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A sustained decrease in plasma fibrinolytic potential following partial liver resection or pancreas resection

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ABSTRACT

Background: Patients undergoing partial hepatectomy have a substantial risk for postoperative venous thrombosis even in the presence of optimal thromboprophylaxis. Recently we demonstrated a hypercoagulable state following a partial hepatectomy which was related to decreased plasma levels of natural anticoagulants and elevated levels of FVIII. The fibrinolytic status following partial hepatectomy has not been studied, but may display unique features as a result of temporarily decreased synthesis of fibrinolytic proteins.
Methods: We included 17 patients undergoing a partial hepatectomy and determined plasma fibrinolytic potential and measured plasma levels of individual fibrinolytic proteins in serial samples taken perioperatively. Results were compared to ten patients undergoing pancreas resection and twenty-four healthy volunteers.
Results and conclusion: Following both partial hepatectomy and pancreas resection plasma fibrinolytic potential decreased at the end of surgery, normalized on post-operative day 1, and decreased again on post-operative day 3 after which the hypofibrinolytic state gradually resolved. The hypofibrinolytic state on day 1 associated with increased plasma levels of PAI-1 in both groups. Plasma levels of plasminogen, α2-antiplasmin and TAFI all decreased following partial hepatectomy and pancreas resection and levels recovered over time. The kinetics of recovery were different for the different proteins and were slower in the liver resection group, resulting in a unique ratio of pro-to-anti-fibrinolytic proteins at each time point. This may explain the hypofibrinolytic status from day 3 onwards. A sustained plasma hypofibrinolytic state in combination with the hypercoagulable state we previously identified may contribute to the increased risk of thrombotic complications after partial liver resection.

1. Introduction

Surgery is a well-known risk factor for venous thrombosis. The mechanisms contributing to thrombotic events in abdominal surgery include stasis and alterations in the hemostatic system induced by tissue damage including increased activation of platelets and reduced levels of anticoagulant factors [1]. In addition, a temporarily impaired fibrinolysis has also been suggested to contribute to surgery-associated thrombosis [2].

We have recently reported on the hemostatic status of patients following a partial liver resection [3]. Shortly after partial liver resection, plasma levels of pro- and anticoagulant proteins are substantially reduced, which is partly related to the defective synthetic capacity of the liver remnant. The reduced levels of procoagulant proteins are reflected by an increase in the prothrombin time (PT). However, the prothrombin time wrongfully suggests a hypocoagulable state in these patients. We used thrombomodulin-modified thrombin generation testing of plasma samples taken during and after partial liver resection to obtain a more accurate measure of the hemostatic status. These studies showed that patients following a partial liver resection are in a hypercoagulable state, despite the prolonged PT [3]. Indeed, the risk of venous thrombosis following a partial liver resection is substantial even in the presence of optimal thromboprophylaxis, and the risk increases with the extent of the resection [4–7].

The status of the fibrinolytic system following a partial liver resection has not been systematically explored. Next to the 'general' postoperative fibrinolytic shutdown, which is a result of a temporary increase in plasma levels of plasminogen activator inhibitor type 1 (PAI-1) [8], additional changes in the fibrinolytic system as a result of a reduction of synthetic capacity of the liver may be expected.

Here we assessed plasma levels of fibrinolytic proteins and the overall fibrinolytic capacity as determined by a plasma-based clot lysis assay in patients following a partial liver resection. In addition, we studied patients undergoing a pancreas resection which is a surgical procedure of a similar extent, but without a decrease in post-operative synthetic capacity of the liver. A decreased fibrinolytic potential as determined by this assay has been consistently shown to be a risk factor for venous thrombosis [9–12].

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2. Materials and methods

2.1. Patients

In this study 17 patients who underwent a right ($n = 15$) or extended right ($n = 2$) hemi-hepatectomy were included. The control group consisted of 10 patients who underwent a pylorus preserving pancreaticoduodenectomy (PPPD). All of these patients were included in the University Medical Center Groningen in the Netherlands. Informed consent was obtained from the patient before inclusion. Exclusion criteria were age younger than 18 years, pre-existing coagulation disorders, pre-operative anti-coagulation, and the use of non-steroidal *anti*-inflammatory drugs or aspirin 1 week before surgery. The study was approved by the medical ethical committee. All patients underwent surgery according to standard surgical and anaesthetic procedures. Laboratory values were compared to a group of 24 healthy individuals recruited from our hospital. These healthy individuals were used to determine reference values for the various tests performed.

Blood samples were collected at the following time points: after induction of the anaesthesia, at the end of surgery and on post-operative days 1, 3, 5, 7 and 30. Blood samples from each patient were drawn from a dedicated arterial line after induction of anaesthesia and at day 1, and by vena puncture on postoperative days 3–30 and collected in vacuum tubes containing 3.8% tri-sodium citrate as an anticoagulant, at a blood to anticoagulant ratio of 9:1. None of the patients received anticoagulants prior to surgery, and all received standard thromboprophylaxis (LMWH, Nadroparin 2850 Units once daily) until patients are mobilised. On all post-operative days, blood was drawn just prior to the LMWH infusion. Samples were centrifuged at 2000*g* and 10,000*g* at 18 °C for 10 min. Plasma was immediately snap frozen and stored at −80 °C until use.

2.2. Assays of individual proteins

Plasminogen and α2-antiplasmin levels were measured on an automated coagulation analyzer (ACL 300 TOP) with reagents and protocols from the manufacturer (Instrumentation Laboratory, Breda, the Netherlands). PAI-1 antigen levels were measured using a commercially available ELISA from R&D Systems (Abingdon, UK). The levels of thrombin-activatable fibrinolysis inhibitor (TAFI) were measured on an automated coagulation analyzer (BCS-XP) using a commercially available kit (Pefakit, Pentapharm, Basel, Switzerland).

2.3. Overall plasma fibrinolytic potential

The overall plasma fibrinolytic potential was determined using an in-house assay as previously described [10]. In short, 50 μl plasma was mixed with a solution containing phospholipid vesicles (40% L-alpha-dioleoylphosphatidylcholine, 20% L-alpha-dioleoylphosphatidylserine, and 40% L-alpha-dioleoylphosphatidylethanolamine, final concentration 10 μM), tissue factor (Innovin, Siemens Healthcare Diagnostics, final dilution 1:1000), tissue-type plasminogen activator (tPA, Actilyse, Boehringer Ingelheim B.V., Ingelheim am Rhein, Germany, 56 ng/ml), and $CaCl_2$ (final concentration 17 mM), diluted in HEPES buffer (25 mM HEPES [*N*-2-hydroxytethylpiperazine-*N*′-2ethanesulfonic acid], 137 mM NaCl, 3.5 mM KCl, 3 mM CaCl2, 0.1% bovine serum albumin, pH 7.4) and pipetted in a 96-well microtiter plate. After mixing, the plate was incubated at 37 °C in a spectramax 340 kinetic microplate reader (Molecular Devices corporation) and the optical density was monitored every 20 s at 405 nm, resulting in a clot-lysis turbidity profile. The clot lysis time (CLT) was derived from this clot-lysis profile and defined as the time in minutes from the midpoint of the clear to the maximum turbid transition, representing clot formation, and from the midpoint of the maximum turbid to the clear transition, representing the lysis of the clot.

2.4. Statistical analysis

All calculations were performed using GraphPad Prism software (San Diego, CA, USA) and IBM SPSS statistics 22 (New York, NY, USA). Values are expressed as means (with s.d.), medians (with interquartile ranges), or numbers (with percentages) as appropriate. Differences between baseline values of CLT or individual proteins, and follow-up values were evaluated by mixed linear models. To determine differences between independent groups a *t*-test or Mann-Whitney *U* test, as appropriate, was used. Differences between patient values and healthy controls were compared using Kruskal-Wallis H-test (with Dunn's post-test). p-Values < 0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

Fifteen patients who underwent a partial hepatectomy and two patients that underwent an extended right hemi-hepatectomy were included in this study, as were 10 patients who underwent a PPPD, and 24 healthy controls. The patient characteristics are displayed in Table 1. The most common indication for the partial hepatectomy was metastasis from colon cancer. For the PPPD group the most common indication was pancreatic cancer. There were no cases of thrombosis in the 30 days after surgery in the patient groups.

3.2. Fibrinolytic proteins

We first determined plasma levels of individual fibrinolytic proteins (Fig 1).

Plasma levels of plasminogen were 99% (94–107) [median (interquartile range)] in the controls, 90% (81–96) in the patients undergoing partial hepatectomy ($p > 0.05$ compared to controls) and 86% (79–99) in the patients undergoing PPPD at the start of surgery ($p > 0.05$ compared to controls). In patients undergoing partial hepatectomy, plasminogen levels decreased to 40% (30–46) on postoperative day 3 ($p < 0.0001$ compared to baseline) and increased to baseline levels on postoperative day 30. In patients undergoing a PPPD plasma levels of plasminogen decreased to 48% (38–65) on postoperative day 1 ($p < 0.001$ compared to baseline) and increased to baseline levels on postoperative day 5. The recovery of plasminogen levels was faster in the PPPD group as the level of plasminogen was still significantly lower in the partial hepatectomy group on post-operative day 3, 5 and 7 compared to the PPPD group($p = 0.0002/0.01/0.007$, respectively).

α2-antiplasmin levels were 95% (89–102) in the controls, and baseline α2-antiplasmin levels were 85% (80–92) in patients undergoing partial hepatectomy ($p > 0.05$ compared to controls), and 82% (78–89) in patients undergoing PPPD ($p > 0.05$ compared to controls). α2-antiplasmin levels decreased to 52% (45–69) at the end of surgery ($p < 0.0001$ compared to baseline) in patients undergoing partial hepatectomy and recovered to baseline levels on postoperative day 5. In the PPPD group α2-antiplasmin levels decreased to 60% (41–65) at the end of surgery ($p < 0.0001$ compared to baseline) and then increased to a maximum of 106% at postoperative day 5 ($p < 0.001$ compared to baseline). The recovery of α2-antiplasmin was faster in the PPPD group as the α2-antiplasmin levels were significantly lower in the partial hepatectomy group on post-operative day 3, 5 and 7 compared to the PPPD group ($p = 0.0002/0.007/0.002$, respectively). Recovery of α2-antiplasmin started earlier and was faster than the recovery of plasminogen.

TAFI plasma levels were 85% (79–92) in the control group, and baseline levels were 85% (74–100) in patients undergoing a partial hepatectomy ($p > 0.05$ compared to controls) and 78% (66–96) in the patients undergoing a PPPD ($p > 0.05$ compared to controls). TAFI levels decreased to 50% (39–64) on post-operative day 1 ($p < 0.0001$ compared