Acta Diabetol 32: 53–56, 1995

ACTA
DIABETOLOGICA

Baclofen, a gamma-aminobutyric acid-b receptor agonist, delays diabetes onset in the non-obese diabetic mouse

P. E. Beales[1], M. Hawa[1], A. J. K. Williams[1], M. C. Albertini[1, 2], A. Giorgini[1,2], P. Pozzilli[1, 3]

[1] Department of Diabetes and Metabolism, St. Bartholomew's Hospital, London EC1A 7BE, UK
[2] Chimica Biologica, University of Urbino, Urbino, Italy
[3] Endocrinologia (I), II Clinica Medica, University of Rome 'La Sapienza', Rome, Italy

Received: 15 February 1994 / Accepted in revised form: 18 November 1994

Abstract. Glutamic acid decarboxylase (GAD) is the enzyme responsible for the synthesis of gamma-aminobutyric acid (GABA). GAD has been identified as a 64-kDa antigen expressed in pancreatic beta-cells, to which autoantibodies are generated prior to the onset of type 1 (insulin-dependent) diabetes mellitus. GAD may therefore be an initiating factor in beta-cell destruction. We administered baclofen, a GABA-B receptor agonist, to non-obese diabetic (NOD) mice in an attempt to down-regulate GAD expression and thereby reduce the incidence of diabetes. Twenty-four female NOD mice were given baclofen in their drinking water at a final dose of 50 mg/kg body weight daily from weaning to 30 weeks of age. Twenty-four sex- and litter-matched mice were used as controls. At 30 weeks there was no difference in the incidence of diabetes in the treated group compared with the controls. However, there was a significant delay in the onset of diabetes in the treated group ($P< 0.001$, parallelism test). The degree of insulitis and the GAD activity in the pancreas per mg of protein were unchanged by baclofen treatment with respect to controls. These results suggest that baclofen may be effective in delaying diabetes onset in NOD mice by stimulating GABA activity, as this neurotransmitter, localised in the islets, may modulate insulin secretion and the antigen expression associated with it.

Key words: Non-obese diabetic mouse – Gamma-aminobutyric acid – Glutamic acid decarboxylase – Baclofen

Introduction

Type 1 (insulin-dependent) diabetes mellitus is characterised by a selective destruction of pancreatic beta-cells, resulting in a lack of endogenous insulin secretion [1]. Autoimmune mechanisms are known to play an important part in the development of this disease [2]. The best currently available markers of beta-cell autoimmunity are circulating antibodies against islet antigens such as islet cell antibodies (ICA) and insulin autoantibodies (IAA) [3, 4]. Antibodies against the enzyme glutamic acid decarboxylase (GAD) have also been reported [5] and these may represent a very early marker in subjects destined to develop clinical type 1 diabetes [6]. There is now clear evidence that GAD is expressed not only in the GABA-ergic neurones of the brain, but also in pancreatic beta-cells, where it catalyses the decarboxylation of glutamic acid to form GABA [7]. It has also been suggested that GAD in pancreatic islets may be subject to up-regulation following a local insult, be it environmental or immunological, which might accelerate the onset of type 1 diabetes [8].

There have been several attempts to prevent diabetes in animal models of the disease [9]; perhaps one of the most interesting is reducing the antigen expression associated with beta-cells [10]. GAD-like immunoreactivity has been reported in the pancreata of mice [11], and interfering with the autoimmune response towards GAD results in a reduction of antigen expression by beta-cells, as recently reported [12, 13]. Therefore, in an attempt to down-regulate GAD expression and thereby prevent or at least reduce the incidence of diabetes, we tested a GABA-B receptor agonist, baclofen, by administering it to NOD mice starting from an early age.

Materials and methods

The NOD mouse spontaneously develops a form of insulin-dependent diabetes which closely resembles that found in humans [14]. Lymphocytic infiltration of the pancreatic islets (insulitis) is observed from the age of 4 weeks, with progressive destruction of beta-cells and onset of diabetes from 10 weeks of age [15]. Unlike human type 1 diabetes, a notable sex difference is seen, with a preponderance of female mice developing diabetes. The NOD/Ba mouse colony established at St. Bartholomew's Hospital Medical College, London, in 1987 was originally derived from Dr. E. Leiter's laboratory (Bar Harbour, Maine, USA). There is a stable cumulative incidence of diabetes of 55% in females and 15% in males at 30 weeks of age [16]. The colony is housed in a purpose-built area and main-

Correspondence to: P. Beales

tained strictly according to international [17] and UK [18] guidelines.

Baclofen (Lioresal), a GABA-B receptor agonist normally used for the relief of muscle spasticity, acts principally on the central nervous system by stimulating the GABA-B receptors and reducing GAD and transaminase [19]. GAD activity is responsible for GABA synthesis, which is also reduced. It is normally used at a maximum dose of around 2 mg/kg body weight in humans, but for this experiment we decided to administer a much higher dose to NOD mice in an attempt to maximise down-regulation of GAD, although it has been reported that baclofen does not affect GAD activity under various experimental conditions in vitro [20].

Two groups of female mice from our NOD mouse colony were used in this study. Twenty-four were given baclofen at a final dose of 50 mg/kg body weight daily in their drinking water from weaning (at 3 weeks of age) to 30 weeks of age. A further 24 age-, sex- and litter-matched mice were used as controls. A standard maintenance diet (FFG[M]) was provided ad libitum, and the food intake and body weight were measured weekly.

Mice were screened weekly for diabetes from 10 weeks of age by means of urinary glucose testing (Diabur-Test 5000, Boehringer Mannheim, Germany). The occurrence of diabetes was diagnosed on finding a repeated level of glucosuria equal to or greater than 56 mmol/l and further confirmed by random blood-glucose testing.

Histological studies

At 30 weeks of age, the non-diabetic survivors were killed and their pancreata removed, snap-frozen in liquid nitrogen and stored at 70°C until required. Cryostat sections were then prepared as follows: a 5-μm section was cut and put on a microscope slide. Approximately 300 μm were then cut and discarded, after which another section was cut and put on the slide. This was repeated until 10 sections per pancreas were obtained. This process allowed the identification of 10–30 islets per pancreas; the separation between samplings meant that each section contained different islets. For morphological analysis sections were stained with haematoxylin and eosin and examined in a 'blind' controlled manner at ×250 magnification using a microscope with an ocular grid. Islet infiltration was scored as: no infiltration (grade 0); peri-insulitis (grade I) in which about 10% of the islet area was infiltrated by a peripheral ring of lymphocytes around the islet (more than 100 lymphocytes were usually present); medium insulitis (or grade II) in which more than 10% but less than 50% of the islet area was infiltrated, and lymphocytes were present in higher numbers; severe insulitis (or grade III) in which more than 50% of the islet area was infiltrated, and in this case lymphocytes would be present in sufficient numbers to alter the normal architecture of the islet. The number of infiltrated and non-infiltrated islets was noted for each pancreas and an index calculated, by multiplying the number of islets in each category by the grade of infiltration (0-III) and dividing by the total number of islets observed.

GAD assay

The remaining pancreatic tissue not used for the determination of insulitis was assayed for GAD using a method based on that described by Albers and Brady [21] and modified by Christie et al. [22]. Briefly, tissue was placed in a Teflon-glass homogeniser in 1 ml of buffer containing 1 mmol/l 2-aminoethyl isothioronium bromide hydrobromide, 0.2 mmol/l pyridoxal phosphate and 1 mmol/l benzamidine. Following homogenization, the material was spun in a microcentrifuge at 12 000 × *g* at 4°C, the supernatant then removed and the pellet further extracted using 2% Triton-x114. GAD activity was then determined, using pooled aqueous and detergent phase supernatants, as follows: duplicate 50-μl aliquots were incubated overnight at 37°C with 30 μl of 5 mmol/l L-glutamic acid and 0.125 μCi [1-^{14}C]-L-glutamic acid in homogenization buffer. $^{14}CO_2$ released during the reaction was adsorbed onto filter paper soaked with 50 μl of 1mol/l hyamine hydroxide and quantified (using a beta-counter) by liquid scintillation spectrometry. The protein content of the extracts was determined using a DC protein assay kit (Bio-rad, Herts, UK) and the GAD activity corrected per mg of protein. Salivary glands were also processed in the same way as control tissue.

Results

As one of the side-effects of baclofen administration is a reduction in muscle tone, it was considered important to monitor the weight of the mice, particularly in view of the high dose used. However, between 3 and 8 weeks of age (the time of maximum growth and before any weight loss or polyphagia due to diabetes is likely to occur), no significant differences were found in the food intake of baclofen treated mice (23.9 ± 0.51 g/mouse/week; mean ± SEM) or their controls (23.8 ± 0.59 g). Although slightly reduced, the body weight of treated animals (18.2 ± 0.43 g) was not statistically different from that of the controls (19.0 ± 0.45 g). No impairment of motor function was observed, despite the high doses used, suggesting that some degree of tolerance had developed to the drug [19].

Overall diabetes incidence was unchanged in baclofen-treated mice, compared with controls at 30 weeks of age, using a log-rank test on a life-table [23] (Fig. 1). However, there was a change in the time of onset of diabetes as cal-

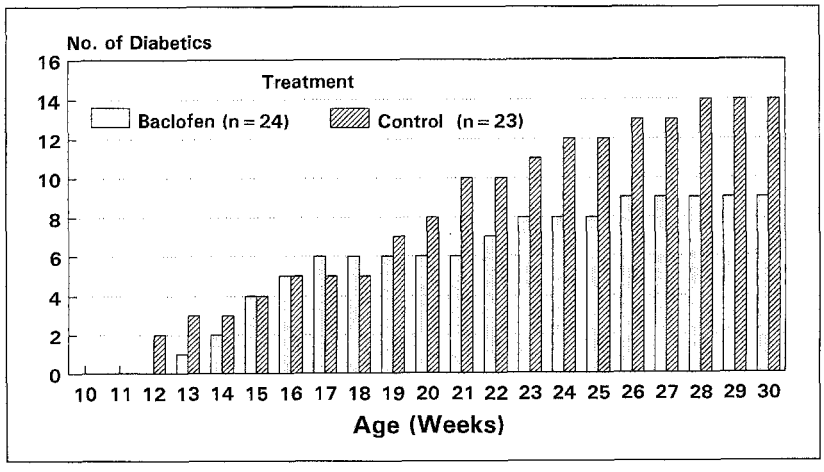

Fig. 1. Diabetes incidence in non-obese diabetic (NOD) mice treated with baclofen (n=24) and controls (n=23, see text). Baclofen given at a dose of 50 mg/kg body weight daily. Diabetes incidence p=ns; time of diabetes onset $p<0.001$, (parallelism test)

culated using the parallelism test ($P< 0.001$), a modification of the analysis of co-variance test which is more appropriate for identifying differences between curves of incidence [24]. One mouse in the control group died at 10 weeks of age from causes unrelated to diabetes, but this was corrected for by using life-table analysis.

The index of the degree of insulitis of mice not developing diabetes did not significantly differ between treated (1.23 ± 0.43; mean ± SEM; n=15) and control (0.96 ± 0.27; n=8) mice.

Finally, GAD activity per mg of protein was low in the salivary tissue used as a control in both treated (505 ± 73; n=24) and untreated (435 ± 70; n=20; NS) animals. As expected, GAD activity was higher in pancreatic tissue [22]; there was, however, no significant difference between treated (1159 ± 69; n=23) and untreated (1300 ± 130; n=19) groups. Results are expressed as mean disintegrations $^{14}CO_2$ per minute per mg of protein ±SEM. Disintegrations $^{14}CO_2$ are proportional to the levels of GAD activity present in the tissue.

Discussion

In this study we have shown that administering baclofen to NOD mice from weaning can delay the onset of diabetes. However, it did not protect the animals from eventually developing the disease and did not down-regulate GAD activity sufficiently to block the autoimmune process at the doses used. The fact that this compound had some effect in delaying the onset of the disease suggests that either a slight reduction in GAD or modulation of GABA activity in the islets may interfere with the process leading to beta-cell destruction and clinical diabetes. In the first case a small reduction in antigen expression early in life may be insufficient to prevent disease progression and eventual clinical diabetes in NOD mice but may still favour the generation of clones of toleragenic T cells to the initiating target antigen. More likely, as GABA has been detected in the beta-cells localised in a population of synaptic-like microvesicles [25] and secreted in a regulated fashion, our findings suggest that baclofen may modulate GABA secretion in the islets, thus suppressing insulin and the antigens associated with its secretion. A similar finding has been made with insulin administration, which may suppress endogenous insulin secretion, thereby reducing the expression associated with hormone release and blocking diabetes insurgence [26]. One of the potential advantages of using baclofen to reduce antigen expression is that this compound has already been widely used in humans, and its side-effects are well documented and understood. It can also be used on a long-term basis, as would be necessary for diabetes prevention in man. In NOD mice we did not observe significant changes in food intake or body weight, suggesting that this agent could be considered for the treatment of children in strictly monitored trials.

In conclusion, these findings support the concept that modulation of GABA activity is a relevant factor in the generation of an immune response towards beta-cells. We therefore suggest that attempts to modify GABA activity should be considered as a means of preventing diabetes. Baclofen, by virtue of its ability to delay the onset of diabetes in NOD mice, should be considered as a possible agent for use in man.

Acknowledgements. This work was supported by a grant from the Joint Research Board, St. Bartholomew's Hospital. P.E.B. is supported by the Fo.Sa.N. Foundation of Italy. The donation of baclofen from Ciba-Geigy is gratefully acknowledged. We are also grateful to Mr. K.J. Mansfield, Ms. J. Haynes and Ms. L.A. Burr for technical assistance and Ms. E. Robertson and Ms. K. Anthony for secretarial help. The analysis of the data by Prof. P. Cugini using the parallelism test is also gratefully acknowledged.

References

1. Atkinson M, Maclaren NK, What causes diabetes? Sci Am 262:61–71, 1990
2. Bonifacio E, Bingley PJ, Dean BM, Shattock M, Dunger D, Gale EAM, Bottazzo GF, Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. Lancet 335:147–149, 1990
3. Gianani R, Pugliese A, Bonner-Weir S, Schiffrin AJ, Soeldner JS, Erlich H, Awdeh Z, Alper CA, Jackson RA, Eisenbarth GS, Prognostically significant heterogeneity of cytoplasmic islet cell antibodies in relatives of patients with type 1 diabetes. Diabetes 41:347–353, 1992
4. Wilkin TJ, Autoantibodies as mechanisms, markers and mediators of B-cell disease. Diabetes Metab Rev 7:105–121, 1991
5. Baekkeskov S, Aanstot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P, Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. Nature 347:151–156, 1990
6. Atkinson MA, Maclaren NK, Scharp DW, Lacy PE, Riley WJ, 64,000 Mr autoantibodies as predictors of insulin-dependent diabetes. Lancet 335:1357–1360, 1990
7. Okada Y, Toniguchi H, Shimada C, High concentrations of GABA and high glutamate decarboxylase activity in rat pancreatic islets and human insulinoma. Science 194:620–622, 1976
8. Deaizpurua HJ, Harrison LC, Glutamic acid decarboxylase in insulin-dependent diabetes mellitus. Diabetes Metab Rev. 8:133–147, 1992
9. Boitard C, Timsit J, Sempe P, Bach JF, Experimental immunoprevention of type diabetes mellitus. Diabetes Metab Rev 7:15–33, 1991
10. Atkinson MA, Maclaren NR, Lucchetta R, Insulitis and diabetes in NOD mice reduced by prophylactic insulin therapy. Diabetes 39:933–937, 1990
11. Gilon P, Tappas M, Remacle C, Localization of GAD-like immunoreactivity in the pancreas and stomach of the rat and mouse. Histochemistry 96:355–365, 1991
12. Kaufman DL, Clare-Salzler M, Tian J, Forsthuber T, Ting GSP, Robinson P, Atkinson MA, Sercarz EE, Tobin AJ, Lehmann PV, Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. Nature 366:69–72, 1993
13. Tisch R, Yang XD, Singer SM, Liblau RS, Fugger L, McDevitt HO, Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. Nature 366: 72–75, 1994
14. Pozzilli P, Signore A, Williams AJK, Beales PE, NOD mouse colonies around the world – recent facts and figures. Immunol Today 14:193–196, 1993
15. Signore A, Pozzilli P, Gale EAM, Andreani D, Beverley PCL, The natural history of lymphocyte subsets infiltrating the pancreas of NOD mice. Diabetologia 32:282–289, 1989

16. Mansfield KJ, Beales PE, Williams AJK, Pozzilli P, The breeding, housing and life-maintenance of the non-obese diabetic mouse. Animal Technol 43:29–37, 1992
17. Principles of laboratory animal care. (NIH publication number 83–25, revised) 1985
18. Animals (Scientific Procedures) Act, 1986. Her Majesty's Stationery Office, London, 1986
19. Gianutsos G, Moore KE, Tolerance to the effects of baclofen and gamma-butyrolacetone on locomotor activity and dopaminergic neurons in the mouse. J Pharmacol Exp Ther 207:859–869, 1978
20. Rimvall K, Martin DL, Increased intracellular gamma-aminobutyric acid selectively lowers the level of the larger of two glutamate decarboxylase proteins in cultured GABAergic neurons from rat cerebral cortex. J Neurochem 58:158–166, 1992
21. Albers RW, Brady RO, The distribution of glutamic decarboxylase in the nervous system of the rhesus monkey. J Biol Chem 234:926–928, 1959
22. Christie MR, Brown TJ, Cassity D, Binding of antibodies in sera from type 1 (insulin dependent) diabetic patients to glutamate decarboxylase from rat tissues. Evidence for antigenic and non-antigenic forms of the enzyme. Diabetologia 35:380–384, 1992
23. Pocock SJ, Analysis of survival data. In: Clincial trials – a practical approach. Wiley, Chichester, pp 224–233, 1991
24. Cugini P, Leone G, Sepe M, De Palma L, Parallelism test on microcomputers for statistically comparing regression lines of bivariate data sets. Comput Methods Prog Biomed 33:109–116, 1990
25. Reetz A, Solimena M, Matteoli M, Folli F, Takei K, De Camilli P, GABA and pancreatic β-cells: colocalization of glutamic acid decarboxylase (GAD) and GABA with synaptic-like microvesicles suggests their role in GABA storage and secretion. EMBO J 10:1275–1284, 1991
26. Zhang ZJ, Davidson I, Eisenbarth G, Weiner HL, Suppression of diabetes in non-obese diabetic mice by oral administration of porcine insulin. Proc Natl Acad Sci 88:10252–10256, 1991

Diabetologia
Clinical and Experimental Diabetes and Metabolism
Journal of the European Association for the Study of Diabetes (EASD)

Vol. 38, No. 2, 1995

Indexed in Current Contents

DBTGAJ 35 (2) 129–254 (1995)
Printed on acid-free paper