42.

OXYGENATION OF CULTURED PANCREATIC ISLETS

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1. INTRODUCTION

The rich vascularization of the pancreatic islets was already noted in early studies performed by Langerhans [1]. The dense glomerular-like islet angioarchitecture assures that no portion of an islet is more than one cell away from arterial blood [2,3]. The blood perfusion of the pancreatic islets is also very high and constitutes 7-10% of the whole pancreatic blood flow, despite the islets contributing only 1-2% to the pancreatic volume [4,5]. This high blood perfusion of pancreatic islets results in a tissue oxygen tension of 40 mmHg [6], which is approximately twice as high as in the kidney cortex [7]. This may reflect the need for adequate glucose sensing of the pancreatic β-cells and may facilitate disposal of secreted islet hormones. Pancreatic islets also normally have a very high demand on metabolic activity and oxygen consumption to meet the varying needs for insulin secretion [8,9].

Prior to transplantation, islets are isolated and cultured for a couple of days. Transplantation of cultured islets is preferred compared to transplantation of freshly isolated islets, since culture reduces islet immunogenicity [10] and culture also supplies time to find the most suitable recipient. In order to optimize the condition of the islet graft, it is very important to limit the dysfunction and necrosis/apoptosis of islet cells during the pre-transplantation culture. Endogenous islets are used to a high level of oxygenation *in vivo*, and one must therefore supply enough oxygen *in vitro* to protect the cultured islets from hypoxia. It is well-known that culture of especially large islets results in a central necrosis due to limitations in oxygen diffusion *in vitro*. Studies of islet oxygenation *in vitro* have been limited by a lack of available experimental techniques and in former studies only mathematical calculations of islet cell oxygenation have been performed [11].

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Low oxygen tension in cells and tissue may be detected by the use of biochemical markers such as 2-nitroimidazoles [12]. Under low oxygen conditions (pO_2 less than 10 mmHg), bioreductive metabolism of 2-nitroimidazoles proceeds inversely proportional to the oxygen tension, eventually generating reactive adducts that bind to macromolecules in the cells [13]. The 2-nitroimidazole-derived side chains can then be recognized by antibodies and used to assess tissue areas/cells low in oxygen tension. In this study we evaluated the oxygenation of cultured islets with the 2-nitroimidazole pimonidazole, which is known to accumulate in tissues with an oxygen tension less than 10 mmHg.

2. METHODS

2.1. Animals

Adult male C57BL/6 mice (~30 g) and Wistar-Furth rats (~300 g) were purchased from B&K, Sollentuna, Sweden. The animals had free access to water and pelleted food throughout the course of the study. All experiments were approved by the local animal ethics committee of Uppsala University.

2.2. Islet Isolation and Culture

Pancreatic islets from mice and rats were prepared by collagenase digestion and cultured free-floating in groups of 150 islets in 5 ml RPMI 1640 medium supplemented with 11.1 mmol/l glucose, 10% (vol/vol) fetal calf serum, 0.17 mmol/l sodium benzylpenicillinate and 0.17 mmol/l streptomycin at 95%air/5%CO_2 [14]. The medium was changed every second day.

Human islets were kindly provided by Professor Olle Korsgren, Department of Clinical Immunology, Uppsala University Hospital, Sweden. Approximately 10 000 human islets were cultured in 10 ml RPMI 1640 medium supplemented with 5.6 mmol/l glucose, 10% (vol/vol) human serum, 0.17 mmol/l sodium benzylpenicillinate and 0.17 mmol/l streptomycin at 95%air/5%CO_2. The medium was changed every second day.

2.3. Evaluation of Oxygen Dependent Accumulation of Pimonidazole

Rat islets were cultured for 1, 5 or 10 days. The islets were split in two experimental groups, where one group was incubated with a normal amount of medium (5 ml) and the second group was incubated with an increased amount of medium (15 ml). After 24h of culture, all culture dishes were incubated, in the presence of pimonidazole (200 μmol/l), at 95%air/5%CO_2 for 2 hours. The rationale for this experimental set-up was that excess non-stirred culture medium markedly increases the diffusion distance of oxygen from air to the pancreatic islets and, thus, decrease the oxygenation of isolated pancreatic islets with resulting increased frequency of central islet necrosis [15].

In order to evaluate if oxygen dependent accumulation of pimonidazole occur in islets *in vivo,* rats were anesthetized with thiobutabarbital sodium (120 mg/kg body weight i.p.; Inactin; Research Biochemicals International, Natick, MA), placed on an operating table maintained at body temperature (37°C) and tracheostomized. Polyethylene catheters were inserted into the right femoral artery and vein. The arterial catheter was pre-heparinized to avoid blood clot formation and was connected to a blood pressure transducer (PDCR 75; Groby, UK) to monitor blood pressure. The abdominal cavity of the animals was opened by a midline incision and the pancreas was exposed and its surface islets visualized, as previously described [16].