Abstract

Transplantation of insulin producing tissue is the only therapeutic method so far allowing type 1 diabetic patients to achieve long-term independence of insulin administration. Transplantation of isolated pancreatic islets (PI) into liver represents a safer but less effective alternative to whole-organ transplantation. This is caused by a significant loss of transplanted islets within a short time after transplantation due to the instant blood-mediated inflammatory reaction (IBMIR). This reaction is triggered by molecules of tissue factor, abundantly expressed on the surface of PI cells. Tissue factor activates directly the coagulation pathway leading to platelet aggregation, complement activation, and infiltration of islet graft by leukocytes which results in a destruction of up to 60 % of transplanted tissue, and a formation of ischemic areas of downstream lying liver tissue. Tissue factor also stimulates angiogenesis which makes it necessary for revascularization of PI after transplantation. Inhibition of tissue factor gene in isolated PI using RNA interference provides an opportunity for short-term suppression of tissue factor expression, leading to protection of PI in the early post-transplantation period without hindering the connection of capillaries to the recipient vascular system later on. Increasing the portion of successfully engrafted tissue would bring a higher efficiency of PI transplantation as a therapeutic method, meaning a significantly better availability of transplant therapy.

In the first part of the presented work, we analyzed the gene expression in isolated rat PI and chose the Ppia gene as a suitable endogenous control for subsequent experiments focused of inhibition of tissue factor expression. In the second part, a new experimental model for *in vivo* imaging of posttransplant liver ischemia in rat was developed, utilizing arterial ligation and providing a useful tool for monitoring of IBMIR level. In the last part of the work, RNA interference was used to inhibit the tissue factor expression in isolated rat PI using microporation and lipofection as a transfection method, and *in vivo* efficacy was evaluated after transplantation into diabetic rats. It was found that after transplantation of PI treated with anti-

tissue factor siRNA, liver ischemia was significantly reduced compared to native islets. Using lipefection, the transplantation outcome was comparable with native islets, diabetes compensation achieved was fast and stable, and the siRNA treated islets even showed a slight advantage over native islets in earlier onset of normoglycemia.

Key words: pancreatic islet (PI) transplantation, instant blood-mediated inflammatory reaction (IBMIR), liver ischemia, tissue factor, RNA interference, siRNA