Fructose-1,6-bisphosphate Preserves Murine Pancreatic Islet Cell Viability In Culture

Grun, L.K.¹; Sesterheim, P.¹; Oliveira, J. R.²; Saitovitch, D.¹; Guma, F. C. R.¹; Barbé-Tuana, F. M.¹,³

¹Dep de Bioquímica, ICBS-UFRGS, RS, Brazil; ²Faculdade de Medicina, PUCRS, RS; ³Dep. de Bioquímica, Centro Universitário La Salle, RS, Brazil

INTRODUCTION: Diabetes mellitus type 1 (DM1) is an autoimmune syndrome characterized by organ-specific selective destruction of β-cells in the pancreatic islets. Trying to minimize loss associated with the procedure of isolation/purification of pancreatic islets, one of the strategies is incubating the islets with different cytoprotective molecules. It has been verified the protective action of fructose-1,6-bisphosphate (FBP) associated with an anti-inflammatory effect, stimulating glycolysis and preventing the formation of free radicals. In this work, our objective was to evaluate the cytoprotective effect of FBP on murine pancreatic islets maintained in cell culture.

MATERIAL AND METHODS: C57BL/6 mice were euthanatized by cervical dislocation and submitted to trichotomy and abdominal laparotomy in U. The pancreas was distended with collagenase type V and subsequently removed. The digested tissue was filtered and the collected islets were separated by isopycnic centrifugation in Ficoll gradient (Cellgro). The isolated cells were maintained in culture for 24h with CMRL supplemented with 10% fetal bovine serum and different FBP concentrations. Viability was determined by staining the cells with fluorescein diacetate (FDA) and propidium iodide (PI). The images of islets stained with FDA / PI were acquired with the FV1000 Confocal Microscope (Olympus). The viability was calculated by quantifying the positive PI area in relation to the total area of the islet, and the differences among groups were determined by one-way ANOVA test followed by post-hoc Duncan. RESULTS AND DISCUSSION: Statistical analysis showed that incubation of pancreatic islets in high concentrations of FBP (1.25 mM, 2.5 mM, 5 mM) preserves cell viability of pancreatic islets compared with the control group (P<0.05), confirming the hypothesis of the cytoprotective action of FBP.

CONCLUSIONS: The development of cytoprotective molecules which may enhance or preserve in vitro cultures for different periods is vital in helping the transplantation of pancreatic islets.

Key words: Cell culture, DM1, fructose-1,6-bisphosphate, pancreatic islets, viability
Support: CNPq, UFRGS, PUCRS