Glucagon Receptor Antagonist LGD-6972 Is Efficacious in Streptozotocin-Induced Diabetic Mice

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ABSTRACT

Recent studies indicate that glucagon action plays an essential role in insulin dependent diabetes. Glucagon receptor (GCGR) knockout mice are resistant to diabetes even after high-dose streptozotocin (STZ) treatment eliminated virtually all insulin production. We examined whether pharmacological antagonism of the GCGR would have similar effects in insulin deficient mice. LGD-6972 is a potent and selective antagonist of the GCGR that has previously demonstrated activity in pre-clinical models of T2DM. Male BALB/c mice were injected with multiple low doses of STZ and left untreated until hyperglycemia was confirmed. Hyperglycemic mice were orally dosed with LGD-6972 or vehicle for 28 days. Non-fasting blood glucose was significantly reduced in LGD-6972 treated mice (average = 80 mg/dL throughout the experiment). Larger effects were observed on fasting blood glucose (Vehicle: 382 mg/dL, LGD-6972: 234 mg/dL, non-diabetic controls: 118 mg/dL). An oral glucose tolerance test revealed a large increase in glucose levels and a slow recovery to baseline levels in STZ mice relative to non-diabetic controls, consistent with an insulin deficient state. LGD-6972 treated mice had lower glucose levels than STZ mice throughout the glucose tolerance test (average decrease of 100 mg/dL) but displayed a similar delayed return to baseline. Terminal measurements of HbA1c, 3-hydroxybutyrate, and free fatty acids (FFA) were significantly elevated in STZ mice indicating prolonged hyperglycemia and ketoacidosis. LGD-6972 partially restored HbA1c and 3-hydroxybutyrate to non-diabetic levels but did not alter FFA levels.

These data indicate that pharmacological inhibition of GCGR may be an effective treatment for T1DM and could potentially be used in an insulin-sparing regimen. Pharmacological inhibition was not able, however, to completely normalize the hyperglycemic state, unlike the effects observed with GCGR knockout mice. Further studies to investigate these differences may be useful.

INTRODUCTION

- Glucagon and insulin play counter-regulatory roles in glucose homeostasis.
- In normogylcemic subjects, glucagon is elevated during periods of fasting to mobilize glucose stores.
- Recent studies suggest that unopposed glucagon action is largely responsible for the hyperglycemia observed in type 1 diabetes(1).
- Recent evidence suggests that disruption of glucagon signalling completely normalizes glucose levels in streptozotocin injected mice, a model of type 1 diabetes(1,2).
- We have discovered a novel, selective, orally bioavailable glucagon receptor antagonist and investigated its efffects on an animal model of type 1 diabetes.

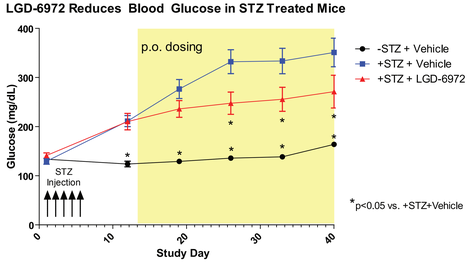
RESULTS

Glucagon Receptor Binding and cAMP Inhibition

LGD-6972 potently displaced human glucagon in a radiolabelled binding assay (IC_{50}=5 nM). cAMP production was measured in glucagon stimulated hepatocytes from human and mouse donors. LGD-6972 potently inhibited cAMP in humans (IC_{50}=0.5 nM), with reduced potency vs. mouse (IC_{50}=150 nM).

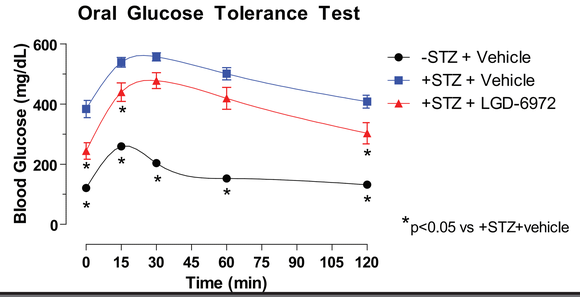
Glucose reduction in streptozotocin-injected mice

Male BALB/c mice were injected with multiple low doses of STZ (40 mg/kg IP for 5 days). After hyperglycemia developed, mice were administered 30 mg/kg LGD-6972 or vehicle for 28 days (PO, QD). Non-fasting blood glucose was monitored weekly. LGD-6972 significantly improved hyperglycemia but was unable to completely normalize glucose levels.

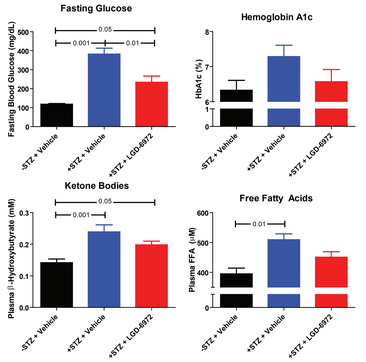


Oral glucose tolerance test in streptozotocin-injected mice

Male BALB/c mice were injected with multiple low doses of STZ (40 mg/kg IP for 5 days). After hyperglycemia developed, mice were administered 30 mg/kg LGD-6972 or vehicle (PO, QD). During the 3rd week of treatment an oral glucose tolerance test was performed. LGD-6972 significantly reduced glucose levels but did not restore glucose to non-diabetic levels.

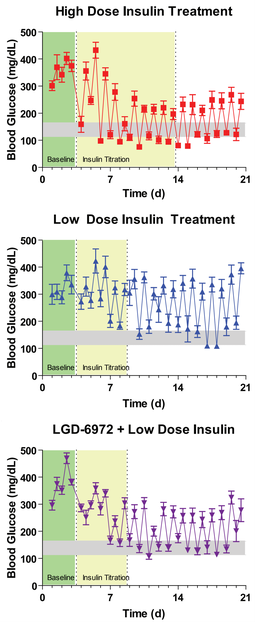


At the conclusion of the study, fasting glucose, hemoglobin A1c, ketone bodies, and free fatty acids were measured. STZ treatment increased all 4 parameters above non-diabetic control levels. LGD-6972 partially restored values to non-diabetic levels.

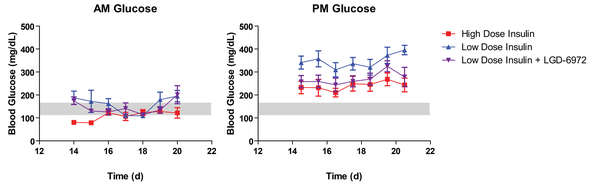


Insulin and LGD-6972 combination treatment

Male BALB/c mice were injected with multiple low doses of STZ (40 mg/kg IP for 5 days). After hyperglycemia developed, mice were sorted into groups and administered low dose insulin, high dose insulin, or low dose insulin + 30 mg/kg LGD-6972. A long acting insulin (Levemir®) was dosed SQ BID and LGD-6972 was dosed PO QD. Non fasting blood glucose was monitored in the morning (after insulin injection) and afternoon (before insulin injection). Insulin doses were titrated during the first 7-10 days to achieve mean morning glucose measurements within the non-diabetic range (shown with gray horizontal bar). Final insulin doses were 0.04 U/day and 0.07 U/day for low and high dose, respectively. All three treatments significantly reduced morning and afternoon glucose from baseline levels although significant glucose volatility was seen with this insulin dosing regimen.



LGD-6972+low dose insulin was more effective than low dose insulin alone, significantly reducing afternoon peak glucose levels (and glucose volatility). Similar efficacy could be achieved with a higher insulin dose, however increased incidence of hypoglycemica was observed with high dose insulin. With optimized dosing paradigms, LGD-6972 may be useful in an insulin sparing regimen for patients with Type 1 diabetes.



Treatment	AM Glucose: Total Number of Measurements	AM Glucose: Incidence of Hypoglycemia (<100 mg/dL)	AM Glucose: %	PM Glucose: Total Number of Measurements	PM Glucose: Incidence of Hyperglycemia (>300 mg/dL)	PM Glucose: %
Hi Dose Insulin	56	27	48%	56	12	21%
Low Dose Insulin	49	7	14%	49	32	65%
Low Dose Insulin + LGD-6972	56	8	14%	56	20	36%

References

1. Unger and Cherrington. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. **J Clin Invest**, 2012, 4-12.
2. Lee, *et al*. Metabolic manifestations of insulin deficiency do not occur without glucagon action. **Proc N Acad Sci**, 2012, 14972-14976.

SUMMARY

- LGD-6972 is a highly potent and selective glucagon receptor antagonist
- LGD-6972 significantly reduced fasting and non-fasting glucose in a model of type 1 diabetes
- LGD-6972 reduces basal glucose levels in diabetic mice but does not fully normalize glucose tolerance
- LGD-6972 reduces ketone bodies and free fatty acids in diabetic mice
- Combination therapy with insulin and LGD-6972 was more effective than insulin alone in diabetic mice
- LGD-6972 is a promising agent for the treatment of T2DM that may also have utility in Type 1 diabetic patients

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