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Gastric fundus submucosa as a site for islets transplantation: An experimental study

Gustavo Heluani Antunes de Mesquita[a,*], Yuri Justi Jardim[a], Leandro Ryuchi Iuamoto[a], Fabio Yuji Suguita[a], Felipe Futema Essu[a], Lucas Torres Oliveira[a], Alberto Meyer[b,c], Alessandra Crescenzi[c], Vinícius Rocha-Santos[c], Flavio H.F. Galvão[c], Wellington Andraus[b], Eleazar Chaib[c], Luiz Augusto Carneiro D'Albuquerque[b,c]

[a] Faculdade de Medicina FMUSP, Universidade de São Paulo, São Paulo, SP, Brazil
[b] Departamento de Gastroenterologia, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brazil
[c] Divisão de Transplante de Fígado, LIM 37, Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brazil

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ABSTRACT

Background: Islets of Langerhans transplantation is a promising alternative for glycemic control in patients with type 1 diabetes. The graft site is a factor that has large impact on the functioning of this transplant, and the stomach appears to be a promising location. Our objective is to describe a new experimental model for the grafting of Islets of Langerhans in rat stomachs.
Methodology: Islets of Langerhans were extracted from 45 isogenic male rats of the Lewis lineage and transplanted into 9 isogenic rats of the Wistar lineage; 5 in the gastric body submucosa, and 4 in the gastric fundus submucosa. Normoglycemia was defined as two successive measurements of < 250 mg/dL. No immunosuppression was used. The two groups glycemia control improvement were compared with *t*-student test.
Results: The results obtained following the transplantation of the islets in 9 rats showed between 995 and 2310 islets transplanted (mean of 1367). The rats from the gastric submucosa group had a better glycemic level improvement, with a confidence equal to 83.94%.
Conclusion: Islets graft into the gastric fundus submucosa is a viable model with potential for adequate glycemic control. This model gives potential for new perspectives and future studies in this area.

1. Introduction

An estimated 4% of the global population has diabetes mellitus, among which around 10% have type 1 diabetes [1–3]. Data from 2015 estimate that 1 in every 11 adults has diabetes mellitus, reaching a total of 415 million carriers worldwide.

Around 5–10% of patients suffer intense and unexpected fluctuations in glycemic levels, resulting in serious clinical complications, including retinopathy and neuropathy. In these cases, pancreatic transplantation can be an option, and is already in clinical practise. Transplantation of Islets of Langerhans is a promising alternative, which is less invasive and has a lower rate of morbimortality.

Research into islet transplantation is mainly focussed on obtaining more efficient techniques for isolating and purifying islet cells, discovering the best site for the graft and guaranteeing the immunoprotection of the islets [4], bearing in mind that the survival rate of the cells still remains low.

The site of the graft has a large influence on the functioning of the transplantation. The liver is still considered the most appropriate location of the implant in clinical practise, however immunological, anatomical and physiological factors have contributed to a need for locations with better results [5]. Currently the most common experimental site is the renal capsule, which is the subject of 70% of published research [6,13].

However, it has been observed that the renal capsule is not the ideal location to carry out the graft [7,8] owing to an unfavourable

* Corresponding author. Faculdade de Medicina FMUSP, Universidade de São Paulo, Dr. Nicolau de Souza Queirós Street, number 406, apartment 92, Vila Mariana, São Paulo, SP, Brazil.
E-mail addresses: gustavo.heluani@gmail.com (G.H.A. de Mesquita), justijardim@gmail.com (Y.J. Jardim), Leandro.iuamoto@gmail.com (L.R. Iuamoto), fabioysuguita@gmail.com (F.Y. Suguita), felipefutema@hotmail.com (F.F. Essu), lt.oliveira92@gmail.com (L.T. Oliveira), alberto.meyer@hc.fm.usp.br (A. Meyer), alecrescenzi@gmail.com (A. Crescenzi), dr_vinicius@uol.com.br (V. Rocha-Santos), fgalvao@usp.br (F.H.F. Galvão), wellingtonandraus@gmail.com (W. Andraus), eleazarchaib@yahoo.co.uk (E. Chaib), luizcarneirodalbuque@me.com (L.A.C. D'Albuquerque).

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microenvironment for islet survival, which is related to diabetic nephropathy [5]. Furthermore the subcapsular space is not large enough to accommodate a large number of cells.

In this sense, the stomach would appear to be a promising location for islet transplantation, as it is well vascularized, easy to approach and has lower levels of immune response compared to other options [7,9,10].

Our aim is to describe an experimental model for islets of Langerhans graft in the stomachs of rats and to evaluate its functionality.

2. Methods

2.1. Study design

Isogenic male rats from the Lewis line were used as donors of Islets of Langerhans, weighing between 200 and 250 g, products of the Laboratory of Liver Transplantation and Surgery (LIM/37) of the School of Medicine at the University of Sao Paulo, Brazil. The animals were maintained in polypropylene cages, receiving food and water ad libitum, at an ambient temperature of 22 ± 2 ºc, an air humidity of 55 ± 5% and a cycle of 12 h of light and 12 h of darkness. The air in the vivarium is recycled, purified and expelled 21 times per hour, passing through absolute filtration of a HEPA type.

The rats used as recipients of the transplantation were isogenic males of the Wistar line, weighing between 200 and 250 g, of the same origin as the donors and maintained under the same conditions of treatment and conservation. Diabetes was induced in the recipient rats (Wistar) using intravenous streptozotocin (STZ) (175–200 mg/kg; Sigma-Aldrich, Poole, UK) a week prior to transplantation. No immunosuppression was used.

The procurement of viable islets for a successful graft was carried out by removing the pancreas of 5 Lewis rats for each Wistar rat to receive the graft. The recipient rats were divided in two groups: one control group, which received the graft in the gastric body submucosa, and the case group, which received the graft in the gastric fundus submucosa.

2.1.1. Donor operations

The Lewis rat islet donors were sedated intraperitoneally with ketamine chlorohydrate and xylazine chlorohydrate (0.2 mL/100 g of body mass). A medial abdominal incision was then made in order to expose the biliopancreatic duct. Before making the ligature, the animal was exsanguinated. Following cardiac arrest the biliopancreatic duct was catheterized and 6 mL of type 5 collagenase solution (Sigma-Aldrich) was injected, at a concentration of 0.7 mg/mL dissolved in Hank's solution, through which pancreatic distension was observed (Fig. 1). Pancreatectomy was carried out when the pancreas was fully filled with collagenase. The pancreas was then removed and placed in a conical tube with 5 mL of Hank's solution and kept on ice.

2.1.2. Digestion of the pancreas

Following the isolation of the pancreas, enzymatic digestion was carried out in a water bath at 37ºc for 20 min. After 20 min, 20 mL of frozen RPMI medium with 10% bovine fetal serum was introduced in order to interrupt the process of digestion. Multiple washes were carried out, and then the pancreas was filtered through a steel mesh with 600 µm pores.

2.1.3. Isolation of the islets with Histopaque

A premade solution was used containing polysaccharose and adjusted to a density of 1,077 g/mL, denominated Histopaque (Sigma-Aldrich). The sediment containing the islets was obtained via centrifuge according to the manufacturer's recommendations for use. The interface cells between the solutions were removed and washed multiple times. To count the cells, a culture plate was introduced, with a ratio of 150 µl of cells in 150 µl of ditizone solution (Sigma-Aldrich).

2.1.4. Recipient operations

The recipient Wistar rats were sedated intraperitoneally with ketamine chlorohydrate and xylazine chlorohydrate (0.2 mL/100 g of body mass). A medial abdominal incision of 2 cm was then made in order to expose the stomach. An opening in the large curvature was made (1 cm), through which it was possible to access the stomach submucosa (Fig. 2).

In the control group, the graft was made with two parallel injections of the solution containing the islets in different portions of the gastric body submucosa. In the case group, the injections were made into the gastric fundus submucosa.

2.1.5. Glycemia measurements

Series measurements of blood sugar were obtained via the rodents' tails using a glucometer (Accu-Chek, da Roche, Burgess Hill, UK). The maximum reading of the glucometer was 600 mg/dL. The determination of glucose was carried out prior to the administration of STZ, immediately following surgery, and daily over the subsequent days (up to graft rejection). Diabetes was confirmed when the blood sugar levels reached over 250 mg/dL.

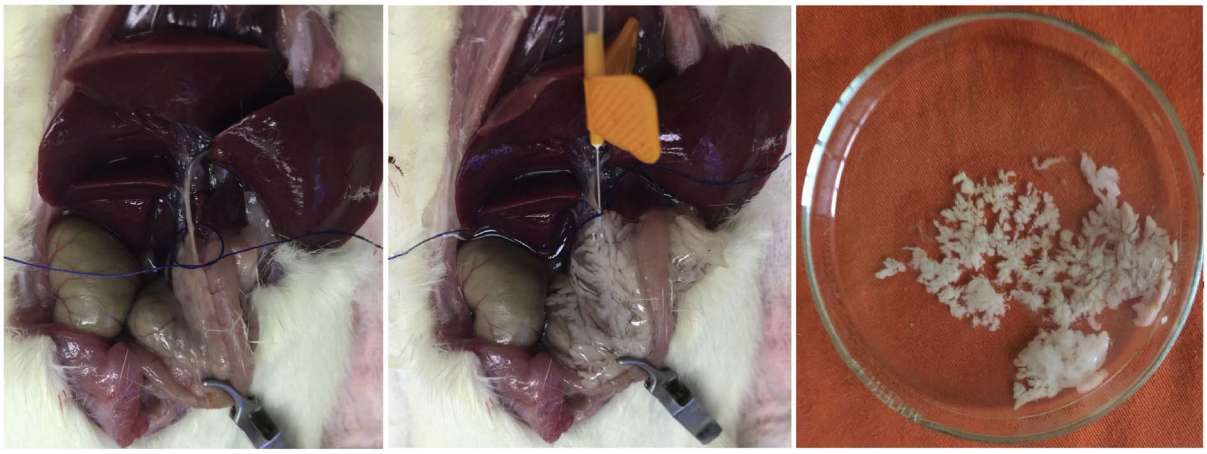

Fig. 1. Details of the surgery of donor rats. (A) Clamp to prevent the overflow of collagenase for the intestine and knot involving the pancreatic duct ready to fix the scalp. (B) Injection of collagenase solution into the biliopancreatic duct through the scalp fixed by the knot. Note the filling of the pancreatic parenchyma with collagenase. (C) pancreas removed from the rat following collagenase injection.

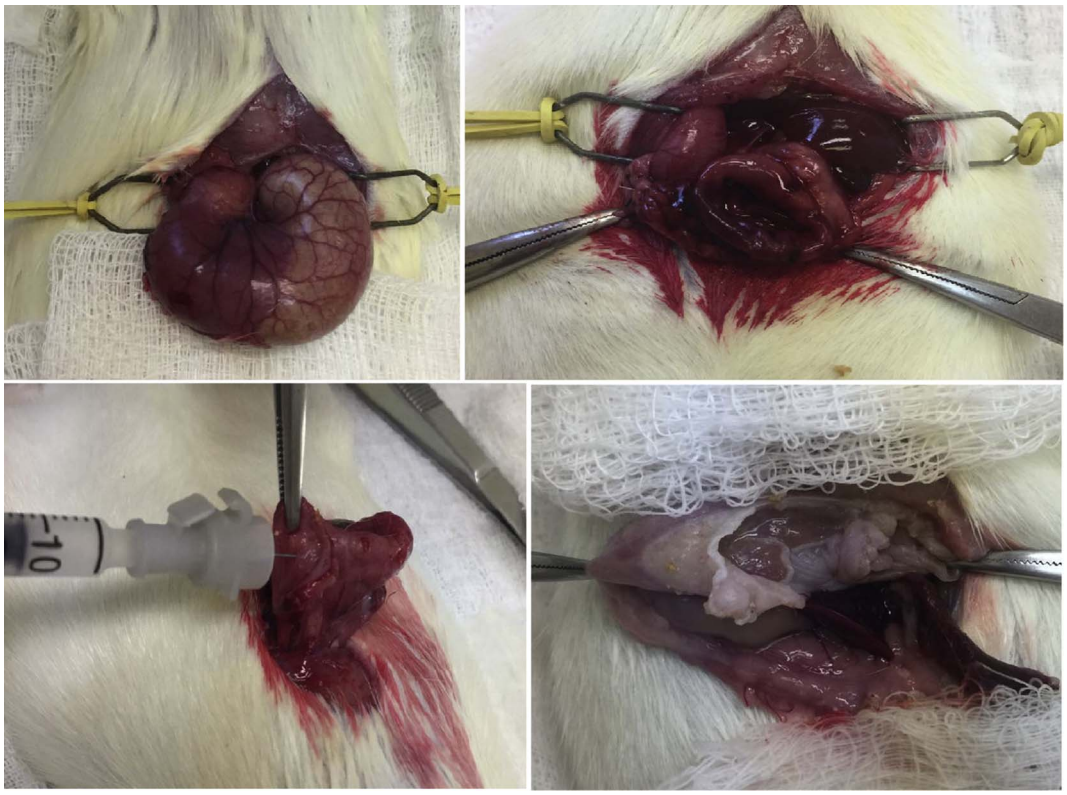

Fig. 2. Details of surgery in recipient rats. (A) stomach of the recipient rat exposed by the abdominal incision. (B) Opening made in the large gastric curvature, at the base. (C) Graft of islets in the submucosa of the gastric base of the recipient rat. (D) Necropsy of one of the rats showing the graft location (gastric fundus).

2.1.6. Assessment of graft function

The main dysfunction was failure to reach blood sugar levels of lower than 250 mg/dL for two consecutive measurements following islet transplantation. Loss of function was defined as 2 consecutive measurements > 250 mg/dL. Normoglycemia was defined as 2 successive measurements of < 250 mg/dL [6].

2.1.7. Assessment of clinical parameters

As well as glycemia and graft function examination, body mass, survival and number of islets transplanted was recorded for each Wistar rat.

2.1.8. Statistical analysis

The two groups were compared using as parameter the rats glycemia. The glycemic improvement index (E) was calculated for all rats using the medium glycemia before transplantation (M1) and medium glycemia after transplantation (M2) in the formulae E = (M1 + 0,5)/(M1 + M2 + 1). The two groups were compared using *t*-student test and had its results distributed in a normal distribution pattern.

2.1.9. Ethical committee approval

All experiments were approved on University of São Paulo School of Medicine Ethical Committee and were in accordance to the relevant guidelines and regulation. All experiments followed ARRIVE criteria.

2.1.10. Results

The pancreas was taken from 45 Lewis rats to carry out the study. The results obtained following the islet transplantation into the gastric submucosa in 9 rats showed a number of islets transplanted of between 995 and 2310 (mean of 1367). In the gastric fundus submucosa group (rats 5 to 9) all rats showed satisfactory glycemic control for at least 2 days; 1 rat (Rat 2) died possibly from hypoglycemia. In the gastric body submucosa group (rats 1 to 4) 2 rats did not present islets function and only one rat presented function for more than one day.(See Table 1)

As result of the statistical analysis, we have notice a confidence equal to 83.94% on the statement that the control improvement in average is smaller than the case improvement. Fig. 3 illustrates the differences in the normal logistic densities of E of the two arms.

3. Discussion

A good site for the graft of Islets of Langerhans should have the following characteristics: reduced inflammatory response to graft, adequate vascularization, easy approach for the carrying out the transplantation and for subsequent monitoring, good capacity to produce insulin in physiological conditions and with lower immunological response [8]. Such conditions form the ideal microenvironment for the functioning of transplanted islets, avoiding rejection and prolonging the useful life of the graft.

The stomach submucosa, the site chosen for our study, has already shown a reduced inflammatory response in other situations [9]. It is a well vascularized region, is easy to approach for the graft and subsequent analysis, which in larger animal models or humans could be done via endoscopy [9,10]. Furthermore, it has shown good results for glycemic control and reduced immunological response [7,9–11].

Liu X. et al. [11], carried out an alotransplant in the portal vein and the gastric subserosa as a graft site in Lewis rats. The rats with subserosa gastric transplantation had satisfactory glycemic control, even when compared to rats without diabetes, while rats with transplantation on the portal vein did not have satisfactory glycemic control. At the beginning of this study we also used the gastric subserosa, and were unable to obtain positive results. This could be explained by the absence of adequate vascularization in this layer of the stomach, which is why we focussed on the submucosa for the development of our experimental model.

In 2007 Caiazzo et al. [7] used diabetic pigs to compare the renal capsule to the submucosal layer as a graft site in minipigs, operated via laparotomy, obtaining better islet function in the gastric submucosa.

Table 1
Present data from islets received, glycemia and weight measurement for each recipient rat, divided in two groups. The bold and underline numbers indicate the glycemia measurements included in the normoglycemia criteria.

Rat Group		Number of Islets	Days	Day -4	Day -3	Day -2	Day -1	Tx = 0	Day 1	Day 2	Day 3	Day 4	Day 5
Gastric Body Submucosa Group	Rat 1	995	Weight(g)	260	254	248	262	252	238	229	229		
			Glycemia (mg/dL)	590	558	491	> 600	562	**236**	352	375		
	Rat 2	900	Weight(g)	290	291	29	287	297	283	261	266	279	278
			Glycemia (mg/dL)	370	368	410	394	413	**218**	**248**	251	321	401
	Rat 3	1650	Weight(g)	280	279	281	280	279	272	279	265	264	
			Glycemia (mg/dL)	392	410	405	421	444	**171**	314	376	387	
	Rat 4	1100	Weight(g)	273	272	272	278	279	271	268	266		
			Glycemia (mg/dL)	476	468	456	467	456	302	398	401		
Gastric Fundus Submucosa Group	Rat 5	1200	Weight(g)		285	281	292	300	296	302			
			Glycemia (mg/dL)		129	441	459	445	365	457			
	Rat 6	2050	Weight(g)	269	265	270	275	291	248	230	218	255	258
			Glycemia (mg/dL)	125	393	455	435	451	**183**	**172**	**77**	296	322
	Rat 7	2050	Weight(g)	268	264	264	267	270	261	261	(died)		
			Glycemia (mg/dL)	138	508	544	512	495	**97**	**148**			
	Rat 8	2310	Weight(g)			230	226	244	236	244	239	243	263
			Glycemia (mg/dL)			128	327	345	**155**	**208**	**140**	**140**	**175**
	Rat 9	2100	Weight(g)		250	246	257	254	240	247	253	271	
			Glycemia (mg/dL)		135	373	328	372	**131**	**146**	**211**	**245**	

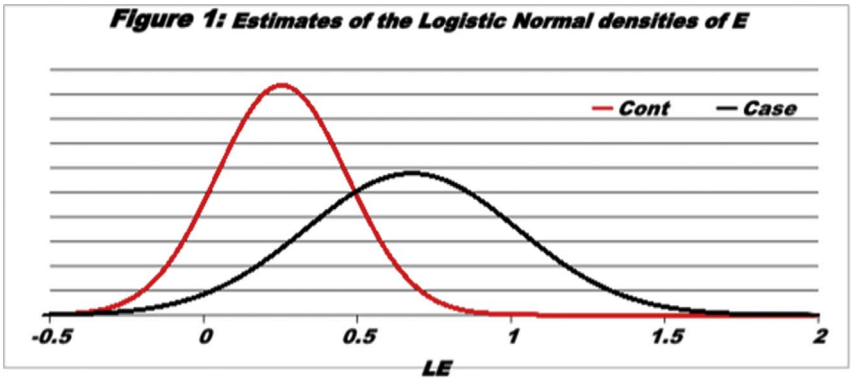

Fig. 3. Illustrates the differences in the normal logistic densities of E of the two arms.

Echeverri et al. [9] obtained better results when compared to the portal vein in a study involving the implant of islets in the gastric submucosa via endoscopy, with lower levels of immediate inflammatory response, better glycemic control and a higher survival rate of islets. Wszola et al. [10] demonstrated glycemic control and lower doses of insulin necessary for diabetic pigs with a graft of islets in the gastric submucosa via endoscopy when compared to a control group. All of the studies described procedures of graft in the submucosa layer of the stomach in diabetic pigs, without specifying the anatomical location in which this graft is best performed; this was an important aspect of our study.

The results in the group with graft in the body of the stomach were not especially encouraging because of poor early glycemic control. Different anatomical parts of the stomach present different histological compositions, which is associated with the functioning of the graft; in the cardia region, there is a predominance of mucous-secreting cells; in the antrum there is a predominance of mucous-secreting cells but also enteroendocrine cells, such as gastrin-secreting G cells; in the body and principally in the gastric fundus parietal cells predominate, secreting intrinsic factor and chloridric acid.

Furthermore, the different sections of the stomach present varying patterns of contraction according to their function [12]. In terms of motility, the fundus makes up part of the proximal stomach, which in periods of fasting presents a high basal tone, and in feeding times relaxes to accommodate the food, a process known as gastric accommodation. The distal portion of the stomach, on the other hand, has a slow wave activity and peristaltic contraction in both fasting and feeding periods.

Our hypothesis is that these elements influenced our early satisfactory results in the functioning of the transplant of pancreatic islets. We believe that such results are related to the histological differences and motility of the gastric fundus, which differentiates this anatomical portion from the rest of the stomach. This paper is the first to consider both the layer and the anatomical location in the stomach. It's also important to restate that no immunosuppression was used, therefore it was expected for the grafts exhibit brief time functionality.

Despite the small number of animals operated, we felt it unnecessary to continue the experiment, owing to the consistently positive results in early glycemic control, following the graft in the fundus, when compared to the control group. Our experimental model showed that islets transplantation into the gastric fundus submucosa is a viable approach and its results corroborate for the hypothesis of a better functionality in the gastric fundus submucosa. However, further investigation using more animals, immunosuppression, standarized number of islets and comparison to other sites, are necessary to evaluate the real applicability of gastric fundus submucosa as graft site.

4. Conclusion

The graft of islets into the gastric fundus submucosa is a viable model in aiding glycemic control. This model points towards potential for further research on this topic.

Ethical approval

The study was approved on Faculdade de Medicina da USP Ethical Committee on Animal Use (reference number: 083/14).

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Author contribution

GHAM, YJJ, LRI – Responsible for writing the main text. Participated in the study design and experiments and literature review.

FYS, FFE, LTO – Participated in the scientific review of the article and literature review.

AM, VRS, FHFG, WA, EC, LACD – Participated in the study design and scientific review.

AC - Responsible for islets processing and biochemical part of the methodology.

Conflicts of interest

The authors declare no conflicts of interest.

Guarantor

Luiz Augusto Carneiro D'Albuquerque.

Unique identifying number (UIN)

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

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