Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Interleukin-6 gene transfer reverses body weight gain and fatty liver in obese mice

Yongjie Ma, Mingming Gao, Hao Sun, Dexi Liu *

Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia, GA 30602, USA

ARTICLE INFO

Article history:
Received 9 October 2014
Received in revised form 16 January 2015
Accepted 21 January 2015
Available online xxxx

Keywords:
IL-6
Obesity
Weight loss
Insulin resistance
Energy expenditure

ABSTRACT

Interleukin-6 (IL-6) is a multifunctional protein and has a major influence on energy metabolism. The current study was designed to assess the therapeutic effect of overexpression of *Il-6* gene through gene transfer on high fat diet-induced obese mice. Hydrodynamic delivery of 1 μg pLIVE-IL6 plasmid per mouse into C57BL/6 obese mice resulted in peak level at 10 ng/ml of circulating IL-6 1 day after gene transfer and above 1 ng/ml thereafter for a period of 6 weeks. Persistent *Il-6* gene expression did not affect food intake but induced a significant reduction in body weight and improved obesity-associated hepatic steatosis. *Il-6* gene delivery enhanced thermogenic gene expression and elevated protein levels of phosphorylated STAT3, PGC1α and UCP1 in brown adipose tissue. *Il-6* overexpression elevated mRNA levels of lipolysis genes, triggered phosphorylation of STAT3, AMPK, and ACC, and increased expression of genes involved in fatty acid oxidation in skeletal muscle. IL-6 did not affect macrophage infiltration but maintained the M2 macrophage population in adipose tissue. Collectively, these results suggest that overexpression of the *Il-6* gene by hydrodynamic gene delivery induces weight loss and alleviates obesity-induced fatty liver and insulin resistance, supporting the notion that gene transfer is a valid approach in managing obesity epidemics.

1. Introduction

Interleukin-6 (IL-6), produced by immune and non-immune cells, is a multifunctional cytokine. IL-6 is commonly known as a pro-inflammatory cytokine that regulates immunoreaction and acute immune response. More recent studies have shown that IL-6 has an anti-inflammatory activity as it increases the production of IL-10, IL-1 receptor antagonist (IL-1ra), and soluble TNF-receptors [1–3]. In addition to modulating the immune system, IL-6 has also attracted particular attention for its pivotal role in regulating energy expenditure, body composition and peripheral lipid metabolism [4–6].

The effects of IL-6 on nutrient homeostasis and obesity remain controversial and unresolved. IL-6 is generally considered an obesity-related inflammatory factor and mediator for insulin resistance as elevated circulating IL-6 is correlated with adiposity and insulin resistance in humans [7–9]. However, substantial evidence suggests that IL-6 exerts a beneficial influence in this context. Physical exercise promotes secretion of IL-6 from skeletal muscle and the blood concentration of IL-6 increases about 100-fold, improving insulin sensitivity [10,11], while IL-6 knockout mice developed obesity, systemic insulin resistance and inflammation [4,12], which could be partially reversed by intra-cerebriventricular injection of the IL-6 protein [4]. Meanwhile, direct delivery of adeno-associated viral vectors containing the *Il-6* gene into the rat hypothalamus or mice carrying the human *Il-6* gene suppressed weight gain and visceral adiposity [13,14], indicating that IL-6 exerts anti-obesity effects in rodents. Moreover, short-term intracerebroventricular injections of the recombinant IL-6 protein and a peripheral increase in *Il-6* gene expression induced weight loss and improved insulin sensitivity in mice [15,16]. Although these studies support the potential application of IL-6 in fighting obesity, the systemic effects of prolonged elevation of IL-6 level in obese mice remain unknown.

Here we preserve sustained *Il-6* expression in obese mice by hydrodynamic gene delivery and demonstrate that long-term maintenance of IL-6 levels significantly induced weight loss in obese mice by enhancing lipolysis and energy metabolism, resulting in improved insulin sensitivity and maintenance of glucose homeostasis as well as a reduction in fat accumulation in the liver. *Il-6* gene transfer did not affect macrophage infiltration, but countered the obesity-related inflammatory signal by enhancing the expression of M2 macrophage gene markers and related anti-inflammatory factor genes. Our results indicate the long-term beneficial effects of IL-6 elevation on obesity and suggest the potential application of *Il-6* gene transfer as a means of controlling obesity and obesity-related metabolic disorders.

* Corresponding author at: Department of Pharmaceutical and Biomedical Science, University of Georgia College of Pharmacy, 450 Pharmacy South, 250 West Green Street, Athens, GA 30602, USA. Tel.: +1 706 542 7385; fax: +1 706 542 5358.
E-mail address: dliu@uga.edu (D. Liu).

http://dx.doi.org/10.1016/j.bbadis.2015.01.017
0925-4439/

2. Methods and materials

2.1. Plasmid construction

A mouse *Il-6* open reading fragment was obtained from OriGene (Rockville, MD) and reconstructed into a pLIVE plasmid vector (Mirus Bio, Madison, WI) at the *NheI* and *XhoI* cutting sites. The insertion in the new plasmid (pLIVE-IL6) was confirmed using DNA sequencing. The pLIVE-IL6 and empty plasmids were purified using CsCl–ethidium bromide density-gradient ultracentrifugation and kept in saline (0.9% sodium chloride). Purity of the plasmids was verified with absorbency ratio at 260 and 280 nm and by 1% agarose gel electrophoresis.

2.2. Animal treatment

C57BL/6 mice (male, 10-week old) were purchased from Charles River Laboratories (Wilmington, MA) and housed under standard conditions with a 12 h light–dark cycle. All procedures performed on animals were approved by the Institutional Animal Care and Use Committee at the University of Georgia, Athens, Georgia. Obese mice (50 ± 2 g) were developed by feeding animals for 20 weeks with high fat diet (HFD) (60% kJ/fat, 20% kJ/carbohydrate, 20% kJ/protein, Bio-Serv, Frenchtown, NJ). Regular mice were fed with a standard laboratory chow diet (20% kJ/fat, 60% kJ/carbohydrate, 20% kJ/protein, LabDiet, St Louis, MO). Food consumption was determined by measuring the difference between the amount provided and the amount left in every three days. Food intake per mouse was calculated based on the amount consumed divided by time and the number of mice per cage. The procedure for hydrodynamic gene delivery in obese mice was performed according to an established procedure [17,18] with some modification. Briefly, an appropriate volume of saline solution (equivalent to 8% lean mass of an obese mouse) containing 1 μg pLIVE-IL6 or empty plasmid was injected through the tail vein within 5–8 s. Mice were continually fed an HFD for 6 weeks. Body weight of each mouse was measured on an electronic balance, and the body composition was analyzed using EchoMRI-100 (Echo Medical Systems, Houston, TX) once per week. The same procedures were also performed on age-matched male mice with chow feeding. Blood was collected at the desired time points using a Microvette-CB300-LH from Fisher Scientific (Pittsburgh, PA) and plasma IL-6 concentrations were determined using ELISA kits from eBioscience (San Diego, CA). Rectal temperature of the mice at desirable time points was measured using a specially designed Thermocouple Meter from Kent Scientific Corp (Torrington, CT).

2.3. Evaluation of glucose homeostasis and insulin sensitivity

Insulin concentrations were determined using a Mercodia Insulin ELISA kit from Mercodia Developing Diagnostics (Winston Salem, NC) following the provided protocol. For the glucose tolerance test (GTT), mice were injected intraperitoneally with glucose at 1.5 g/kg body weight after 6 h fasting. Blood samples were taken at varying time points and glucose concentrations were determined using glucose test strips and TUREtrack glucose meters from Nipro Diagnostics Inc. (Fort Lauderdale, FL). For the insulin tolerance test (ITT), mice fasted for 4 h and blood glucose levels were measured after an intraperitoneal injection of insulin (0.75 U/kg) from Eli Lilly (Indianapolis, IN). HOMA-IR values were calculated based on the formula: (fasting insulin [μU/ml] × fasting glucose [mmol/l])/22.5.

2.4. Histochemical and Oil Red O analysis

After mice were sacrificed, the liver, WAT, and BAT were collected, fixed in 10% formalin and embedded in paraffin. Hematoxylin and eosin (H&E) staining was performed using tissue sections at a thickness of 6 μm. Frozen liver sections (8 μm) were stained with 0.2% Oil Red O reagent (Electron Microscopy Sciences, Hatfield, PA) in 60% isopropanol for 15 min, washed three times with phosphate buffered saline and counter-stained with hematoxylin for 1 min. A microscopic examination was performed and photographs were taken under a regular light microscope.

2.5. Blood and hepatic lipid analysis

A quantitative determination of liver lipids was performed following an established procedure [19,20]. Briefly, liver tissues (100–200 mg) were homogenized in phosphate buffered saline (1 ml), mixed with 5 ml of organic solvent (chloroform: methanol = 2:1, vol/vol) and incubated overnight at 4 °C. The mixture was centrifuged and the organic phase collected. The collected fraction was dried and lipids were re-dissolved in 1% Triton X-100. The amounts of cholesterol, triglyceride (Thermo Fisher Scientific Inc., Waltham, MA) and free fatty acids (Wako Bioproducts, Richmond, VA) were determined following the manufacturers' instructions. The same kits were also used for blood lipid analysis.

2.6. Gene expression analysis by real time PCR

Total RNA was isolated from mouse livers, the pancreas, WAT and BAT using the TRIZOL reagent (Life Technologies, Grand Island, NY) or RNeasy Lipid Tissue Mini Kit (QIAGEN, Valencia, CA) according to the

Table 1
Primer sets for real time PCR analysis of gene expression.

Name	Forward sequence	Reverse sequence
Pparγ1	GGAAGACCACTCGCATTCCTT	GTAATCAGCAACCATTGGGTCA
Pparγ2	TCGCTGATGCACTGCCTATG	GAGAGGTCCACAGAGCTGATT
Cd36	CCTTAAAGGAATCCCCGTGT	TGCATTTGCCAATGTCTAGC
Fabp4	AAGGTGAAGAGCATCATAACCC	TCACGCCTTTCATAACACATTCC
Mgat1	TGGTGCCAGTTTGGTTCCAG	TGCTCTGAGGTCGGGTTCA
Pparα	TGTCGAATATGTGGGGACAA	AATCTTGCAGCTCCGATCAC
Acox1	CCGCAACCTTCAATCCAGAG	CAAGTTCTCGATTTCTCGACGG
Cpt1a	CTCCGCCTGAGCCATGAAG	CACCAGTGATGATGCCATTCT
Cpt1b	GGTCTCTTCTTCAAGGTCTG	CGAGGATTCTCTGGAACTGC
Srebp-1c	CCCTGTGTGTACTGGCCTTT	TTGCGATGTCTCCAGAAGTG
Acc-1	GCCTCTTCCTGACAAACGAG	TGACTGCCGAAACATCTCTG
Fas	AGAGATCCCGAGACGCTTCT	GCCTGGTAGGCATTCTGTAGT
Scd-1	TTCTTACACGACCACCACCA	CCGAAGAGGCAGGTGTAGAG
Cyp7a1	AACGGGTTGATTCCATACCTGG	GTGGACATATTTCCCCATCAGTT
Hmgcr	CTTGTGGAATGCCTTGTGATTG	AGCCGAAGCAGCACATGAT
Pepck	AAGCATTCAACGCCAGGTTC	GGGCGAGTCTGTCAGTTCAAT
G6pase	CGACTCGCTATCTCCAAGTGA	GTTGAACCAGTCTCCGACCA
Insulin1	CACTTCCTACCCCTGCTGG	ACCACAAAGATGCTGTTTGACA
Insulin2	GCTTCTTCTACACACCCATGTC	AGCACTGATCTACAATGCCAC
Hsl	GCTTGGTTCAACTGGAGAGC	GCCTAGTGCCTTCTGGTCTG
Atgl	CAACGCCACTCACATCTACGG	TCACCAGGTTGAAGGAGGGAT
Ucp1	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATTT
Ucp2	GCGTTCTGGGTACCATCCTA	GCTCTGAGCCCTTGGTGTAG
Ucp3	ATGAGTTTTGCCTCCATTCG	GGCGTATCATGGCTTGAAAT
Pgc-1α	GAAGTGGTGTAGCGACCAATC	AATGAGGGCAATCCGTCTTCA
Pgc-1β	TTGTAGAGTGCCAGGTGCTG	GATGAGGGAAGGGACTCCTC
Dio2	AATTATGCCTCGGAGAAGACCG	GGCAGTTGCCTAGTGAAAGGT
Cidea	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
Elovl3	TTCTCACGCGGGTTAAAAATGG	GAGCAACAGATAGACGACCAC
F4/80	CCCCAGTGTCCTTACAGAGTG	GTGCCAGAGTGGATGTCT
Cd68	CCATCCTTCACGATGACACCT	GGCAGGGTTATGAGTGACAGTT
Cd11b	ATGGACGCTGATGGCAATACC	TCCCCATTCACGTCTCCCA
Cd11c	ACGTCAGTACAAGGAGATGTTGGA	ATCCTATTGCAGAATGCTTCTTTACC
Mcp-1	ACTGAAGCCAGCTCTCTCTTCCTC	TTCCTTCTTGGGGTCAGCACAGAC
Tnfα	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Il-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Cd163	TCCACACGTCCAGAAACAGTC	CCTTGGAAACAGAGACAGGC
Cd206	CAGGTGTGGGCTCAGGTAGT	TGTGGTGAGCTGAAAGGTGA
Il-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Il-4	GGTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
Il-4rα	TCTGCATCCCGTTGTTTTGC	GCACCTGTGCATCCTGAATG
Il-13	CCTGGCTCTTGCTTGCCTT	GGTCTTGTGTGATGTTGCTCA
Il-13rα1	TCAGCCACCTGTGACGAATTT	TGAGAGTGCAATTTGGACTGG
Acadl	TCTTTTCCTCGGAGCATGACA	CAGACCTCTCTACTCACTTCTCCAG
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

Please cite this article as: Y. Ma, et al., Interleukin-6 gene transfer reverses body weight gain and fatty liver in obese mice, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbadis.2015.01.017