CNX-013-B2, A PPAR Pan-Activator Rexinoid, Provides Robust Control Of Glucose, HbA1c, Triglyceride And LDL-C In db/db Mice And Does Not Cause Hemodilution And Edema In Rodents

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Abstract

Retinoid X Receptor (RXR) is a member of the nuclear receptor family of transcription factors that functions as a 'sensor' receptor by binding specific liphophilic ligand and modulates gene expression. RXR is considered 'promiscuous' as it forms heterodimers with several other nuclear receptor family members. Treatment of 6 weeks old db/db mice orally with 2.5, 5.0 and 10 mg/kg CNX-013-B2 qd (9am) for 8 weeks resulted in a reduction of 20 – 28% FPG, 11 – 30% PPG, 3 – 24% Glucose AUC_{24hrs}, 1.4 – 2.4% HbA1c, 14 – 21% serum triglyceride, 33 – 42% LDL-C and 25 - 35% in HOMA-IR (Panel 3). There was no change in food intake or increase in body weight. CNX-013-B2 significantly increased Succinate Dehydrogenase activity of gastrocnemius muscle by ~40% and also significantly reduced CDK5 mediated phosphorylation of PPARγ at Ser-273 in mesentric adipocytes. At 10 mg/kg dose mRNA expression of Sterol Response Element Binding Protein 1c (SREBP1c), Thyroid Hormone Receptor β (THRB), Aceyl-CoA oxidase 1 (ACOX) and MDR2 in liver, SREBP1c and Myocyte enhanced factor 2c (Mef2c) in muscle, PPARγ and UCP2 in inguinal adipose tissue was increased.

At 10 and 30 mg/kg dose levels rosiglitazone caused edema and hemodilution, including a reduction in RBC number and hemoglobin, while CNX-013-B2 did not cause any change in the parameters compared to untreated control Wistar rats. Long term treatment of Wistar rats with 25, 50 and 100 mg/kg of CNX-013-B2, po, for 28 days did not cause any test article-related adverse effect on clinical chemistry, gross pathology, physiological behavior, body weight, organ weights, micropathology and urinary parameters.

In C57BL/6j mice on high fat diet CNX-013-B2 treatment enhanced exercise endurance and also increased muscle creatine phosphate content. In conjunction with exercise CNX-013-B2 synergistically reduced body weight. Further CNX-013-B2 reduced adiposity in bones.

In contrast to Rosiglitazone, CNX-013-B2 treatment did not lead to edema, hemodilution and weight gain.

Methods

Study in db/db mice: Six weeks old db/db mice were orally treated with 2.5, 5.0, 10 and 15 mg/kg CNX-013-B2 qd (9am) for 8 weeks. Fasting and fed glucose, 24h glucose profile, serum TG, body weight and food intake were measured during study and HbA1c, serum LDL, organ weights, muscle SDH activity and organ-wise mRNA expression of selected genes were determined at the end of the treatment.

Vascular permeability and edema in Wistar rats: Wistar rats were treated with either CNX-013-B2 or Rosiglitazone at doses of 10 and 30 mg/kg b.wt, po for 4 weeks. Weekly Body weight & daily feed consumption was recorded. Hematological parameter was estimated at end of week 4 of treatment. Animals were cannulated at the end of the study to perform Evans Blue dye leakage determination.

Endurance study in C57 mice: 6 week old male C57BL/6J mice were fed high fat diet (HFD - D12492; 60% kcal from fat) for 11 weeks and then treated with 5, 10 and 20 mg/kg CNX-013-B2, qd, po for 4 weeks. Untrained control and treated animals were subjected to treadmill running till exhaustion. For the exercise-B2 synergy study animals in the treatment group received 10 mg/kg CNX-013-B2, qd, po. All animals were subjected to 5 days a week exercise training once a day till exhaustion.

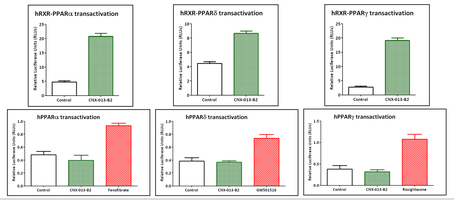
Oral repeat dose toxicity studies in Wistar rats: For the 28 day toxicity study Wistar rats were treated with 25, 50 and 100 mg/kg CNX-013-B2 (po) and in the subsequent 90 day toxicity study rats were treated at 100mg/kg. Body weight and food intake were measured weekly. Clinical chemistry, hematology, gross pathology, organ weights, micropathology and urinary parameters were recorded at the end of the treatment.

RT-PCR and Western Blotting: After study termination, cDNA and proteins were prepared from liver, adipose and muscle and used for RT-PCR (SYBR green chemistry) and western blot (Chemiluminescence) based measurements.

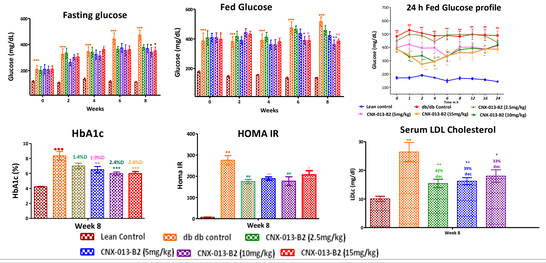
Transactivation assay: Over expression constructs of human PPAR isoforms either alone or with hRXRα were expressed in HEK293 cells along with PPRE reporter construct. Post 24 treatments with CNX-013-B2 (1μM), reporter activity was measured.

Bone adiposity in DIO mice: Seventeen weeks old male C57BL/6J mice DIO mice (11 weeks on HFD D12492; 60% kcal from fat) and age matched mice on chow diet (10% kcal from fat) were treated with 5, 10 and 20 mg/kg CNX-013-B2, qd, po or Rosiglitazone 25 mg/kg, po, od for 8 weeks . Decalcified bone sections were subjected for H&E staining to demonstrate bone adiposity.

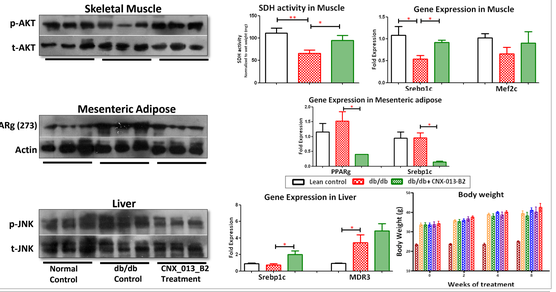
1 *CNX-013-B2 does not activate human PPAR isoforms directly*



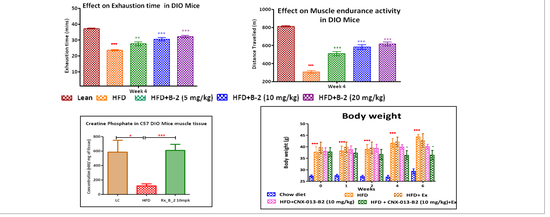
2 *CNX-013-B2 provides robust glycemic and lipid control*



3 *CNX-013-B2 has significant effects in muscle, adipose and liver; prevents weight gain*

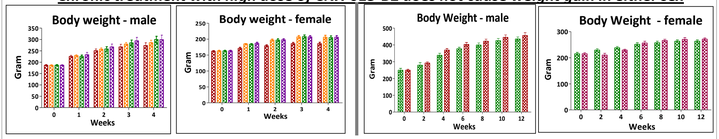


4 *CNX-013-B2 enhances endurance and in synergy with exercise reduces body weight*

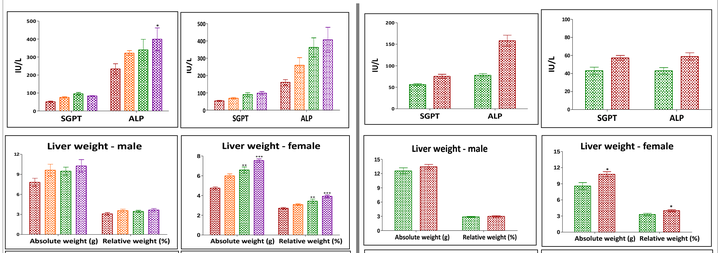


5 28 day Tox / 90 day Tox

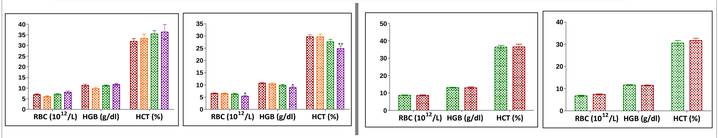
Chronic treatment with high dose of CNX-013-B2 does not cause weight gain in either sex



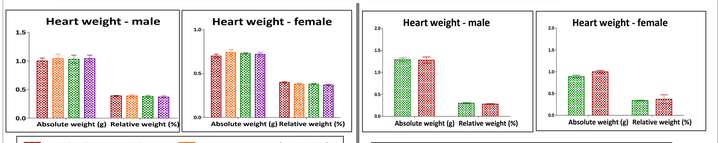
Chronic treatment with high dose of CNX-013-B2 does not cause hepatomegaly or hepatotoxicity



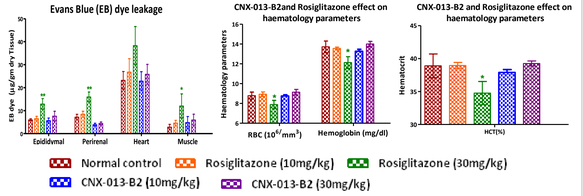
Chronic treatment with high dose of CNX-013-B2 does not cause Anemia and Hemodilution



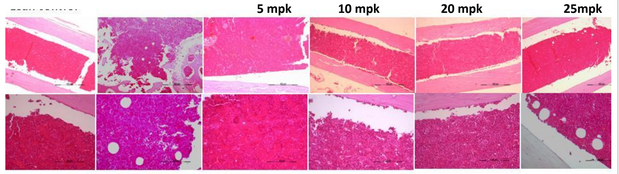
Chronic treatment with high dose of CNX-013-B2 does not increase heart weight



6 *Unlike Rosiglitazone treatment with CNX-013-B2 does not lead to Edema, Fluid Retention and Anemia*



CNX-013-B2 reduces adiposity in bones and has low risk of causing bone fractures



Summary

- CNX-013-B2 is a PPAR-pan activator and also modulates activity of other important heterodimer nuclear receptor partners in a manner favorable for the control of risk factors of the metabolic syndrome.
- Pharmacological action of CNX-013-B2 is devoid of the serious side effects of Glitazones that lead to ischemic cardiovascular events.
- It has the potential to be the 'next generation' oral anti-diabetic
  - Improves insulin sensitivity, provides robust glycemic and lipid control, reduces body weight and has cardio protective benefits.
- CNX-013-B2 significantly improves muscle physiology and function and has the hallmarks of an 'exercise mimetic'.
- In long term toxicity studies CNX-013-B2 displayed excellent 'drug like properties' and is being progressed into IND-enabling studies.
- CNX-013-B2 is being assessed for its therapeutic impact on other disease indications such as multiple sclerosis, Alzheimer's, Cachexia and cancer.