Mechanism for the Instant Blood-Mediated Inflammatory Reaction in Rat Islet Transplantation

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ABSTRACT

Objective. The purpose of this study was to investigate the mechanisms involved in the development of instant blood-mediated inflammatory reaction (IBMIR) triggered by pancreatic islet transplantation in an in vitro system.

Materials and Methods. Pancreatic islets were prepared and blood was taken from male and female Wistar rats. An in vitro heparinized tubing loop model on a rocking device that was placed in a 37°C incubator was used to generate blood flow inside the loops. Rat blood was added to the device, and after a 60-minute incubation period, 800 islet equivalents were added to the system. Rat blood samples were taken for immediate hematologic analysis before circulation, before the addition of islets, and 5, 15, 30, and 60 minutes after the addition of islets. After 60 minutes, the blood in the tubing was filtered, and the filtrate was stained using hematoxylin and eosin for microscopic analysis.

Results. Following the addition of islets, the counts of platelets, white blood cells, and mononuclear cells were significantly decreased at 60 min ($P < .05$), and the plasma levels of thrombin-antithrombin complexes and complement C3a were significantly increased ($P < .05$). At 60 minutes after introduction of the islets, the thrombi and tissue in the remaining blood in the device consisted of a few islets with serious structural damage and incomplete capsules, and there were many microthrombi with a large number of infiltrating neutrophils around the islets.

Conclusion. Our in vitro model of IBMIR was characterized by platelet consumption, activation of the coagulation and complement cascade systems, and leukocytic infiltration.

TRANSPLANTATION of the pancreatic islets of Langerhans has become an effective approach for treating diabetes; however, many islets lose function soon after islet transplantation, which not only leads to increased demand for insulin, but also markedly reduces the efficacy of transplantation. The instant blood-mediated inflammatory reaction (IBMIR) [1] after islet transplantation is an important cause of failure of a large number of grafts. The purposes of this study were to establish an in vitro circulation model that simulates IBMIR, and to perform a preliminary investigation of the mechanisms for its occurrence.

MATERIALS AND METHODS

Animals

A closed colony of standard male and female Wistar rats, weighing 250–300 g, was purchased from the Laboratory Animal Center of China Medical University. All experiments were carried out in accordance with National Research Council guidelines and approved by the ethics committee of China Medical University.

Methods

Isolation and Purification of Pancreatic Islets. Five rats each time were used to obtain pancreatic islets after anesthetizing rats with pentobarbital anesthetic. The method for isolation and

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purification was published previously [2]. Type V collagenase (1.5 mg/kg body weight) was perfused via the bile duct into the rat pancreas, and then the pancreas was excised, pooled, and placed into an oscillating water bath in a tube at 37°C for 12–15 minutes for digestion; then it was filtered through an 800 mesh stainless steel mesh and centrifuged through a discontinuous gradient (25%, 23%, 20.5%, and 11% Ficoll and Hanks; 4°C, 200*g* for 10 min). The islet cell fractions with density between 23% and 11% were collected and washed with Hanks solution three times. The pancreatic islets were counted, the islet purity was assessed, and they were then preserved on ice for use.

Preparation of an In Vitro IBMIR Circulation Model. Fifteen rats (5 rats each time; total 3 times) were used to prepare the in vitro circulation model based on a previously published method [2]. A 390-mm polyethylene tube with a diameter of 6.3 mm was heparinized (dose of heparin, 0.5 μg/cm^2). A heparinized three-way connecter was used to link the two ends of the tube to form a closed loop. Rat blood (2 mL) was injected via the third inlet of the three-way connecter, the loop system was placed on a rocking apparatus in a water bath at 37°C for 60 minutes, and then 800 equivalents of fresh islets (1 equivalent = the volume of an islet with a diameter of 150 μm) were injected and the blood containing the islets was allowed to circulate in the system for 60 minutes.

Test Parameters

Rat blood samples were collected before introduction into the in vitro circulation device, before the addition of pancreatic islets, and at 5, 15, 30, and 60 minutes after the addition of pancreatic islets. Routine blood counts were performed for platelets, neutrophils, lymphocytes, and monocytes. An enzyme-labeled method (Enzygnost TAT Siemens Healthcare Diagnostics Products, Marburg, Germany) was used to determine the plasma concentration of thrombin-antithrombin complex (TAT), and an enzyme-linked immunosorbent assay (Bender MedSystems, Vienna, Austria) was used to quantify the plasma complement 3a (C3a) concentration. After 60 minutes of circulation, the contents of the loop were filtered with an 800 mesh stainless steel mesh and the macroscopic thrombi and tissues on the filter were stained using hematoxylin and eosin (HE) for histological evaluation.

Statistical Analysis

Statistical analyses were conducted using SPSS, version 16.0 (SPSS, Chicago, Ill, United States). All data are expressed as means ± standard deviation. The *t* test was used for comparisons. $P < .05$ was considered statistically significant.

RESULTS

Condition of Purified Pancreatic Islets

On microscopic evaluation, the prepared pancreatic islets appeared opaque, with a round or oval shape; the islet purity was >90% and the cell viability rate was >85%.

Time Course of Platelet and White Blood Cell Counts and Percentage Monocytes, Before and After Simulated Islet Transplantation Into an In Vitro Rat Model

There were no significant differences in the measured blood parameters in blood samples assessed before introduction into the blood circulating device and after 60 minutes of circulation without islets in the device, although the platelet count was slightly reduced ($P = .0353$; Table 1; Fig 1). The mean platelet counts at 15, 30, and 60 minutes after introduction of pancreatic islets into the circulating device were significantly lower than the platelet counts measured in fresh blood samples before they were added to the device (all $P < .05$). The mean white blood cell counts and monocyte counts at 15, 30, and 60 minutes after introduction of islets into the device were significantly lower than the counts measured in fresh blood samples (all $P < .05$). There were no differences in the percentages of lymphocytes in blood samples before the 30 minutes after the circulation. The mean lymphocytes counts decreased at 60 minutes.

Time Course of TAT and Complement C3a Plasma Concentrations Before and After Simulated Islet Transplantation Into an In Vitro Rat Model

There were no significant differences in the TAT and C3a plasma concentrations of blood samples assessed before introduction into the blood circulating device and after 60 minutes of circulation in the device (Table 2). The TAT concentrations at 15, 30, and 60 minutes after introduction of pancreatic islets were significantly higher than the concentrations in fresh blood samples before introduction into the device (all $P < .05$). The mean C3a plasma concentration at 30 and 60 minutes after introduction of islets was

Table 1. Time Course of Blood Components Before and After Simulated Islet Transplantation Into an In Vitro Rat Model (Mean ± SD)

Time	*n*	Platelet Count (× 10^9/L)	White Blood Cell Count (× 10^9/L)	Monocyte Count (× 10^9/L)	Percentage of Lymphocytes (%)
Before Circ	15	298 ± 45	9.1 ± 1.3	0.48 ± 0.11	70 ± 9
Circ after 60 min					
0 min	15	264 ± 39	8.9 ± 1.2	0.49 ± 0.12	69 ± 10
		$P = .0353$	$P = .6649$	$P = .8137$	$P = .7756$
5 min	15	251 ± 18	7.8 ± 1.3	0.41 ± 0.14	68 ± 9
		$P = .0008$	$P = .0106$	$P = .1390$	$P = .5477$
15 min	15	161 ± 35	6.9 ± 1.2	0.37 ± .13	65 ± 8
		$P = .0000$	$P = .0000$	$P = .0185$	$P = .1190$
30 min	15	101 ± 27	5.1 ± 1.2	0.30 ± 0.12	66 ± 10
		$P = .0000$	$P = .0000$	$P = .0002$	$P = .2593$
60 min	15	48 ± 12	4.2 ± 1.0	0.25 ± 0.10	62 ± 9
		$P = .0000$	$P = .0000$	$P = .0000$	$P = .0216$

Abbreviation: Circ, circulation.