SHORT COMMUNICATION

Treatment with a proton pump inhibitor improves glycaemic control in *Psammomys obesus*, a model of type 2 diabetes

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Abstract

Aims/hypothesis Gastrin has been implicated in islet growth/neogenesis, and proton pump inhibitors (PPIs) have been shown to increase endogenous gastrin levels in animals and humans. Therefore, we investigated the effect of PPIs in a model of type 2 diabetes, *Psammomys obesus*.
Methods *P. obesus* (morning blood glucose [mBG] 16.9 ± 0.6 mmol/l) were treated with vehicle or different doses (1–15 mg/kg) of lansoprazole for 17 days.
Results Treatment with lansoprazole resulted in up to ninefold dose-dependent increases in endogenous gastrin levels ($p<0.05$ for 10 mg/kg lansoprazole vs vehicle). There was a significant reduction in mBG levels in all animals in the high-dose lansoprazole groups during the 17 day treatment period, whereas there was no significant improvement in mBG in animals in the vehicle groups. The mBG at end of study was 18.2 ± 2.1, 8.7 ± 2.2 ($p<0.01$), and 6.1 ± 2.3 ($p<0.001$) mmol/l for vehicle and lansoprazole 10 and 15 mg/kg, respectively. The animals treated with 15 mg/kg lansoprazole, compared with vehicle, had a 2.3-fold increase in the intensity of insulin staining in beta cells ($p=0.0002$) and 50% higher beta cell mass ($p=0.04$).

Conclusions/interpretations The PPI lansoprazole had significant glucose-lowering effects in an animal model of type 2 diabetes, an effect that is most likely mediated through an increase in endogenous gastrin levels.

Keywords Animal model · Beta cell mass · Gastrin · Glycaemic control · Proton pump inhibitor · Type 2 diabetes

Abbreviations
BCM   Beta cell mass
BW   Body weight
mBG   Morning blood glucose
PPI   Proton pump inhibitor
SPA   Scintillation proximity assay

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Introduction

The consistent finding of reduced functional pancreatic beta cell mass (BCM) not only in type 1 diabetes but also in type 2 diabetes has increased the focus on finding new treatments that may increase and/or cause regeneration of functional BCM. Gastrin has been shown to increase proliferation of human and rodent duct-like pancreatic cells in culture, and administration of gastrin stimulates beta cell neogenesis and expansion of the BCM in rodents [1, 2]. During treatment with proton pump inhibitors (PPIs), endogenous gastrin levels are increased in humans and rodents, and in a recent publication Suarez-Pinzon et al. showed that PPI treatment of mice in a model of type 1 diabetes resulted in an almost threefold increase in endogenous gastrin levels and improvement in glycaemia [3].

Since no data have been published on the effect of gastrin or PPIs in type 2 diabetes, we decided to investigate

whether treatment with the PPI lansoprazole would have beneficial effects on glycaemic control in a type 2 diabetes animal model, the gerbil *Psammomys obesus*. When this gerbil is fed a high-energy diet, morning blood glucose (mBG) levels are distinctly increased, with subsequent/concomitant loss of beta cell function and mass [4].

Methods

Animals Male and female *P. obesus* (Harlan, Jerusalem, Israel) were fed low-energy (10 kJ/g) chow until the age of 14–15 weeks, after which they were transferred to an ad libitum high-energy (13 kJ/g) diet during which body weight (BW), mBG and HbA_{1c} were monitored for 4 weeks. The animals that showed increased mBG levels, defined as mBG >10 mmol/l on two consecutive readings, were used in the treatment study.

   P. obesus were treated with vehicle ($n=5$) or 1 mg/kg ($n=8$), 3 mg/kg ($n=11$) or 10 mg/kg ($n=7$) lansoprazole in the first study. At the start of the experiment there were 10–12 animals in each group, except for the 10 mg/kg group, which was smaller due to lack of compound. During the study, five animals in the vehicle group and two in the 1 mg/kg group had to be killed due to severely elevated mBG and ketoacidosis. Two animals, one from the 3 mg/kg group and one from the 10 mg/kg group, died due to dosing into the lungs. They were removed from all the analyses. The second study consisted of animals dosed with vehicle ($n=8$) or 10 mg/kg ($n=8$) or 15 mg/kg ($n=8$) lansoprazole. Both studies were conducted over 2.5 weeks, during which mBG, BW and HbA_{1c} were monitored, and gastrin levels were measured at the end of the study. Pancreases from the groups receiving 10 and 15 mg/kg were evaluated histologically for insulin content and BCM.

   The study was approved by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

Formulation of lansoprazole Lansoprazole (L8533; Sigma-Aldrich, Broendby, Denmark) was suspended in vehicle (0.6% carboxymethylcellulose, 0.2% $NaHCO_3$) to the final dose volume of 2 ml/kg.

mBG and HbA_{1c} Blood samples were collected from the tail, and mBG and HbA_{1c} measured as previously described [5].

Endogenous gastrin Gastrin levels were measured in samples collected from the aorta using a competition scintillation proximity assay (SPA). Briefly, SPA beads, coated with anti-rabbit antibody, were incubated with rabbit anti-gastrin antibody and ^{125}I-labelled Tyr12-gastrin (human). Sample, standard or control was added to the plates, followed by 10 µl of antibody rabbit-anti-gastrin serum

(#2609 11/10-73; gift from J. Rehfeld, University Hospital, Copenhagen, Denmark) diluted in assay buffer, 10 µl tracer ($[^{125}I$-$Tyr^{12}]$-gastrin I [human]), 0.37 MBq/ml NEX176 (Amersham, Cardiff, UK) and 10 µl bead solution (Anti-Rabbit Coupled Polystyrene SPA RPQ0742; GE Healthcare, Umeå, Sweden). The plates were mixed and left for 6–24 h, after which they were centrifuged and counted (LEADseeker; GE Healthcare).

Pancreas histology The pancreases were examined histologically, as previously described [5]. Briefly, pancreases were fixed in paraformaldehyde and sectioned according to the smooth fractionator principle with $F=1/4$ of the fragments in each of the two capsules [6]. The sections were stained for insulin to visualise beta cells. BCM was evaluated stereologically in two to three sections with the observer blinded to the origin of the sections. The insulin staining intensity was graded semiquantitatively on a scale of 0–5, with 0 being equal to islets characterised by absence of insulin staining while 5 equals islets with insulin staining as seen in normal rat pancreas. The grading was carried out with the observer blinded to the origin of the sections.
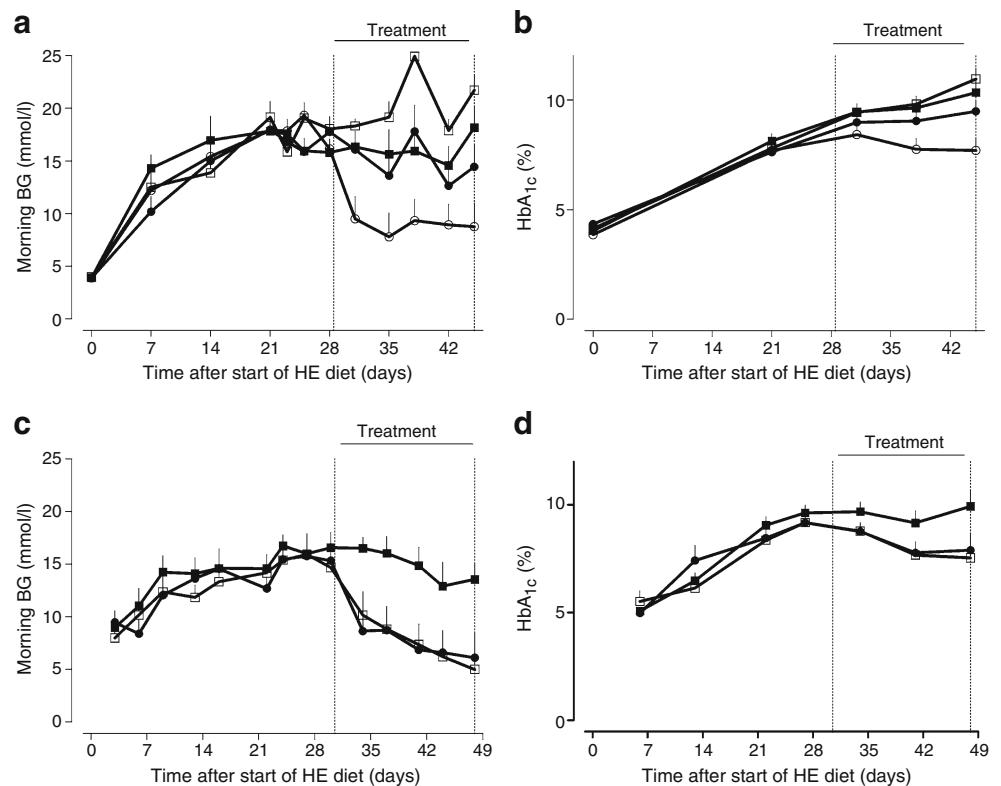
Statistical analysis Data were analysed using non-parametric (Kruskal–Wallis) ANOVA with Dunn's multiple comparisons as post test or a non-parametric paired *t* test (Wilcoxon). $p \leq 0.05$ was considered significant. Data are presented as mean±SEM.

Results

Glycaemic control In the first experimental setup, 2.5 weeks of treatment had no significant effects on mBG in the vehicle and low-dose lansoprazole (1 and 3 mg/kg) groups. mBG at end of study was 18.2±2.1, 21.7±1.5 and 14.4±2.2 mmol/l for vehicle ($n=5$) and lansoprazole 1 mg/kg ($n=8$) and 3 mg/kg ($n=11$), respectively (Fig. 1a). The animals treated with the highest dose (10 mg/kg) had significantly lower mBG (8.7±2.2 mmol/l, $p<0.01$) at the end of the study. At start of treatment, there were no significant differences in mBG and HbA_{1c} for the different treatment groups (Fig. 1a, b). At the end of the study period, HbA_{1c} levels in the animals treated with the highest dose (10 mg/kg) of lansoprazole were significantly lower (7.7±0.6%) than with vehicle (10.3±0.5%, $p<0.05$) or with 1 mg/kg (11.0±0.5, $p<0.05$) or 3 mg/kg lansoprazole (9.5±0.5%, $p<0.05$; Fig. 1b).

   In a subsequent study, primarily focusing on pancreas histology and a higher dose of lansoprazole, similar results were obtained (Fig. 1c, d). There was no reduction in mBG

Fig. 1 Morning blood glucose (BG) (**a, c**) and HbA$_{1c}$ (**b, d**) in *Psammomys obesus* before and during treatment with vehicle or different doses of lansoprazole. **a, b** Black squares, vehicle (*n*=5); white squares, lansoprazole 1 mg/kg (*n*=8); black circles, lansoprazole 3 mg/kg (*n*=11); white circles, lansoprazole 10 mg/kg (*n*=7). **c, d** Black squares, vehicle (*n*=8); white squares, lansoprazole 10 mg/kg (*n*=8); black circles, lansoprazole 15 mg/kg (*n*=8). HE, high energy. Data are expressed as mean±SEM

in the vehicle-treated group (*n*=8); mBG at end of study was 13.6±1.5 mmol/l. In the lansoprazole 10 mg/kg group mBG was significantly reduced to 5.0±1.4 (*p*<0.001) and in the 15 mg/kg group to 6.1±2.3 mmol/l (*p*<0.001 compared with vehicle) (Fig. 1c). Likewise, HbA$_{1c}$ in the animals treated with 10 and 15 mg/kg of lansoprazole was significantly lower (6.9±0.3% and 7.0±0.3% respectively, *p*<0.01) than in the vehicle-treated animals (10.4±0.7%; Fig. 1d).

BW During the study, no difference was observed in BW gain between the groups (data not shown).

Endogenous gastrin After 17 days of treatment with lansoprazole (1, 3 and 10 mg/kg), endogenous gastrin levels were dose-dependently increased by up to ninefold in the animals treated with 10 mg/kg (*p*<0.05 vs vehicle, Fig. 2a); no further increase was seen in the 15 mg/kg group (Fig. 2b).

BCM and intensity of insulin staining Stereological quantification of BCM revealed that the animals treated with 10 and 15 mg/kg lansoprazole had 40% (NS) and 50% (*p*= 0.04) higher BCM, respectively, compared with vehicle-treated animals (Fig. 2c).

Immunohistochemical analysis of the pancreas showed a 2.3-fold increase in the intensity of the insulin staining in the lansoprazole-treated animals compared with the vehicle-treated animals (*p*=0.0002; Fig. 2d).

Discussion

Our results show that the PPI lansoprazole increases endogenous gastrin levels in *P. obesus*, glycaemic control
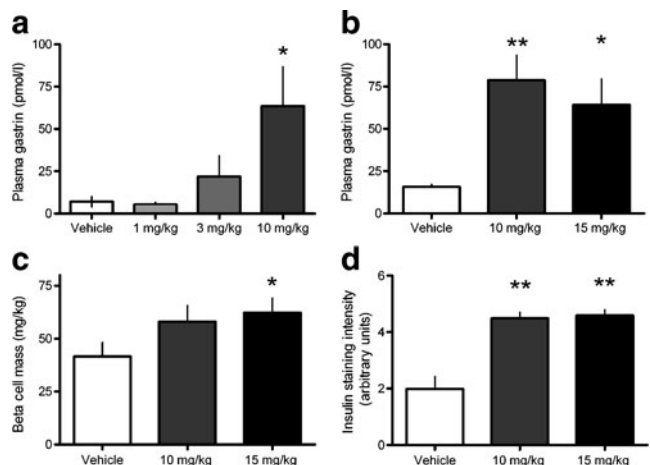

Fig. 2 Plasma gastrin levels (**a, b**), beta cell mass (**c**) and intensity of insulin staining in pancreatic sections (**d**) from *Psammomys obesus* after 17 days of treatment with vehicle or different doses of lansoprazole. **a** White bar, vehicle (*n*=5); very pale grey bar, lansoprazole 1 mg/kg (*n*=8); pale grey bar, lansoprazole 3 mg/kg (*n*=11); dark grey bar, lansoprazole 10 mg/kg (*n*=7). **b** White bar, vehicle (*n*=5); dark grey bar, lansoprazole 10 mg/kg (*n*=7); black bar, lansoprazole 15 mg/kg (*n*=8). **c, d** White bar, vehicle (*n*=5); dark grey bar, lansoprazole 10 mg/kg (*n*= 7); black bar, lansoprazole 15 mg/kg (*n*=8). Data are expressed as mean±SEM. **p*<0.05 vs vehicle-treated animals; ***p*<0.01 vs vehicle-treated animals

is almost normalised and BCM and insulin content of the pancreas are increased. Several studies have shown that PPIs elevate gastrin levels in both rodents and humans, and in this study we show this also to be the case in *P. obesus*. It has been shown that treatment with gastrin alone can induce formation of new beta cells under different conditions [1, 2]; however, from the present study we cannot determine whether the increased beta cell mass is due to an increase in neogenesis and/or proliferation or a decrease in apoptosis. Further studies that directly address these mechanisms are warranted also in light of the possibility that rodents have a higher capacity for regeneration of BCM than humans.

Although we did not directly measure beta cell function, there was a significant reduction in mBG and HbA_{1c}, which are both clinically relevant variables to measure for any agent that influences beta cell function or BCM.

Even though it is known that PPIs stimulate gastrin production, treatment with PPIs will result in very different pharmacokinetic profiles as compared with gastrin injections. Therefore, it could not be expected that PPIs would have the same effect on expansion of beta cell mass and function as has previously been demonstrated with gastrin. Whereas gastrin, in the doses used in the in vivo rodent studies, has a short half-life in rodents (less than 10 min; T. B. Bödvarsdóttir, L. Pridal, unpublished results), which results in a high exposure for a short time period, PPI treatment, in contrast, will result in a chronic elevation of gastrin levels to a few times above normal. A direct comparison of gastrin and PPIs is needed in order to clarify whether PPI treatment is superior to gastrin or vice versa. But, since PPIs are readily available as oral agents with a long-standing record of safety, the data presented here from lansoprazole-treated *P. obesus* warrant further studies of the clinical benefits of PPIs in type 2 diabetes.

In conclusion, the PPI lansoprazole has significant glucose-lowering effects in an animal model of type 2 diabetes, and thus PPIs might have the potential to become a new class of safe glucose-lowering agents, provided these findings can be extrapolated to other PPIs and humans.

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