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Research report

NESS038C6, a novel selective CB1 antagonist agent with anti-obesity activity and improved molecular profile

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HIGHLIGHTS

- NESS038C6 produced a significant weight loss in DIO mice fed with a fat diet.
- Chronic treatment with NESS038C6 improved cardiovascular risk factors.
- NESS038C6 regulated the molecular pathways between hypothalamus and fat tissue.
- NESS038C6 upregulated metabolic enzymes and PPAR-α mRNA in the liver.
- Our compound upregulated monoaminergic transporters and neurotrophic factors mRNA.

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ABSTRACT

The present work aims to study the effects induced by a chronic treatment with a novel CB1 antagonist (NESS038C6) in C57BL/6N diet-induced obesity (DIO) mice. Mice treated with NESS038C6 and fed with a fat diet (NESS038C6 FD) were compared with the following three reference experimental groups: DIO mice fed with the same fat diet used for NESS038C6 and treated with vehicle or the reference CB1 antagonist/inverse agonist rimonabant, "VH FD" and "SR141716 FD", respectively; DIO mice treated with vehicle and switched to a normal diet (VH ND).

NESS038C6 chronic treatment (30 mg/kg/day for 31 days) determined a significant reduction in DIO mice weight relative to that of VH FD. The entity of the effect was comparable to that detected in both SR141716 FD and VH ND groups.

Moreover, if compared to VH FD, NESS038C6 FD evidenced: (i) improvement of cardiovascular risk factors; (ii) significant decrease in adipose tissue leptin expression; (iii) increase in mRNA expression of hypothalamic orexigenic peptides and a decrease of anorexigenic peptides; (iv) expression increase of metabolic enzymes and peroxisome proliferator-activated receptor-α in the liver; (v) normalization of monoaminergic transporters and neurotrophic expression in mesolimbic area. However, in contrast to the case of rimonabant, the novel CB1 antagonist improved the disrupted expression profile of genes linked to the hunger-satiety circuit, without altering monoaminergic transmission.

In conclusion, the novel CB1 antagonist compound NESS038C6 may represent a useful candidate agent for the treatment of obesity and its metabolic complications, without or with reduced side effects relative to those instead observed with rimonabant.

1. Introduction

Obesity has become a major public health concern in industrialized countries. Worldwide there are 1.1 billion overweight people with a BMI between 25 kg/m^2 and 30 kg/m^2 and 312 million with a BMI >30 kg/m^2 [20]. A projection for the year 2030 estimates that 366 million people will suffer from obesity [54]. In addition, abdominal obesity is significantly associated with various metabolic abnormalities, including insulin resistance, impaired glucose tolerance/type-2 diabetes, and atherogenic dyslipidaemia

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with low high-density lipoprotein (HDL) cholesterol, high triglycerides, and increased small dense low-density lipoprotein (LDL) cholesterol [45,54,59]. These metabolic disorders may be attributed in part to increased endocannabinoid activity [55]. The selective cannabinoid 1 (CB1) receptor antagonist/inverse agonist rimonabant has been shown to reduce body weight, waist circumference, insulin resistance, triglycerides, dense LDL, and blood pressure, and to increase HDL and adiponectin concentrations in both non-diabetic and diabetic overweight/obese patients [62]. Despite rimonabant withdrawn from European market in 2008, principally due to its adverse effects on CNS, including depression and anxiety, the development of anti-obesity drugs targeting CB1R has been recently relaunched. The new strategies are principally based on the discovery of novel CB1 antagonist compounds selectively acting at peripheral level in order to eliminate CNS side effects, and maintaining therapeutic benefits in metabolic syndrome and associated diseases [65].

In this work, we tested the effects of a new CB1 antagonist, NESS038C6, on the metabolic pathways in a mouse model of diet-induced obesity (DIO mice). The novel CB1 derivative was compared to rimonabant. We first measured the impact of NESS038C6 on body weight, caloric intake and body mass index. Moreover, we examined the effect of CB1 blockade on both biochemical blood parameters and mRNA expression of metabolic markers in the liver and in the visceral fat associated to the hypothalamic pathway of food intake. In addition, we determined in the several mesolimbic areas the gene expression of monoamine transporters and neurotrophic factors involved in the metabolic syndrome.

2. Materials and methods

2.1. Chemicals

NESS038C6 (*N*-piperidinyl-7-bromo-1-(2′,4′-dichlorophenyl)-4,5-dihydro-1H-thieno[2,3-g] indazol-3-carboxamide) was synthesized according to the previously reported procedure described in US Patent 7,485,730 [33]. The compounds showed CB1 and CB2 affinity expressed as Ki of 4,47 and 36,75 nM, respectively. CB1 antagonism behavior was previously highlighted for NESS038C6 by both isolated organ assays and in vivo test based on rat intestinal motility (data not shown). CB1 antagonist/inverse agonist *N*-piperidinyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (rimonabant) was purchased by KEMPROTECH Limited, Middlesbrough, UK.

For in vivo assays, daily prepared formulations were employed by solubilization of the compounds in 0.9% Saline containing Tween 80 (Sigma Aldrich, Milan, Italy), ratio of 8:1 (v/v).

2.2. Animals

The experiments were performed according to the UE (CEE N°86/609) guidelines for the care and use of experimental animals.

A total of 48 male C57BL/6N DIO mice (30–40 g) were purchased from Charles River (Calco, Lecco, Italy) at 10 weeks of age. The mice were housed one animal per cage in environmentally controlled conditions (22° ± 1 °C, and maintained on a reverse 12:12 h light/dark cycle) and fed ad libitum with a high fat diet (D12492, 60% fat, 20% carbohydrate, 20% protein, total 5.24 kcal/g; Research Diets Inc., New Brunswick) for 1 week. DIO mice were then weighed and divided into 4 groups of 12 animals each and exposed to a 31 days chronic treatment:

"VH FD": DIO mice fed with diet D12492 and treated with vehicle (Tween 80 in saline solution, ratio of 8:1 v/v);

"NESS038C6 FD": DIO mice fed with diet D12492 and treated with NESS038C6 (30 mg/kg);

"SR141716 FD": DIO mice fed with diet D12492 and treated with rimonabant (10 mg/kg);

"VH ND": DIO mice switched to a normal chow (D12450B, 10% fat, 70% carbohydrate, 20% protein, total 3.85 kcal/g; Research Diets Inc., New Brunswick) and treated with vehicle.

2.3. Chronic treatment

Rimonabant, NESS038C6, and vehicle were administered by oral gavage once per day. Rimonabant and NESS038C6 were administered at a dose of 10–30 mg/kg/day, respectively. The dosage of rimonabant was chosen according to previously reported data [34], while that of NESS038C6 was selected on the basis of the value of the CB1 Ki compare to that of the reference compound. Individual body weight, caloric intake and cage food consumption were recorded daily. Body length was measured at the start and at the end treatment for the calculation of body mass index (BMI) according to the formula reported elsewhere [14]. At the end of the experimental period, blood samples were collected for immediate assessment of serum biochemical parameters. The brain regions (hypothalamus, prefrontal cortex, amygdala, nucleus accumbens, hippocampus), the liver and white total adipose tissues (epididymal, lumbar, and perirenal) were removed, weighed and immediately stored at −80 °C until RNA analysis.

Table 1
Oligonucleotides TaqMan® MGB probe genes with assay ID used for semi-quantitative real-time PCR amplifications in the the brain and peripheral tissues.

Genes	Tissues
Cannabinoid receptor 1, Mm00432321_s1, CB1	Hypothalamus, Liver, Visceral fat
Neuropeptide Y, Mm00445771_m1, NPY	Hypothalamus
Agouti-related protein, Mm00475829_g1, AGRP	Hypothalamus
Cocaine and amphetamine regulated transcript, Mm00489086_m1, CART	Hypothalamus
Proopiomelanocortin, Mm00435874_m1, POMC	Hypothalamus
Serotonin transporter, Mm00439391_m1, SERT	Nucleus accumbens, Amygdala, Prefrontal cortex
Norepinephrine transporter, Mm00436661_m1, NET	Nucleus accumbens, Amygdala, Prefrontal cortex
Dopamine transporter, Mm00438388_m1, DAT	Nucleus accumbens, Amygdala, Prefrontal cortex
Brain derived neurotrophic factor, Mm04230607_s1, BDNF	Hippocampus
Nerve growth factor, Mm00443039_m1, NGF	Hippocampus
Insulin receptor, Mm00439694_m1, INS-R	Hypothalamus
Leptin receptor, Mm 00440181_m1, LEP-R	Hypothalamus
Adiponectin, Mm01343606_m1, ADIPOQ	Visceral fat
Leptin, Mm 00434759_m1, LEP	Visceral fat
Peroxisome proliferator-activated receptor-α, Mm00440939_m1, PPAR-α	Liver
Peroxisome proliferator-activated receptor-γ, Mm01184322_m1, PPAR-γ	Visceral fat
Fatty acid synthase, Mm00662319_m1, FAS	Liver, Visceral fat
Glucokinase, Mm01183091_m1, GLUK	Liver
Pyruvate kinase, Mm00443090_m1, PYK	Liver

2.4. Blood analysis

Blood samples (300 μL, approximately) were collected from orbital sinus and centrifuged at 1000 g for 10 min. Triglycerides (TG), glucose (GLU), total cholesterol (CHO), and transaminase ALT and AST, were determined in the serum samples by a KEYLAB LiquiVet® Analyzer using kits from BPC BioSed srl (Castelnuovo di Porto, Rome, Italy).

2.5. RNA extraction and cDNA synthesis

Frozen dissected central and peripheral tissues were rapidly thawed on ice. Total RNA was extracted by Trizol (Life technologies, Monza, Italy) following manufacturer instructions. The extraction procedure was an improvement of the RNA isolation method by Chomczynski and Sacchi [7]. RNA concentration and purity were estimated by absorbance at 260–280 nm. After digestion with DNAse, RNA (0.5 μg) was retrotranscribed into a cDNA molecule by SuperScript® VILO™ cDNA Synthesis Kit (Life technologies, Monza, Italy). An aliquote (3.5 μl) of cDNA was then amplified by Real time PCR.

2.6. Real time PCR

Real time PCR was used to evaluate mRNA expression levels of genes in the brain and peripheral tissues showed in Table 1. Beta-actin was used as housekeeping gene. TaqMan® MGB probe genes were amplified in single, parallel reactions to normalize any variation of RNA or cDNA quality or quantity. PCR was performed in duplicate in 96 wells optical plates (Life technologies, Monza, Italy) by the ABI Prism 7000 Sequence Detection instrument (Life technologies, Monza, Italy). PCR conditions were: 95 °C, 10 min; 50 cycles 95 °C, 15 s and 60 °C, 1 min. Relative quantification was performed using the comparative C(T) method also referred to as the $2^{(-\Delta\Delta CT)}$ method.