Ileal interposition for the treatment of diabetes in rats: repercussion on beta cells mass

Tratamento do diabetes pela interposição ileal em ratos: Repercussão na massa de células beta

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ABSTRACT

Purpose: Research with the aim of studying the effect of ileum interposition in hyperglycemia and pancreatic beta cell mass in nonobese diabetic rats. **Methods:** We used 18 Wistar rats randomly divided into 3 groups of 6 each. A non-diabetic control group, a diabetic control group, and diabetic with ileal interposition group. Induction of diabetes was underwent with streptozotocin. The beta cell mass was quantified by an indirect method by dividing the serum C-peptide (ng/mL) for blood glucose (mg / dL). We used variance (ANOVA) and Tukey's test for analysis of specific differences, considering the significance $p < 0.05$. **Results:** The blood glucose of diabetic animals with ileal interposition was significantly lower (94.5 ± 5.6mg/dL) than in diabetic control rats (245 ± 4.8 mg / dL) ($p < 0.05$). The C-peptide levels showed significantly higher in diabetes interposition group (0.58 ± 0.06 ng / mL) than in control diabetes group (0.42 ± 0.03 ng / mL), suggesting a greater response to pancreatic stimulation in that group ($p < 0.05$). The ratio of C-peptide and glucose levels showed a higher beta cells mass in diabetic interposition animals (0.61) than in control diabetes (0.004), and the difference was significant ($p<0.05$). **Conclusion:** The results showed that the ileal interposition contributed to the reduction of blood glucose and to increase the mass of functioning beta cells in diabetic rats.
Key words: Diabetes. Ileum interposition. Streptozotocin. Beta cells. Rats.

RESUMO

Objetivo: Pesquisa com o objetivo de estudar a ação da interposição do íleo terminal na hiperglicemia e na massa de células beta pancreáticas em ratos diabéticos não obesos. **Métodos**: Foram utilizados de 18 ratos Wistar, divididos aleatoriamente em 3 grupos de 6 cada. Um grupo controle não diabético, um grupo (controle diabético) submetido à indução do diabetes com estreptozotocina, e outro grupo diabetes submetido à interposição ileal. A massa de células beta foi quantificada por método indireto através da divisão dos níveis séricos de peptídeo-C (ng/mL) pela glicemia (mg/dL). Usados os testes de variância (ANOVA) e Tukey, para análise de diferenças específicas, considerando-se significância de $p<0.05$. **Resultados**: A glicemia dos animais diabéticos com interposição ileal foi significativamente reduzida (94,5±5.6mg/dL), quando comparada com o grupo controle diabético (245±4,8mg/dL) ($p<0,05$). O peptídeo-C revelou níveis significativamente maiores nos animais do grupo diabetes interposição (0,58±0,06ng/mL) do que no grupo controle diabetes (0,42±0,03ng/mL), sugerindo maior resposta à estimulação pancreática neste grupo ($p<0,05$). A razão entre peptídeo-C e glicemia revelou diferença significante ($p<0,05$), observando-se maior massa e função de células beta nos animais diabetes interposição (0,61) do que nos controle diabetes (0,004). **Conclusão**: Os resultados obtidos permitem concluir que a interposição ileal contribuiu para a redução da glicemia e para o aumento da massa de células beta funcionantes em ratos diabéticos.

Descritores: Diabetes. Interposição. Íleo. Estreptozotocina. Células beta. Ratos.

Introduction

Diabetes mellitus (DM) has great importance in Brazil, because it affects 7.6% of individuals between 30 and 69 years[1,2], with increasing prevalence particularly in urbanized population. The increasing prevalence is a worldwide phenomenon in the United States and a study of 18,825 adults aged >20 years showed a prevalence of 5.1% of diagnosed DM, and 2.7% of non diagnosed DM[3]. The DM causes cardiovascular, neurological, renal and eye complications and is among the 10 leading causes of morbidity and mortality in Brazil[4], resulting in high costs to the health system[5]. The correct treatment decreases morbidity and mortality, improves quality of life, and reduce costs[6,7].

Type 2 diabetes is the most common form of diabetes in humans and is a combination of genetic and acquired factors that impair the function of pancreatic beta cells and tissue sensitivity to insuline[8]. There is evidence that beta-cell dysfunction is crucial for the development and progression of this form of diabetes[9,10]. Studies in patients and in isolated pancreatic islets have demonstrated quantitative and qualitative defects in insulin secretion, stimulated by glucose. Thus, there has been growing interest in the possibility of preservation of beta cells to prevent diabetes, or to prevent the progressive deterioration of glycemic control observed after diagnosis of the disease over the years [10].

Assuming that the beta-cell dysfunction is the key to the development and progression of type 2 diabetes, there is evidence that the beginning and triggering of disease can be slowed and the blood glucose control can be affected by certain therapeutic measures. Ideally, these beneficial effects are associated, at least in part, to the maintenance of beta cell function. For this to occur, strategies must be developed through drugs, cells and islets transplantation, or other appropriate measures. In addition, efforts should be undertaken to better understand what changes in the beta cells are present[11].

Obesity and diabetes mellitus (DM) have reached epidemic proportions, and the scientific world has studied new methods of treating these metabolic disorders[12]. Remission of diabetes has been observed after surgical procedures that affect the entero-insular axis, as is the case of bariatric surgery[13,14,] with improvement in glucose homeostasis independent of weight loss[15,16]. Bariatric surgery offers the control of diabetes and co-morbidities, leading to a change in lifestyle, having a role of surgery metabólica[17]. With the increasing number of bariatric surgeries, technical modifications have been explored to minimize the morbidity and associated malabsorption. However, the improvement of diabetes after bariatric surgery may be related to other factors that do not occur in non obese diabetic individuals. This applies to the large reduction in calorie intake and very fast reduction of fat tissue mass. The possibility of surgical intervention interfere with the beta cells mass has been studied[18].

The ileal interposition involves alteration of the intestinal tract through the transposition of a segment of distal ileum for the zone of proximal jejunum. This surgery, together with the sleeve gastrectomy, has resulted in post-operative glucose control. As it does not involve bypass of small bowel and nutrients, changing in food absorption is small[18,19]. The hypothesis inherent in the model, initially proposed by Mason, is that the early stimulation of the ileum is an important component in the mediation and release of hormones that can improve type 2 diabetes[19]. Some studies have demonstrated improved glucose homeostasis in diabetic patients and in postoperative of animal models of ileal interposition[18,20].

Possible factors involved include the increase of hormones produced by the ileum, such as glucagon-like peptide-1 (GLP-1), which is significantly elevated after ileal

interposition[20]. Since GLP-1 and its receptor antagonists promote the proliferation of pancreatic beta cells and reduce apoptosis, is likely that ileal interposition results in proliferation of beta cells mass[21,22.23].

O tratamento com estreptozotocina resulta na destruição de células beta e causa severa hiperglicemia em ratos[24]. No presente projeto será utilizada dose de estreptozotocina suficiente para destruição parcial de células beta[25].

Treatment with streptozotocin results in the destruction of beta cells and causes severe hyperglycemia in rats[24]. In the present study we used a streptozotocin dose enough to partial destruction of beta cells[25]. Assuming that the ileal interposition results in increased secretion of GLP-1, the protocol for this project worked with the hypothesis that the interposition will extend the beta cell mass and glucose tolerance in rats. The incretins, including GLP-1, are hormones secreted in the digestive tract and make the production of insulin more efficient[20]. The gastrointestinal bypass prevents and cures diabetes type II, stimulating the release of GLP-1 from the terminal ileum when glucose and fat come to this intestinal region[25].

There is no consensus about which test is best to monitor beta cell mass and function in patients with diabetes and after islet transplantation[26]. Many tests give variable results, are time consuming and difficult to perform. The low variability, high reproducibility and close relationship between the serum C-peptide with insulin secreted into the portal system, makes it an important tool to monitor beta cell function[27]. The measurement of C-peptide has been routinely performed before and after transplantation of pancreatic islets to document the survival of transplanted islets[28]. Plasma levels of C-peptide values depend on glucose, so that they may indicate good allograft function if the glucose is normal, but may be too low if glucose is high. Calculations involving C-peptide values in relation to blood glucose in type 2 diabetes, as well as in transplants of the pancreas and islets have been made with measurements in urine and serum[29-31]. In this study we used the ratio between C-peptide and glucose (CP/G), which corrects the calculations according to their glycemic control[32].

Based on the above concepts and the fact that this is an important issue, and that this is an issue poorly understood, in this study we induced diabetes in rodents and examened the action of the ileum transposition in the mass of pancreatic beta cells and treatment of hyperglycemia in non-obese rats.

Objetive

To examine the effect of ileal interposition in the evolution of glycemia in diabetic rats. Through an indirect method, to quantify the beta cells mass before and after ileal interposition.

Methods

Animals and diet

Non-diabetic Wistar rats 3 months old, were housed in individual polypropylene cages and maintained with free access to water and food (Labina - Purina ®). The protocol followed all the precepts of the Brazilian Law No. 11.794/08, which addresses the use of animals in research.

Experimental protocol

After acclimatization in the laboratory for 7 days, the animals were weighed. An initial dosage of glicemia was performed and the animals were randomly distributed according to the protocol in three groups: non-diabetic control group (n = 6), diabetic control (n = 6); diabetic interposition (n = 6).

Diabetes induction

The groups of diabetic rats were treated with a single dose of streptozotocin (STZ) (Sigma-Aldrich ®) (35 mg / kg dissolved in citrate buffer pH 4.5) to induce hyperglycemia. With this dose, not all beta cells were destroyed and theoretically this model did not create insulin-dependent diabetes. 48 hours after the use of STZ, blood glucose was measured by manual Glucometer (Accu-chek, Roche ®, Germany).

Surgery

After defined hyperglycemia between 200 and 300 mg/dL, the rats fasted for 12 hours and they were anesthetized with thiopental (20 mg/kg) and ketamine (30 mg/kg) IM. Using aseptic technique, 3 cm midline laparotomy was performed in all of them, the cecum was exposed and the terminal was ileum identified. In the diabetes interposition group, the ileum was cut to 2 cm and 12 cm from the ileocecal valve, and an ileal loop 10 cm in length was prepared, keeping the blood supply intact. Ileo-ileal anastomosis was performed to reconstruct intestinal transit. The jejunum was transected 10 cm from the Treitz angle, the previously isolated ileal loop was interposed between the two ends of the jejunum by an isoperistaltic anastomosis, using 6-0 Prolene ® (Ethicon), with the aid of surgical microscope 10x (DFV, São Paulo, Brazil). Rats body weight was determined and recorded once a week after surgery and all animals were observed for 30 days.

Quantification of indirect functional beta cells mass

Completed 30 days of evolution, glucose concentrations were determined by the Accu-Chek (Roche, Germany). Measurement of C-peptide was performed by radioimunoassay. The ration between C-peptide and glucose levels was performed using the following formula:

$$\text{C- peptide/glucose ratio} = \frac{\text{C-Peptide (ng/mL)x100}}{\text{Glucose (mg/mL)}}$$

All calculations were performed using measurements of C-peptide and serum glucose obtained from the same blood sample.

Morbidity and Mortality

All rats were examined daily from the clinical point of view during the experiment. Diarrhea, pain signals, water and food intake and weight control were measured.

Statistical Analysis

The data were submitted to analysis of variance (ANOVA). The Tukey test was applied to analyze specific differences. Data were expressed as mean±standard deviation, considering the significant differences at $p<0.05$.

Results

There was a significant difference in body weight among the 3 groups. All 18 rats survived for 30 days. Group control diabetes animals had a mean weight loss of 85±15.1 g and the diabetes interposition group had a mean weight loss of 73±13.4 g over the 30 days ($p<0.05$). Non diabetes control group gained a mean of 65±6.2 g.

Table 1 shows the results of serum glucose and C-peptide, as well as tests to investigate the statistically significant differences between diabetic and diabetic control ileal transposition, as well as between the control non-diabetic and diabetic control rats.

Table 1 – Effect of ileal interposition in serum biochemical parameters in non-diabetic control rats, diabetic control and diabetic rats undergoing ileal interposition.

Parameter	Non-diabetic control	Diabetic control	Diabetic ileal interposition
Glucose mg/dL	6.2±2.56*	245±4.8	94.5±5.6 **
C-peptide ng/mL	0.65±0.08 *	0.42±0.03	0.58±0.06**

Data expressed as mean±standard deviation * Values significantly different compared with the diabetic control group * P <0.05, ** Values with significant difference compared with control non-diabetic and diabetic control group ** P <0.05.

The analysis of Table 1 to means that the blood glucose, in diabetic animals which ileal interposition was performed, was significantly reduced, when compared with non-operated diabetic animals ($p<0.05$). The same profile was observed in relation to determination. Significantly higher levels of C-peptide were observed in the diabetic interposition group than in diabetic control rats, suggesting increased pancreatic stimulation response ($p<0.05$).

Evaluation of beta cells mass

Table 2 – Effect of ileal interposition on functional beta cell mass, calculated by the C-peptide/glucose ratio in non diabetic control, diabetic control and diabetic ileal interposition rats.

Parameter	Non-diabetic control	Diabetic control	Diabetic ileal interposition
Peptídeo-C /Glicose	0.94	0.004	0.61*

*P<0.05. Values significantly different comparing with diabetic control group.

The C-peptide and glucose ratio was higher in diabetic ileal interposition group (0.61), when compared with diabetic control rats (0.004), and the difference was significant ($p<0.05$). Thus, we can infer that the ileal interposition contributed to increase the beta cell mass (Table 2).

Discussion

The bariatric and metabolic surgery emerged to control morbid obesity and to treat comorbidities such as type 2 diabetes mellitus. It is known that these surgical techniques imply on restriction of food intake, malabsorption and increased release of intestinal glucagon[20]. On early times the improvement of metabolic alterations after bariatric surgery was exclusively attributed to weight loss. However, studies have shown

that most obese and diabetic patients undergoing surgical treatment had an improvement in type 2 diabetes long before a significant weight loss[33,34]. The origin of this observation is related to increased release of incretins by intestine L cells, especially GLP-1. L cells are found mainly in the ileum and proximal colon and they are part of the enteroendocrine system, which is involved in the mechanism of hunger/satiety control, gastrointestinal motility, peripheral insulin sensitivity, glucose and lipid metabolism[35]. Anatomical abnormalities resulting from the ileal interposition stimulate the release of some hormones, mainly GLP-1, because of the early perfusion of nutrients in the distal ileum[36].

Thus, the ileal interposition promotes the release of incretins, without causing disabsorptive syndrome. The present study found significant improvement in blood glucose in the diabetic group after ileal interposition, compared with diabetic control group. This fact corroborates the findings of Culnan et al[37], in which the early stimulation of the ileum after ileal interposition improved glucose tolerance, insulin sensitivity and muscle glucose uptake in Zucker obese rats, without changing the absorption of nutrients. Similarly, clinical study interposing a segment of 50 cm ileum distal to the Treitz angle, showed the same endocrine stimulation, with reduction in serum glucose[38].

Strader et al[39] demonstrated weight reduction associated with increased levels of GLP-1 in rats. This effect is possibly related to the anorectic effect of this hormone and peptide YY, an incretin hormone also produced by L cells.

Regarding the measurement of indirect effects of incretins on the mass of functioning beta cells of animals, dosage of C-peptide in the systemic circulation has been used. C-peptide is produced by beta cells and secreted into the bloodstream. For a long time, the C-peptide was considered important in the biosynthesis of insulin, but it has minimal biological activity[40]. However, it remains an excellent parameter to evaluate the function of pancreatic beta cells. It is equimolecular with insulin secretion, has longer half life than insulin and a negligible hepatic clearance. Many researchers prefer C-peptide concentrations instead of insulin, to detect changes in insulin secretion by beta cells[27,32,40].

The ileal interposition used in our study to treat diabetes in the animal model used in this study revealed a positive effect on the measurement of C-peptide. The animals of the diabetes interposition group showed levels of this peptide significantly higher than the diabetic control rats. Likewise, the analysis of the C-peptide/glicemia ratio showed a significant difference between groups ileal interposition diabetes and diabetes control. This finding validates the finding that ileal interposition results in increased beta cell mass in rats[21-.23].

The results of this study, which is part of a research line, corroborated with the literature, demonstrate the great importance of the issue, in view of the effective potential of this surgical technique in treating diabetes and preventing its complications. Other parameters of immunogenetics, cell proliferation and hormone examination are part of further research to validate the preliminary results of this work.

Conclusion

The results showed that the ileal interposition was beneficial for the evolution of blood glucose and contributed to increased beta cell mass in diabetic rats..

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