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Effect of Acarbose, Sitagliptin and combination therapy on blood glucose, insulin, and incretin hormone concentrations in experimentally induced postprandial hyperglycemia of healthy cats

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ABSTRACT

Acarbose (AC) and Sitagliptin (STGP) are oral hypoglycemic agents currently used either alone or in conjunction with human diabetic (Type 2) patients. AC has been used with diabetic cats, but not STGP thus far. Therefore, the objective of this study was to determine the potential use of AC or STGP alone and in combination for diabetic cats, by observing their effect on short-term post-prandial serum glucose, insulin, and incretin hormone (active glucagon-like peptide-1 (GLP-1) and total glucose dependent insulinotropic polypeptide (GIP)) concentrations in five healthy cats, following ingestion of a meal with maltose.

All treatments tended ($p < 0.10$; 5–7.5% reduction) to reduce postprandial glucose area under the curve (AUC), with an accompanying significant reduction ($p < 0.05$, 35–45%) in postprandial insulin AUC as compared to no treatment. Meanwhile, a significant increase ($p < 0.05$) in postprandial active GLP-1 AUC was observed with STGP (100% higher) and combined treatment (130% greater), as compared to either AC or no treatment. Lastly, a significant reduction ($p < 0.05$) in postprandial total GIP AUC was observed with STGP (21% reduction) and combined treatment (7% reduction) as compared to control. Overall, AC, STGP, or combined treatment can significantly induce positive post-prandial changes to insulin and incretin hormone levels of healthy cats. Increasing active GLP-1 and reducing postprandial hyperglycemia appear to be the principal mechanisms of combined treatment. Considering the different, but complementary mechanisms of action by which AC and STGP induce lower glucose and insulin levels, combination therapy with both these agents offers great potential for treating diabetic cats in the future.

An effective treatment strategy for Type 2 diabetes includes targeting of the incretin system, comprising of molecules such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). However, both molecules are rapidly degraded by dipeptidyl peptidase-4 (DPP-4) (Drucker, 2007). Intestinal breakdown of carbohydrates is mediated by α-glucosidase, which is located in the brush border of the proximal small intestinal epithelium. α-GI (α-glucosidase inhibitor) can prevent the degradation of disaccharides, resulting in a slower absorption rate of sugars, thereby lowering postprandial blood glucose levels and suppressing excessive insulin secretion after food ingestion (Juretić et al., 2003).

Sitagliptin (STGP) is a highly selective DPP-4 inhibitor in humans and mice (Hemmeryckx et al., 2014), providing 24-hour DPP-4 inhibition when administered once daily (Herman et al., 2007). In human diabetic (Type 2) patients, STGP combined with an α-GI, has been shown to improve glycemic control (Iwamoto et al., 2010). Moreover, Aoki et al. (2010) demonstrated that combined therapy with a starch blocker (miglitol) and STGP, can reduce postprandial plasma glucose and insulin concentrations in non-diabetic humans. Acarbose (AC) is an α-GI, when combined with insulin and diet, can improve glycemic control in diabetic cats (Greco, 1997; Mazzaferro et al., 2003). However, the effects of a combination of α-GI and DPP-4 inhibitors on blood glucose, insulin and incretin levels in cats are unknown.

As such, the main objective of this study was to evaluate the effectiveness of AC, STGP, and combination therapy on postprandial glucose, insulin and incretin levels in healthy cats. Starch has less of an effect on postprandial glucose and insulin responses in cats than in dogs or humans (de-Oliveira et al., 2008). Therefore, in order to evaluate dynamic changes in blood glucose, insulin, and incretin concentrations in response to a physiologically informative route of administration, we have determined these parameters in response to a meal with maltose.

Five (3 castrated males and 2 spayed females; 3.5–5.9 kg body weight (BW); 3 body condition score (BCS); 1–4 years old) adult domestic cats, maintained in our laboratory for research, were used in this study. The BCS was determined on a five point scale: 1, thin; 2, lean; 3, optimal; 4, obese; and 5, gross. Cats were fed on a C/D dry diet (Hill's Colgate, Tokyo, Japan) twice a day and caloric intake was set at

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half of 1.4 × RER ($BW^{0.75} \times 70$) (Thatcher et al., 2010), whereby RER represents a resting energy requirement. Approval for the work was given by the Nippon Veterinary and Life Science University Animal Research Committee.

Four weeks prior to starting our study, cats were acclimated to the C/D dry diet and daily feeding schedule. The study was conducted for four weeks, composing of four 1-wk periods, with the same 5 animals undergoing different treatments on a rotating weekly basis. Cats were fed on only a C/D dry diet twice a day and caloric intake was set at half of 1.4 × RER without maltose and oral hypoglycemic drugs for the preceding six days of each wk period. On the last day of each 1-wk period, caloric intake was set at half of 1.2 × RER ($BW^{0.75} \times 70$) for their complete feeding within 1 h following a 24 h fast. Concurrent with diet feeding, cats received treatment as either no medication, Acarbose (AC, Glucobay, Bayer Holding Ltd., Tokyo, Japan), Sitagliptin (STGP, JANUVIA, Merck & Co., Inc., Tokyo, Japan) or combined at each weekly period. The dose of AC and STGP orally administered was 12.5 mg and 4.2 mg, respectively, and these amounts were referenced for clinical use in diabetic cats and T2DM patients, respectively (Aoki et al., 2010; Mazzaferro et al., 2003). Concurrent with feeding, in order to mimic postprandial hyperglycemia, we added maltose powder (Maltose Monohydrate, Wako Pure Chemical Industries Ltd, Osaka, Japan) into the diet, by mixing maltose at 5 g/kg (previously referenced in a human study (van Can et al., 2012)) of wet food (Select Protein, Royal canin Japon, Tokyo, Japan), which replaced a quarter of 1.2 × RER ($BW^{0.75} \times 70$) calories with cats being fed the rest of the calories as C/D dry diets. We confirmed that 100% of all the food was eaten within 60 min for all five cats, and also checked for side effects (hypoglycemia or gastrointestinal problem) in all cats for up to three days after feeding and treatments.

Blood samples were collected by bleeding 1.5 ml of blood from the jugular vein of cats, 0.5 h prior to and 0.5, 1, 2, 4, 6, and 10 h post-feeding of the diets. Blood samples for glucose and insulin determination were collected into polypropylene tubes and allowed to clot at room temperature for 15 min. Subsequently, blood samples were centrifuged (1700 ×*g*) at 4 °C for 10 min to separate the serum. Blood samples for active GLP-1 and total GIP assaying were collected into ice-cooled Vacutainer® EDTA-plasma tubes. Immediately after collection of GLP-1 blood samples, an appropriate amount (10 μl per milliliter of blood) of DPP-4 inhibitor reagent solution (DPP-IV Inhibitor; Millipore Headquarters, Missouri, USA) was added, according to the manufacturer's instructions. Samples were immediately centrifuged at 1000 *g* for 10 min at 4 °C and 2000 *g* for 15 min at 4 °C to obtain plasma for active GLP-1 and total GIP assaying, respectively. Serum and plasma samples were immediately stored at −80 °C until further use.

Serum glucose and insulin concentrations were measured as previously described (Mimura et al., 2013). Plasma samples were used to determine GLP-1 and GIP concentrations, which were measured using a commercial Glucagon-Like Peptide-1 (Active) 96-Well Plate ELISA Kit for mammals (Millipore Headquarters, Missouri, USA) validated for use with felines (Hoenig et al., 2010) and Rat/Mouse GIP (Total) 96-well plate (Millipore Headquarters, Missouri, USA) (intra-assay coefficient of variation was 3.0% and excellent linearity (R^2 value of 0.9966) after serial dilution of feline pooled plasma was demonstrated in our laboratory), respectively, according to the manufacturer's protocols.

Data are presented as the median [min, max]. Total area under the curve (AUC) was estimated as the post-prandial summary variable and calculated by the trapezoidal rule in units of concentration × hours. Significance was determined using Friedman repeated measures ANOVA on ranks and Student–Newman–Keuls Method for pairwise multiple comparison procedures for comparison of AUC or temporal analysis of glucose, insulin, GLP-1, and GIP concentrations between the control and treatments. The significance level was set at $p < 0.05$ and all tests were run in Sigmaplot 11.0 (Build 11.2.0.11, Systat Software Inc., CA, USA).

In our study, there was a tendency ($p < 0.10$) for all treatments to reduce median glucose AUC (GAUC) as compared to control (932.25 [889.25, 985.00] mg/dl·h^{-1}) (Fig. 1a). AC, STGP, and combined treatment produced GAUC values of 870.25 [820.00, 907.00] mg/dl·h^{-1}, 880.75 [834.00, 925.00] mg/dl·h^{-1}, and 848.25 [778.75, 905.00] mg/dl·h^{-1}, respectively. Because only healthy animals were used in our study, glucose regulation is working properly, and thereby minimizing any pronounced effect from the compounds, which would be more evident with diabetic animals. Alternately, all treatments resulted in significant ($p < 0.05$) reductions to median insulin AUC (IAUC) values as compared to control (23.23 [14.42, 31.43] ng/ml·h^{-1}). AC, STGP, and combined treatment produced IAUC values of 14.84 [13.65, 16.66] ng/ml·h^{-1}, 14.38 [13.79, 21.88] ng/ml·h^{-1}, and 13.08 [8.95, 16.18] ng/ml·h^{-1}, respectively (Fig. 1b).

Overall, AC and STGP alone, appeared to be similarly effective for reducing postprandial glucose and insulin in healthy cats, strangely enough. AC can delay the absorption of digested carbohydrates from the small intestine, but has no direct effect on glucose-dependent insulin secretion (Iwamoto et al., 2010) per se. Alternatively, STGP enhances endogenous incretin activity (Herman et al., 2007), increasing glucose-dependent postprandial insulin secretion (Karasik et al., 2008). As such, one would expect to see increased insulin secretion with STGP as part of its mechanism of action, but not AC. However, since non-diabetic healthy cats were used, STGP might not have been able to induce higher insulin secretion as compared to control since all animals were healthy and capable of insulin secretion and regulation. Interestingly though, combined treatment (AC + STGP) was able to further reduce blood GAUC and IAUC values as compared to either compound alone. We speculate that one possible explanation for the reduced postprandial insulin response observed lies with a reduction in plasma glucagon levels associated with increased circulating GLP-1 levels (Bock et al., 2010). Reduced postprandial glucagon levels have been reported with the use of two other DIPP-4 inhibitors, alogliptin and vildagliptin (Moritoh et al., 2010).

As expected, mean postprandial plasma active GLP-1 AUC (GLPAUC) values of STGP (233.36 [129.93, 533.68] pmol/ml·h^{-1}) and combined treatment (209.19 [162.81, 503.35] pmol/ml·h^{-1}) was significantly ($p < 0.05$) greater than that of AC (104.74 [95.13, 406.06] pmol/ml·h^{-1}) or control (103.29 [64.74, 406.68] pmol/ml·h^{-1}) (Fig. 1c). In fact, there was almost no difference between control and AC GLPAUC values, which is expected since AC should have no effect on GLP-1 amount; whereas being a DPP-4 inhibitor, STGP treatment should result in an increased level of active GLP-1.

With regards to mean total plasma GIP AUC (GIPAUC) values, STGP (1969.41 [1478.59, 3511.36] pg/ml·h^{-1}) and combined treatment (2324.63 [1456.87, 3568.49] pg/ml·h^{-1}) significantly ($p < 0.05$) reduced, whereas AC (2392.51 [1954.20, 4547.19] pgl/ml·h^{-1}) tended ($p < 0.10$) to reduce as compared to control (2502.83 [1997.69, 4905.68] pgl/ml·h^{-1}) (Fig. 1d). The reduction in GIPAUC observed with STGP and combined treatment appears to be counterintuitive to the mechanism of action of DPP-4 inhibition. Interestingly, Bock et al. (2010) and Deacon et al. (2002) have reported that STGP treatment results in decreased total GIP and GLP-1 concentrations in humans. One possible explanation for these results is that the increased level of active GLP-1 and GIP from DPP-4 inhibition, produces an inhibitory effect on the L and K cells respectively, implicating a possible negative feedback inhibition of enteroendocrine secretion, resulting in a negative feedback inhibition of total GIP secretion (Herman et al., 2006).

Finally, STGP treatment was generally well-tolerated in this study while AC medication was associated with increased incidences of loose stool in three of five cats. Therefore, side effects of both drugs for cats should warrant further discussion and investigation with a more appropriate numbers of cats.

In conclusion, AC and STGP were both effective, using their own unique mechanistic pathways, producing similar results in cats, as what has been documented in humans, when used alone. Moreover, AC, STGP, or combined treatment can significantly induce positive post-prandial changes to the insulin and incretin hormone levels of healthy cats, thereby providing useful information for managing and