Lessons from Leptin's Molecular Biology: Potential Therapeutic Actions of Recombinant Leptin and Leptin-Related Compounds

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Abstract: Leptin, a peptide secreted by the white adipose tissue, circulates to the central nervous system and signals the status of body energy stores, regulating feeding behavior and energy balance. As human obesity is characterized by hyperleptinemia and leptin resistance, increasing leptin sensitivity is an attractive target for obesity treatment.

Key Words: Obesity, obesity treatment, leptin, leptin resistance, lipodystrophy.

INTRODUCTION

Leptin is a 16 kDa protein mostly produced by white adipose tissue that is secreted into the circulation, crosses the blood-brain barrier through receptor-mediated mechanisms and activates its specific receptor in central nervous system nuclei. Importantly, the activation of leptin receptors in the arcuate nucleus of the hypothalamus reduces feeding behavior and increases metabolic expenditure that favors negative energy balance.

Plasma leptin concentration parallels adipose tissue mass and is substantially increased in obesity. Despite marked hyperleptinemia, obese subjects tend to overeat. Furthermore, treatment of common human obesity with recombinant leptin is marginally effective. Altogether, these observations indicate that obesity is a state of relative leptin resistance. Indeed, several molecular mechanisms have been associated with leptin resistance.

Weight loss is associated with significant reductions in serum concentrations of leptin. However, endocrine-metabolic compensatory mechanisms impair weight maintenance after weight loss. Preliminary results suggest that recombinant leptin treatment may oppose these compensatory mechanisms and may facilitate weight management in formerly obese subjects.

Congenital and acquired lipodystrophy syndromes are characterized by hypotrophy of adipose tissue and toxic accumulation of lipids in non-adipose tissues. Patients with lipodystrophy exhibit insulin resistance, type 2 diabetes, hepatic and skeletal muscle steatosis. Ectopic accumulation of lipids and ensuing lipotoxicity have been associated with reduced serum concentrations of leptin (*i.e.* leptin insufficiency).

In this article, we review the central nervous system bases of leptin signaling and resistance, and potential leptin-sensitizing strategies to treat obesity. The therapeutic use of recombinant leptin in weight management of formerly obese subjects and in the treatment of lipodystrophy patients are also revisited.

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1. LEPTIN SIGNALING

Leptin receptors (OB-Rs) belong to the cytokine type I receptor family that also includes various receptors for interleukins. Six isoforms of the receptor (*i.e.* OB-Ra through OB-Rf) are synthesized through alternative splicing and classified as short, long and secretory. The OB-Ra and OB-Rc are short and mostly expressed in the choroid plexus and in the microcirculation. These receptors have been related with leptin transport through the blood-brain barrier. The soluble secretory isoform OB-Re binds leptin in the circulation and may limit the activation of OB-Rb receptors by circulating unbound leptin [1]. The long form receptor (*i.e.* OB-Rb) possesses a long intra-cytoplasmatic domain that contains diverse active motifs allowing interaction and phosphorylation of down-stream signaling proteins. The OB-Rb receptor is mainly expressed in the arcuate nucleus of the hypothalamus and other neighboring brain regions triggering diverse signal pathways that partly mediate feeding behavior and energy metabolism. Notably, three intracellular signaling pathways are activated through leptin bound to OB-Rb: 1) the Janus kinase 2 (JAK2)-signal transducer and activator of transcription protein type 3 (STAT3), or JAK-STAT pathway, 2) the phosphoinositide-3-kinase (PI3K) pathway and 3) the mitogen-activated protein kinase complex / extracellular regulated kinase pathway (MAPK/ERK) [2].

Leptin binding to OB-Rb induces homodimerization of the receptor and generates tetrameric OB-Rb-leptin complexes that promote recruitment and auto-phosphorylation of JAK2. In addition, several tyrosine residues (Tyr) located at the intracellular domain of OB-Rb are phosphorylated. Importantly, phosphorylation of Tyr^{1138} has been shown to be essential for recruitment of STAT3, which is then phosphorylated by JAK2. Phosphorylated STAT3 dimerizes, migrates to the nucleus, and activates transcription of genes encoding for enzymes that participate in lipid metabolism such as carnitine palmytoil transferase 1 (CPT 1), acetyl coenzyme A carboxylase (ACC), acyl-coenzime A oxidase (ACO) and fatty acid synthase (FAA) [3].

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STAT3 has been shown to promote pro-opiomelanocortin (POMC) expression in the arcuate nucleus in mice [4]. POMC is the precursor of α-melanocyte stimulating hormone (α-MSH) that acts mainly on the melanocortin receptor type 4 (MC-4 receptor), inhibiting feeding behavior and increasing thermogenic metabolism. Therefore, the JAK-STAT pathway appears to be associated with regulation of energy homeostasis through melanocortinergic mechanisms.

The activation of PI3K has been associated with insulin-leptin system cross talk. Both leptin and insulin receptors activation, through distinct mechanisms, phosphorylates insulin receptor substrate proteins (IRSs). This finding may partly explain leptin's ability to recruit GLUT-4 in isolated muscle cells and to increase insulin sensitivity in normal rats [5]. In addition, PI3K inhibition abrogates leptin-induced hyperpolarization of neuropeptide Y /Agouti related protein (NPY/AgRP) neurons and inhibits in part leptin-induced suppression of feeding behavior. Interestingly, pharmacologic antagonism of PI3K in the central nervous system has also been shown to inhibit leptin-dependent increases in sympathetic outflow to renal nerves in rats [6]. Thereby, leptin mechanisms that depend on the PI3K pathway might play an important role in the regulation of cardiorenal functions.

The MAPK complex activates ERK, increasing the expression of the egr-1 transcription factor and c-fos, that may participate in cell proliferation. Leptin-dependent activation of MAPK complex has also been associated with apoptosis, induction of nitric synthase, and vascular remodeling [2]. Additionally, *in vitro* studies have shown that β-integrins potentiate ERK-dependent signaling pathways in large, mature adipocytes as compared to small ones, suggesting that ERK could play an important role in the adaptation of adipose functions to cell size [7].

Indeed, ERK signaling can be activated by two pathways that depend on Src homology-containing tyrosine phosphatase (SHP2). One of these pathways does not depend on the phosphorylation of tyrosine residues after activation of the OB-Rb by leptin. The second pathway involves the binding of SHP2 to tyrosine residue 985 (Tyr^{985}) in the OB-Rb receptor that leads to the phosphorylation of SHP2. Both pathways require SHP2 phosphatase activity. The activation of these pathways promotes the expression of egr-1 transcription factor in murine hypothalamus. The hypothalamic actions of egr-1 transcription factor remain to be fully established [8].

Recently, Dr. Kamal Rahmouni (personal communication from author) has shown that leptin-induced sympathoactivation of brown adipose tissue in mice is associated with MAPK-dependent mechanisms. Thus, MAPK signaling might play an important role in leptin-dependent regulation of thermo-genic metabolism in mice.

1.1. Neuronal Pathways Associated with Leptin Signaling

Leptin signals interact with several downstream neural pathways that contribute to its hyporexigenic and metabolic effects. Importantly, leptin acts on neurons that secrete NPY, AgRP, and α-MSH [9].

Leptin receptors are densely located at the arcuate nucleus of the hypothalamus, which is considered the main site of action of leptin and an important site of energy homeostasis regulation [10]. NPY neurons are also abundant in the arcuate nucleus and project mainly to the paraventricular nucleus and lateral hypothalamus. NPY binds to 6 different G-protein-coupled receptors denominated Y-1 through Y-6. Activation of NPY receptors Y-1 through Y-5 is known to increase appetite and reduce energy expenditure.

Leptin administration into the central nervous system of rats reduces the expression of hypothalamic NPY mRNA [11]. Also, the leptin-deficient obese *ob/ob* mouse overexpresses hypothalamic NPY mRNA [12], suggesting that leptin is physiologically relevant to the modulation of NPY in the central nervous system. Indeed, NPY may play an important role in the development of obesity in the *ob/ob* mice since NPY gene knockout in the *ob/ob* mice, substantially attenuates the obese phenotype [13].

Surprisingly, knocking out NPY and its Y-5 receptor does not alter energy expenditure or abrogates weight gain in mice [14, 15]. Presumably, redundant orexigenic systems might compensate for the lack or reduction of NPY signaling and prevent starvation.

The actions of the melanocortin system on feeding behavior and energy homeostasis have been well established. Leptin increases neuronal α-MSH secretion and inhibits AgRP release. α-MSH binds to central nervous system MC-3/4 receptors, increasing metabolic expenditure and suppressing feeding behavior. The anorectic effect of α-MSH and other melanocortin agonists is mainly mediated through the activation of the MC-4 receptor. Underpinning its physiological importance, mutations of the MC-4 receptor induce obesity and hyperphagia in mice and humans [16, 17]. Importantly, antagonism of MC-4 receptors substantially decreases leptin-induced suppression of food intake [18], indicating that the satiety effect of leptin might be partially dependent on the activation of downstream α-MSH neurons.

α-MSH, along with adrenocorticotrophin (ACTH) and γ-MSH are by-products of the cleavage of POMC. Leptin receptors co-localize with POMC-neurons in the arcuate nucleus that project mainly to the paraventricular nucleus. The synthesis and release of α-MSH from arcuate nucleus POMC neurons are modulated by leptin. Indeed, central nervous system administration of leptin increases POMC mRNA [19]. Moreover, selective knockout of leptin receptors in POMC neurons induces obesity in mice [20].

AgRP is an endogenous antagonist of MC-4 receptors and, like NPY, increases food intake. Many arcuate neurons co-express AgRP, NPY and leptin receptors and project axons mainly to the paraventricular nucleus and dorsomedial hypothalamus. AgRP also reduces energy expenditure and thermogenic metabolism [21]. Transgenic mice overexpressing AgRP in the central nervous systems and other tissues develop obesity, indicating an important role of AgRP in the regulation of body weight [22].

Leptin also regulates expression of AgRP in the central nervous system. Leptin perfusion of isolated hypothalamic cells suppresses AgRP secretion [23]. *In vivo*, leptin admini-

stration to leptin-deficient *ob/ob* mice substantially reduces AgRP mRNA expression [24]. Also, high levels of leptin suppress hypothalamic AgRP after feeding [25].

Leptin may interact with other neuropeptides to regulate feeding behavior. Notably, selective deletion of leptin receptors in POMC neurons only produces a mild obesity in mice, whereas *db/db* mice with non-selective deletion of the leptin receptor develop severe obesity [20].

Cocaine/amphetamine-regulated transcript (CART) neurons are expressed in the arcuate nucleus and thought to inhibit feeding behavior, despite evidence showing that CART neuron sub-populations might also increase feeding after NPY stimulation [26]. Although several CART peptides have been isolated, CART receptor(s) remains unknown. Leptin administration to leptin-deficient *ob/ob* obese mice increases the expression of CART mRNA, suggesting that CART might contribute to the downstream leptin-dependent hypoorexigenic signals [27].

The lateral hypothalamus abundantly expresses melanin concentrating hormone (MCH), that is the agonistic ligand of MCH-1 receptor. The activation of MCH-1 receptor is associated with strong inhibition of feeding behavior. Overexpression of MCH and MCH-1 receptors in the arcuate and paraventricular nuclei was also demonstrated in rats with dietary obesity [28]. Importantly, leptin-deficient *ob/ob* mice overexpress MCH, suggesting that leptin is physiologically relevant for the regulation of MCH expression. Furthermore, knocking out the MCH gene in *ob/ob* mice (*i.e.* leptin-MCH double knockout) substantially improves obesity and glucose intolerance, and reduces glucocorticoid serum concentrations [29]. Nevertheless, the improvement of metabolic syndrome in leptin-MCH double knockout mice was not followed by significant reductions in food intake. The improvement in obesity was attributed to increased locomotion and augmented resting metabolic activity. Leptin also suppresses MCH-1 receptor expression [30].

Anandamide and 2-arachidonoyl glycerol are endocannabinoids that activate cannabinoid type 1 receptors (CB-1 receptors) in hypothalamic and extra-hypothalamic nuclei [31]. The stimulation of CB-1 receptors promotes feeding behavior, whereas the knock out of this receptor in mice reduces food intake [32]. In addition, CB-1 receptor knockout reverses the anorectic effect of cannabinoid antagonists [32]. It has been demonstrated that leptin-deficient *ob/ob* mice, leptin-resistant *db/db* mice and obese *fa/fa* Zucker rats exhibit increased hypothalamic endocannabinoids expression. Furthermore, hypothalamic expression of anandamide and 2-arachidonoyl glycerol decreased in leptin-deficient *ob/ob* mice after leptin treatment. These results suggest that leptin might tonically inhibit hypothalamic endocannabinoids [32].

1.2. Molecular Bases of Leptin Resistance

Leptin resistance and compensatory hypererleptinemia are commonly observed in animal and human obesity. Leptin resistance has been defined as attenuation of leptin's hyporexigenic and lipopenic signals. Also, the magnitude of compensatory hyperleptinemia has been used as a surrogate index of leptin resistance and adiposity [33]. Several post-receptor signaling mechanisms have been associated with leptin resistance. Notably, leptin-dependent activation of JAK-STAT pathway increases the expression of suppressor of cytokine signaling 3 (SOCS3), that binds to JAK2 and blocks the docking domain of STAT3, limiting STAT3 phosphorylation and subsequent dimerization.

Leptin reconstitution rapidly increases expression of SOCS3 in leptin-deficient *ob/ob* mice, but not in *db/db* mice that lack functional OB-Rb. Also, *in vitro* studies indicated that SOCS3 expression abrogates leptin signaling [34]. This mechanism was later confirmed in isolated Chinese hamster ovary cells where leptin-dependent activation of SOCS3 inhibited phosphorylation of STAT3 [35]. Conversely, RNA interference attenuating SOCS3 expression amplifies the JAK 2 and STAT3 signals by increasing the phosphorylation of these proteins, *in vitro* [36]. Furthermore, mice with specific SOCS3 knock out in the brain have increased leptin sensitivity and are resistant to diet-induced obesity [37]. Therefore, SOCS3 presumably provides physiologic negative feedback that regulates leptin-dependent JAK-STAT pathway activation. This concept is in line with experimental findings showing that reduction in leptinemia by fasting corresponds to decreased mRNA and protein expression of SOCS3 in the arcuate and dorsomedial nuclei of the rat hypothalamus [38]. Increased expression of SOCS3 in response to chronic hyperleptinemia of obesity might contribute to leptin resistance. For instance, the profoundly leptin-resistant yellow agouti obese mouse (Ay mouse) overexpresses SOCS3 in the hypothalamus [39]. To assess the role of SOCS3 on leptin resistance of aging, hypothalamic SOCS3 mRNA expression was measured before and after induction of hyperleptinemia by gene transfer. Baseline hypothalamic SOCS3 mRNA expression was 3 times greater in older rats. Importantly, the expression of SOCS3 mRNA tripled in aging rats after development of hyperleptinemia [40]. However, conflicting results have been reported in rats with dietary-induced obesity that do not overexpress SOCS3 as compared to controls despite hyperleptinemia [41].

Protein tyrosine phosphatase 1B (PTP1B) may also play an important role in leptin resistance. JAK2 tyrosine residues are dephosphorylated by PTP1B which inhibits STAT3 phosphorylation and dimerization. Indeed, mice lacking PTP1B (*i.e.* $PTP1B^{-/-}$ mice) show higher leptin-induced STAT3 phosphorylation in the hypothalamus that might explain weight gain resistance in response to high fat diet and increased energy expenditure. Exogenous leptin administration also produces exaggerated weight loss and enhances suppression of feeding behavior in $PTP1B^{-/-}$ mice [42]. Another phosphatase that might contribute to leptin resistance is the Src homology-containing tyrosine phosphatase (SHP2). By docking at Tyr^{985} of the phosphorylated OB-Rb, SHP2 dephosphorylates JAK2, and down regulates leptin-induced activation of JAK-STAT pathway [43].

Conflicting with these findings, it has been shown that the conditional deletion of SHP2 gene in mice increased rather than improved leptin resistance. These SHP2-deficient mice develop diabetes, hepatic steatosis and early onset obesity with increased serum concentrations of leptin, insulin, and triglycerides. Interestingly, mice deficient in SHP2 do not overeat, suggesting that SHP2-dependent mechanisms regulate metabolic rate rather than feeding behavior [44].

The potentiation of leptin-resistance in SHP2-deficient mice is thought to partly depend upon activation of leptin-stimulated ERK pathway [44]. It has also been speculated that SHP2 could compete with SOCS3 for the docking site at Tyr^{985} of the OB-Rb. Thereby SHP2 could potentially antagonize the inhibitory action of SOCS3 on the JAK-STAT pathway [8].

Saturation of leptin transporters (*i.e.* short isoforms of leptin receptor OB-Ra and OB-Rc) across the blood-brain barrier is associated with leptin resistance and hyperleptinemia in mice and humans [45, 46]. Indeed, the ratio between leptin concentrations in the cerebrospinal fluid and plasma is about four times higher in lean as compared to obese human subjects despite much higher leptinemia in obese subjects. Therefore, the access of leptin to the cerebrospinal fluid in obese subjects appears to be impaired, causing functional leptin resistance [46].

2. LEPTIN IS MARGINALLY EFFECTIVE FOR TREATMENT OF COMMON HUMAN OBESITY

Human leptin deficiency is a rare genetic condition associated with severe obesity, voracious appetite and metabolic abnormalities. Farooqi *et al.* [47] have treated three children with congenital leptin deficiency with low doses of recombinant methionyl human leptin (approximately 0.01 mg/kg lean body weight), for 6 to 48 months, achieving variable leptin serum concentrations. Importantly, leptin treatment induced sustained reductions in weight loss due to substantial reduction in fat mass. The reduction in energy intake ranged between 45% and 84%. Also, serum concentrations of insulin, cholesterol, triglycerides, and LDL-cholesterol declined and HDL-cholesterol increased gradually throughout leptin treatment. Leptin has also been used successfully in the treatment of adult patients with congenital leptin deficiency. Recombinant leptin treatment dramatically improved morbid obesity, feeding behavior, type 2 diabetes and hypogonadism in these subjects. Notably, the body mass index of these adults with congenital leptin deficiency decreased from 51±2 to 27±2 kg/m^2 after 18 months of recombinant leptin treatment [48].

Despite initial enthusiasm, a small randomized, double-blind, multi-center, escalating dose clinical trial has failed to show unequivocal benefit of leptin in the treatment of polygenic environmental human obesity [49]. In this study, lean and obese subjects were treated daily with placebo or 4 doses of subcutaneous recombinant leptin (0.01; 0.03; 0.1; 0.3 mg/kg) for 4 weeks. Afterwards, obese subjects continued the treatment for 20 weeks. Besides subcutaneous treatment injections, obese subjects followed an individualized diet to decrease daily calorie intake by 500 kcal. The main outcomes of the study were changes in body weight, body fat and incidence of adverse effects. After 4 weeks of treatment, the investigators reported significant reductions in weight and adiposity in lean and obese subjects. The overall dose-dependent weight loss in lean and obese subjects ranged between -0.4±2 kg with placebo and -1.9±1.6 kg with leptin 0.1 mg/kg. The weight loss in response to leptin 0.3 mg/kg was -1.5±2 kg. Obese subjects continued to lose weight during 24 weeks of leptin treatment. The weight loss after 24 weeks ranged between -0.7±5.4 kg with leptin 0.01 mg/kg and -7.1±8.5 kg with leptin 0.3 mg/kg. There was a corresponding increase in leptinemia across escalating doses of leptin, despite weight loss that would be expected to reduce serum leptin. For instance, serum leptin increased from 15 ng/ml at baseline to 480 ng/ml after 24 weeks of leptin 0.3 mg/kg. This result shows adequate leptin bioavailability after subcutaneous administration. Weight loss did not correlate with baseline leptinemia and was mainly attributed to fat mass reduction, as measured by dual x-ray absorptiometry. Notably not all obese subjects lost weight with leptin treatment. This lack of response could have been due to the development of antileptin antibodies, as observed in 71% of obese subjects treated with leptin for 24 weeks. Nevertheless, it is unlike that these antibodies neutralized leptin actions, given that no correlation between lack of weight loss and development of antileptin antibodies was observed.

A significant number of eligible subjects in the placebo group (8/20 subjects) and in the highest leptin dose group (10/18 subjects) did not complete the 24-week study. To address this issue, the investigators conducted a "last observation carried forward" (LOCF) analysis and showed that the weight loss ranged between -0.7±4.6 kg with leptin 0.01 mg/kg and -3.3±6.7 kg with leptin 0.3 mg/kg. Thus, the LOCF analysis indicated a more modest effect of the highest dose of leptin on the weight loss of obese participants. The reasons why eligible participants declined further participation are not clear. Increased rates of local reactions associated with subcutaneous administration of study drugs might have contributed to participant withdrawal. Notably, 56% to 76% of obese participants per treatment group developed some sort of injection site reaction such as ecchymosis, erythema and pruritus. These reactions did not differ between placebo and the escalating doses of leptin, even though treatment discontinuation was more frequent among subjects receiving the highest dose of leptin.

The authors highlighted that the variability of weight loss response to leptin might reflect a state of partial leptin resistance. Higher doses of leptin could potentially increase weight loss in subjects with common obesity by overcoming partial leptin resistance. However, given that a 32 fold increase in serum leptin concentrations was observed after 24 weeks of leptin 0.3 mg/kg, it is unlike that further increases in leptin doses will substantially potentiate weight loss.

2.1. Recombinant Ciliary Neurotrophic Factor and CBT-1452: Leptin Mimetics that May Overcome Leptin Resistance

The ciliary neurotrophic factor (CNTF) is a 23 kDa peptide present in glial cells and in several populations of motor neurons that stimulates the differentiation of neuronal and non-neuronal cells. The actions of CNTF are mediated by a multi-unit receptor composed of an α-subunit (CNTFRα), and two signal transducing subunits: gp130 and leukemia inhibitory factor receptor (LIFR). Like leptin, CNTF also reduces weight in animals and humans. Indeed, activation of CNTFRα promotes the heterodimerization of gp130 and LIFR, initiating JAK-STAT signals that are also activated by leptin receptors. Importantly, CNTF has been shown to induce weight loss and improve glucose metabolism not only in leptin-deficient *ob/ob* mice, but also in *db/db* mice that

lack functional leptin receptors, and in leptin-resistant mice with dietary obesity.

Therefore, CNTF could potentially induce weight loss and improve metabolic abnormalities despite leptin resistance of polygenic human obesity. Indeed, early phase III clinical trials have confirmed that recombinant CNTF (Axokine™, Regeneron Pharmaceuticals Inc.) promotes weight loss in human obese subjects. Although CNTF-treated subjects lost approximately 4 kg more than the placebo group, 30% of trial participants developed neutralizing antibodies anti-CNTF and did not lose weight. [50]. In a 12-week phase II clinical study, recombinant CNTF reduced body weight in type 2 diabetics and tended to improve glucose and other metabolic variables [51]. Also, the safety and tolerability of pegylated CNTF has been studied in phase I clinical trials. Pegylation may increase the serum concentrations and half-life of CNTF, and may reduce its immunogenicity. However, preliminary results showed low availability of pegylated CNTF and a high incidence of local reactions at the injection site [51].

Recently, Kokoeva *et al*. [52] reported that CNTF induces hypothalamic neuronal differentiation in *ob/ob* mice and in mice with dietary obesity. These newborn neurons express POMC, α-MSH and respond to leptin by increasing STAT signals that favor negative energy balance. Interestingly, CNTF-dependent weight loss is sustained after discontinuation of CNTF treatment. This response is partly explained by CNTF actions on neuronal plasticity. Furthermore, in contrast with previous findings, it is possible that CNTF acts in the brain through molecular mechanisms unrelated with leptin. Indeed, CNTF and leptin induce distinct patterns of acute gene expression in different central nervous system sites in mice and rats. Also, CNTF appears to possess inflammatory properties distinct from leptin [53]. In May 2006, recombinant CNTF is not listed in Regeneron's pipeline [54]. However, these recent findings may revamp the enthusiasm for future studies with recombinant CNTF in the treatment of obesity and maintenance of weight loss.

Cambridge Biotechnology Ltd. (acquired by Biovitrum in March, 2005) has developed CBT1452, a small molecular weight leptin receptor agonist that promptly crosses the blood-brain barrier through receptor-independent mechanisms [55]. Importantly, CBT1452 is administered orally, increases metabolic rate and substantially decreases feeding behavior and body weight in leptin-resistant rats with diet-induced obesity [51]. Since February 2006, Biovitrum has been conducting toxicology tests with its leptin receptor agonist [55].

2.2. PTP1B Inhibitors May Increase Leptin Sensitivity

PTP1B removes phosphate groups from JAK2 phosphotyrosine residues and inhibits leptin-dependent activation of JAK-STAT pathway. Likewise, PTP1B dephosporylates tyrosine residues at activated insulin receptors and impairs phosphorylation of insulin receptor substrate 1 (IRS-1). Indeed, reduced expression of PTP1B increases leptin and insulin sensitivity [56]. Few studies suggest a variable role for PTP1B in the development of insulin and leptin resistance in humans. It has been demonstrated that PTP1B activity is increased in visceral as compared to subcutaneous adipose tissue in obese subjects. Conversely, PTP1B expression and phosphatase activity are reduced in obese diabetic subjects [57]. Still, PTP1B may be an important mediator of leptin and insulin resistance in obesity and diabetes and an attractive pharmacological target in the management of these conditions.

Pharmaceutical industries have been working intensively to design a potent, specific and bioavailable PTP1B inhibitor. For instance, Wyeth developed a PTP1B inhibitor, ertiprotafib, that reached the initial stages of clinical development (Fig. (**1**)). Unfortunately, phase II clinical trials with ertiprotafib were discontinued because of insufficient efficacy and dose-related adverse effects [58]. ISIS-113715 is a PTP1B oligonucleotide antisense developed by Isis Pharmaceuticals Inc. Pre-clinical studies have shown that hyperinsulinemia and total body fat in Zucker *fa/fa* rats treated with ISIS-113715 were reduced, even though body weight did not change. In mice with diet induced obesity, ISIS-113715 reduced weight gain, increased metabolic rate and improved glucose tolerance. Additionally, administration of ISIS-113715 in obese rhesus monkeys substantially improves insulin resistance and decreases hyperinsulinemia. Regrettably, this study did not provide information about body weight variation [59].

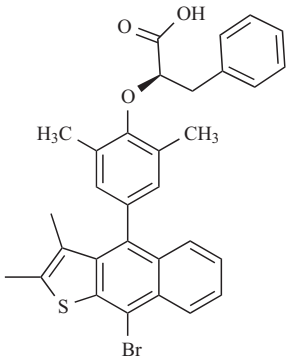

Fig. (1). The structure of the PTP1B inhibitor ertiprotafib.

ISIS-113715 is currently being evaluated for the treatment of type 2 diabetes in phase II placebo-controlled clinical trials. Preliminary data confirm the efficacy of parenteral ISIS-113715 to dose-dependently reduce hemoglobin A1C and fasting glicemia in never-treated type 2 diabetics. The overall tolerability of the drug was satisfactory and the treatment has not been associated with hypoglycemic events, metabolic acidosis or weight gain for the 6-week trial duration. No further information about weight variability during the study was disclosed [60]. A myriad of peptide and non-peptide PTP1B inhibitors are currently undergoing pre-clinical development. Cell permeability (given the polar nature of these drugs) and selectivity have been major challenges in the development of effective PTP1B inhibitors. It is hoped that these problems can be offset by the development of effective prodrugs [57].

3. LEPTIN TREATMENT MAY STABILIZE WEIGHT IN FORMERLY OBESE SUBJECTS

Due to leptin resistance and sub-optimal efficacy in clinical trials, native and pegylated leptin molecules have been

abandoned as potential treatments for weight loss. Nevertheless leptin might be useful in weight management of formerly obese subjects. Weight loss is associated with reductions in energy expenditure. Reduced sympathetic activity, as reflected by decreased urinary excretion of cathecolamines, and reduced thyroid function are correlated with decreased energy expenditure of weight loss [61]. These compensatory responses might explain in part why dieters usually fail to maintain weight loss for long periods and regain weight.

Even though variation in leptinemia does not correlate with changes in body weight [62], Rosenbaum *et al*. [63] have shown that leptin reverses some of the metabolic compensatory responses of weight loss. Under tightly controlled experimental conditions, 7 obese subjects volunteered for an in-hospital weight reduction program aiming at 10% weight loss. In average, leptinemia decreased from 48 ± 6 ng/ml to 34 ± 6 ng/ml (p-value: < 0.05) after successful weight loss over 6 to 8 weeks of liquid hypocaloric diet. During the weight maintenance phase of the study, low doses of recombinant leptin (0.08 – 0.14 mg/kg of fat mass/day) injections were administered subcutaneously twice daily for 5 weeks. Leptin doses were individually calculated to restore pre-weight loss circulating leptin, achieving 52 ± 7 ng/ml. Importantly, low-dose leptin treatment restored pre-weight loss energy expenditure, skeletal muscle work efficiency, sympathetic nervous tone and thyroid hormones serum concentrations. These results underscore the potential use of leptin as a clinically effective weight stabilizer in formerly obese subjects.

4. LEPTIN REVERSES TOXIC EFFECTS OF ECTOPIC LIPID ACCUMULATION IN HUMAN LIPODYSTROPHY SYNDROMES

Presently, the most promising therapeutic venue for recombinant leptin is in the treatment of human congenital and human immunodeficiency virus (HIV)-associated lipodystrophies. One of the physiologic actions of leptin is to facilitate fatty acid oxidation by promoting the expression of peroxisomal proliferation-activated receptor α (PPARα) [64]. In experimental models, impairment of leptin signaling due to profound leptin resistance causes ectopic accumulation of triglycerides in non-adipose tissues such as skeletal muscles, pancreas and heart. Ectopic accumulation of lipids in these tissues induces ceramide synthesis and activates inducible nitric oxide synthase, promoting apoptosis. The toxic effect of lipid accumulation in skeletal muscle and pancreas cause insulin resistance and β-cell dysfunction, respectively, and could contribute to the pathogenesis of type 2 diabetes. Also, cardiac lipotoxicity has been associated with experimental myocyte dysfunction and congestive heart failure.

The physiologic relevance of leptin action to normal pancreatic β-cell function has been demonstrated through experiments with obese *fa/fa* Zucker rats. These rats exhibit non-functional leptin receptors and are, therefore, profoundly leptin-resistant and obese. In addition to obesity, *fa/fa* Zucker rats develop diabetes due to pancreatic dysfunction that is likely related with 50-fold increases in triglyceride content in islet β-cells. Importantly, *in vitro* leptin treatment decreases intracellular triglyceride by 87% in *fa/fa* Zucker rat's pancreatic tissue expressing functional leptin receptor after adenoviral transfection [65, 66]. The reduction of lipid accumulation in pancreatic cells was followed by substantial improvement of glucose-stimulated insulin secretion [66] and reduction of inducible nitric oxide expression [65]. Presumably, lipoapoptosis can be minimized through reductions in inducible nitric oxide expression.

Similar mechanisms also appear to operate in the *fa/fa* Zucker rat's heart. Zhou *et al*. [67] have demonstrated an age-dependent increase in myocardial triglyceride content, which was accompanied by overt steatotic infiltration of the heart. Cardiac triglyceride accumulation in these animals was associated with reduced expression of enzymes responsible for fatty acid oxidation: acyl-CoA-oxidase (ACO) and carnitine palmitoyltransferase 1 (CPT-1). The myocyte expression of PPARα, the transcription factor for ACO and CPT-1, was also decreased in obese *fa/fa* Zucker rats. Likewise, the myocyte levels of ceramide and inducible nitric oxide synthase were increased 3 and 4 times relative to controls, respectively. Furthermore, DNA laddering, an index of apoptosis, reached 20 times the normal levels. Importantly, 20-week old *fa/fa* Zucker rats exhibited echocardiographic evidence of ventricular dysfunction characterized by augmented end-systolic volume and reduced fractional area shortening. As expected, troglitazone, a potent PPARα agonist, minimized the molecular, histological, and functional cardiac abnormalities in *fa/fa* Zucker rats. This last result clearly underscores the relevance of PPARα-dependent mechanisms to the pathogenesis of cardiac disease in leptin-resistant obese *fa/fa* Zucker rats.

The toxic effect of lipid accumulation in non-adipose tissues is clearly observed in human lipodystrophy syndromes. Hypotrophy of adipose tissue and very low serum levels of adipocyte-derived leptin are hallmarks of human lipodystrophies. In addition to adipose tissue hypotrophy, lipodystrophies are characterized by the development of profound insulin resistance, type 2 diabetes, dyslipidemia (predominantly hypertriglyceridemia), hepatomegaly due to severe non-alcoholic hepatic steatosis, skeletal muscle steatosis, proteinuric nephropathy and pituitary dysfunction. Oral *et al*. [68] demonstrated for the first time that leptin was effective in the treatment of metabolic and hepatic complications of congenital lipodystrophy. In this study, 9 lipodystrophy patients were treated for 4 months with recombinant methionyl human leptin (0.03-0.04 mg/kg/day) to increase serum levels from 1.3 ± 0.3 ng/ml to 11.1 ± 2.5 ng/ml. In average, serum triglycerides decreased by 60% and hepatic volume reduced by 28%. Notably, the reduction in absolute glycated hemoglobin value was 1.9%. Decreased glycated hemoglobin was followed by interruption or substantial reduction of anti-diabetic treatment.

Subsequent case series reports confirmed the efficacy of recombinant methionyl human leptin treatment to improve most clinical complications of congenital lipodystrophy syndromes [69-74]. Current clinical experience with recombinant leptin in lipodystrophy syndromes indicates sustained effectiveness after 12 months with significant reductions of triglyceridemia, LDL and total cholesterol, liver volume, glycated hemoglobin and lesser need of anti-diabetic therapy [75].

Human immunodeficiency virus (HIV) infection is also associated with lipodystrophy syndromes, particularly during highly active antiretroviral therapy (HAART). The development of HAART-induced lipodystrophy has been associated with inhibition of PPARγ and sterol regulatory element binding protein 1 (SREBP1) expression in the peripheral adipose tissue [76]. Unlike rare congenital lipodystrophy syndromes, the prevalence of HIV-associated lipodystrophy can reach 84% in patients on protease inhibitors [77]. Notably, HAART-induced lipodystrophy correlates with increased cardiovascular risk [78].

HIV-associated lipodystrophies are phenotipically diverse and can vary from generalized lipoatrophy to lipohyperthrophy. Recently, Lee *et al*. [77] demonstrated that recombinant leptin might be clinically effective in the management of patients with HIV-related lipoatrophy. In a randomized, double blind, placebo-controlled, cross-over, proof-of-concept study, 7 HIV patients with HAART-induced lipoatrophy were treated with recombinant leptin for 2 months either before or after 2 months of placebo. The eligibility for participation included baseline leptin levels < 3 ng/ml and fasting triglyceridemia > 300 mg/ml. Five participants completed the study whereas two subjects were dropped due to intermittent hypertriglyceridemia greater than 1000 mg/ml that was present before enrollment.

Pharmacokinetic studies have shown that the average leptinemia increased from 0.9 ± 0.5 ng/ml at baseline to 16 ± 1 ng/ml after 4 hours of administration of recombinant leptin 0.04 mg/kg/day. Importantly, univariate intention-to-treat analysis showed a significant association between recombinant leptin treatment and reductions in plasma insulin and improved insulin sensitivity. Nevertheless, only significance trends between leptin treatment and these primary outcomes were achieved with multivariate data analysis.

Recombinant leptin treatment was also independently associated with reduced total and truncal fat mass and total cholesterol after multivariate adjustments for baseline visceral adiposity and administration order of treatments. Altogether, these results warrant future larger scale clinical trials addressing the effectiveness and safety of recombinant therapy in HIV patient with HAART-induced lipodystrophy, a condition by far more prevalent than congenital lipodystrophy.

5. SUMMARY AND CONCLUSIONS

Leptin resistance is strongly associated with common polygenic obesity in humans and might explain the disappointing clinical results of recombinant leptin to treat obese subjects. Pharmacologic strategies to improve leptin sensitivity or overcome leptin resistance are currently being developed and might generate breakthrough treatments for obesity in the future.

Nevertheless, recombinant leptin treatment may facilitate weight management in formerly obese subjects by opposing compensatory endocrino-metabolic mechanisms that follows weight loss. Moreover, preliminary clinical results strongly suggest that recombinant leptin treatment might minimize the lipotoxic complications of ectopic lipid accumulation in patients with congenital and HAART-induced lipodystrophy.

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