Thrombin-Activatable Fibrinolysis Inhibitor Is Activated in an Instant Blood-Mediated Inflammatory Reaction After Intraportal Islet Transplant

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Abstract

Objectives: Activated thrombin-activatable fibrinolysis inhibitor is a coagulation factor in some thrombotic diseases. However, available data on whether thrombin-activatable fibrinolysis inhibitor is activated in islet transplant are limited. In this study, changes of plasma-activated thrombin-activatable fibrinolysis inhibitor levels in instant blood-mediated inflammatory reaction after islet transplant were assessed.

Materials and Methods: Plasma concentrations of thrombin-antithrombin complex, D-dimer, C-peptide, and activated thrombin-activatable fibrinolysis inhibitor were assessed at 0 minutes, 30 minutes, 1 hour, 6 hours, 12 hours, and 24 hours after an intraportal islet transplant using rats via an enzyme-linked immunosorbent assay, or solid-phase, 2-site chemiluminescent immunometric assay. We recovered the liver at 1 hour after the transplant for histologic examination.

Results: Thrombin-antithrombin complex, C-peptide, and activated thrombin-activatable fibrinolysis inhibitor levels increased immediately after we stopped islet infusion, and their peak levels occurred at 1 hour after islet infusion. D-dimer levels increased continually after islet infusion was stopped, and peaked 24 hours after infusion. Histologic examination of the liver 1 hour after islet infusion revealed frequent portal venous thrombi, with entrapped islets. The entrapped islets showed a disrupted morphology.

Conclusions: Activated thrombin-activatable fibrinolysis inhibitor was generated and peaked 1 hour after islet transplant according with activating coagulation, indicating that thrombin-activatable fibrinolysis inhibitor is activated and accumulated at levels in instant blood-mediated inflammatory reaction was sufficient to affect fibrinolysis.

Key words: Diabetes mellitus, Instant blood-mediated inflammatory reaction, Islet transplant, Thrombin-activatable fibrinolysis inhibitor

Introduction

Intraportal islet allotransplant has become promising therapy for patients with type 1 diabetes mellitus.1 However, the procedure’s success is hindered by the islet loss that occurs immediately after transplant, leaving a final islet cell survival of around 20% to 40% of a healthy nondiabetic subject.2 A major cause of early islet loss is instant blood-mediated inflammatory reaction (IBMIR), which occurs rapidly when islets come in direct contact with blood after infusion of the portal vein. It is characterized by coagulation activation, inflammatory cell infiltration, and subsequent insulin dumping. Inflammatory thrombosis is driven by a tissue factor and manifests itself clinically with formation of thrombi with entrapped islets.3,4

The most recently identified coagulation factor is activated thrombin-activatable fibrinolysis inhibitor (TAFIa).5,6 Upon activation by thrombin/thrombomodulin, TAFI becomes the active TAFI (TAFIa), and modulates fibrinolysis in vivo by cleaving C-terminal lysine residues from...
partially degraded fibrin. Because these residues serve as plasminogen binding sites, removal of them by TAFIa reduces local concentrations of plasmin at the site of the clot. One of the properties of plasmin is anticoagulation. Thrombin-activatable fibrinolysis inhibitor serves to inhibit plasmin recruitment at the forming thrombus; thus, TAFIa is a potent inhibitor of clot lysis, protecting fibrin clots against fibrinolytic attack. Some studies have shown that increased TAFIa is a risk factor for ischemic stroke, acute myocardial infarction, and venous thrombosis. Although not all thrombotic disorders will cause activation of TAFI. Coagulation activation in a baboon model of low-dose E. coli induces sepsis not accompanied by activated TAFI, while continuous TAFI activation up to 8 hours, is seen in coagulation activation seen by high-dose E. coli. However, the available data on activation of TAFI in IBMIR are limited. In this study, we assessed the changes of plasma TAFIa levels after islet transplant.

Materials and Methods

Preparation of rat islets
Animals were supplied by the experimental animal center of Tongji University in Shanghai, China. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (publication no. 85-23, revised 1985) and is approved by the Ethics Committee of Tongji University. Pancreatic islets were obtained from adult male Sprague-Dawley rats, weighing 250 to 300 grams, by collagenase V (Sigma-Aldrich, St. Louis, MO, USA); digestion and discontinuous Ficoll density gradient centrifugation (MEDIATECH, INC., Herndon, VA, USA). After washing and handpicking, islets were cultured for 24 hours in RPMI-1640 (Gibco [now Invitrogen Corporation], Carlsbad, CA, USA) medium supplemented with 10% fetal bovine serum in a humidified 5% CO₂ incubator at 37°C. Islet purity was assessed by dithizone (Sigma) staining after isolation. Islet viability was assessed via fluorescence staining with acridine orange and propidium iodide (Sigma). Islet isolations with > 90% viability and > 90% purity were used. Before transplant, islets were washed 3 times and suspended in phosphate buffered saline (PBS).

Animal experiment design
Seventy-two normal Sprague-Dawley rats received 30 islet grafts. Receptors were randomly divided into 2 groups (experimental and control). Each group was further divided into 6 subgroups with 6 rats in each subgroup to obtain blood before infusion and at 30 minutes, 1 hour, 6 hours, 12 hours, and 24 hours after infusion. Rats in the experimental group received intraportal injection of islets at 800 IEQ/rat, and those in the control group received only PBS.

Plasma analysis
When they were killed, rats were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg). Blood was drawn from the inferior vena cava into tubes containing 3.8% sodium citrate (9 parts blood, 1 part anticoagulant). Plasma was obtained by centrifugation and stored at -80°C. Plasma thrombin-antithrombin complex (TAT) level was determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Behringwerke, Marburg, Germany). D-dimer level was measured with ELISA kits (American Diagnostica Inc., Greenwich, CT, USA). C-peptide level was measured using solid-phase, 2-site chemiluminescent immunometric assay kits (Mercodia AB, Uppsala, Sweden). Plasma TAFIa levels were measured with ELISA kits (American Diagnostica Inc.).

Histopathologic examination
Livers were recovered 1 hour after islet infusion. Liver biopsy samples were fixed in paraformaldehyde. Sections of the liver biopsies then were stained with hematoxylin and eosin stain.

Statistical analyses
The data are presented as mean values ± standard deviations. The analysis of variance (ANOVA) test was used to assess statistical significance at different times within groups; the paired t test was used to assess statistical significance between 2 groups at the same time. Values of $P < .05$ were considered statistically significant.

Results

Expression of TAT, D-dimer, C-peptide, TAFIa during islet transplant
Reflecting the activation of coagulation, TAT levels increased immediately after islet infusion was
stopped, and the peak level in the series was reached after only 1 hour, and then decreased gradually. After 12 hours, the levels returned to baseline values (Figure 1A). Reflecting the activation of fibrinolysis, D-dimer levels increased continually after islet infusion was stopped and peaked at 24 hours after infusion (Figure 1B). C-peptide was released immediately after infusion. The levels peaked 1 hour after infusion and declined thereafter, but never reached baseline levels (Figure 1C). TAFIa levels increased immediately after islet infusion was stopped, and peaked 1 hour after infusion, and then decreased rapidly. After 6 hours, levels had returned to baseline values (Figure 1D). No obvious changes in the levels of TAT, D-dimer, C-peptide, and TAFIa were observed in the vehicle-treated control rats.

Histologic evaluation of the liver after intraportal islet transplant

Plasma analyses showed that activation of coagulation and release of C-peptide occurred mainly during the first hour after infusion, so the time point of histologic examination for the liver was chosen 1 hour after islet infusion. Results revealed frequent portal vein thrombi with entrapped islets, and the entrapped islets showed a disrupted morphology (Figure 2).

Discussion

The general characteristics of IBMIR are activation of coagulation and disruption of normal islet morphology 1 hour after islet infusion. Reflecting the magnitude of the IBMIR, activation of coagulation indicated by elevated TAT peaked 1 hour after islet infusion. The generation of D-dimer further strengthens the appearance of the coagulation after transplant. In parallel with coagulation, a rapid liberation of C-peptide and disruption of islet morphology were observed during the first hour, suggesting that transplanted islets were damaged severely during the thrombotic reaction. Release of C-peptide as a result of glucose stimulation is highly unlikely, because the glucose concentration in nondiabetic rat receptors is normal. Therefore, IBMIR after islet transplant may be responsible for this rapid release of C-peptide.

This is the first study to report on the kinetics of TAFIa plasma concentrations during the development of IBMIR after an islet transplant. This rapid and continuous increasing of TAFIa concentration during the first hour after infusion is consistent with the increasing level of TAT, which might be indicative of higher levels of thrombin/thrombomodulin complex generated after islet transplant, this is thought to be the physiologic TAFI activator. Although definite proof of a causal relation is currently unavailable, it is reasonable to interpret this temporal profile as a rapid increase of TAFIa during the first hour after transplant, indicating that TAFIa favors islet clotting because of its antifibrinolytic activity.

To obviate the effects of IBMIR, some methods of regulating early coagulation, including systemic administration of anticoagulants such as heparin, melagatran, dextran sulfate, and nacystelyn, have been shown to prevent islet-induced coagulation and
reduce islet loss to a certain extent. However, it is difficult to apply these anticoagulants in the clinical environment because systemic administration is associated with an increased risk of severe bleeding. Therefore, there is an urgent need to explore new therapeutic targets for treating IBMIR.

Previous studies have shown that endogenous thrombolyis is enhanced, and thrombosis is reduced, by inhibiting TAFIa in different thrombus models. Additionally, inhibition of TAFIa represents a potentially subtle adjustment of the clotting and lysis balance, without affecting the coagulation cascade. Ideally, this subtlety may reduce the risk of bleeding when using TAFIa inhibitor, compared with other potential mechanisms for treating thrombotic disease. Because administration of TAFI inhibitor does not produce an increase in bleeding time in a rat-transsection bleeding model, TAFIa would be a safe and effective drug for inhibiting thrombosis in IBMIR. TAFIa peaks 1 hour after infusion then declines to its baseline level, suggesting that 1 hour after infusion might be the best intervention time for IBMIR.

Taken together, the present study shows that TAFIa is generated and peaks 1 hour after islet transplant in accord with activation of coagulation, indicating that TAFI is activated and accumulates at levels in which IBMIR is sufficient to affect fibrinolyis. Taking into account that inhibition of TAFIa may enhance endogenous thrombosis, while leaving the coagulation system intact, TAFIa might be an innovative drug target for eliminating the effects of IBMIR.

References


