Inhibition of Thrombin Abrogates the Instant Blood-Mediated Inflammatory Reaction Triggered by Isolated Human Islets

Possible Application of the Thrombin Inhibitor Melagatran in Clinical Islet Transplantation

Lisa Özmen,[1] Kristina Nilsson Ekdahl,[1,2] Graciela Elgue,[1] Rolf Larsson,[1] Olle Korsgren,[1] and Bo Nilsson[1]

A thrombotic/inflammatory reaction is elicited when isolated islets of Langerhans come in contact with ABO-compatible blood. The detrimental effects of this instant blood-mediated inflammatory reaction (IBMIR) provide a reasonable explanation for the observation that an unexpectedly high number of islets, from several donors, are needed to produce normoglycemia in transplant patients with type 1 diabetes. In this study, the hypothesis that a specific thrombin inhibitor, Melagatran, could reduce IBMIR in an in vitro model in which human islets are exposed to ABO-compatible blood was tested. The administration of Melagatran abrogated IBMIR dose-dependently. Islets exposed to blood, in the absence or presence of 0.4 µmol/l Melagatran, exhibited a loss of integrity and were found to be trapped in macroscopic clots containing platelets and CD11b^{+} leukocytes. At concentrations from 1 to 10 µmol/l, Melagatran inhibited both coagulation and complement activation. Also, platelet and leukocyte activation and consumption were decreased. Islet morphology was maintained with almost no platelets adhering to the surface, and infiltration by CD11b^{+} leukocytes was considerably reduced. In conclusion, Melagatran significantly reduced IBMIR in this model system. This protective effect indicates that thrombin plays a pivotal role in IBMIR and suggests that thrombin inhibition can improve the outcome of clinical islet transplantation. *Diabetes* 51:1779–1784, 2002

From the [1]Department of Radiology, Oncology and Clinical Immunology, Division of Clinical Immunology, the Rudbeck Laboratory, University Hospital, Uppsala, Sweden; and the [2]Department of Chemistry and Biomedical Science, University of Kalmar, Kalmar, Sweden.

Address correspondence and reprint requests to Bo Nilsson, MD, Department of Radiology, Oncology and Clinical Immunology, Division of Clinical Immunology, the Rudbeck Laboratory, University Hospital, S-75185 Uppsala, Sweden. E-mail: bo.nilsson@klinimm.uu.se.

A major breakthrough was made in the use of clinical islet transplantation as a cure for type 1 diabetes when Shapiro et al. (1) demonstrated that insulin independence could be obtained using a glucocorticoid-free immunosuppressive regimen. However, islets from more than one donor were needed to achieve insulin independence (1). In a follow-up study, it was estimated that the metabolic capacity in these patients corresponded to only 20% of that in healthy subjects (2). Because glucocorticoids had not been used and thus could not have been responsible for any adverse effects on islet function, the authors concluded that some other adverse process(es) must be involved in the loss of islet tissue.

We have previously reported that a thrombotic/inflammatory reaction is elicited when islets come in direct contact with blood in an in vitro tubing loop model. Similar reactions have also been observed in clinical islet transplantation (3) and in vivo in an allogenic pig model of intraportal islet transplantation (4). The detrimental effects of this instant blood-mediated inflammatory reaction (IBMIR) could provide a reasonable explanation for the relatively low success rate in clinical islet transplantations and could explain the need for islets from several donors to obtain normoglycemia in the transplantation series performed by Shapiro and co-workers (1,2).

The initial event in IBMIR is a rapid loss of platelets, with a simultaneous secretion of β-thromboglobulin (β-TG) that indicates platelet activation. At the same time, activated platelets bind to the islets and contribute to a continuous fibrin formation that eventually generates a capsule containing platelets and infiltrating CD11b^{+} leukocytes. Extensive generation of the coagulation and complement activation products thrombin-antithrombin (TAT) and FXIa-antithrombin (FXIa-AT) complexes, C3a, and sC5b-9 indicate that both the complement and coagulation cascade systems have been activated. Taken together, these reactions point toward a platelet-amplified reaction.

A strategy to prevent or decrease IBMIR could be of utmost importance in minimizing islet loss in the immedi-

ate posttransplantation period. Several steps in the process of IBMIR could serve as targets for pharmacological intervention as a means of preventing the reaction and promoting engraftment of the islets. One obvious candidate drug that affects platelet function is acetylsalicylic acid. However, in doses required to inhibit platelet aggregation completely, we have not observed any effects on IBMIR in vitro in the tubing loop model (unpublished observations).

Another approach to affect platelet function is to inhibit thrombin. Thrombin, a serine proteinase generated from the zymogen prothrombin, is considered to be the key enzyme in the clotting reaction. Thrombin is a potent platelet activator, acting via the specific cell-surface receptors PAR-1 and PAR-4 (5,6). Thrombin-activated platelets provide an appropriate surface for the assembly of coagulation complexes involved in the amplification of the clotting cascade (7). Several established thrombin inhibitors are available, including unfractionated heparin (8) and hirudin (9). These drugs, however, are limited in terms of their efficacy and safety because of a narrow therapeutic window and poor penetration into the thrombus (10).

In the present study, we examined the effect of a reversible and highly specific low-molecular-weight inhibitor of thrombin, Melagatran (Astra Zeneca, Hässle, Gothenburg, Sweden) (11–15). Because of its small size (430 Da) and high affinity, the drug has the ability to inhibit thrombin rapidly also within the developing clot (16). Melagatran has been used extensively in clinical studies and shown to have a wide therapeutic window, with minimal bleeding complications (12,16). By using this inhibitor in an in vitro tubing loop model, we have been able to investigate the role of thrombin in the thrombotic reaction elicited when human islets come in contact with ABO-compatible blood. Our findings indicate that thrombin may play an important role in the IBMIR reaction that occurs under these conditions and that Melagatran may be effective in reducing the adverse effects of IBMIR in clinical islet transplantation.

RESEARCH DESIGN AND METHODS

Islet isolation. Pancreata were obtained from cadaver donors after consent was obtained either from the organ donor registry or from relatives. The organs were obtained from three female and two male normoglycemic donors (aged 59–85 years, three with blood group O and two with blood group A). The islets were isolated at the Division of Clinical Immunology at the University of Uppsala using a modification of previously described semiautomated digestion-filtration methods (17–19). This isolation procedure was followed by further purification on a continuous density gradient in a refrigerated COBE 2991 centrifuge (COBE Blood Component Technology, Lakewood, CO). The islets were placed in untreated culture flasks and maintained in suspension culture at 37°C (5% CO_2) for 1–5 days. The culture medium (CMRL 1066; ICN Biomedicals, Costa Mesa, CA) was changed every other day. The volume and purity of the islets were determined by microscopic sizing on a grid after staining with diphenylthiocarbazone. The purity of the islet preparations used in this study ranged from 40 to 95% (70 ± 6.3%). Viability was assessed in terms of insulin secretion in response to a glucose challenge in a dynamic perfusion system (in 1.67 mmol/l glucose, then in 16.7 mmol/l, and finally back to 1.67 mmol/l) in the presence and absence of 10 μmol/l Melagatran.

Heparin treatment. All materials that came in contact with whole blood were furnished with a Corline heparin surface (Corline, Uppsala, Sweden) according to the manufacturer's recommendation (20). The surface concentration of heparin was 0.5 μg/cm², corresponding to ~0.1 unit/cm², with an antithrombin binding capacity of 2–4 pmol/cm².

Preparation of blood. Fresh human blood, obtained from healthy volunteers who had received no medication for at least 14 days, was collected in surface-heparinized 60-ml syringes (18 gauge, Microlance; Becton Dickinson, Franklin Lakes, NJ). The cannulae of the syringes were connected to surface-heparinized silicon tubing. During sampling, the syringes were rotated continuously.

Tubing loops as a model. A modification of the model previously described was used (4,20,21). This device consisted of loops made of polyvinyl chloride (diameter, 6.3 mm; length, 390 mm) whose inner surface was furnished with immobilized heparin. The tubing was held together with a specially designed heparinized connector. A circular loop was formed when the ends of the connector were tightly pushed into the lumen of the tubing ends.

A rocking apparatus, placed in a 37°C incubator, was used to generate blood flow inside the loops. The loops were rocked at a setting that prevented the blood from coming in contact with the connectors. Up to 12 devices could be rocked at the same time. Ten 60-min islet experiments were performed, with islets isolated from five different donors.

Melagatran, dissolved in 5 mmol/l citrate buffer containing 0.15 mol/l NaCl (pH 5.5), was tested at 0, 0.4, 1, 4, and 10 μmol/l. For each experiment, two loops of fresh human blood without islets, one containing CMRL 1066 (GibcoBRL) and one containing 10 μmol/l Melagatran, were included as controls. Fresh ABO-compatible human blood from the same donor (5 ml) was added to each loop. The loops were placed on the rocking device for a 10-min preincubation with Melagatran or citrate buffer. Thereafter, the loops were opened, and 150 μl CMRL 1066, with or without 4 μl islets (~4,000 islet equivalents), was added to the loops and followed by another 60-min incubation on the rocking device at 37°C. Blood glucose levels were measured with a glucometer (Glucometer Elite; Bayer Diagnostics, Leverkusen, Germany) before the perfusion.

After every perfusion, the loop contents were filtered through 70-μm-diameter filters (Filcons, Cuptype; DAKO, Glostrup, Denmark). Both macroscopic blood clots and tissue recovered on the filters were frozen in liquid nitrogen for immunohistochemical staining. For immunohistochemical comparison, nonperfused islets were also frozen and stained by the same procedure. The remaining filtered blood was collected in 4.1 mmol/l EDTA (final concentration) and used for hematologic analysis (platelets, lymphocytes, monocytes, and granulocytes) and assays of coagulation activation (prothrombin fragments 1 and 2, TAT and FXIa-AT), complement activation (C3a and sC5b-9), platelet activation (β-TG), and insulin. Samples taken at 0 min were also included. In these samples, the blood was not added to the tubing loop but was instead transferred immediately to the EDTA-containing tubes. The blood samples were centrifuged at 4°C at 3,290g for 20 min, and the plasma was collected and stored at −70°C until analyzed.

Activation of complement in wells of microtiter plates. The direct effect of Melagatran on the complement system was studied using a method in which serum was incubated in the wells of microtiter plates (22). Serum (100 μl) was added to each well of a 96-well microtiter plate together with Melagatran at a final concentration of 0, 0.4, 1, 4, or 10 μmol/l. After a 30-min incubation at 37°C, the serum was transferred to tubes containing EDTA (final concentration, 10 mmol/l). These samples were stored at −70°C before analysis of the complement fragment C3a (see below).

To detect bound C3 fragments, we washed the wells of the microtiter plates with phosphate-buffered saline containing 0.05% (vol/vol) Tween 20 and incubated with 100 μl of horseradish peroxidase (HRP)-conjugated anti-C3c (DAKO) for 60 min at 37°C. The binding of antibody was detected by the addition of 1,2-phenylenediamine dihydrochloride (Buchs, Fluka, Switzerland), and the staining was monitored at 492 nm.

Blood and plasma analysis. Platelet counts and differential leukocyte counts were analyzed on a Coulter ACT-diff analyzer (Beckman Coulter, Miami, FL) using EDTA-treated blood.

Enzyme immunoassays

Prothrombin fragments 1 and 2 and TAT. Plasma levels of prothrombin fragments 1 and 2 and TAT were quantified using commercially available enzyme immunoassay (EIA) kits (Enzygnost Prothrombin, fragments 1 and 2; TAT, Behringswerke, Marburg, Germany). The values are given as nanomoles per liter and micrograms per liter, respectively.

FXIa-AT. Complexes between FXIa and AT were measured in plasma according to the method of Sanchez et al. (23). Values are expressed as micromoles per liter.

β-TG. β-TG was analyzed in EDTA plasma using Asserachom B-TG (Diagnostica Stago, Asnières-sur-Seine, France). The values are expressed as international units per milliliter.

C3a. EDTA plasma was analyzed as previously described (24). Monoclonal antibody 4SD17.3 was used as capture antibody. Bound C3a was detected with biotinylated polyclonal anti-C3a followed by HRP-conjugated streptavidin (Amersham, Buckinghamshire, U.K.). Zymosan-activated serum, calibrated against a solution of purified C3a, served as a standard, and the values are given as nanograms per milliliter.
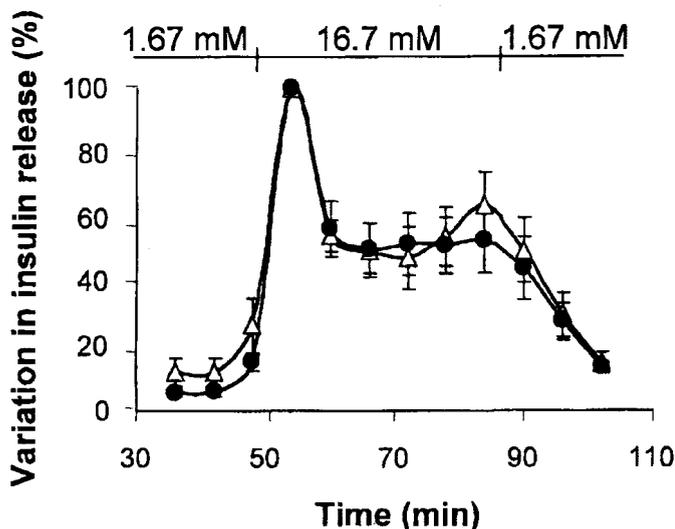
FIG. 1. Islet function tested in a dynamic perfusion system. Islets from three different pancreata were cultured in the presence (△) and absence (●) of 10 μmol/l Melagatran for 3 days. The figure shows insulin release from the islets after stimulation with glucose (1.67, 16.7, and 1.67 mmol/l).

sC5b-9. Plasma was analyzed using a modification of the EIA described by Mollnes and colleagues (24,25). EDTA plasma was added to microtiter plates coated with monoclonal antibody anti-neoC9. sC5b-9 was detected by polyclonal anti-C5 antibodies (DAKO), followed by HRP-conjugated anti-rabbit immunoglobulin (DAKO). Zymosan-activated serum, defined as containing 40,000 arbitrary units (AU/ml), served as the standard.

Insulin. Plasma concentrations of insulin were analyzed before and after islet perfusion with a commercial EIA kit (DAKO). Values are given as milliunits per liter

Immunohistochemical staining. Islets and macroscopic clots recovered on filters after perfusion with blood and various concentrations of Melagatran were collected in an embedding medium (Tissue-Tek; Miles, Eckhart, IN) and snap-frozen in liquid nitrogen. Islets were sectioned and subsequently stained with HRP-conjugated mouse anti-human CD41a (R&D Systems, Abingdon, U.K.) and anti-CD11b (Clone 2LPM 19c; DAKO).

Statistical analysis. Because of individual variation in blood cell counts and in plasma parameters, the changes were calculated either as a percentage of the values obtained in the medium control loop (for blood cells) or as a ratio of the values obtained in the experimental loop and in the islet loop with no Melagatran. All results are expressed as mean ± SEM. Mean values were compared using Friedman ANOVA (Analyse-It, Version 1.44, Software LTD, Leeds, U.K.). The significance was determined at $\alpha = 0.05$.

RESULTS

Islet function is not affected by Melagatran. To confirm that Melagatran had no adverse effect on islet function, we cultured islets at 10 μmol/l Melagatran for 3 days and then tested them in a dynamic perfusion system in which insulin secretion was assessed after stimulation with glucose. Melagatran showed no effect on islet function (Fig. 1).

Perfusion of human islets with fresh human ABO-compatible blood in the tubing loop model. Glucose concentrations in the blood before islet perfusion ranged from 4.5 to 7.4 mmol/l. After a 60-min incubation of fresh nonanticoagulated human blood without islets in the control tubing loops, a slight drop was seen in the blood cell counts when compared with the 0-min samples (Table 1). In addition, increases (3- to nearly 50-fold) in the coagulation (TAT, prothrombin fragments 1 and 2, and FXIa-AT), platelet (β-TG), and complement (C3a and sC5b-9) parameters were seen (Table 1). All of these alterations, however, were in absolute values small and considered to
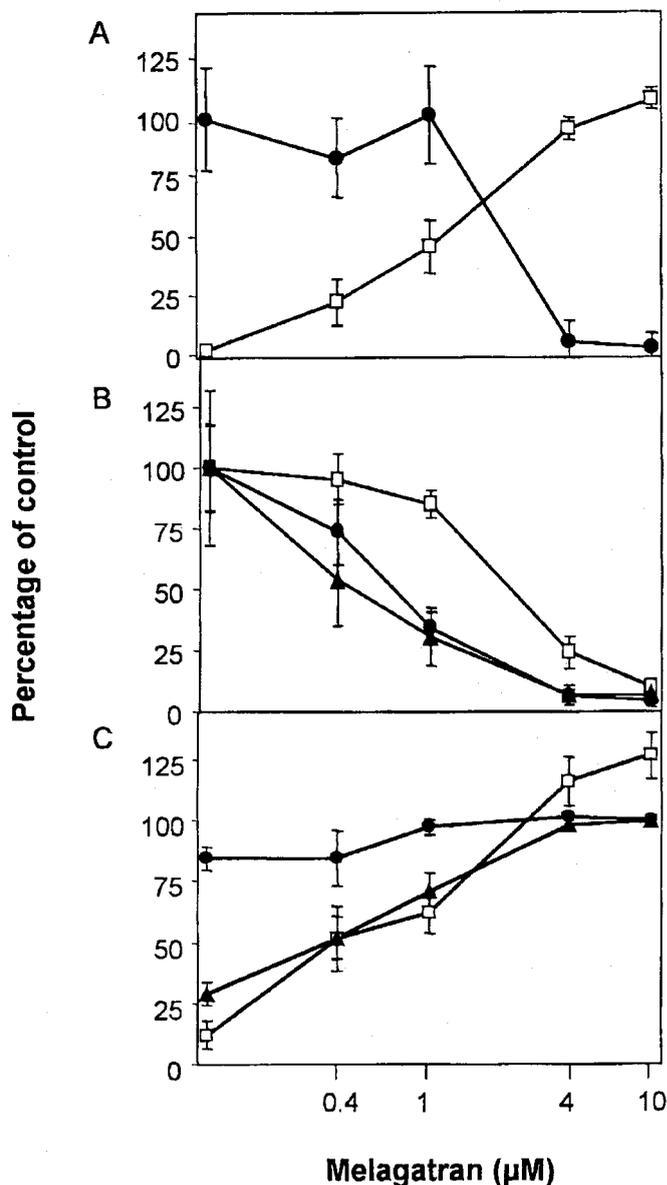

FIG. 2. Human islets incubated in the tubing loop model filled with ABO-compatible blood containing Melagatran at concentrations ranging from 0 to 10 μmol/l. *A:* Changes in platelet particle count (□) and the release of β-TG (●). *B:* Changes in prothrombin fragments 1 and 2 (□), TAT (●), and FXIa-AT (▲). *C:* Changes in lymphocyte (●), monocyte (□), and PMN (▲) particle counts. The EIA values represent data minus the medium control (no islets or Melagatran) after 60 min and are expressed as percentage of the values for the untreated control containing human islets but no Melagatran. The cell counts are expressed as percentage of the values for the medium control (no islets or Melagatran).

be normal background changes resulting from interactions of the blood with the tubing surface and the fluid-air interphase.

In tubing loops without Melagatran, nearly all of the platelets were consumed after 60 min (Table 1), concurrently with a pronounced secretion of β-TG. Granulocytes and monocytes were also consumed after 60 min, whereas lymphocytes were essentially unaffected. In addition, in the absence of Melagatran, macroscopic clotting was seen and was accompanied by a significant rise in TAT, prothrombin fragments 1 and 2, and FXIa-AT complex levels. Marked increases in the complement activation product

TABLE 1
Blood cell counts, coagulation, and complement parameters before and after 60 min of human islet perfusion with fresh ABO-compatible blood

| | 0 min | 60 min | | |
| | | | Islets | |
	No islets*	No islets*	No Melagatran	10 µmol/l Melagatran
n	10	10	10	10
Platelets ($\times 10^9$/l)	240 ± 11	160 ± 7.6	2.4 ± 1.1†	170 ± 8.2‡
Lymphocytes ($\times 10^9$/l)	2.2 ± 0.1	2.0 ± 0.1	1.7 ± 0.1	2.0 ± 0.1‡
Monocytes ($\times 10^9$/l)	0.4 ± 0.0	0.5 ± 0.1	0.1 ± 0.0†	0.6 ± 0.1‡
Granulocytes ($\times 10^9$/l)	4.0 ± 0.3	3.8 ± 0.3	1.0 ± 0.1†	3.7 ± 0.3‡
TAT (µg/l)	9.4 ± 2.2	470 ± 87	16000 ± 2900†	890 ± 160‡
Prothrombin F1+2 (nmol/l)	1.5 ± 0.2	25 ± 5.1	1000 ± 180†	110 ± 28‡
FXIa-AT (µmol/l)	0.02 ± 0.0	0.4 ± 0.1	14 ± 2.6†	0.9 ± 0.6‡
β-TG (IU/ml)	520 ± 130	1300 ± 180	4000 ± 450	1300 ± 200‡
C3a (ng/ml)	120 ± 15	530 ± 62	950 ± 140†	510 ± 50‡
sC5b-9 (AU/ml)	24 ± 3.4	130 ± 16	180 ± 31	140 ± 17.0
Insulin (mU/l)	12 ± 4.3	10 ± 4.0	460 ± 140†	1500 ± 350‡

Data are means ± SEM. *Control containing blood and medium, but no islets. †Significant difference when compared with the control loop after 60 min of perfusion. ‡Significant difference when compared with the islet loops with no Melagatran.

C3a and in plasma insulin levels were also observed; also, sC5b-9 increased but to a lesser extent.

Melagatran inhibits IBMIR in a dose-dependent manner. Melagatran diminished cell consumption and cascade system activation in a dose-dependent manner (Table 1; Fig. 2). An effect on most parameters was observed already at concentrations as low as 0.4 µmol/l. The cell counts were fully restored at ≥4 µmol/l (Fig. 2*A* and *C*). The release of β-TG was also normalized at the same dosage (Fig. 2*A*). The coagulation parameters TAT and FXIa-AT decreased to nearly the same level as the medium control at 4 µmol/l Melagatran, but inhibition of prothrombin fragments 1 and 2 formation required higher doses of Melagatran (Fig. 2*B*). In parallel with the FXIa-AT and TAT complexes, the complement activation product C3a was also inhibited by Melagatran (Fig. 3).

To investigate whether Melagatran had any direct effect on the complement system, we tested its effects in human serum in which complement was activated on the polystyrene surface of microtiter plate wells (Fig. 3). Melagatran had no effect on the binding of C3 fragments to the polystyrene surface or on the generation of C3a, indicating that it does not directly affect the complement serine proteases.

Immunohistochemical staining of human islets after perfusion in the tubing loop model. After 60 min of perfusion, the islets were consistently found to be embedded in clots. Immunohistochemical staining with anti-CD41a monoclonal antibody showed a capsule of fibrin and platelets surrounding the islets. In addition, there was an accumulation of CD11b^{+} polymorphonuclear cells (PMN) and monocytes in the thrombi, many of which were seen to penetrate into the islets (Fig. 4*A* and *B*).

In contrast, islets that had been incubated with fresh human blood in the presence of 10 µmol/l Melagatran showed no signs of clot formation. There were almost no CD41^{+} platelets surrounding the islet surface (Fig. 4*C*), and only a few CD11b^{+} cells were found to have penetrated into the islets (Fig 4*D*). No CD41^{+} or CD11b^{+} staining was observed in control islets that had not been exposed to blood (not shown).

DISCUSSION

In the present study, we demonstrated that Melagatran is a potent inhibitor of the thrombotic IBMIRs that occur in vitro when human islets come in contact with ABO-compatible blood. In this system, the inhibition of thrombin by Melagatran completely blocked the generation of prothrombin fragments 1 and 2 and of TAT. Production of prothrombin fragments 1 and 2 directly reflects the cleavage of prothrombin to thrombin, whereas the binding of TAT mirrors the proteolytic function of thrombin (26). Notably, the concentration of Melagatran needed to inhibit the generation of prothrombin fragments 1 and 2 was higher than that for TAT, suggesting that the thrombin activity was relatively more affected than was the generation of thrombin. Similarly, other parameters reflecting the proteolytic function of thrombin, such as FXIa-AT generation and platelet activation, were affected to the same
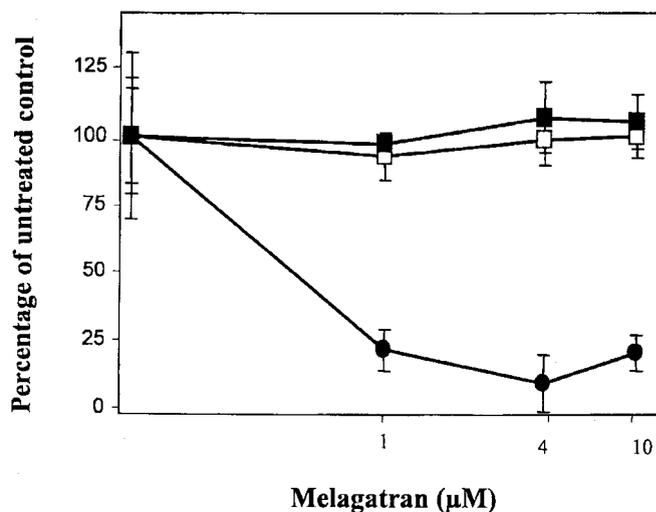

FIG. 3. Effect of Melagatran on the complement system. Changes in the generation of soluble C3a (□) and bound C3 fragments (■) in a serum model with no islets present, and changes in soluble C3a (●) in the tubing loop model containing islets in ABO-compatible blood with Melagatran at concentrations of 0–10 µmol/l.
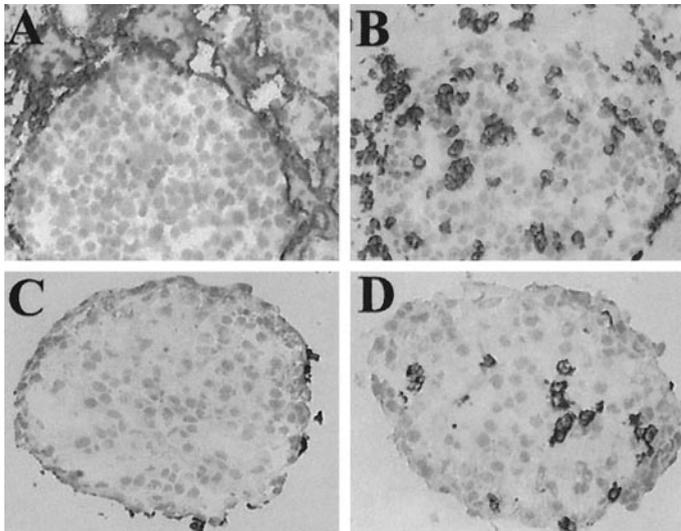
FIG. 4. Human islets incubated in tubing loops filled with ABO-compatible blood containing Melagatran at 0.4 μmol/l (*A* and *B*) or 10 μmol/l (*C* and *D*). The figure shows the distribution of platelets and leukocytes after staining with monoclonal anti-CD41a (*A* and *C*) and anti-CD11b$^+$ (*B* and *D*), respectively.

extent as TAT. A possible explanation for this discrepancy between actual thrombin generation and thrombin function lies in the fact that thrombin is generated by two pathways: the tissue factor pathway (extrinsic pathway) and an amplification loop involving the intrinsic pathway (27). Whereas the intrinsic pathway is driven directly by thrombin and therefore can be inhibited by Melagatran, the tissue factor pathway is initially independent of thrombin. However, in later stages, this pathway of coagulation activation is amplified by thrombin-activated platelets. This difference might explain why Melagatran was able to inhibit the generation of prothrombin fragments 1 and 2 only at the highest concentration, when no further platelet activation occurred.

Notably, complement activation was also completely abrogated by Melagatran. This effect was evident even at a low concentration (1 μmol/l) of Melagatran. To rule out the possibility that Melagatran nonspecifically inhibited complement serine proteinases, we applied a serum system in which complement was activated on a polystyrene surface. In this model, with no islets present, concentrations of up to 10 μmol/l Melagatran showed no inhibitory effect on complement activation. Because Melagatran abrogated the complement activation observed in blood containing islets, it seems that the clotting reaction of IBMIR was responsible for this activation. Complement activation in IBMIR seems to play a central role in regulating islet infiltration by CD11b$^+$ cells because sCR1 in combination with heparin can inhibit this leukocyte infiltration (4). However, heparin alone, even in high doses (not clinically applicable), is unable to abrogate complement activation and leukocyte infiltration (4). Notably, despite the combined treatment of sCR1 and heparin in high concentration, extensive platelet accumulation and fibrin formation around the islets were observed. In contrast, Melagatran alone had a pronounced inhibitory effect not only on complement and coagulation activation but also on the number of platelets that adhered to the islet surface and the number of infiltrating leukocytes.

Administration of Melagatran in the loop model abrogated platelet consumption as well as PMN and monocyte consumption. Our immunohistochemical analyses showed that platelet binding was considerably diminished, although trace amounts of platelets surrounding the islets were still observed. The effect of these remaining platelets is, however, not necessarily a disadvantage. Animal studies have shown that after transplantation, at least 1 week elapses before revascularization of the islets (28). Platelets contain a number of important growth factors, such as platelet-derived growth factor, vascular endothelial growth factor, and fibroblast growth factor (29,30), that may support revascularization and islet engraftment in the liver. This possibility is supported by previous studies demonstrating that blood-borne metastatic cells are dependent on platelet adhesion as they bind to the endothelium of distant organs and invade and colonize these sites (30). Moreover, platelet adherence to tumor cells and the capacity to form metastases are attenuated in P-selectin–deficient mice (31). In clinical islet transplantation, when islets are embolized into the portal vein, a wreath of adhering platelets might, in a similar way, support their engraftment and survival in the liver tissue. Hence, a complete blockade of IBMIR is not necessarily the optimal strategy for intraportal islet transplantation; instead, a fine-tuned balance between successful islet engraftment and thrombosis might be preferred.

Our data indicate that Melagatran at the highest concentration tested (10 μmol/l) did not exert any toxic effect on the islets or abrogate glucose-induced insulin secretion. Consequently, the unexpectedly high insulin release registered in the loop with both Melagatran and islets present (Table 1) was not related to any toxic effect of Melagatran. Similarly, a high level of insulin release was observed in our previous study (4) in which IBMIR was inhibited by the combined effects of heparin and sCR1. A potential explanation for the high insulin levels observed in the loops where clotting was prevented is the mechanical force, created by the blood flow in the loops, exerted on the islets. In contrast, in loops with no Melagatran added, the islets are buried in blood clots, which protect the islets from mechanical stress.

In the present study, the most striking effects of Melagatran were seen at 1–4 μmol/l, a range that is approximately 3–10 times higher than that used in patients being treated for deep venous thrombosis (16). An in vitro system generates more thrombin and consumes more prothrombin than would be the case in the dynamic in vivo situation. Thus, inhibition of thrombin in vitro usually requires much higher concentrations of an inhibitor. The concentration of Melagatran required to abrogate IBMIR in clinical islet transplantation therefore must be estimated from data in preclinical animal studies. Melagatran can also be given orally as a prodrug (32). Under these conditions, the concentration in the portal vein, into which the islets are injected, will be higher than the rest of the circulation.

In conclusion, the present study indicates that thrombin is the main driving force in platelet, coagulation, and complement activation during the process of IBMIR. Our results suggest that Melagatran, an effective inhibitor of thrombin activity, is a possible candidate drug for protecting the islets from IBMIR in clinical islet transplantation.

Forthcoming animal studies should indicate whether Melagatran alone is able to control IBMIR in vivo or whether the thrombin inhibitor should be combined with some additional drug.

REFERENCES

1. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238, 2000
2. Ryan EA, Lakey JR, Rajotte RV, Korbutt GS, Kin T, Imes S, Rabinovitch A, Elliott JF, Bigam D, Kneteman NM, Warnock GL, Larsen I, Shapiro AM: Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes* 50:710–719, 2001
3. Sever CE, Demetris AJ, Zeng Y, Tzakis A, Fung JJ, Starzl TE, Ricordi C: Islet cell allotransplantation in diabetic patients. Histologic findings in four adults simultaneously receiving kidney or liver transplants. *Am J Pathol* 140:1255–1260, 1992
4. Bennet W, Sundberg B, Groth CG, Brendel MD, Brandhorst D, Brandhorst H, Bretzel RG, Elgue G, Larsson R, Nilsson B, Korsgren O: Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? *Diabetes* 48:1907–1914, 1999
5. Kahn ML, Nakanishi-Matsui M, Shapiro MJ, Ishihara H, Coughlin SR: Protease-activated receptors 1 and 4 mediate activation of human platelets by thrombin. *J Clin Invest* 103:879–887, 1999
6. Coughlin SR: Thrombin signalling and protease-activated receptors. *Nature* 407:258–264, 2000
7. Walsh PN: Platelet coagulant activities and hemostasis: a hypothesis. *Blood* 43:597–605, 1974
8. Hirsh J, Fuster V: Guide to anticoagulant therapy. Part 1: heparin. American Heart Association. *Circulation* 89:1449–1468, 1994
9. Markwardt F: The development of hirudin as an antithrombotic drug. *Thromb Res* 74:1–23, 1994
10. Weitz JI, Hudoba M, Massel D, Maraganore J, Hirsh J: Clot-bound thrombin is protected from inhibition by heparin-antithrombin III but is susceptible to inactivation by antithrombin III-independent inhibitors. *J Clin Invest* 86:385–391, 1990
11. Elg M, Gustafsson D, Deinum J: The importance of enzyme inhibition kinetics for the effect of thrombin inhibitors in a rat model of arterial thrombosis. *Thromb Haemost* 78:1286–1292, 1997
12. Elg M, Gustafsson D, Carlsson S: Antithrombotic effects and bleeding time of thrombin inhibitors and warfarin in the rat. *Thromb Res* 94:187–197, 1999
13. Gustafsson D, Antonsson T, Bylund R, Eriksson U, Gyzander E, Nilsson I, Elg M, Mattsson C, Deinum J, Pehrsson S, Karlsson O, Nilsson A, Sorensen H: Effects of Melagatran, a new low-molecular-weight thrombin inhibitor, on thrombin and fibrinolytic enzymes. *Thromb Haemost* 79:110–118, 1998
14. Mehta JL, Chen L, Nichols WW, Mattsson C, Gustafsson D, Saldeen TG: Melagatran, an oral active-site inhibitor of thrombin, prevents or delays formation of electrically induced occlusive thrombus in the canine coronary artery. *J Cardiovasc Pharmacol* 31:345–351, 1998
15. Eriksson BI, Carlsson S, Halvarsson M, Risberg B, Mattsson C: Antithrombotic effect of two low molecular weight thrombin inhibitors and a low-molecular weight heparin in a caval vein thrombosis model in the rat. *Thromb Haemost* 78:1404–1407, 1997
16. Eriksson H, Eriksson UG, Frison L, Hansson PO, Held P, Holmstrom M, Hagg A, Jonsson T, Lapidus L, Leijd B, Stockelberg D, Safwenberg U, Taghavi A, Thorsen M: Pharmacokinetics and pharmacodynamics of Melagatran, a novel LMW thrombin inhibitor, in patients with acute DVT. *Thromb Haemost* 81:358–363, 1999
17. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW: Automated method for isolation of human pancreatic islets. *Diabetes* 37:413–420, 1988
18. Brandhorst H, Brandhorst D, Brendel MD, Hering BJ, Bretzel RG: Assessment of intracellular insulin content during all steps of human islet isolation procedure. *Cell Transplant* 7:489–495, 1998
19. Lakey JR, Warnock GL, Shapiro AM, Ao Z, Kneteman NM, Rajotte RV: Intraductal collagenase delivery into the human pancreas using syringe loading or controlled perfusion. *Cell Transplant* 8:285–292, 1999
20. Gong J, Larsson R, Ekdahl KN, Mollnes TE, Nilsson U, Nilsson B: Tubing loops as a model for cardiopulmonary bypass circuits: both the biomaterial and the blood-gas phase interfaces induce complement activation in an in vitro model. *J Clin Immunol* 16:222–229, 1996
21. Larsson R, Elgue G, Larsson A, Ekdahl KN, Nilsson UR, Nilsson B: Inhibition of complement activation by soluble recombinant CR1 under conditions resembling those in a cardiopulmonary circuit: reduced up-regulation of CD11b and complete abrogation of binding of PMNs to the biomaterial surface. *Immunopharmacology* 38:119–127, 1997
22. Nilsson UR, Storm KE, Elwing H, Nilsson B: Conformational epitopes of C3 reflecting its mode of binding to an artificial polymer surface. *Mol Immunol* 30:211–219, 1993
23. Sanchez J, Elgue G, Riesenfeld J, Olsson P: Studies of adsorption, activation, and inhibition of factor XII on immobilized heparin. *Thromb Res* 89:41–50, 1998
24. Nilsson Ekdahl K, Nilsson B, Pekna M, Nilsson UR: Generation of iC3 at the interface between blood and gas. *Scand J Immunol* 35:85–91, 1992
25. Mollnes TE, Riesenfeld J, Garred P, Nordstrom E, Hogasen K, Fosse E, Gotze O, Harboe M: A new model for evaluation of biocompatibility: combined determination of neoepitopes in blood and on artificial surfaces demonstrates reduced complement activation by immobilization of heparin. *Artif Organs* 19:909–917, 1995
26. Zawilska K: Markers of intravascular activation of coagulation. *Acta Haematol Pol* 25:27–33, 1994
27. Ofosu FA, Liu L, Freedman J: Control mechanisms in thrombin generation. *Semin Thromb Hemost* 22:303–308, 1996
28. Menger MD, Yamauchi J, Vollmar B: Revascularization and microcirculation of freely grafted islets of Langerhans. *World J Surg* 25:509–515, 2001
29. Dunn IF, Heese O, Black PM: Growth factors in glioma angiogenesis: FGFs, PDGF, EGF, and TGFs. *J Neurooncol* 50:121–137, 2000
30. Jensen RL: Growth factor-mediated angiogenesis in the malignant progression of glial tumors: a review. *Surg Neurol* 49:189–195, 1998 (Discussion p. 196)
31. Kim YJ, Borsig L, Han HL, Varki NM, Varki A: Distinct selectin ligands on colon carcinoma mucins can mediate pathological interactions among platelets, leukocytes, and endothelium. *Am J Pathol* 155:461–472, 1999
32. Gustafsson D, Nystrom J, Carlsson S, Bredberg U, Eriksson U, Gyzander E, Elg M, Antonsson T, Hoffmann K, Ungell A, Sorensen H, Nagard S, Abrahamsson A, Bylund R: The direct thrombin inhibitor Melagatran and its oral prodrug H 376/95: intestinal absorption properties, biochemical and pharmacodynamic effects. *Thromb Res* 101:171–181, 2001