CNX-011-67, A Novel Orally Available GPR40 Agonist, Enhances Glucose Stimulated Insulin Secretion (GSIS) and Significantly Reduces Fasting and Non-Fasting Hyperglycemia- Studies *In Vitro* And In a Preclinical Model of T2DM

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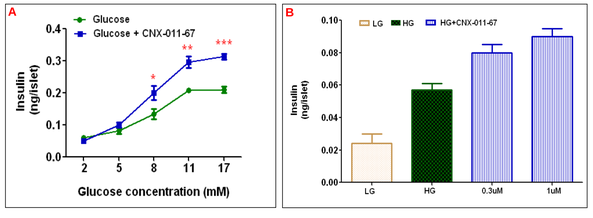
Introduction

GPR40 is a G-protein receptor coupled mainly with $G_{q q 11}$ which activates phospholipaseC resulting in the production of IP3 and DAG. In humans, GPR40 is expressed mainly in pancreas, brain and macrophages. GPR40 is reported to play an important role in glucose and fat mediated insulin secretion. It is now established that GPR40 does not mediate lipotoxicity. Activation of GPR40 by small molecule is reported to enhance insulin secretion.

We report the development and application of a highly selective potent and safe GPR40 agonist, CNX-011-67, which exhibits unique properties to improve beta cell function under conditions of metabolic stress. CNX-011-67 improves glucose sensitivity of β-cells and enhances glucose stimulated insulin secretion and content in multiple species including human T2DM islets. In a pre-clinical model of T2DM, CNX-011-67 has demonstrated good anti-diabetic activity.

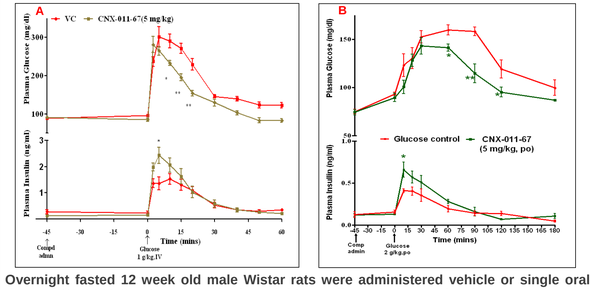
Results

1 CNX-011-67 enhances glucose responsiveness and insulin secretion in isolated rat and human islets



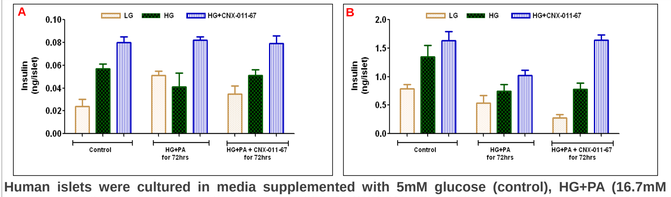
Isolated rat (A) and human(B) islets were cultured in RPMI medium containing 10%FBS. Insulin secreted after incubation for 120 min in KRBH buffer containing LG (2mM glucose), HG (11mM glucose) with or without CNX-011-67 was measured.

2 CNX-011-67 enhances first phase insulin secretion in wistar rats



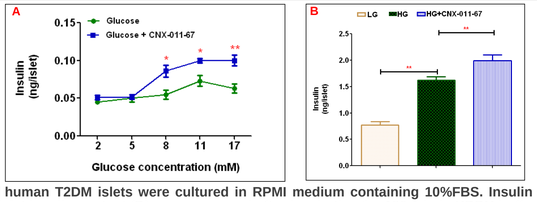
Overnight fasted 12 week old male Wistar rats were administered vehicle or single oral dose of CNX-011-67 at 5 mg/kg body weight. Insulin and glucose levels were measured during IVGTT (A) and OGTT (B) at indicated intervals.

3 CNX-011-67 enhances GSIS and insulin content in human islets under chronic metabolic stress



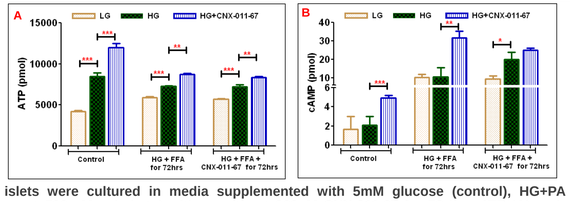
Human islets were cultured in media supplemented with 5mM glucose (control), HG+PA (16.7mM glucose + 500 µM palmitate) with or without 300nM CNX-011-67 for 72 hrs. Insulin secreted (A) and islet insulin content (B) were measured after 120 min.

4 CNX-011-67 restores glucose responsiveness, GSIS and insulin content in human T2DM islets



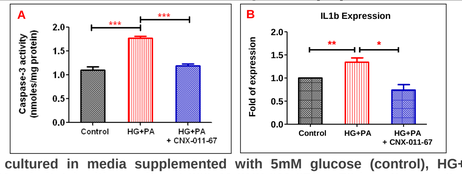
Isolated human T2DM islets were cultured in RPMI medium containing 10%FBS. Insulin secreted (A) and islet insulin content (B) were measured after incubation for 120 min in KRBH buffer. [LG-2mM glucose, HG-11mM glucose].

5 CNX-011-67 enhances Islet ATP and cAMP levels



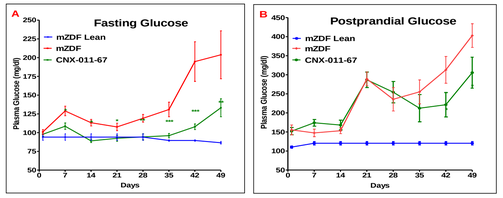
Rat islets were cultured in media supplemented with 5mM glucose (control), HG+PA (16.7mM glucose + 500 µM palmitate) with or without 300nM CNX-011-67 for 72 hrs. ATP (A) and cAMP (B) levels were measured 60 min after transferred to KRBH medium containing 2 and 11mM glucose.

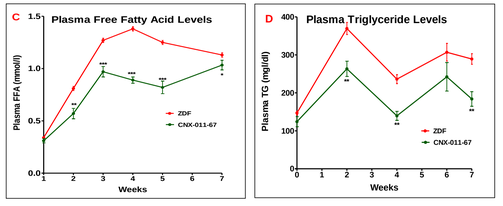
6 CNX-011-67 reduces β-cell apoptosis

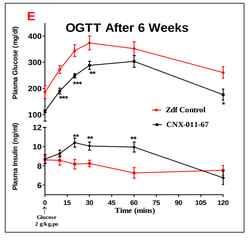


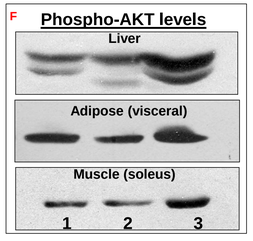
Rat islets were cultured in media supplemented with 5mM glucose (control), HG+PA (16.7mM glucose + 500 µM palmitate) with or without 300nM CNX-011-67 for 72 hrs. Caspase3 activation (A) and IL1b mRNA (B) were measured.

7 CNX-011-67 delays onset of hyperglycemia and significantly controls multiple metabolic parameters in male ZDF rats







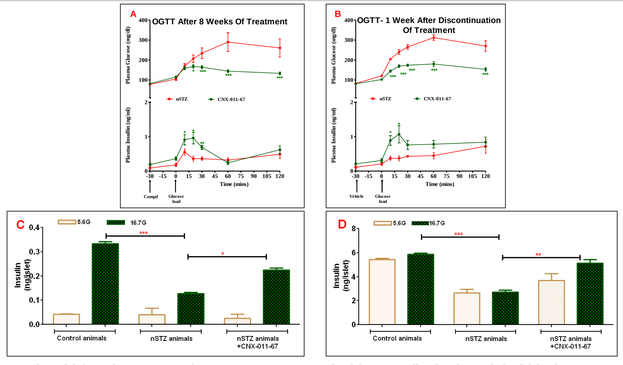


G Physiological parameters

Parameters	Treatment Groups		
	Lean	ZDF	CNX-011-67
HbA1c (%)	3.65 ± 0.06	5.50 ± 0.3	5.18 ± 0.11
Plasma Fructosamine (µM)	92.47 ± 10.68	236.66 ± 19.08	111.25 ± 25.98**
HOMA-IR	2.16 ± 0.74	64.26 ± 6.18	41.82± 2.59 *
HOMA-β	117.3 ± 39.3	1520.2 ± 227.3	2206.79±418.69

6 weeks old mZDF rats were treated with 5 mg/kg body weight bid of CNX-011-67. Fasting and postprandial glucose, free fatty acids and triglycerides were measured at the indicated intervals. OGTT was performed after 6 weeks. Phospho-AKT levels were measured in liver, adipose and muscle of ZDF lean(1), mZDF control(2) and mZDF treated with CNX-011-67(3).

8 CNX-011-67 enhances glucose responsiveness and insulin content in adult n2-STZ rats



18 weeks old female n2-STZ Wistar rats were treated with 15 mg/kg body weight bid of CNX-011-67. Plasma insulin and glucose levels were estimated during OGTT (3g/kg glucose load) at 8th week (A) and one week after discontinuation of treatment (B).

Isolated islets were assayed for insulin secretion (C) and islet insulin content (D) after incubation for 120 min with indicated concentrations of glucose.

Summary

- CNX-011-67 is a highly potent and selective GPR40 agonist having no risk of hypoglycemia and has excellent pharmacokinetic and safety profile.
- Agonist enhances glucose metabolism, insulin secretion and islet insulin content even under conditions of stress including chronic glucolipotoxicity.
- Acute treatment with CNX-011-67 enhances glucose responsiveness, insulin secretion and islet insulin content in T2DM islets also.
- Treatment with CNX-011-67 reduces β-cell apoptosis under glucolipotoxicity.
- The studies in mZDF rats showed
  - Robust control of both fasting and post-prandial glycemic levels
  - Improvement in β-cell health and phasic insulin secretion and an attendant delay in the onset and progress of T2DM
  - Significant reduction in the levels of both serum FFA and TG.
  - Improvement in the peripheral action of insulin
- CNX-011-67 is presently in IND enabling studies under GLP conditions.

References

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