The dual FXR/TGR5 agonist INT-767 reduces visceral fat mass, promoting preadipocyte brown differentiation, mitochondrial function and insulin sensitivity in a rabbit model of high fat diet-induced metabolic syndrome

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Introduction and objectives

Expanding brown adipose tissue is a potential therapeutic strategy to counteract insulin resistance and metabolic syndrome (MetS). Farnesoid X receptor (FXR) and takeda G protein-coupled receptor 5 (TGR5) activation enhances insulin sensitivity, suggesting the capacity of FXR/TGR5 agonists to promote brown differentiation in adipose tissue.

The aim of this study is to investigate the effect of a FXR/TGR5 agonist on visceral adipose tissue (VAT) by using a rabbit model of high fat diet (HFD)-induced MetS.

Methods

We employed a recently established animal model of high fat diet (HFD)-induced MetS, characterized by insulin resistance, hypertension, atherogenic dyslipidemia and VAT accumulation (Filippi et al., 2009; doi: 10.1111/j.1743-6109.2009.01467.x). Subgroups of MetS rabbits were treated with increasing doses of the dual FXR/TGR5 agonist INT-767 (3, 10, 30mg/Kg, orally, daily, 5 days a week for 12 weeks). Rabbits fed with a regular diet (RD) were taken as control. We studied the effects of HFD and in vivo INT-767 treatments on VAT function and the adipogenic potential of rabbit preadipocytes (rPAD) isolated from VAT of regular diet (RD), HFD, and INT-767-treated HFD rabbits. VAT was studied by immunohistochemistry, western blot, and RT-PCR. Isolated rPADs were cultured for 10 days in differentiation medium (to evaluate the spontaneous adipogenic potential) or were exposed in vitro to a differentiating mixture (Insulin, dexameethazone, isobutylmethylxantine, DMI), known to favor while adipogetic phenotyping.

Results

Beneficial metabolic effects of INT-767 treatments in HFD-induced MetS model

INT-767, at all doses tested, significantly reduced VAT mass, even below the RD level. INT-767 reduced dose dependently several parameters related to MetS, such as hyperglycemia, glucose intolerance and hypercholesterolemia. At all tested doses of INT-767 we found increased HDL levels, compared to either RD or HFD groups. HFD-induced hypertriglyceridemia was reduced by all INT-767 treatments, without reaching statistical significance when compared to HFD. However, INT-767 restored in all the treated groups triglyceride levels up to the RD level. In contrast, HFD-induced increase in MAP was not affected by any treatment. Prevalence of MetS (three or more factors higher than two standard deviations of values recorded in RD rabbits) was decreased from 63.6% in HFD to 40%, 0% and 0% in INT-767 3, 10, 30 mg/Kg, respectively.

INT-767 treatment improves mitochondrial function and enhances in preadipocytes expression of genes involved in brown adipogenesis and mitochondrial biogenesis.

Quantitative RT-PCR analysis

Moreover, in vivo treatments of HFD rabbits with different doses of INT-767 increased mRNA expression of several genes involved in brown adipogenesis (UCP1, CIDEA, BMP4, BMP7, HOXC9, TMEM26, LGI8), mitochondrial biogenesis (Tfam, NRF1), membrane respiratory chain (SLC25A12, NDUF3, NDUF8, SDHb, pro-fusion (MFN2), pro-fission (FIS1) proteins of mitochondria and cGMP signaling (GCa, GCb, PKG1).

In vivo INT-767 treatment on mitochondrial ultrastructure in rPAD. This analysis was performed using transmission electron microscopy.

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Insulin sensitivity in DIM-induced rPADs.

Glucose uptake in response to insulin increased dose dependently, with the expected EC50 in all rPADs (shared EC50=1.65±0.6 nM). However, the maximal effect of insulin was significantly reduced in HFD rPADs (ECmax=114.2±5.8%, as compared with RD (ECmax=180.1±9.5%, p=0.002). Interestingly, all INT-767 treatments increased insulin EC50 when compared to HFD (EC50 INT-767 3 mg/Kg=174.9±1%, p=0.027; EC50 INT-767 10mg/Kg=153.6±7.6, p=0.01; EC50 INT-767 30mg/Kg=150.9±7.4, p=0.01), although the EC50 of both INT-767 10 mg/Kg and INT-767 30 mg/Kg groups was still lower than that of the RD rabbits (p=0.006).

INT-767 treatment counteracts HFD-induced VAT remodelling

The dual FXR/TGR5 agonist INT-767 ameliorates the metabolic profile and reduces visceral adiposity by improving insulin sensitivity and promoting brown differentiation in visceral adipose tissue.

Conclusions

The dual FXR/TGR5 agonist INT-767 ameliorates the metabolic profile and reduces visceral adiposity by improving insulin sensitivity and promoting brown differentiation in visceral adipose tissue.