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Original Paper

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TransEndoscopic Gastric SubMucosa Islet Transplantation (eGSM-ITx) in pigs with streptozotocine induced diabetes – technical aspects of the procedure – preliminary report

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Summary

Background: Islets and pancreas transplantation have become standard treatments of patients with diabetic complications. However pancreas transplantation is associated with high incidence of complications and the long-term results of islet transplantation are still unsatisfactory. Loss of pancreatic islets grafts is caused not only by immunological reactions but also due to the site of grafting and IBMIR. Gastric submucosal space could be an alternative site for transplantation. The aim of this study was to assess the possibility of endoscopic islets transplantation into the gastric submucosa-its efficacy and potential complications.

Material/Method: 20 Landrace pigs weighing 19–24 kg were obtained for the study. Seven animals were controls (C-group) and 13 formed the transplantation group (TX group). In both groups diabetes was induced by streptozotocine (stz) infusion at a dose of 200 mg/kg. At 7 days post stz infusion pigs of both groups underwent endoscopy-in group C to assess the feasibility of gastroscopic examination under general anaesthesia in pigs with diabetes and to study the influence of basiliximab infusion on pigs, in the Tx-group to perform endoscopic submucosal islet transplantation (eGSM-ITx). Immunosuppression consisted of tacrolimus 0.2 mg/kg and sirolimus 6 mg/m^2. At 7 days post transplantation, control gastroscopy was performed to assess the gastric mucosa and to obtain biopsies for histopathology. 10 to 30 days after eGSM-ITx, magnetic resonance (MRI) scan was performed. Stomach and pancreas were obtained at autopsy for histopathology. Glycemia was assessed twice daily during the experiment. For 10 days after diabetes induction (up to three days after eGSM-ITx) in both groups, insulin was given to reach glycemia between 150–200 mg/dl, after that period insulin was given only when glycemia exceeded 600 mg/dl.

Results: There were no differences in insulin requirement and glycemia up to the day of eGSM-ITx between the groups. Tx-group animals received a mean of 6000±3170 IEQ/kg. Tx-group animals had a significantly lower insulin requirement and significantly lower mean glycemia since the first day post transplantation. C-group animals all required insulin once daily to keep glycemia below 600 mg/dl. There were no signs of perforation, ulceration or bleeding after eGSM-ITx on gastroscopy and histopathological examination. MRI scans revealed unspecific thickening of gastric wall at sites of islet deposition.

Conclusions: Transendoscopic islets transplantation into gastric submucosa is feasible and a safe procedure in an experimental animal setting. Its potential for clinical application in human subjects needs further studies.

Key words: **endoscopic islets transplantation • gastric submucosal space • diabetes • streptozotocine • basiliximab • pigs**

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BACKGROUND

Diabetes and its complications are a growing medical problem in most countries. It is more frequently seen in developed countries where excessive carbohydrate intake is more common [1,2]. Diabetes mellitus is a metabolic syndrome: type II is associated with reduced insulin production by Langerhans islets, whereas in type I – there is a complete cessation of islet function due to immunological reasons [3]. New era of diabetes treatment began when Banting and Best extracted insulin from canine pancreas in 1921 [4]. This discovery allowed for prolongation of life of diabetic patients and at the same time revealed long-term diabetic complications such as nephropathy, retinopathy or micro- and macroangiopathy. Another breakthrough in managing diabetic complications came with pancreas transplantation [5]. At approximately the same time, in 1965, Moskalewski of Warsaw Medical University demonstrated for the first time a successful islets isolation from the pancreas of a guinea pig [6]. This opened a new perspective for patients with complicated diabetes. After early islets transplantation, first in animals [7] then in humans [8] results were not encouraging. Later, improvement in isolation techniques by Ricordi [9] and the immunosupression protocol from Edmonton [10] provided new perspectives for the future.

Islets transplantation has become a promising technique of transplantation for patients with unstable diabetes complicated by severe hypoglycemic attacks. Islets are routinely administered into the portal vein, both in allo and autotransplantation. In patients who undergo autotransplantation, the islets gradually lose their function [11]. Loss of islet function is not only due to immunological reasons (allotransplantation only) but also due to the site of grafting and Instant Blood Mediated Inflammation Reaction (IBMIR) and apoptosis [12]. For the above reasons many centres have begun searching for more advantageous sites for graft islets [13–15]. Stomach and the gastric submucosal space are well vascularized, with a poor immunological status and easy access. Autotransplantation into the gastric submucosa has shown promising results [13] and has been considered a possible alternative site for islets transplantation.

The aim of this study was to assess the possibility and technical aspects of endoscopic islets transplantation into the gastric submucosa and to determine its efficacy and potential complications.

Material and Methods

Animals

20 female Landrace pigs weighing 19–24 kg (mean 21.5 kg), aged 2 months were obtained for the study. 7 animals were controls (C group)and 13 formed the transplantation group (TX group). All animals were kept in separate cages and in accordance with European Community regulations on animal maintenance and care. All animals were fed twice daily a standard diet of up to 3% of body weight. All procedures were accepted by the Ethical Committee of Warsaw Medical University and Ministry of Science.

Induction of the diabetes

In both groups diabetes was induced by streptozotocine (stz) infusion at 200 mg/kg. The animals were fasted overnight before induction of diabetes. Water was provided continuously. Venous access was obtained through an ear vein and the infusion of stz in 100 ml of 0.9% NaCl was given over 10 minutes. After diabetes induction the pigs were fed to prevent hypoglycemia. The glycemia level was checked twice daily up to the end of the study. Induction of diabetes was considered successful when glycemia exceeded 300 mg/dl and the animal needed insulin administration to keep glycemia below 600 mg/dl. For 10 days after diabetes induction (up to three days after eGSM-ITx) in both groups, insulin was given to reach glycemia between 150–200mg/dl, after that period insulin was given only when glycemia exceeded 600 mg/dl – to prevent ketoacidosis. C-peptide levels were collected before and after induction of diabetes and after transplantation. All are kept in –70°C. Analysis will be performed in further studies.

Organ procurement

Pancreata were obtained from a slaughterhouse (Zychowicz, Daleszyce, Poland). Pancreata were retrieved from pigs weighing 120–200 kg. Exsanguination was performed by incision of the carotid artery and vein. After 10±2 min of warm ischemia all abdominal organs were placed on sterile ice and the pancreas was excised and perfused with 500 ml of UW solution in order to flush out the blood and lower organ temperature. Afterwards, all retrieved pancreata were kept by the 2-layer method with perfluorocarbon and UW solution. Mean cold ischemia time until the start of the isolation procedure was 200±100 min.

Isolation procedure

Mean pancreas weight after dissection of vessels and before of infusion of digestion solution was 145 grams. Isolation was performed with use of Collagenase NB-8 (Serva) and Neutral Protease (Serva) in 6 cases and Liberase (Roche) in 2 cases. Enzyme concentration was 3.1 PZ-U/g pancreas for Collagenase NB-8 and 0.72 DMC-u/g pancreas for Neutral Protease. Liberase concentration was 1.38 mg/mL. Cannulated pancreatic ducts were manualy infused with enzyme solution in volume of 180 ml. After infusion pancreas was cut into 8-10 pieces. Mean time of digestion was 14 min at the temperature of 37°C with a heating circuit and gentle manual agitation of Ricordi Chamber. After that free islets had appeared in the sample, the circuit was cooled and the dilution was started. Dilution was performed for 20 to 50 minutes depending on pancreas dispersion. Newborn calf serum in concentration of 10% was used as an enzyme inhibitor. Islets and exocrine tissue debris were collected, centrifuged and suspended in 500 ml of CMRL 1066, then the sample of 100 µl was taken to dithizone staining to assess the results of isolation. Then all material was centrifugated at 4°C for 3 min and the pellet was suspended in 50 to 100 ml of CMRL 1066 – solution which was immediately ready for transplantation. Islets purification was not performed in all cases.

Immunosupression regimen

Immunosupression consisted of tacrolimus 0.2 mg/kg and sirolimus 6 mg/m^2. Immunosupression was started one day prior to transplantation. 3 days post e-GSM-ITx tacrolimus and sirolimus concentrations were checked and the doses were modified in order to reach levels of tacrolimus – 10–15 ng/ml and sirolimus 8–10 ng/ml.

In the first series (2 pigs), 5 mg of basiliximab/pig was infused during the endoscopic procedure.

Endoscopic and transplantation (eGSM-ITx) procedures

At 7 days post stz infusion pigs of both groups underwent endoscopy. In groupC to assess the feasibility of gastroscopic examination under general anesthesia in pigs with diabetes and to study the influence of basiliximab infusion on pigs. In Tx-group to perform transendoscopic gastric submucosal islet transplantation (eGSM-ITx).

eGSM-ITx was performed under general anesthesia with intubation. Blood oxygen saturation and heart rate were controlled continuously. Blood glucose level was measured before, during and at least 3 times at intervals of 10 minutes post eGSM-ITx. In cases of hypoglycemia, 5% glucose was administred iv. During anesthesia 20 mg of pantoprazole iv was administered prior to gastroscopy. Pantoprazole was continued during the rest of the experiment – 40 mg p.o./pig. No antibiotic prophylaxis was used. Gastroscopy was performed with an endoscope with a working channel of 3.2 mm (Olympus Europe). eGSM-ITx was performed with the use of endoscopic needles (Olympus Endotherapy nr. DSN-180-10; Gauge 19, 3 French; Figure 1). Needle was introduced into the gastric submucosal space at an angle of 20° to 90° to mucosal surface. Full length of the needle (5 mm) was placed in the submucosa. Then 2 to 4 ml of 0.9% NaCl was infused in order to check whether the needle is in the right position and to assure that there will be no leakage of islets infused into the submucosa. In the initial 7 animals, 10 ml of Islets suspension in Ringer's lactate solution followed by 2 ml of saline (in order to flush out all islets from the working channel) were infused slowly within 2 minutes (approximately 5 to 10 infusions). In another 5 animals 5 ml of Islets suspension in Ringer's lactate solution followed by 2 ml of saline were infused slowly within 2 minutes (approximately 10 to 20 infusions). Sites for transplantation were chosen randomly throughout the stomach starting from the antrum along both curvatures. Infusion sites were spaced at least 3 cm from each other to prevent large mucosa detachment. eGSM-ITx was performed within one hour after the isolation procedure was completed. The video from that procedure can be viewed on a website [16].

At 7 days post transplantation, control gastroscopy was performed to assess the gastric mucosa and to take biopsies for histopathology.

10 to 30 days after eGSM-ITx magnetic resonance (MRI) scans were performed. Stomach and pancreas were obtained at autopsy for morphological examination and histopathology.

Statistical analysis

A comparison of means between two groups was performed using a Wilcoxon and T-student tests. Within the survey a critical level for hypothesis testing was set at 0.05.

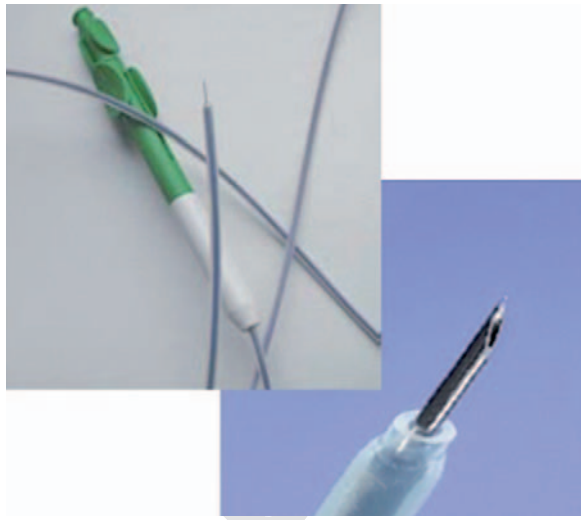

Figure 1. Endoscopic needle used for eGSM-ITx.

Results

Basiliximab infusion

2 pigs experienced pulmonary edema and cardiac arrest following basiliximab infusion of 5mg iv (one from Txgroup and one from the control group) – following this basiliximab use was discontinued.

Induction of diabetes

Mean fasting glycemia before stz administration was 107 mg/dl in the control group and 117 mg/dl in the Tx-group (p=NS). Pig mean weight in both groups was 22.14±2.26 vs 20.77±±1.57 (p=NS). In one case in the Tx-group diabetes induction was unsuccessful – in this case the stz was administered intraperitoneally due to poor venous access (in all other cases stz was given iv) the animal was withdrawn from further study. In all other 19 animals induction of diabetes was successful. There were no differences in insulin requirement and glycemia up to the day of eGSM-ITx between the groups (Figures 2,3).

eGSM-ITx results

Tx-group animals received a mean of 6000±3170 IEQ/kg. Tx-group animals had a significantly lower insulin requirement and significantly lower mean glycemia since the first day post transplantation (Figures 2,3). C-group animals all required insulin once daily to keep glycemia below 600 mg/dl.

eGSM-ITx complications

In the immediate period (up to 12 hours) post eGSM-ITx glycemia dropped significantly and in

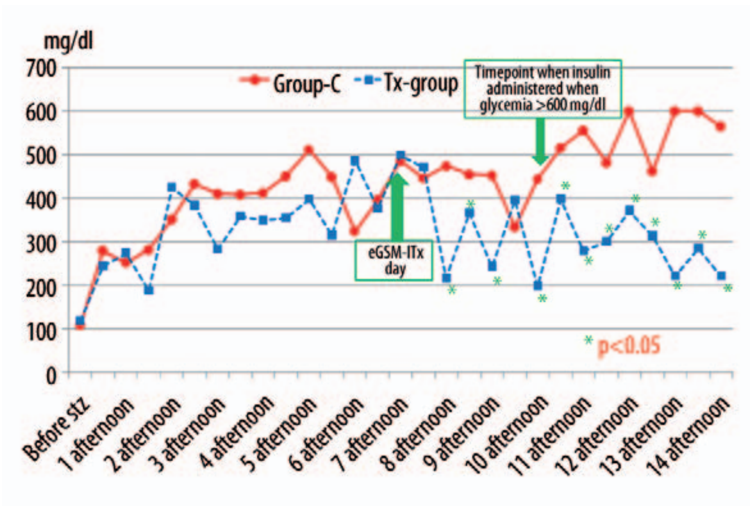

Figure 2. Mean glycemia within 7 days after induction of diabetes (p=NS) and after eGSM-ITx (p<0.05).

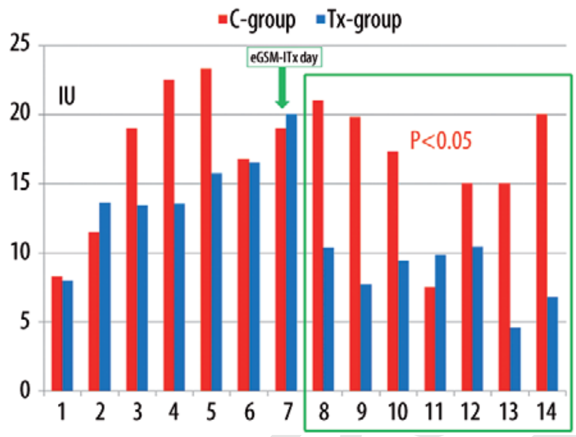

Figure 3. Mean daily insulin intake after diabetes induction (p=NS) and eGSM-ITx (p<0.05).

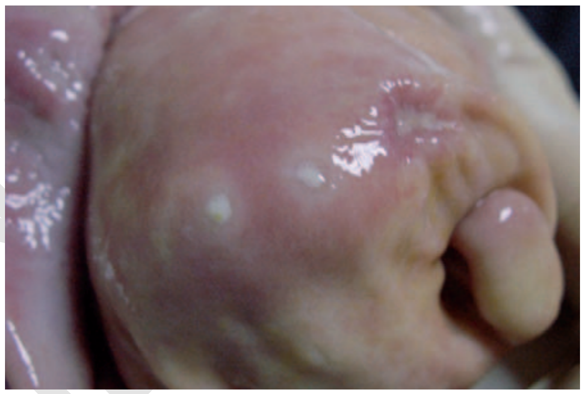

Figure 4. Morphological examination of gaster, 4 days after taking biospes.

5 cases there was a need for 5% glucose infusion in order to prevent hypoglycemia. After that period hypoglycemia below 60 mg/dl did not occur. In four cases diarrhea occurred, presumably due to immunosuppressive drugs administration – reduction of sirolimus doses in all cases resolved the problem. In one case an incompletely formed abscess of the tail was observed (where samples of blood were taken to measure blood sugar levels) – this was treated successfully with enteral antibiotics.

Control gastroscopy

There were no signs of perforation, ulceration or bleeding after the eGSM-ITx on control gastroscopy. In one case, 4 days post biopsy in control gastroscopy, on morphological examination, after euthanasia, there were 3 small ulcerations at sites from which biopsies were obtained (Figure 4). In one case, on histopathological examination, an abscess and necrosis, at the site of islets infusion, was present.

MRI results

In MRI scans revealed unspecific thickening of gastric wall observed at sites of islet deposition. There was an enhancement of signal after intravenous infusion of the contrast.

Discussion

To the best of our knowledge it is the first time that results of endoscopic allotransplantations of islets into gastric submucosal space are presented. Its usefulness has been postulated recently [17] and Caiazzo et al. have presented good results of autotransplantation to gastric submucosa in minipigs through open laparotomy [13]. With our experiment we have demonstrated the technique of endoscopic procedure and the problems which may occur during and after allotransplantation. Some of them, such as abscess of the tail or diarrhea could be foreseen – all transplantologists are familiar with such problems. Others, such as abscesses and necrosis within the gastric wall are new. In this study, the use of prophylactic antibiotics was discarded on purpose, to increase the chances of seeing such difficulties. On the other hand it was helpful and forced us to change the technique of infusion from quite-large volume (10 ml) deposition to smaller ones (5 ml). The insulin requirements and glycemia were definitely better in Tx-group of animals although not all animals immediately established satisfactory glicyemic control. This might be due to the relatively small (6000 Eq) number of transplanted islets. Surprisingly, very good glycemic control (glycemia below 150 mg/dl) was not associated with the number of islets equivalents transplanted (data not shown). It might be a problem of MHC matching which was not performed in this experiment or due to other, not completely understood, processes. Furter histopathological analysis of material are on the run. Nevertheless, eGSM-ITx appears to be a procedure which establishes insulin-independence in pigs over a limited period of time. Further analysis of potential clinical applications should be continued. Its potential for clinical application in human subjects needs further studies.

Conclusions

- Transendoscopic islets transplantation into gastric submucosa is feasible and a safe procedure in an experimental animal setting.
- Glycemia and insulin requirements in the group of animals which received eGSM-ITx are significantly lower.

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