolytic parameters studied for the 60 patients are shown in Fig. 1.

There was a highly significant rise in the fibrinogen levels and a prolongation of euglobulin lysis time postoperatively ($p < 0.001$); t-PA antigen decreased on the third postoperative day when compared to the preoperative level ($p < 0.03$) with no differences on days 1 and 7. PA-inhibitor showed a rapid increase of short duration on the first postoperative day ($p < 0.001$) with no changes on days 3 and 7. α_2-AP increased slightly on day 1 ($p < 0.03$) and markedly on days 3 and 7 ($p < 0.001$).

No correlation was found between plasma PA-inhibitor activity and t-PA antigen levels.



r. 2 Pre- and postoperative levels of fibrinogen, ELT, t-PA antigen, -inhibitor and α_2-AP in patients with (−) and without (----) postopera- e DVT. Mean ± SEM is reported. *p <0.01

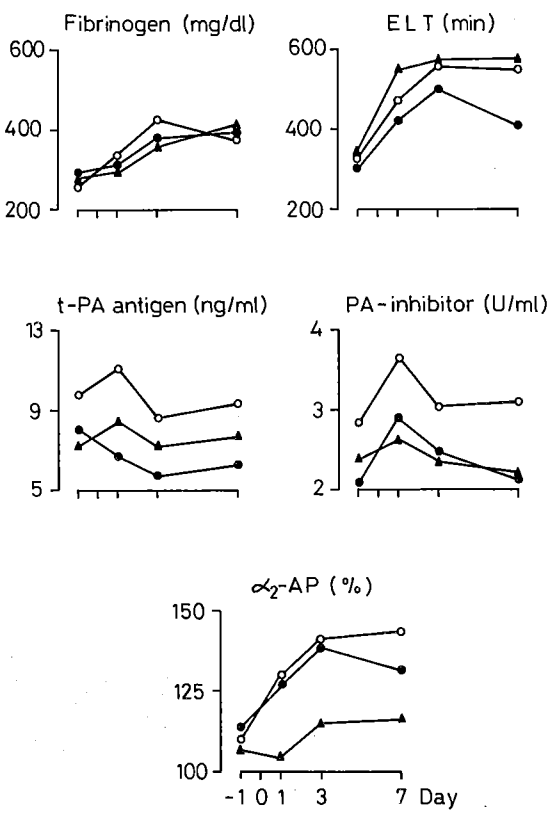


Fig. 3 Pre- and postoperative levels of fibrinogen, ELT, t-PA antigen, PA-inhibitor and α_2-AP in control group (○), aspirin group (●) and heparin-DHE group (▲)

Correlation with Isotopic Deep Vein Thrombosis

The pre- and postoperative levels in the fibrinolytic parameters for patients with and without DVT are shown in Fig. 2. There were no significant differences between the groups as regards the postoperative levels of fibrinogen, ELT and α_2-AP. Moreover, t-PA antigen and PA-inhibitor activity did not correlate with the postoperative DVT. However, PA-inhibitor levels were significantly higher preoperatively ($p < 0.01$) in patients with DVT (mean 2.8) than in those without DVT (mean 2.2).

Influence of Prophylaxis

As shown in Fig. 3 significant differences were observed when control, aspirin and heparin-DHE groups were compared in relation to the changes observed postoperatively. Fibrinogen and ELT showed similar evolution in the three groups. However, whereas in the control group there was a significant decrease in t-PA antigen on the 3rd postoperative day, an increase in PA-inhibitor on the 1st day and an increase in α_2-AP throughout the postoperative period with respect to the preoperative level, no differences in these parameters were observed in the heparin-DHE group. Moreover, lower t-PA antigen and higher PA-inhibitor levels were observed in the aspirin group with respect to the control group.

Discussion

Reported results on fibrinolytic parameters during the postoperative period are conflicting. A postoperative fibrinolytic shut-down and a reduced fibrinolytic activity have been related to the development of DVT (2, 4, 22). In this study impairment of fibrinolysis as assessed by reduced t-PA levels and increased PA-inhibitor and α_2-AP was observed (Fig. 1) and thus a postoperative fibrinolytic shut-down was present. However, no differences in the laboratory parameters studied were found in relation to the incidence of postoperative DVT (Fig. 2). This finding agrees with previous reports (22, 23, 24). We have found significantly higher preoperative PA-inhibitor levels in patients with postoperative DVT. The clinical relevance of this difference, not previously reported in the literature, is at present unknown but it seems that preoperative PA-inhibitor levels in plasma may have a predictive value and therefore be useful for identification of patients requiring prophylaxis of postoperative DVT.

When prophylaxis was administered significant postoperative changes were observed both in aspirin and heparin-DHE groups when compared to the control group (Fig. 3). A significant decrease in the t-PA antigen levels on the seventh postoperative day and an increase in PA-inhibitor on the third postoperative day with respect to the preoperative value were demonstrated in the aspirin group but not in the control group. The effect of aspirin on fibrinolysis has not been well established yet. Some authors have demonstrated an increase in fibrinolytic activity after ingestion of aspirin (25, 26); in contrast, Ghezzo et al. (27) have shown a decrease in fibrinolysis. A "paradoxical" thrombotic effect of aspirin was demonstrated under experimental conditions (28). Our results are in agreement with those recently reported by Levin et al. (29), who have shown that aspirin inhibits vascular plasminogen activator activity in vivo. No significant differences in t-PA antigen, PA-inhibitor and α_2-AP were found postoperatively in the heparin-DHE group when compared to the preoperative levels. The exact meaning of these findings related to heparin-DHE administration is unknown. Several studies show contradictory results regarding the effect of heparin on fibrinolysis (30, 31, 32).

The present study shows that the reduced overall fibrinolytic activity after total hip replacement is not due exclusively to the reduction in t-PA levels and may be explained by the increase in PA-inhibitor activity. However, these changes do not correlate with the development of postoperative DVT. The possibility that PA-inhibitor may be a useful predictive marker needs further evaluation.

Acknowledgements

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