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Abstract

Glucagon-like peptide-1 (GLP-1) analogues and inhibitors of its degrading enzyme, dipeptidyl peptidase IV (DPPIV), are interesting therapy options in human diabetics because they increase insulin secretion and reduce postprandial glucagon secretion. Given the similar pathophysiology of human type 2 and feline diabetes mellitus, this study investigated whether the DPPIV inhibitor NVP-DPP728 reduces plasma glucagon levels in cats. Intravenous glucose tolerance tests (ivGTT; 0.5g/kg glucose after 12h fasting) and a meal response test (test meal of 50% of average daily food intake, offered after 24h fasting) were performed in healthy experimental cats. NVP-DPP728 (0.5-2.5mg/kg IV or SC) significantly reduced glucagon output in all tests and increased insulin output in the ivGTT. Follow-up studies will investigate the potential usefulness as therapy in diabetic cats.
Short Communication

The dipeptidyl peptidase IV inhibitor NVP-DPP728 reduces plasma glucagon concentration in cats

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Abstract

Glucagon-like peptide-1 (GLP-1) analogues and inhibitors of its degrading enzyme, dipeptidyl peptidase IV (DPPIV), are interesting therapy options in human diabetics because they increase insulin secretion and reduce postprandial glucagon secretion. Given the similar pathophysiology of human type 2 and feline diabetes mellitus, we investigated whether the DPPIV inhibitor NVP-DPP728 reduces plasma glucagon levels in cats. We performed intravenous (IV) glucose tolerance tests (ivGTT; 0.5g/kg glucose after 12h fasting) and a meal response test (test meal of 50% of average daily food intake, offered after 24h fasting) in healthy experimental cats. NVP-DPP728 (0.5 – 2.5 mg/kg IV or SC) significantly reduced glucagon output in all tests and increased insulin output in the ivGTT. Follow-up studies will investigate the potential usefulness for therapy in diabetic cats.

Keywords: Glucagon-like peptide-1, Dipeptidyl peptidase IV, Glucagon, Cat, Diabetes mellitus.
Diabetes mellitus is one of the most common endocrinopathies in cats. Current treatment mainly relies on insulin and feeding low carbohydrate diets. Effective lowering of blood glucose levels requires aggressive insulin therapy, bearing the risk of insulin-induced hypoglycemia. Glucagon-like peptide-1 (GLP-1) is released by endocrine intestinal cells in response to eating. GLP-1 is an interesting treatment option in type 2 diabetics because it potentiates glucose-induced insulin secretion, improves beta-cell proliferation, reduces beta-cell apoptosis and also decreases postprandial glucagon secretion. GLP-1's effects are blunted in hypoglycemia, therefore avoiding dangerously low glucose levels (Nauck, 1998; Holst, 2002; Nielsen et al., 2004). Endogenous GLP-1 is degraded rapidly by dipeptidyl peptidase IV (DPPIV). Its half-life in rodents is 1–1.5 min (Nauck, 1998). GLP-1 action can be enhanced by delaying its degradation using DPPIV inhibitors, such as NVP-DPP728 (1-[2-[5-cyanopyridin-2-yl]amino]ethylamino] acetyl-2-cyano-(S)-pyrrolidide monohydrochloride) (Balkan et al., 1999).

Hyperglucagonemia is a metabolic hallmark in human type 2 diabetics (Ritzel et al., 1995; Holst, 2002) but also diabetic cats (Tschuor et al., 2006). We therefore investigated if NVP-DPP728 reduces plasma glucagon in cats. The doses (0.5 – 2.5 mg/kg) were adapted from effective doses in rodents and humans (Ahren et al., 2002; Ahren and Hughes, 2005). Blood glucose and insulin were also measured because GLP-1 increases glucose-induced insulin secretion in rodents and humans (Nauck, 1998; Holst, 2002; Nielsen et al., 2004).

All procedures were approved by the Veterinary Office Zurich. Twelve healthy male castrated domestic short-hair cats (Harlan, 16-24 months old, body weight 4.9 ± 0.4 kg) were
kept under a 12 h light/dark cycle and fed canned food (Purina Veterinary Diet: Diabetes Management, Nestlé Purina) twice daily to maintain stable body weight. Cats were sedated with ketamine (Ketaminol, Veterinaria; 5-7 mg/kg, IM) and midazolam (Dormicum, Roche; 0.2 mg/kg, IM), followed by propofol anesthesia (Propofol, Fresenius Kabi; 6-7 mg/kg, IV) to implant a jugular catheter (Seldinger, 1953). Cats were subjected to two intravenous (IV) glucose tolerance tests (ivGTT) and a meal response test. All tests were performed with six cats using a cross-over design and 4 weeks between trials.

Blood glucose was measured with an automated photometric test (COBAS MIRA, Roche). Insulin and glucagon were measured in EDTA plasma containing aprotinin (Trasylol, Bayer; 500KIU/mL plasma), by radioimmunoassay (RIA) (insulin: Linco Porcine Insulin RIA Kit, glucagon: ICN Biomedicals) validated before use (Zini et al., in press). Area under the curve (AUC) was calculated above baseline of each animal. This was used to compute average AUC. Values are given as mean ± SEM. Experiments were analyzed by paired Student's t-tests with P < 0.05 considered significant.

Cats were fasted overnight before ivGTT. After basal blood sample, saline or NVP-DPP728 (dissolved in saline) were injected IV (0.5 mg/kg). In a second experiment saline or NVP-DPP728 were injected subcutaneously (SC, 1 mg/kg). Thirty min (IV) or 40 min (SC) later, D-glucose (0.5 g/kg, 50% dextrose solution, Kantonsapotheke Zürich, Switzerland) was injected over 30 s via the jugular catheter. A lower dose of glucose was used than recommended to achieve maximal insulin secretion (Hoenig et al., 2002) to test whether inhibition of GLP-1 degradation further increases glucose-induced insulin secretion. Eight blood samples were
obtained from 3 to 180 min after glucose. In the first test, plasma glucose in controls increased from 4.6 ± 0.2 mmol/L to 27.6 ± 3.5 mmol/L 3 min after glucose administration. Glucose levels and time course were unaffected by NVP-DPP728 (0.5 mg/kg IV). Insulin peaked 15 min after glucose (37.4 ± 5.8 µIU/mL versus 9.2 ± 0.5 µIU/mL [baseline]). Total insulin output (AUC) over 15 min increased significantly (P < 0.05) by about 20% by NVP-DPP728. Glucose time-dependently decreased plasma glucagon with the lowest level reached after 3 min (214 ± 30 pg/mL versus 367 ± 65 pg/mL [baseline]). This decrease was stronger after NVP-DPP728 (201 ± 29 pg/mL versus 471 ± 85 pg/mL [baseline]). Overall, NVP-DPP728 significantly reduced glucagon output (Fig. 1).

In the second ivGTT, glucose increased from 4.4 ± 0.1 mmol/L to 28.9 ± 4.6 mmol/L 3 min after injection. Insulin peaked 15 min after glucose (36.8 ± 5.5 µIU/mL versus 8.6 ± 0.4 µIU/mL [baseline]). Insulin output (AUC over 15 min) increased significantly (P < 0.05) by about 25% after NVP-DPP728 (1 mg/kg SC). Glucose significantly decreased plasma glucagon. This was significantly more pronounced after NVP-DPP728 (Fig. 1).

Cats were fasted for 24 h before the meal response test. After basal blood sample, saline or NVP-DPP728 (2.5 mg/kg) were injected SC. Pilot tests indicated that a higher dose of NVP-DPP728 is necessary than during ivGTT, possibly because glucose may be a stronger acute stimulus for the pancreas than a meal. Forty min later, the test meal (50% of average daily food intake) was offered which was consumed within 10 min. Blood was sampled at meal ending (0 min) and for the following 5 h, and treated as above. No postprandial hyperglycemia was present, irrespective of treatment. Insulin was significantly higher (18.7 ± 1.6 µIU/mL versus 8.6
± 0.4 µIU/mL [baseline], n = 6) 15 min after meal ending. Total insulin secretion (AUC) was unaffected by NVP-DPP728. Plasma glucagon significantly increased after the test meal (1 h: 224 ± 32 pg/ml versus 171 ± 29 pg/ml [baseline]). Total glucagon output over 1 h after the test meal was significantly reduced by NVP-DPP728 (Fig. 2). Despite a clear trend, significance was not reached at individual time points or for glucagon output over 5 h.

The present study shows that plasma glucagon in cats was lower after single peripheral injection of the DPPIV inhibitor NVP-DPP728. This was observed in both ivGTT's and the meal response test. Insulin output was increased during the ivGTT. Even though GLP-1 was not directly measured here due to the lack of a specific assay, we presume that the reduction in glucagon and increase in insulin by NVP-DPP728 was due to reduced degradation of endogenous GLP-1, similar to what is known from other species (Balkan et al., 1999).

GLP-1 reduces glucagon secretion in rodents and humans and normalizes diabetic hyperglucagonemia (Ritzel et al., 1995; Holst, 2002). We provide indirect evidence that similar mechanisms may be active in cats because inhibition of DPPIV lowered plasma glucagon under all test conditions. Because hyperglucagonemia is present in diabetic cats (diabetic cats at baseline: approx. 1300 pg/mL versus 350 pg/mL in healthy controls; Tschuor et al., 2006), our findings suggest that further studies should test DPPIV inhibitors for the treatment of feline diabetes. Higher doses seem to be required for SC than IV administration of NVP-DPP728. This may be due to different bioavailability. DPPIV inhibitors can also be administered orally, but effective doses would have to be tested.
Our study has some limitations. First, one may argue that the effects of NVP-DPP728 on plasma glucagon were rather small and therefore potentially of little relevance. Of note, however, we used young healthy cats of normal body weight. We believe that the effects of NVP-DPP728 on glucagon may be stronger in diabetic cats because GLP-1's biological actions generally are more pronounced during hyperglycemia than during normoglycemia (Nauck, 1998; Nielsen et al., 2004). This needs to be tested in follow-up studies. Lack of postprandial hyperglycemia may also explain why NVP-DPP728 did not increase insulin levels during the meal response test.

Second, GLP-1 levels were not directly measured because a specific assay for feline GLP-1 is not available. Nonetheless, data from other species suggest that NVP-DPP728's effect relies on inhibition of GLP-1 degradation.

In summary, we provide evidence that inhibition of GLP-1 degradation may lower plasma glucagon in cats. GLP-1 analogues or DPPIV inhibitors may therefore bear therapeutic potential in diabetic cats. At the doses tested, NVP-DPP728 produced no adverse side effects.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the concept of the paper.

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References


Legends to figures

Fig. 1: In two independent experiments, NVP-DPP728 (0.5 mg/kg IV or 1 mg/kg SC, n = 6 for all groups) significantly reduced plasma glucagon in ivGTT’s in 12 h-fasted cats, as assessed by AUC during the first 15 min after glucose. * significantly different from respective control (P < 0.05).

Fig. 2: NVP-DPP728 (2.5 mg/kg SC) significantly reduced plasma glucagon in a meal response test in six 24 h-fasted cats, as assessed by AUC 1 h after the end of meal. * significantly different from control (P < 0.05).
Fig. 2

![Bar graph showing glucagon levels with error bars. The graph compares control and NVP DPP728 groups. The NVP DPP728 group has a statistically significant increase in glucagon levels marked with an asterisk (*)].

- **Glucagon (pg/ml x 60min)**
  - control
  - NVP DPP728

*Statistically significant difference.*