Role of GAD peptides p217 and p290 in the repair of INS receptor in salivary tissues of type 1 diabetic mice

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Abstract: Glutamate decarboxylase or glutamic acid decarboxylase (GAD) is a protein associated with autoimmune diseases, including type-1 diabetes. This disease is primarily associated with the occurrence of a specific isoform: GAD65. Conversely, some specific peptides of this protein may block autoimmunity in diabetes. In this respect, understanding the relationship between GAD and the development of diabetes is important, and it is necessary to understand the role of each GAD peptide to design effective autoimmune diabetes treatments. The purpose of the present study was to analyze the effects of treatment with GAD-derived peptides p217 and p290 on INS receptors in the salivary epithelium of nonobese diabetic (NOD) animals. Three groups of 7 mice each were studied: I, BALB/c mice (control); II, NOD mice; and III, NOD mice treated with peptides p290 and p217. Groups I and II only received buffered saline solution. Glucose levels were measured daily during the 21 days of the experiment. After the study, the animals were euthanized and the parotid and submandibular glands were removed for the analysis of INS-R by fluorescence microscopy. Therapy with two peptides together was associated with reduced glucose levels in NOD mice and intense INS-R expression in both salivary organs. Our approach of combining GAD p217 and p290 peptides contributed to hormonal balance and promoted the repair of INS-R.

Keywords: Glutamic acid decarboxylase, insulin, insulin receptor, nonobese diabetic mice.

INTRODUCTION

Like many other organ systems, the salivary glands are targets of insulin (INS) and are affected by diabetes, which causes numerous complications (Fushini, 1980; Ho, 1990; Cagnon et al., 2000; Chavez et al., 2000; Carvalho et al., 2003; Pace et al., 2003; Shirai et al., 2004; Caldeira and Cagnon, 2008; Kumar, 2004). INS is related to cellular maintenance, and its action depends on its ability to bind its receptors (Ullrich et al., 1985; Goldfine, 1987; White and Kahn 1994; Caldeira and Cagnon, 2008). INS receptors (INS-R) are heterotetrameric proteins comprised of alpha and beta subunits with intrinsic kinase activity, and first interaction between INS and its receptor requires the alpha subunits (Patti and Kahn, 1998).

Type 1 diabetes affects glandular organs and their homeostasis. This disease is initiated by chronic inflammation, leading to pancreatic beta-cell damage that results in general INS deficiency and hyperglycemia. Similarly, tissue damage affecting the salivary glands is also mediated by an autoimmune process primarily involving cytotoxic T cells. Autoantibodies against INS, the protein tyrosine phosphatase (ICA 512), and glutamic acid decarboxylase (GAD) also play roles in these detrimental processes (Karlsson et al., 2000; Fenalti and Buckle, 2010; Metidieri et al., 2012).

Glutamic acid decarboxylase (GAD) is an autoantigen of INS-secreting cells that is expressed in both human patients and nonobese diabetic (NOD) mice (De Aizpurua et al., 1994; Pleau et al., 1995; Yoon et al., 1999). GAD is primarily expressed in the nervous tissue, but this protein is also found in other tissues, including the pancreas. However, the relationship of GAD with this glandular organ is not entirely clear (Petersen et al., 1998; Esclapez and Houser, 1999; Wei and Wu, 2008; Ludvigsson, 2009).

In the last few years, considerable progress has been made in describing and characterizing GAD autoantigens. Several isoforms of GAD have been reported, including GAD 65 (Roep, 1996; Ludvigsson et al., 2008; Fenalti and Rowley, 2008). Despite the relationship of GAD with autoimmune processes, recent studies have suggested that GAD and its peptides can prevent the progression of diabetes (Liu, 2006). GAD protein therapy has been shown to induce an immune tolerance and, as a consequence, can potentially interrupt pancreatic cell destruction (Morales and Thrailkill, 2011). In contrast, other studies have demonstrated that GAD can increase the inflammatory process in models of experimental diabetes (Gauvrit et al., 2004). These conflicting results...
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illustrate the complexity of GAD and the need for additional investigations. Such studies are important, as this approach could be a potential therapy to reverse the damage that diabetes causes in salivary gland epithelial cells.

Therefore, the purpose of this work was to analyze the effects of treatment with the GAD-derived peptides p217 and p290 on INS receptor expression in the salivary epithelium of NOD mice.

MATERIALS AND METHODS

Animal protocol - We obtained 15-week-old mice (females/ n=21/ weighing 20±2.5g) from the Animal Care Centre (CEMIB) of Universidade Estadual de Campinas. The animals were divided in three groups (n=7 each): I, BALB/C control mice; II, NOD mice without treatment; and III, NOD mice treated with GAD peptides p217 and p290. The animals were maintained under standardized conditions at the Animal Experimentation Laboratory, Faculty of Medicine of Jundiaí, FMJ, São Paulo, Brazil.

Groups I and II received 1-ml intraperitoneal injections of phosphate-buffered saline (PBS) alone to emulate the experimental phases. After hyperglycemia was confirmed in group III, each animal received an intraperitoneal injection of 1 ml PBS plus GAD peptides p217 (200µg) associated with p290 (200µg) (peptides purity >98%) (Rheabiotech, São Paulo, Brazil) diluted in IFA (incomplete Freund's adjuvant/100µl). During the 21-day experiment, the animals received three intraperitoneal injections, the first one 7 days after disease confirmation, and then successively on days 14 and 21, similarly to what was previously described (Tisch et al., 1999).

The blood glycaemia levels (mg/dl) were observed on a weekly basis with Accu-Chek test strips (Performa, Roche Diagnostics, Basel, Switzerland). The mean glucose level of control animals was 169mg/dl. Diabetes was defined as a mean glucose level above 660mg/dl. A mean lower glucose level (485mg/dl) was observed in the treated group (III). The mean glucose level in nondiabetic control mice (group I) was 169mg/dl.

Glucose levels

Diabetic animals (group II) had serum glucose levels above 660mg/dl. A mean lower glucose level (485mg/dl) was observed in the treated group (III). The mean glucose level in nondiabetic control mice (group I) was 169mg/dl.

Immunofluorescence microscopy

Submandibular gland

In control animals (group I), a saturated INS-R signal was observed (fig. 1A and table 1). In group II (NOD mice without treatment), receptor labeling was mild, with localization in salivary epithelium (fig. 1B and table 1). In group III, however, INS-R labeling was also saturated and localized in the salivary epithelium, as noted in group I (fig. 1C and table 1).

Parotid gland

Similarly, in parotid tissues of group I (control animals) high INS-R levels were observed in the salivary epithelium (fig. 1D and table 1). The INS-R labeling in untreated animals of group II was mild, with similar localization (fig. 1E and table 1). Similar to what was found in the submandibular gland, INS-R labeling in diabetic mice (group III) was intense in the salivary epithelium (fig. 1F and table 1).

DISCUSSION

NOD mice are a typical model for studying type 1 diabetes and INS effects (Leiter, 1989). Although INS is mainly produced by the pancreas, it may be produced by other tissues. This hormone modulates physiological events in many tissues, including the salivary glands. It is known that INS controls glucose levels and maintains...
The immunofluorescence analysis revealed alterations in INS-R levels in diabetic animals. In contrast, receptor expression was normalized in the treated group, to a level similar to that observed in healthy mice. Others factors, as well as hormones and peptides, can affect cellular homeostasis, including in salivary tissues. Scientific evidence from animal models indicates that changes in these hormones, proteins and their receptors may lead to salivary gland pathology. Several studies have confirmed the influence of these membrane receptors (mainly INS-R) on cellular homeostasis (Caldeira and Cagnon, 2008; Gorjup et al., 2009; Maekawa et al., 2011; Yashida et al., 2011; Csete and Doyle, 2014). Similar findings were observed in the present experiments, suggesting that treatment with GAD peptides led to an increase in INS and modulation of its receptors. In a previous study, we demonstrated that diabetes altered INS-R expression in animals in which glycemic control was achieved with INS treatment (Caldeira and Cagnon, 2008). This finding suggests that there were changes in the production of INS and the interaction of INS with the alpha subunits of INS-R. This may indicate an affinity between endocrine INS and the salivary glands, similar to what occurs in tissues in subjects with type 2 diabetes. This result is in accordance with findings reported for types 1 and 2 diabetes.

Importantly, we found that peptide-based therapy led to INS-R recovery. These results are similar to those observed in previous studies in which experimental treatment with GAD or its peptides could prevent diabetes progression. These authors also emphasized that the peptides p217 and p290 of GAD65 may block the damage to the pancreas caused by hyperglycemic conditions. This process is probably related to the deletion of antigen-specific pathogenic T cells, thus impeding the progression of autoimmune diabetes (Tisch et al., 1999; Chen et al., 2003; You et al., 2004; Liu, 2006).

In autoimmune diabetes, the activation of CD4+ T-lymphocytes promotes cellular destruction through the action of cytotoxic CD8+ T-lymphocytes. In the second stage of pathogenesis, discharge of cytokines by CD4+ T-lymphocytes promotes the formation of oxygen free radicals that maintain tissue destructive inflammatory processes (Calcinaro et al., 1996). GAD65 is a sensitive marker for these events, indicating an autoimmune attack on pancreatic beta cells.

Conversely, the GAD65 protein can protect diabetic animals from this immune destruction, indicating that GAD65 is also important for the maintenance and control of the autoimmune response (Yoon et al., 1999). Despite this, other authors have noted that therapy with GAD and its peptides is only effective in animal models, and the treatment of human autoimmune diseases remains a challenge (Chen et al., 2003; Gauvrit et al., 2004; You et al., 2004; Wherrett et al., 2011).

Another study demonstrated that the GAD peptides p217 and 290 could be fused to other molecules. These fusion proteins can maintain peripheral cellular structures, suggesting new possibilities for the functional recovery of these components (Wang et al., 2009). The approach used in our study may have promoted a fusion between the peptides and the alpha subunits of INS-R, thus restoring the interaction between INS and its receptor.

CONCLUSION

These data strongly suggest that GAD peptides (p217 and p290) are good targets of treating type 1 DM. Thus, therapies that are directed at these peptides response might be of benefit in controlling type 1 diabetes, which is supported by studies that show combining GAD p217 and p290 peptides contributed to hormonal balance and promoted the repair of INS-R in NOD mice. Furthermore, these findings suggest that GAD specific CD4+T-cell promiscuity reflects a novel form of T-cell avidity maturation. Our results also suggest that these fusion proteins may be effective in inhibiting the development of diabetes and promoting the recovery of INS receptor expression in salivary tissues. This treatment contributed to lower glucose levels and possibly repaired hyperglycemia-induced damage to salivary glands, indicating that this peptide combination may be therapeutically effective. Additional studies are needed to elucidate the mechanisms underlying the benefits of the GAD-derived p217 and p290 peptides in relation to glucose control.

ACKNOWLEDGMENTS

This work was supported by the Nucleus of Support to Research and Teaching (NAPED) of the Faculty of Medicine of Jundiaí, SP, Brazil, and Research Foundation of the State of São Paulo (FAPESP) (grant number: 2012/18012-2).

ABBREVIATIONS

GAD: Glutamic Acid Decarboxylase, NOD: Nonobese diabetic, INS R: Insulin Receptor
Fig. 1: Immunostaining of submandibular glands. A: Group I, showing saturated expression of INS-R (arrow). B: Group II, showing mild INS-R expression (arrow). C: NOD mice treated with peptides p217 and p290 (Group III), showing saturated INS-R expression similar to control non-diabetic mice (arrow). Immunostaining of the parotid glands. D: Group I, with saturated expression of INS-R (arrow). E: Group II, with mild INS-R expression (arrow). F: Treated animals of group III, showing saturated INS-R expression similar to control non-diabetic mice (arrow).
Table 1: Semi-quantitative evaluation of the INS-R expression in salivary glands of control non-diabetic mice (group I) and NOD mice without treatment (group II) or treated with GAD peptides p217 plus p290 (Group III).

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<th>Groups</th>
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+ mild; ++: moderate; +++: saturated.

REFERENCES


