TTP399, A Liver Selective Glucokinase Activator (GKA) that Preserves the Physiological Regulation of Glucokinase (GK) by GK Regulatory Protein (GKRP)

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Abstract

GK acts as a glucose sensor to elicit glucose specific responses, primarily from hepatocytes and pancreatic β-cells. Previously identified GKAs evaluated in the clinic for the treatment of Type 2 diabetes demonstrate improved glucose control; however these GKAs show increased incidence of hypoglycemia and hyperlipidemia and an apparent lack of durability. These liabilities have been correlated to hyper-stimulation of the ß-cells and/or the accumulation of lipids in the liver, consistent with the disruption of GK and GKRP interaction. Thus, liver selective GKAs that do not activate GK in ß-cells or affect the GK-GKRP interaction are expected to demonstrate a superior profile. TTP399 is a novel liver selective GKA that has shown normalization of glycemic control in animal models and Type 2 diabetic subjects without inducing hypoglycemia or dyslipidemia.

The purpose of the studies described herein was to evaluate TTP399 effects on GK and GKRP interaction *in vitro* and to examine *in vivo* evidence of hypoglycemia, dyslipidemia or changes in hepatic glycogen /lipid content. Experiments were conducted *in vitro* to evaluate the effects of TTP399 on the nuclear localization of GK at various glucose concentrations in hepatocytes to measure the GK-GKRP interaction. *In vivo*, changes in plasma glucose, liver glycogen and triglycerides (TG) were evaluated after chronic treatment with TTP399 in *ob/ob* mice and minipigs. Our results demonstrate that TTP399 is a *liver*-selective GKA that does not disrupt the interaction between GK and GKRP. Further *in vivo* evidence confirms that at anticipated therapeutic concentrations, TTP399 stimulates the liver to metabolize glucose while inducing little or no insulin secretion, no dyslipidemia and no increase in glycogen or TG in the liver. These results indicate that TTP399 has a superior profile compare to previous GKAs by greatly reducing the risk of hypoglycemia and dyslipidemia while maintaining durability.

Introduction

Glucokinase (GK), also called Hexokinase IV, plays an essential role in blood glucose homeostasis, catalyzing glucose phosphorylation that is the rate-limiting reaction for glycolysis. Glucokinase is expressed in a variety of tissues including liver hepatocytes and pancreatic α and β-cells as well as different areas in the brain. In pancreatic β-cells, GK acts as a glucose sensor establishing the threshold for glucose-stimulate-insulin secretion and may play a role in regulating glucagon secretion from α-cells. In the liver, GK determines the rates of both glucose uptake and glycogen synthesis, thus regulating the balance between hepatic glucose production and consumption, as well as a critical part of the pathway for lipogenesis[1].

Genetic evidence in humans validates GK as the physiological glucose sensor. Mutations that inactivate GK, such as Mody2 (Maturity Onset Diabetes of the Young 2), are associated with hyperglycemia while activating mutations, such as those seen with PHHI (Persistent Hyperinsulinemic Hypoglycaemia of Infancy), result in hypoglycemia. In addition, in humans loss of function mutations in the Glucokinase Regulatory Protein (GKRP) are also associated with increased plasma lipids[2].

In the liver when glucose concentrations are low, GKRP interacts with GK to form an inactive complex that is localized in the nucleus. When glucose concentrations are high, GK dissociates from GKRP, translocates to the cytoplasm thereby initiating glucose disposal (Figure 1). Previous non-liver selective GKAs have been shown to disrupt the GK-GKRP[3] interaction and have also been shown to increase hypoglycemic events. Liver selective activation in GK knockout and diabetic transgenic animal studies suggests that 'selective' activation of liver glucokinase (GK) can provide an alternative and safer mechanism for reduction of blood glucose.

1. Effect of TTP399 on Glucokinase Translocation

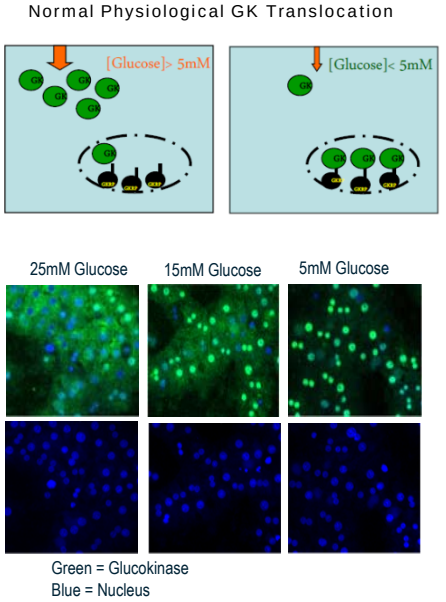
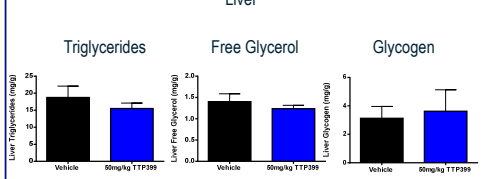


Figure 1. TTP399 does not disrupt the glucose dependent nuclear translocation of GK in rat hepatocytes. Freshly isolated primary hepatocytes from rats were plated in Williams Medium E containing serum. Following overnight attachment, cells were treated with 1 μM TTP399 in the presence of 5, 15 or 25 mM glucose for 1hr followed by detection of GK protein with a rabbit anti-GK antibody (SantaCruz) followed by a goat anti-rabbit Alexa™ 488 (green) and the nucleus using a Hoescht stain (blue) with the InCell© 2000 instrument (General Electric).

3. Effects of 13 weeks dosing of TTP399 in minipigs



- No significant differences were observed in plasma lactate or plasma triglycerides

Figure 3: Effects of 13 weeks of treatment on Normal Minipigs. Naive Gottingen Minipigs® were dosed by oral gavage once daily for 13 consecutive weeks with either vehicle or 50mg/kg TTP399 as indicated with n=8 per group (4 female and 4 male). Liver was homogenized and Plasma was analyzed using standard assays. The mean and standard deviation are reported. No significant changes were observed.

2. Effects of TTP399 following 4 weeks dosing in ob/ob mice

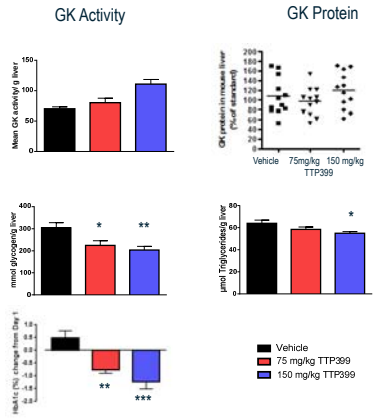


Figure 2. Effects of 4 weeks of TTP399 on liver triglyceride and glycogen levels and HbA1c in ob/ob mice. Following 4 weeks of daily oral dosing of vehicle or TTP399 in *ob/ob* mice (n=14/group), cytosolic liver GK activity was assayed in the presence of 15mM glucose and GK protein from the liver was quantified using western blotting. In addition, HbA1c levels were measured in plasma and triglyceride and glycogen levels from liver samples were also analyzed using standard methods. The mean and SEM are reported. Significance is indicated by *p<0.05, **<0.01, ***<0.001 as compared to vehicle.

4. Effects of 39 weeks of dosing TTP399 in Minipigs

	Change from Day 1 (CFB), following 39 weeks of dosing:							
	ALT (U/L)		AST (U/L)		GGT (U/L)		Total Bilirubin (mg/dL)	
TTP399 dose (mg/kg)	Female	Male	Female	Male	Female	Male	Female	Male
0	4.5	13.5	12.8	26.2	-7.6	-6.6	-0.02	0.03
25	1.5	-2.2	36	20.8	-5.7	0.2	0	-0.03
100	1.4	-0.8	-6.5	24.2	-29.8	-11	0	0
200	-1.4	5	7	11.3	-10.9	-14.7	-0.02	0

	CFB, Cholesterol (mg/dL)		CFB, Triglyceride (mg/dL)	
TTP399 dose (mg/kg)	Female	Male	Female	Male
0	21.2	13.8	28.1	18
25	8.5	-0.8	17.3	20
100	12.5**	1.5*	32.7	10.5
200	-21.8**	-9**	12.9	16.5

	CFB, Glucose (mg/dL)	
TTP399 dose (mg/kg)	Female	Male
0	7	-2
25	5.7	3.5
100	1	3.2
200	-0.4	13.6

	Cmax (ng/mL)		AUC_{0-24} (hr*ng/mL)	
TTP399 dose (mg/kg)	Female	Male	Female	Male
25	3320	3880	18,200	18,700
100	21,100	30,600	118,000	157,000
200	58,000	54,900	462,000	329,000

Tables: Effects of 39 weeks of dosing TTP399 in Normal Minipigs. Naive Gottingen Minipigs® were dosed by oral gavage once daily for 39 consecutive weeks with either vehicle or TTP399 as indicated with n=8-12/group (4-6 female and 4-6 male). Plasma was analyzed using standard assays and the change from baseline to week 39 of the means are reported. TTP399 was detected using standard bioanalytical methods and mean Cmax and AUC (0-24) are reported. Significant changes from vehicle: * p< 0.05, ** p< 0.01.

Conclusions

- ***In vitro GK translocation:***
  - TTP399 does not disrupt the interaction between GK and GKRP
- ***Chronic treatment of TTP399 in ob/ob mice results in:***
  - Increased GK activity without increase in protein levels
  - Lowering of plasma glucose and HbA1c
  - Dose dependent lowering of liver triglycerides (TG) and glycogen
  - No observable signs of hypoglycemia
- ***Following chronic dosing of TTP399 in normal minipigs, there is:***
  - No significant increase in liver glycogen, TG or free glycerol content
  - No changes in plasma lactate or TG
  - No changes in liver function
  - All of the above at plasma concentration of TTP399 over 100-fold higher than those at therapeutic doses in humans
- ***TTP399 stimulates the liver to metabolize glucose***
  - while inducing little or no insulin secretion
  - without causing hypoglycemia
  - without causing dyslipidemia or increasing hepatic glycogen or hepatic TG
- ***Results in animal models confirmed in the clinic*** *(results presented at ADA in 2014 122-OR):*
  - improved glycemic control
  - no increase in plasma lipids
  - no increased incidence of hypoglycemia

References

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For more information on current clinical trials with TTP399 visit: www.MyAgata.com or www.clinicaltrials.gov