Selective Activation of Peroxisome Proliferator-Activated Receptor β/δ holds promise for the treatment of obesity, metabolic syndrome and diabesity

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ABSTRACT

Using a highly selective peroxisome proliferator-activated receptor β/δ (PPARδ) agonist KD3010, we demonstrate that activation of PPARδ shows promise in ameliorating some of the root causes of metabolic derangements observed in obesity, metabolic syndrome and diabesity. Oral administration of KD3010 to mice fed high fat diet (HFD), attenuated diet-induced obesity (DIO), reduced visceral adiposity, adipocyte hypertrophy, plasma triglycerides and non-esterified fatty acid (NEFA), in addition to reversing hyperinsulinemia and improving insulin resistance. The attenuation of weight gain by KD3010 in this model was reversible and repeatable with no effect on satiety. The effects of KD3010 in the DIO model were accompanied by an up regulation of genes involved in fatty acid oxidation, lipid catabolism and energy expenditure in both muscle and adipose tissue of mice. In leptin-deficient *ob/ob* mice, KD3010 attenuated hyperglycemia, hypertriglyceridemia and hyperinsulinemia, and increased HDL cholesterol levels. KD3010 also increased glucose disposal during glucose tolerance testing (GTT) and demonstrated improved insulin sensitivity during insulin tolerance testing (ITT) in both models. The improved insulin sensitization by KD3010 could be explained, in part, by the up regulation of GLUT4 mRNA in both adipose tissue and muscle in these mice. Interestingly, KD3010 also reversed HFD-induced hepatic steatosis and reduced serum transaminases in *ob/ob* mice. These results suggest a potential for KD3010 to improve hepatic dysfunction driven by dyslipidemia. Pharmacokinetic and pharmacodynamic (PK/PD) studies reveal that KD3010 partitions into appropriate tissues to affect the observed pharmacology. KD3010, therefore, affects various facets of metabolic derangement, holding promise for the treatment of obesity, metabolic syndrome and diabesity.

INTRODUCTION

PPARβ/δ, one of the three PPAR isoforms, is a member of the nuclear receptor superfamily, and is expressed in several metabolically active sites such as liver, muscle and fat. Tissue specific overexpression and knock-out studies suggest a role of PPARδ in obesity and metabolic syndrome. Therefore, specific and selective PPARδ ligands hold promise for the treatment of metabolic disorders. We have evaluated the pharmacological efficacy of a highly selective and potent PPARδ ligand KD3010 for the treatment of metabolic syndrome and obesity using two well established murine models. The HFD-induced model of obesity in mice exhibits several features of polygenic metabolic syndrome in humans and the leptin deficient *ob/ob* mice represent a monogenic model of obesity. Based on the observed pharmacology in these models and the PK/PD relationships, we believe that KD3010 holds significant promise for the treatment of metabolic disorders.

KD3010: Selective PPARδ Agonist

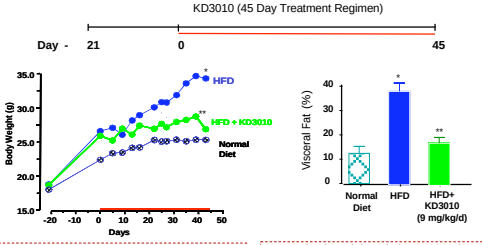
	Full Length PPARδ EC_{50} (nM)	Efficacy	PPARα Selectivity	PPARγ Selectivity	ADRP (THP-1) Efficacy
Human	1.3	80	>1000x	>1000x	95
Mouse	20	90	>500x	>500x	-

	NR Panel (17 receptors) Selectivity	PAN LABS (65 targets) EC_{50} (μM)	GPR40 EC_{50} (μM)
KD3010	>1000x	>10	>10

CONCLUSION

- **In the DIO model, KD3010 attenuates**:
  - weight gain in a reversible and repeatable manner without affecting satiety
  - visceral adiposity and adipocyte hypertrophy
  - hyperinsulinemia and improves peripheral insulin resistance
  - hypertriglyceridemia and attendant hepatic steatosis
- **In the *ob/ob* model, KD3010 improves**:
  - hyperglycemia, insulin resistance and hypertriglyceridemia with minimal effects of body weight gain
  - liver function as reflected in decreases in levels of serum transaminases

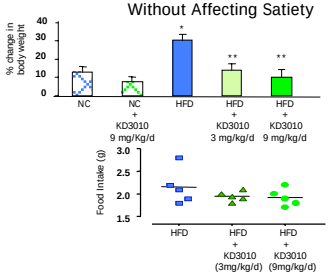
KD3010 Attenuates HFD induced Adiposity



KD3010 (9 mg/Kg/day) attenuates HFD (58% lard) induced weight gain in C57Bl/6j mice
*p ≤ 0.05 vs NC mice, **p ≤ 0.05 vs HFD mice

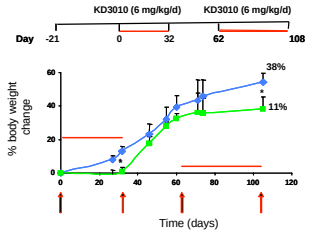
Attenuation in weight gain by KD3010 (9 mg/Kg/day) is accompanied by reduction in visceral fat as measured by Dexa analyses.
*p ≤ 0.05 vs NC mice, **p ≤ 0.05 vs HFD mice

KD3010 Attenuates Diet Induced Weight Gain Without Affecting Satiety



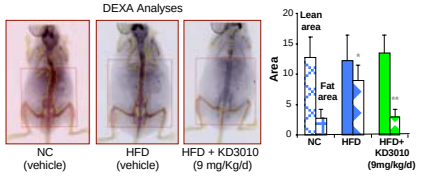
KD3010 induced attenuation in HFD-induced weight gain in C57Bl/6j mice is dose dependent.
KD3010 has no effect on satiety in this rodent model *p ≤ 0.05 vs NC mice, **p ≤ 0.05 vs HFD mice

Attenuation of HFD-induced weight gain by KD3010 is reversible and repeatable



Mice on HFD were treated with KD3010 (6 mg/Kg/day) for 32 days, taken off treatment for 30 days and treatment was reinstated for another 36 days. Attenuation of weight gain by KD3010 was repeatable in a therapeutic regimen. *p ≤ 0.05 vs HFD mice

KD3010 significantly reduces HFD-induced visceral adiposity and adipocyte hypertrophy

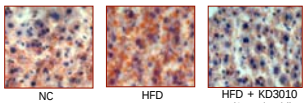


Body mass analyses was performed using the Piximus Dexa scanning. Gating was performed to demarcate the visceral trunk. Quantitation for lean vs fat mass was performed using the Piximus software. HFD increased visceral fat mass in C57Bl/6j mice. KD3010 significantly reduced the visceral fat mass without affect lean body mass *p ≤ 0.05 vs NC mice, **p ≤ 0.05 vs HFD mice

H&E staining of adipocytes from white visceral adipose tissue

Visceral white adipose tissue was removed at sacrifice and fixed in buffered formalin. 6 μM sections were made and stained using the standard H&E staining protocol. Adipocyte size and integrity was assessed using this method. Compared to NC mice HFD increased profound hypertrophy of visceral adipocytes. Treatment with KD3010 reduced HFD-induced adipocyte hypertrophy

KD3010 reduces HFD-induced hepatic steatosis



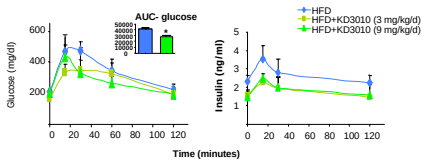
Oil Red 'O' staining was performed using frozen sections (6 μM) from liver slices removed at the termination of experiment. KD3010 blocked HFD-induced hepatic steatosis in C57Bl/6j mice

KD3010 attenuates HFD-induced fasting hypertriglyceridemia

Treatment	Total Cholesterol (mg/dL)	TG (mg/dL)
Normal Chow	91.5 ± 6.4	153.4 ± 57
HFD	229 ± 26 *	289 ± 60 *
HFD -KD3010 (3 mg/kg/d)	194 ± 21.7	211 ± 116
HFD -KD3010 (9 mg/kg/d)	210 ± 22	182 ± 47 **

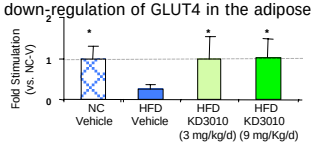
Clinical chemistry analyses of plasma samples collected on day 40 following overnight fast. *p ≤ 0.05 vs NC mice, **p ≤ 0.05 vs HFD mice

KD3010 significantly improves HFD-induced fasting hyperinsulinemia and peripheral insulin resistance



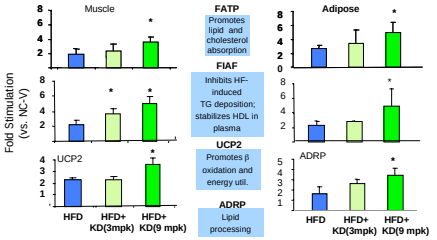
Glucose tolerance Testing (IPGTT) was performed following intraperitoneal administration of 50% dextrose solution (2 mg/Kg). Glucose and insulin levels were monitored prior to glucose bolus (fasting levels) and over time following glucose administration. Compared to vehicle treated miice, KD3010 improves HFD-induced peripheral insulin resistance as demonstrated by lower AUC-glucose (33% reduction) and AUC-insulin (43% reduction). *p ≤ 0.05 vs HFD mice

KD3010 normalizes HFD-induced down-regulation of GLUT4 in the adipose tissue



GLUT4 mRNA level was analyzed byg bDNA technology and GLUT4 specific probes using lysates from visceral white adipose tissue of NC or HFD fed mice treated with KD3010 for 14 days. Five weeks of high fat feeding down regulates GLUT4 mRNA in visceral white adipose tissue which is normalized following treatment with KD3010 for 14 days. GLUT4 levels were normalized to Cyclophillin which served as the house keeping control. *p ≤ 0.05 vs HFD mice

KD3010 induces genes involved in lipolysis and energy utilization in muscle and adipose tissue



mRNA expression of PPARδ responsive genes in muscle and adipose tissue using bDNA analyses and gene specific probes. Tissues were collected on day 45 following termination of treatment. *p ≤ 0.05 vs HFD mice

Pharmacology of KD3010 in leptin deficient *ob/ob* mice

	Glucose (mg/dL)	Insulin (μg/L)	TG (mg/dL)	FFA mEq/L	AST (U/L)	ALT (U/L)
Vehicle	389.4 ± 37.5	7.2626 ± 1	115.5 ± 8.8	1.6436 ± 0.1	353 ± 23.1	381.4 ± 28.8
KD3010 (2mg/kg/d)	288.2 ± 35	4.3901 ± 0.4	85 ± 2.3*	1.6596 ± 0.2	343 ± 63.2	358.4 ± 67.5
KD3010 (10mg/kg/d)	245.2 ± 25*	4.4551 ± 1.4*	80.4 ± 17.3	1.172 ± 0.2	248.8 ± 11.9*	251 ± 14.2*

*p ≤ 0.05 vs vehicle