SUCCESSFUL ISLET ISOLATION REQUIRES ADJUSTMENT OF COLLAGENASE CLASS I AND CLASS II ACTIVITIES

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OBJECTIVES

The enzymatic dissociation of pancreatic tissue by collagenase is the key step in the islet isolation procedure[1]. The successful release of intact islets requires the selective dissociation of acinar tissue without damaging the morphological integrity of islets. This process is mediated by degradation of components of the extracellular matrix by both collagenase and neutral protease (NP)[2]. It was shown that subclasses of purified collagenase differ in their capacities to degrade various types of collagen which result in different efficiencies of class I and class II collagenase to dissociate pancreatic tissue and cleave islets from acinar tissue[3.4]. However, no informations about the optimal ratio between both subclasses are available yet. We evaluated the efficiency of different ratios between collagenase class I and class II (C-ratio) utilizing the rat pancreas as a model for other species.

METHODS

Pancreases were obtained from male Lewis rats and intraductally distended in situ with cold HBSS supplemented with 20 PZ-U of fractionated collagenase NB 1 and 1.0 or 0.4 DMC-U of NP. Rat islets were isolated by stationary digestion at 37°C as previously described[5]. The C-ratio (II over I) was varied between 0.4 and 2.6. Pancreas dissociation was continuously monitored through biopsies taken during digestion. After completion of dissociation islets were purified utilizing a Ficoll-Na-diatrizoate gradient[5]. Islet yield was determined converting islets to islet equivalents (IEQ)[6]. Quality assessment was performed to evaluate islet viability[5] and function after transplantation into diabetic NMRI nude mice.

RESULTS

Utilizing a NP activity of 1.0 DMC-U yield, purity, fragmentation, and viability of islets were not affected by different C-ratios. However, a reduction of NP activity from 1.0 to 0.4 DMC-U revealed significant differences between different C-ratios in terms of digestion time, yield and purity, and was associated

with a significantly increased viability observed in all experimental groups.

The shortest digestion time was found utilizing a C-ratio of 0.7 which correlated with highest islet yield and lowest islet fragmentation. Increasing class I collagenase activity to a C-ratio of 0.4 resulted in a significantly prolonged digestion time that was associated with increased islet fragmentation. Differences in purity between different C-ratios were marginal but nevertheless significant.

Transplantation of rat islets freshly isolated by means of different C-ratios resulted always in immediate and sustained reversal of hyperglycemia in diabetic nude mice until nephrectomy of graft-bearing kidneys was performed.

CONCLUSIONS

The present study indicates that the ratio between class I and class II collagenase activity significantly influences all important parameters of enzymatic islet isolation. The observation that higher amounts of NP activity reduce islet viability confirmed previous findings in the rat[2] and are in agreement with actual studies in the human pancreas. We conclude that maximum release of undamaged islets from acinar tissue requires an exact balance between class I and class II collagenase as well as neutral protease.

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