Abstract

Type 1 diabetes (T1D) is an autoimmune disease in genetically predisposed individuals characterised by selective destruction of the β-cells. Development of diabetes is in the asymptomatic pre-diabetic period characterised by impaired first-phase insulin response and the first clinical symptom is elevated blood glucose (BG). It is still uncertain whether stress or incidental hyperglycaemia can be regarded as predictors for development of T1D or not, even when immunologic and genetic markers for T1D are considered. The aim of this study was to investigate if there was any relationship between elevated BG in 30-day-old anaesthetised pre-diabetic diabetes-prone Bio Breeding (BB-DP) rats and later development of diabetes. Rats anaesthetised by intraperitoneal (ip) injection for islet transplantation displayed significantly higher BG values (Δ1.27 mmol/l, \( p = 8.27 \times 10^{-12} \)) compared to non-anaesthetised non-transplanted rats, indicating that ip injection and/or anaesthesia induce a higher BG level. Linear regression analysis of BG and time of onset of diabetes in transplanted and non-transplanted BB-DP rats revealed no correlation (R² at 0.0075 and 0.0324 and \( p \)-values at 0.56 and 0.23 respectively). We were not able to identify any association or correlation between the induced temporary hyperglycaemia in 30-day-old BB-DP rats and later development of diabetes.

Key words

Anaesthesia-induced hyperglycaemia • Stress-induced hyperglycaemia • Diabetes • BB-DP rat • Anaesthesia

Introduction

Type 1 (insulin-dependent) diabetes (T1D) is an autoimmune disease, in which immune tolerance is broken in genetically predisposed individuals, leading to selective destruction of the β-cells [1]. The destructive process is characterised by auto-antibodies towards β-cell antigens and impaired first-phase insulin response in a non-symptomatic pre-diabetic period. The first clinical symptom is elevated blood glucose (BG), when the β-cell mass is unable to fulfil the body’s requirement for insulin.

Hyperglycaemia can occur under conditions of severe stress, such as trauma, operations and myocardial infarction in individuals without prior history of diabetes [2]. Stress hyperglycaemia is a frequent clinical finding (4–5%) in children admitted to a hospital unit and seems to be associated with severity of the illness [3, 4]. When distinguishing between incidental hyperglycaemia (elevated BG level found by incidence in a non-stressful situation) and stress hyperglycaemia, there seems to be a higher risk for T1D development in children with incidental hyperglycaemia and immunological markers for T1D [4–10].

Previously, we have shown that mononuclear cell infiltration (insulitis) and islet destruction in syngeneically transplanted neonatal diabetes-prone Bio Breeding (BB-DP) rat islets under the kidney capsule of 30-day-old rats are the same, as seen in the islets in pancreas during development of diabetes [11, 12]. Transplantation of 200 islets decreases the diabetes incidence approximately 20% \( \text{per se} \) from 85 to 65% in our strain, but does not change the insulitis process compared to non-transplanted rats [12]. In these studies, we observed that some of these rats anaesthetised for islet transplantation, but prior to any operation had slightly elevated BG values compared to non-anaesthetised rats. It has previously been shown that daily subcutaneous vehicle injections to pre-diabetic (25–30-day-old) BB-DP rats until one week prior to onset...
of diabetes does not influence the BG levels [13]. The
aims of the present study were to compare the first measured
BG in these BB-DP rats anaesthetised for islet transplanta-
tion with age-matched non-anaesthetised BB-DP rats and to test whether an elevated BG in relation to
anaesthesia is predictive for later development of diabetes
in this highly diabetes-prone strain.

Materials and methods

Study design

BG was measured in 30-day-old anaesthetised BB-DP rats prior
to islet transplantation and in non-anaesthetised BB-DP rats fol-
lowed by measurements three times weekly for 90 days or until
development of diabetes. To clarify if the initial BG value is pre-
dictive for development of diabetes in BB-DP rats, the initial BG
value is tested for an association to later development of diabetes
in these diabetes-prone animals.

Animals

Inbred BB/Wor/Mol-BB2 (BB-DP) rats with a spontaneous dia-
betes incidence at 85% were purchased from M&B, Ll.,
Skensved, Denmark and housed under specific pathogen-free
environments. The rats were acclimatised to the animal facility
at Steno Diabetes Center five to six days prior to transplantation
and BG measurements. "Principles of laboratory animal care"
(NIH publication no. 83–25, revised 1985) were followed, as
well as the Danish Law on Research involving Animals, no. 726
from 1993. Furthermore the experiments were approved by the
Danish Council for Animal Welfare under the Ministry of
Justice.

Anaesthesia and islet transplantation procedures

The rats were anaesthetised with ketamine 8.75 mg/100 g
(Ketalar, 50 mg/ml, Warner Lambert/Parke-Davis, Barcelona,
Spain) and xylazine 0.7 mg/100 g (Rompun vet, 20 mg/ml,
Bayer, Leverkusen, Germany) intraperitoneally (ip). After induc-
tion of anaesthesia the rats were weighed and shaved above the
left kidney prior to transplantation of 200 isolated neonatal BB-
DP islets under the kidney capsule of 30–32 day-old BB-DP rats
as previously described [11, 12, 14, 15]. After transplantation
the rats received 0.01 mg buprenorphin subcutaneously (Temgesic
0.3 mg/ml, Schering-Plough, Kenilworth, NJ, USA) when they
woke up after transplantation and the next morning [12]. The
day after transplantation the rats received either an insulin or
placebo implant or nothing (control) to investigate the effects of
prophylactic insulin treatment as previously described [12].
Non-transplanted 30–32-day old BB-DP rats received either an
insulin or placebo implant in local anaesthesia or nothing (con-
trol). No differences in diabetes development were observed
between placebo and control rats [12].

Results

Initial BG

The average BG was 5.45 mmol/l (range 3.2–6.9) in the
non-anaesthetised (non-transplanted) rats and 6.72 mmol/l
(range 4.4–10.4) after induction of anaesthesia in rats
selected for transplantation prior to any surgical proce-
dures. No differences in the BG level between control,
placebo or insulin groups within the non-anaesthetised
group or within the anaesthetised group were observed at
the first BG measured (Table 1). A difference on 1.27
mmol/l (p=8.27x10−12) was observed between the first BG
in non-anaesthetised and anaesthetised rats (Table 1).
There was no difference between BG values in rats devel-
opring or escaping diabetes within the non-anaesthetised
and anaesthetised groups.

No association was found between the first measured
BG higher than the 75, 90 or 95 percentiles of anaes-
thetised and non-anaesthetised rats and later develop-
ment of diabetes. BG in anaesthetised rats at the 75, 90 and 95%
percentile corresponds to 7.4, 8.2 and 8.8 mmol/l, respec-

tively. Linear regression analysis of the first BG and time
of onset of diabetes does not influence the BG levels. The
observation of the rats

The first BG was measured by puncture of the tail tip in
the transplanted rats after induction of anaesthesia but prior to trans-
plantation and in non-transplanted (non-anaesthetised) rats prior
to implantation. The second BG was measured the next morning
in all rats. After the second BG all rats were weighed and the BG
measured three times weekly. BG measurements were performed
at a Cobas Mira Plus, Roche Diagnostic Systems, Basel,
Switzerland using Granitest-250, Merck-Diagnostica,
Darmstadt, Germany. Ten microlitres of blood from the tail tip
were haemolysed in 750 µl Solution for Hemolysis (Roche
Diagnostic Systems, Basel, Switzerland). If a rat had a BG high-

er than 14 mmol/l, the BG was measured the next day and dia-
betes was defined as BG higher than 14 mmol/l for two consec-
tutive days. The rats were sacrificed after 90 days of observation
(120 days of age) or at onset of diabetes by cervical dislocation.

Statistical analyses

Single factor ANOVA test and student’s t-test were used com-
paring BGs. Linear regression analysis was used to examine the
relationship between initial BG and onset of diabetes and Fishers
exact test for comparing the numbers of rats above percentiles.

p values below 0.05 were considered significant.