Molecular and Cellular Endocrinology 412 (2015) 36–43

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Bardoxolone methyl prevents insulin resistance and the development of hepatic steatosis in mice fed a high-fat diet

Danielle Camer [a], Yinghua Yu [a], Alexander Szabo [a,b], Chi H.L. Dinh [a], Hongqin Wang [a], Licai Cheng [a], Xu-Feng Huang [a,*]

[a] Centre for Translational Neuroscience, Faculty of Science, Medicine and Health and Illawarra Health and Medical Research Institute, University of Wollongong, NSW 2522, Australia
[b] ANSTO LifeSciences, Australian Nuclear Science and Technology Organisation, NSW 2234, Australia

ARTICLE INFO

Article history:
Received 24 November 2014
Received in revised form 27 April 2015
Accepted 15 May 2015
Available online 19 May 2015

Keywords:
Bardoxolone methyl
High fat diet
Obesity
Insulin resistance
Hepatic steatosis

ABSTRACT

High-fat (HF) diet-induced obesity is a major risk factor for the development of insulin resistance and hepatic steatosis. We examined the hypothesis that bardoxolone methyl (BM) would prevent the development of insulin resistance and hepatic steatosis in mice fed a HF diet. C57BL/6J male mice were fed a lab chow (LC), HF (40% fat), or HF diet supplemented with 10 mg/kg/day BM orally for 21 weeks. Glucose metabolism was assessed using a glucose tolerance test (GTT) and insulin sensitivity test (IST). Signalling molecules involved in insulin resistance, inflammation, and lipid metabolism were examined in liver tissue via western blotting and RT-PCR. BM prevented HF diet-induced insulin resistance and alterations in the protein levels of protein tyrosine phosphatase 1B (PTP1B), forkhead box protein O1 (FOXO1) and BDNF, and expression of the *insulin receptor (IR)*, *IRS-1* and *glucose-6-phosphatase (G6Pase)* genes. Furthermore, BM prevented fat accumulation in the liver and decreases in the β-oxidation gene, *peroxisomal acyl-coenzyme A oxidase 1 (ACOX)* in mice fed a HF diet. In the livers of HF fed mice, BM administration prevented HF diet-induced macrophage infiltration, inflammation as indicated by reduced IL-6 and signal transducer and activator of transcription 3 (STAT3) protein levels and *TNFα* mRNA expression, and increased *nuclear factor-like 2 (Nrf2)* mRNA expression and nuclear protein levels. These findings suggest that BM prevents HF diet induced insulin resistance and the development of hepatic steatosis in mice fed a chronic HF diet through modulation of molecules involved in insulin signalling, lipid metabolism and inflammation in the liver.

1. Introduction

Obesity is a major risk factor for the development of insulin resistance, type 2 diabetes and hepatic steatosis (Forouhi and Wareham, 2010; Kahn et al., 2006). It is widely accepted that high-fat (HF) diet-induced obesity causes increased fat accumulation, macrophage infiltration and chronic inflammation in peripheral tissues (Weisberg et al., 2003; Xu et al., 2003). Increased fat accumulation and inflammation promote insulin resistance and tissue injury in peripheral tissues involved in glucose and fat metabolism, such as the liver (Weisberg et al., 2003; Xu et al., 2003). A number of studies provide direct evidence demonstrating a link between obesity-associated inflammation and insulin resistance, and hepatic steatosis (Emanuela et al., 2012; Ginsberg, 2006; Qureshi and Abrams, 2007). However, there is a need to develop novel therapeutic approaches targeting hepatic inflammation and to improve obesity-induced insulin resistance and hepatic steatosis.

The activation of inflammatory molecules can promote the expression of the negative regulators of insulin signalling, protein tyrosine phosphatase B (PTP1B) and SOCS3 (Hong et al., 2001; Zabolotny et al., 2008). PTP1B levels are increased in the liver of HF diet-induced obese mice, which contributes to the development of insulin resistance by reducing insulin signalling through inhibition of insulin receptor (IR) and insulin receptor substrate 1 (IRS-1) activation (Goldstein et al., 2000; Lam et al., 2006). SOCS3 is another important molecule which impairs insulin signal transduction in the liver through its inhibition of the binding of IR to IRS-1 (Ueki et al., 2004). Furthermore, activation of hepatic insulin signalling results in the inactivation of forkhead box protein O1 (FOXO1), which

Abbreviations: ACOX, peroxisomal acyl-coenzyme A oxidase 1; BDNF, Brain-derived neurotrophic factor; BM, bardoxolone methyl; CDDO-Im, 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole; FAS, fatty acid synthase; FOXO1, Forkhead box protein O1; HF, high fat; LC, lab chow; GTT, glucose tolerance test; G6Pase, glucose-6-phosphatase; IKKβ, Inhibitor of nuclear factor kappa-B kinase subunit beta; IKKε, Inhibitor of nuclear factor kappa-B kinase subunit epsilon; IR, insulin receptor; IST, insulin sensitivity test; NFκB, Nuclear factor kappa-B; Nrf2, Nuclear factor-like 2; PTP1B, Protein tyrosine phosphatase 1B; SCD1, stearoyl-CoA desaturase 1; STAT3, Signal transducer and activator of transcription 3.

* Corresponding author. Illawarra Health and Medical Research Institute, School of Medicine, University of Wollongong, Northfields Avenue, NSW 2522, Australia. Tel.: +61 02 42214300; fax: +61 02 42214096.
E-mail address: xhuang@uow.edu.au (X.-F. Huang).

http://dx.doi.org/10.1016/j.mce.2015.05.018
0303-7207/

is a transcription factor inhibiting genes such as *glucose-6-phosphatase (G6Pase)* for endogenous glucose production via gluconeogenesis (German et al., 2009; Nakae et al., 2001). When insulin signalling is impaired, through inhibition by PTP1B or SOCS3, and activation of FOXO1, this leads to the promotion of glucose production and a reduction in glucose reuptake, leading to glucose intolerance and insulin resistance in obesity (German et al., 2009; Nakae et al., 2001). Brain-derived neurotrophic factor (BDNF) also plays an import role in insulin action as it has been found to modulate hepatic glucose metabolism via its actions on glucokinase (GK) in obese insulin resistant rats (Kuroda et al., 2003). In the liver, GK enhances glycolysis, resulting in reduced blood glucose levels (Hariharan et al., 1997).

A HF diet is known to cause fat accumulation in the liver, which can progressively worsen to hepatic steatosis (Marchesini et al., 2001). Hepatic lipid homeostasis is regulated by a number of genes that promote lipogenesis, including *ACC*, *FAS* and *SCD1*, and β oxidation, such as *ACOX* (Musso et al., 2009). Hepatic fat accumulation leads to macrophage infiltration which promotes the production of pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumour necrosis factor alpha (TNFα) and IL-1 β (McArdle et al., 2013). Increased IL-6 has been found to enhance inflammatory signalling by increasing signal transducer and activator of transcription 3 (STAT3) levels, which promotes cytokine dependent signalling by increasing the expression of inflammatory genes such as IL-6 (Yang et al., 2007). In addition, inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ) and inhibitor of nuclear factor kappa-B kinase subunit epsilon (IKKε) are important pro-inflammatory signalling molecules upstream of the transcription factor, nuclear factor kappa-B (NFκB), which promote PTP1B and SOCS3 activation (Hong et al., 2001; Napetschnig and Wu, 2013; Zabolotny et al., 2008).

The oleanolic acid synthetic derivative, bardoxolone methyl (BM), has attracted wide attention due to its anti-inflammatory effects (Liby and Sporn, 2012; Reisman et al., 2012; Wang et al., 2011). Its ability to directly up-regulate the potent anti-inflammatory molecule, nuclear factor-like 2 (Nrf2), has demonstrated therapeutic benefits in human clinical trials for treating chronic kidney disease and advanced solid tumours (Hong et al., 2012; Liby et al., 2007; NIH, 2012; Pergola et al., 2011). BM has also been found to directly influence the activity of pro-inflammatory signalling through IKKβ (Ahmad et al., 2006). Furthermore, a recent study demonstrated that 2 week administration of BM decreased hepatic inflammation in diet-induced obese mice (Saha et al., 2010). In addition, previous studies have demonstrated that oral administration of a derivative of BM, 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im), prevents HF diet-induced obesity and attenuates diabetes in mice (Shin et al., 2009; Uruno et al., 2013). In this study, we investigated whether chronic oral BM administration in mice fed a HF diet for 21 weeks could prevent insulin resistance and liver injury in mice fed a HF diet. We also examined signalling molecules involved in insulin resistance, inflammation, and lipid metabolism in liver tissue.

2. Materials and methods

2.1. Animals and HF diet-induced obesity model

Male C57BL/6J mice (12 weeks old) were purchased from the Animal Resource Centre (Perth, Western Australia) and maintained in the animal facility at the University of Wollongong. The experiments were performed in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*. All procedures were approved by the Animal Ethics Committee, University of Wollongong, Wollongong, Australia (AE 12/15). Mice were housed in environmentally controlled conditions (temperature 22 °C, 12 hr light/dark cycle) and 1 week after acclimatisation were randomly divided into 3 groups (n = 7 per group). For the next 21 weeks one group of mice were fed a lab chow (LC) diet (5% of energy as fat; Vella Stock Feeds, Doonside, New South Wales, Australia), and the other two groups a HF diet (40% of energy as fat; SF11-095, Specialty Feeds, Glen Forrest, Western Australia), and mice in the treatment group were fed a HF diet for 21 weeks and an oral daily dose of BM (10 mg/kg) in their drinking water (Table 1) (final average body weight after 21 weeks: LC, 27.15 g; HF, 40.84 g; BM, 28.13 g). HOMA-IR was calculated using the formula, (Fasting Insulin × Fasting Glucose)/22.5.

Table 1
Composition of the high fat and lab chow diets.

	High fat diet	Lab chow diet
Total energy (kcal/100 g)		
Fat	40	5
Carbohydrate	45	75
Protein	15	20

Typical ingredients.
High fat diet: Casein (acid), sucrose, lard, sunflower oil, cellulose, wheat starch, dextrinized starch, minerals, and vitamins.
Lab chow diet: Cereal grains, meat offal meal, fish offal meal, whey powder, vegetable oils, soybean protein, cereal offal, corn offal, minerals, and vitamins.

2.2. Glucose tolerance test

Mice were fasted overnight (16 hrs) before a glucose tolerance test (GTT) was performed to assess glucose clearance, following an intraperitoneal (i.p.) injection of glucose (0.5 g/kg; Sigma-Aldrich, St. Louis, MO). Blood samples were taken from the tail vein before and 30, 60 and 120 minutes following the injection of glucose. Blood glucose was measured using an Accu-Chek glucometer (Roche Diagnostics GmbH Mannheim, Germany).

2.3. Insulin sensitivity test

Mice were fasted for 5 hours before an insulin sensitivity test (IST) was performed to assess glucose clearance, following an i.p. injection of insulin (0.75 U/kg; Sigma-Aldrich, St. Louis, MO). Blood samples were taken from the tail vein before and at 30, 60 and 120 minutes following the injection of insulin. Blood glucose was measured using an Accu-Chek glucometer (Roche Diagnostics GmbH Mannheim, Germany).

2.4. Tissue collection and sample preparations

For tissue analysis, mice were euthanized at week 21 of the experiment. Tissue was dissected from the mice and immediately frozen in liquid nitrogen before being stored at –80 °C.

2.5. Oil Red O staining

Oil Red O staining was used to examine hepatic lipid accumulation as described previously (Kudo et al., 2007). Briefly, frozen liver sections (10 μm) were stained with 0.5% Oil Red O (Sigma-Aldrich) for 15 minutes and then washed. Three fields from three sections of each mouse were viewed under a Leica microscope, and digital photographs were captured. Image J software (http://imagej.nih.gov/ij/download.html) was used to quantify the staining, which corresponds to the percentage of stained lipid droplets on an area of each slide (Mehlem et al., 2013).

2.6. Haematoxylin and eosin (H&E) staining

To determine the degree of liver damage fresh frozen liver sections (10 μm) were stained with haematoxylin and eosin for 30 s each. Three fields from three sections of each mouse were viewed