Effects of bacterial infection on insulin secretory capacity of human adult pancreatic islets

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ABSTRACT

This study aims to determine the origin of bacterial contamination of pancreatic tissue cultures, as well as its influence on insulin secretory activity (expressed as stimulation index [SI]) of the pancreatic islets. Pancreatic tissue was obtained after pancreatectomy in patients who had chronic pancreatitis or benign tumours. Islets were isolated under aseptic conditions by a manual method. Microbiological analyses were performed by standard procedures and the SI was determined on the first and seventh day of cultivation. In cultures contamminated by *Pseudomonas*, SI was 1.58±1.16 on day 1 and 0.22±0.14 on day 7 ($P<0.01$). Cultures contaminated by *Enterobacter* showed an SI of 0.21±0.1 on day 1, which increased to 1.19±0.66 on day 7 ($P<0.01$). In cases of *Staphylococcus* contamination, SI was 0.07±0.05 on day 1 and 0.33±0.21 on day 7 ($P<0.01$). The study shows that cell culture contamination originates from an original pancreatic tissue infection. The presence of bacteria may reduce or increase insulin secretion in cell culture, depending on the type of microorganism, and this can provoke reduced or elevated levels of insulin secretion in recipients, thus increasing the chances for the onset of diabetes.

KEY WORDS: Bacteria.
Diabetes.
Insulin.
Pancreas.

Introduction

Despite the efficacy of insulin therapy as a treatment for type 1 diabetes mellitus (IDDM), clinical complications are common and over the past decade there has been extensive research into pancreatic tissue transplantation as an alternative.

Transplantation of isolated human islets has certain advantages such as simplicity of surgical technique and the possibility of *in vitro* modulation to reduce post-operative immunosuppressive treatment.[1,2]

Human adult islets can be collected from multiple cadaveric donors, but optimal results are obtained by isolating sufficiently large numbers of islets from a single pancreas with good tissue compatibility.[3] In such a case, insulin independence has been reported over a six-year period.[4]

Pretransplantation procedures include islet isolation, purification and *in vitro* cultivation, as well as estimation of graft quality by determining cytological characteristics (islet number and viability by dithizone staining), histological characteristics (by light and electronic microscopy), the degree of islet purification, checking microbiological sterility, and establishing the functional capacity of the islets (perfusion and stimulation index [SI]).[5]

A major problem in transplantation is bacterial contamination of the pancreatic tissue, which may occur in surgery or during transport, and may be of viral, bacterial or fungal origin. Viruses may damage body cells directly or initiate autoimmune attack on the pancreatic islets, which results in type 1 diabetes.

It is thought that enterovirus infection causes β-cell damage by replicating in the pancreatic islets and contributes to the development of type 1 diabetes. The results show a definite islet-cell tropism of enteroviruses in the human pancreas. Some enteroviruses seem to use previously identified cell surface molecules as receptors in β-cells.[6,7]

Analysis of contaminants showed 74% Gram-positive and 21% Gram-negative organisms, and 5% fungi. Length of transport time was significantly associated with bacterial contamination. The rate of microbial contamination during pancreas procurement and transport is high, with *Staphylococcus* species the most common organism. Transmission of infectious pathogens from donor to recipient is a possible source of post-transplantation infection, including after islet transplantation. However, contamination during the islet isolation process is rare.[5] One study on dogs has demonstrated that the lowest rate of glucose elimination was in a group infected with *Staphylococcus intermedius*.[8]

Infection causes marked changes in whole body glucose metabolism as a result of acceleration in endogenous glucose production, due to increased gluconeogenesis.[9] It has been shown that acute infection in humans causes insulin resistance and glucose intolerance.[10] Endotoxin or lipopolysaccharide (LPS) is a potent stimulator of inducible nitric oxide synthase (iNOS). Furthermore, LPS injection can cause hyperglycaemia, insulin resistance and increased iNOS protein expression and activity.[11] One study of rats infected with *Escherichia coli* demonstrated that infected animals were hyperthermic and showed increased rates of glucose metabolism as well as mild hyperlacticacidaemia, and plasma catecholamine concentration was increased by 50–70%.[12] Results of experiments with male rats treated with

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bacterial endotoxin (*Salmonella enteritidis*) suggest that variations in an individual's early life bacterial environment may contribute to differences in glucose homeostasis, insulin action and disease susceptibility later in life.[13]

The aim of this study is to investigate the origins of bacterial contamination in pancreatic islet culture, and its influence on insulin secretion.

Materials and methods

Human adult pancreatic tissue was obtained after pancreatectomy in patients who had chronic pancreatitis or benign tumours.[14] Operative procedures were performed in the Institute for Gastrointestinal Diseases, Clinical Center of Serbia.

For this study, islets from 16 pancreata were obtained (eleven males, five females, age range: 60.84±10.44 years). Cold ischaemia was 70.45±26.41 min; warm ischaemia was 59.40±4.01 min. Average tissue weight was 4.89±0.49g.

Islets were isolated under aseptic conditions by a manual method using collagenase XI (5 mg/mL; Sigma-Aldrich).[15] After semidigestion for 30 min at 37°C, the supernatant was decanted and the cells were washed in HBSS (Aplichem) solution containing 20 mmol/L Hepes and 0.2% fetal calf serum (FCS) (pH 7.4). Islets were not purified in order to avoid cell loss (<50%), and yield was 2000–4000 IEQ/g pancreatic tissue.

The islets were resuspended in RPMI 1640 culture medium (Sigma-Aldrich) containing $Ca(NO_3)_2$ (0.1 g/L), $MgSO_4$ (0.048 g/L), KCl (0.4 g/L), $NaHCO_3$ (2 g/L), NaCl (6 g/L), Na_2HPO_4 (0.8 g/L), glutamine (0.3 g/L), D-glucose (1 g/L), 25 mmol/L Hepes, 10% FCS , 100 units/mL penicillin and 100 µg/mL streptomycin, and transferred to plastic 50 mL culture flasks (Falcon 3013) and incubated at 37°C in 5% CO_2 (95% humidity) for seven days. Functional capacity of the isolated islets was established by a static glucose stimulation assay.

Islets (1000–2000 islets per culture) were incubated for 1 h in low and high glucose concentrations (2.8 mmol/L and 20 mmol/L), respectively, in Hepes-buffered Hank's balanced salt solution on the first and seventh day of cultivation.[16] Supernatant from each sample was collected and stored at –18°C. Insulin content was measured by radioimmunoassay (RIA; sensitivity: 0.60 miu/L, detection range: 0.6–300 miu/L). Insulin release in response to the glucose challenge under static incubation conditions was expressed as the stimulation index (SI), which was calculated as the ratio of insulin released during 1-h incubation with high glucose concentration, to insulin released during 1-h low glucose incubation.

Islets were stained with diphenylthiocarbazone, as described previously.[17] These studies were approved by the Ethical Committee, Clinical Center, Belgrade, Serbia, and written consent was obtained from the patients.

Microbiology was performed using standard procedures at the Institute for Microbiology, Clinical Center, Serbia. Bacterial analysis of pancreatic tissue samples was performed at three points: immediately after obtaining tissue samples, after transport of samples, and at the end of the cultivation period.

All results were expressed as mean±SD. $P<0.05$ was considered to be statistically significant (Student's *t*-test).

Results

Microbiology on specimens prior to their transfer to the laboratory for pancreatic islets culture showed that nine pancreatic samples were contaminated and seven were sterile. In total, 36 contaminated and 26 sterile cultures were examined. Bacterial species found in the cultures were *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Staphylococcus* spp. in 16.6%, 33.3% and 11.1% of cases, respectively.

Figure 1 shows insulin secretion values in response to low and high glucose stimulation for all cultures. Stimulation index for the sterile cultures was 2.15±0.67 on day 1 and 0.73±0.5 on day 7. The SI for all infected cultures was 1.52±0.54 on day 1 and 0.63±0.31 on day 7.

Figure 2 shows SI values for all three types of bacterial contamination. In cultures showing *Pseudomonas* contamination the SI was 1.58±1.16 and 0.22±0.14 on day 1 and day 7, respectively ($P<0.01$). Cultures showing *Enterobacter* contamination on day 1 had an SI of 0.21±0.1, which increased to 1.19±0.66 on day 7 ($P<0.01$). In cases showing *Staphylococcus* contamination the SI was 0.07±0.05 on day 1 and 0.33±0.21 at the end of cultivation period ($P<0.01$).

Discussion

Enterobacter cloacae is part of the normal flora of the gastrointestinal tract of 40–80% of people and is widely distributed in the environment. Like most members of the Enterobacteriaceae, these organisms are capable of causing opportunistic infection in hospitalised or debilitated patients.[18]

Staphylococcus aureus is considered an extracellular pyogenic pathogen and in tissue is found almost exclusively in extracellular spaces. Localised infections, such as soft-tissue abscesses, and life-threatening systemic diseases, such as infective endocarditis, result from the ability of this pathogen to: i) colonise a diverse array of host tissue

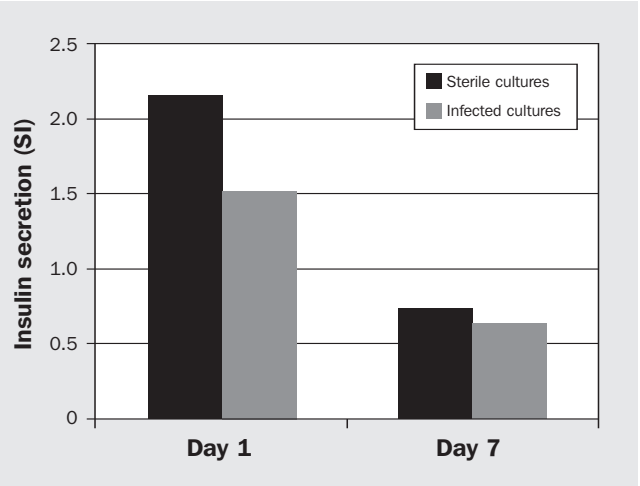

Fig. 1. Insulin secretion (SI) in response to low and high glucose stimulation in sterile and infected cultures. Stimulation index (mean±SD) values in sterile cultures were 2.15±0.67 and 0.73±0.5 on the first and seventh day ($P>0.05$), respectively; and in infected cultures was 1.52±0.54 and 0.63±0.31 ($P>0.05$), respectively.

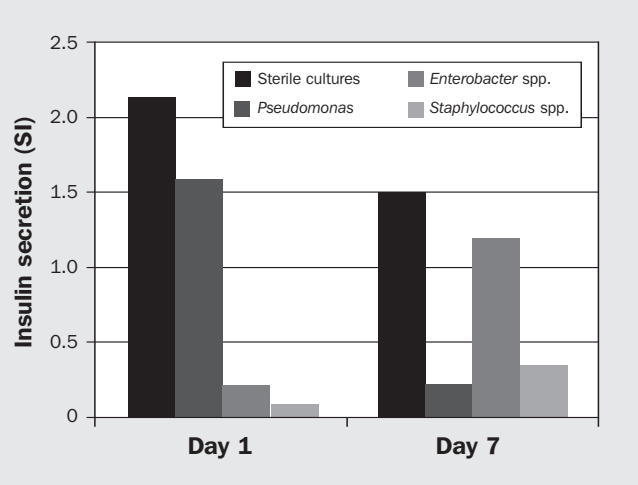

Fig. 2. Stimulation index values (mean±SD) for the three species of bacteria. In *Pseudomonas* cultures, SI on the first and seventh days were 1.58±1.16 and 0.22±0.14, respectively ($P<0.01$); in *Enterobacter* cultures, SI on the first and seventh days were 0.21±0.1 and 1.19±0.66, respectively ($P<0.01$); and in *Staphylococcus* cultures, SI on the first and seventh days were 0.07±0.05 and 0.33±0.21, respectively ($P<0.01$).

surfaces, and ii) elaborate proteases, exotoxins and enzymes that destroy tissue and protect bacteria from the host immune response.[19]

Pseudomonas aeruginosa produces membrane vesicles (MVs) that are released into the culture medium during normal growth. Their release is increased approximately three-fold after exposure of the organism to four times the minimum inhibitory concentration (MIC) of gentamicin. Immunoelectron microscopy and Western blot (immunoblot) analysis of the vesicles demonstrates the presence of B-band lipopolysaccharide (LPS). In addition to LPS, several enzymes (e.g., phospholipase C, protease, haemolysin and alkaline phosphatase) that are known to contribute to the pathogenicity of *Pseudomonas* infections have been found to be present in both vesicle types. Consequently, these vesicles could play an important role in genetic transformation and disease by serving as a transport vehicle for DNA and virulence factors and are presumably involved in septic shock.[20]

On the basis of the microbiology analysis, infection in the cultures originated from infected pancreatic tissue. The tissue studied here was taken from patients who had chronic pancreatitis or a tumour of the head of the pancreas and were undergoing duodenopancreatectomy (i.e., removal of part of the pancreas together with a part of the duodenum). Intestinal flora from the duodenum infects the pancreas during surgery or are already present, having migrated through the pancreatic ducts. The latter occurs because disease weakens the immune system and allows the microorganisms, especially *Enterobacter*, to grow rapidly.[5]

Normally, fluid in the pancreatic ducts is bacteriostatic to some types of *Staphylococcus* and to *Pseudomonas aeruginosa*,[21] and studies suggest that the donor's status and the institution providing the pancreatic tissue have no effect on contamination rate, as contamination generally occurs during transportation or the islet isolation procedure.[7] This was not the case in the present study as all the solutions used during work in the laminar cabinet were sterile, and sterile tissue always resulted in sterile cultures.

When analysing the functional capacity of insulin secretion in response to stimulation with high and low glucose concentrations, the SI values of the sterile cultures were higher than those that were contaminated, both on the first and seventh stimulation days. However, the drop in SI value on the seventh day was much higher in the sterile cultures than in those that were contaminated. The explanation for a decrease in SI could be due to pancreatic islets on the first cultivation day being less sensitive to the actions of existing bacteria, or the islets were already damaged at the very start of cultivation.

Figure 2 shows the effect of each contaminant on islet functional capacity. It was observed that SI on the first day of stimulation was higher in cultures contaminated with *P. aeruginosa* than in those containing *Enterobacter* and *Staphylococcus*, and a substantial increase in the level of contamination with *P. aeruginosa* resulted in a much lower SI on day 7.

In the case of contamination with *Enterobacter* and *Staphylococcus*, islets showed a considerably lower response to stimulation with glucose, but this response improved during cultivation and an increase in SI was noted by day 7. This is explained by the effect of antibiotics (streptomycin and penicillin) in islet culture, which have a bacteriostatic or bactericidal effect on these organisms. However, it has been shown that bacterial competition occurs in the culture whereby *Enterobacter* suppresses the growth of *Pseudomonas* and *Staphylococcus*; therefore, a much higher SI on day 7 in cultures contaminated with *Enterobacter* can be explained.[19,20]

The present results are in accordance with research using a microbial biopolymer (PGB1) extracted from *Enterobacter* sp. and tested in db/db mice.[22] They demonstrated that high PGB1 supplementation prevents the onset and progression of type 2 diabetes by stimulating insulin secretion and enhancing hepatic glucose metabolic enzyme activities.

Type 2 diabetic patients show diminished insulin secretion in response to glucose stimulation (post-prandial hyperglycaemia). Degradation of glycogen in liver leads to a glycotoxic state which increases β-cell apoptosis. However, increased insulin secretion has a negative effect on the physiological functions of β-cells. Insulin resistance involves a decline in insulin action in the body, which eventually results in the loss of insulin secretion, the onset of glucose intolerance and in some cases the development of diabetes.[23] Exposure to multiple pathogens could cause chronic low-grade inflammation, resulting in insulin resistance.[24]

The results presented here suggest that bacterial contamination of pancreatic islet cultures can either reduce or increase functional capacity (i.e., insulin secretion as a response to *in vitro* stimulation with high and low glucose concentrations) depending on the bacterial species. In addition, pancreatic infection can provoke increased or reduced insulin secretion in recipients, thus increasing chances for the onset of diabetes. □

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