

Protective effects of leptin against cerebral ischemia/reperfusion injury (Review)

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Abstract. In recent years, the use of thrombolytic therapy for treating ischemia/reperfusion injury has resulted in damage to the self-regulatory mechanisms of the brain. This is due to the increased production of free radicals, excitatory amino acids and pro-inflammatory cytokines causing secondary damage to the brain. Simple thrombolytic therapy has not been the best approach for treating ischemia/reperfusion injury. Excessive perfusion leads to failure of the body's self-regulatory functions, which in turn increases the area of cerebral edema and aggravates cerebral ischemia. Previous studies have evaluated the satiety hormone leptin as a link between energy expenditure and obesity. Of note, leptin, which is involved in brain development, synaptic transmission and angiogenesis following ischemia/reperfusion injury, has been considered an important factor for treating ischemia/reperfusion injury. The present review outlines the discovery of leptin and discusses its association with cerebral ischemia/reperfusion.

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1. Introduction

Stroke is a partial or complete brain dysfunction syndrome accompanied by acute cerebral circulatory disorders (1). Stroke globally affects millions of individuals each year (2). Of note, its incidence rate exceeds that of heart disease. In particular, stroke has major life-long consequences. Ischemic stroke, accounting for 70-80% of all strokes, is caused by disturbances in cerebral circulation, leading to cerebral ischemia, hypoxia, neuronal apoptosis and necrosis, thereby leading to dysfunctions. Reconstruction or enhancement of blood flow in the ischemic region is the current key treatment strategy for stroke-associated ischemic brain injury. Recent developments in thrombolytic therapy have led to increases in free radical production, with consequent damage to the self-regulation mechanisms of brain tissues (3). The latter may determine secondary damage to cerebral vessels following cerebral ischemia/reperfusion. Of note, excessive perfusion may lead to the failure of the self-regulation mechanisms of brain tissues, thereby increasing the area of brain edema and aggravating cerebral ischemia. At present, ischemia/reperfusion injury is receiving an increased amount of attention; however, neuroprotective drugs currently used for treating neuronal injury caused by cerebral ischemia/reperfusion are unable to repair and perfuse injured neurons (4,5). Of note, clinicians are currently unable to obtain good results owing to the time constraints associated with and several side effects of thrombolytic and traditional medical therapies. The development of a novel drug capable of dealing with the multiple mechanisms involved in ischemia/reperfusion injury is warranted (6). In recent years, leptin has been linked to the occurrence and development of cerebrovascular diseases. The metabolism and mechanism of action of leptin is similar in animals and humans; however, the effective dose of leptin used in animals is not feasible for use in humans due to drug pharmacokinetics (7,8). Leptin receptor binding reportedly regulates energy metabolism, respiration, neuroendocrine function, immune inflammation, and afferent nerve and neuron regeneration. An obesity-associated gene

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encoding leptin (OB-Rs), to which leptin binds, are widely distributed in humans and rodents. In particular, OB-Rs are distributed in the arcuate nucleus of the hypothalamus and the lateral hypothalamus as well as in the ventromedial, paraventricular, supraoptic and ventral perimammary nuclei (9). Of note, leptin receptors were determined to be distributed in the cortex, hippocampus and striatum in a murine model of ischemia/reperfusion with leptin pre-conditioning (Fig 1) (10,11). Leptin receptors in locations including the striatum have protective roles (12). Studies have demonstrated considerable reductions in excitatory amino acids (EAAs) (13,14), oxygen free radicals, inflammatory factors (15-17), mitochondrial damage and apoptosis in leptin-preconditioned groups compared with those in untreated groups (Fig. 2). Leptin has an important role in decreasing excitatory neurotransmitter levels, protecting the mitochondria, decreasing superoxide and free radical formation, increasing anti-inflammatory factor and -apoptotic protein levels, decreasing apoptotic protein levels, and avoiding the traditional role of protecting the brain from single and potential side effects. In the present review, the protective mechanisms of leptin on the brain are summarized.

2. Production and metabolism of leptin

The leptin gene (OB gene) was originally produced by two mutant mouse strains (ob/ob and db/db; leptin-deficient and leptin-receptor-deficient, respectively) developed by the Jackson Laboratory (Bar Harbor, ME, USA). These diabetic mice (db mice) had obesity, polyphagia and polyuria as comorbidities (18). Obese mice (ob mice) encode an appetite suppressor present in the blood, which was detected by conjoined symbiotic experiments. db mice lack the corresponding receptor was discovered by conjoined symbiosis experiments (19). In 1994, Zhang *et al* (20) located the human OB gene encoding the peptide hormone leptin to chromosome 7 (7q31.3) via positional cloning. Subsequently, in 1995, Halaas *et al* (21) reported on the synthesis of the leptin protein. The metabolism of leptin may be summarized as follows (22): Leptin is a 16-kDa non-glycosylated protein encoded by the OB gene, which is located on the human chromosome 7 and the mouse chromosome 6. The precursor of leptin is a protein with 167 amino acids. During its secretion into the blood, the signal peptide, composed of 21 hydrophilic amino acid residues at the amino end, is hydrolyzed to form a polypeptide chain composed of 146 amino acids (Fig. 1A). It is primarily synthesized by the white adipose tissue, and leptin transport in the brain is identical in humans and mice. Due to its large size, leptin cannot passively cross the blood-brain barrier (BBB). Therefore, it is transported across via a regulated saturable transport system (23,24). Although the molecular identity of this transporter system remains elusive, it appears to act independently from leptin receptors. Studies (8,25,26) have reported three mechanisms that aid the transport of leptin across the BBB: Unidirectional Saturable (Leptin transport to cerebrospinal fluid is saturated but not unlimited) transport of leptin across the BBB; direct access of the leptin receptor neurons to the circulation via projections close to fenestrated capillaries (perivascular space) in circumventricular organs (e.g., median eminence, area postrema and organum vasculum); and transport of leptin by tanycytes into the cerebrospinal fluid

within the ventricular space. Leptin has strong hydrophilicity and is mostly excreted by the kidneys. These mechanisms serve as the basis for the administration of leptin through the lateral ventricle to enhance its neuroprotective effects.

3. Leptin receptors

Leptin receptors (OB-Rs) are expressed in several organs and tissue types, including the hypothalamus, pituitary gland, lymph nodes, liver, lungs, uterus, adipose tissues, kidneys, pancreas, stomach and gonads. Astrocytes are known to express various isoforms of the leptin receptor (18-20). OB-R splicing produces six isoforms of the leptin receptor, named as OB-Ra-f (Fig. 1B). Of those six, OB-Rb is the longest receptor, and OB-Ra and OB-Rc are widely distributed and are predominant in the brain's choroid plexus and microvasculature. The distribution of leptin receptors is central to the regulation of leptin transport, protein binding and the free leptin in the blood through the BBB. The distribution of leptin receptors regulate the amount of leptin passing through the BBB (27). OB-Re exclusively contains extracellular domains, circulating as a soluble receptor. By contrast, other OB-Rs have identical N-terminals as well as intracellular membranes and extracellular domains. OB-Rd and OB-Rf, which are types of single transmembrane receptors, only have partial functions. Among other OB-Rs, they are widely expressed in the testicles, brain, liver, heart and lung tissues. OB-Rb (Fig. 3), a leukocyte type-6 receptor encompassing an intracellular region, is considered the only functional receptor involved in cellular signal transduction (comprising intracellular, membrane and extracellular regions). The extracellular region is a single transmembrane signal transduction protein comprising two cytokines, combining the characteristic sequences (cytokine receptor homology domains separated by an immunoglobulin-like domain) and a fibronectin III region. The membrane and intracellular regions are characterized by the same 29 amino acids of a highly conserved Box1 motif rich in proline, two conservative Box2 motifs and three conservative tyrosine residues (Tyr985, Tyr1077 and Tyr1138) (28). Similarly, OB-Rb expresses various isoforms of the leptin receptor and is prevalent in numerous areas of the mammalian brain, including the cortex, hippocampus and striatum within neurons and astrocytes, and in Schwann cells (29). Leptin receptors undergo mutagenesis in the hypothalamus, hippocampus and prefrontal cortex of db/db mice. Such an occurrence may be responsible for the decreased neuroprotective functions of leptin following ischemia/reperfusion injury. While leptin receptors do not possess intrinsic enzyme activity, they may bind to tyrosine kinase in the cytoplasm. Furthermore, (19,30) in the brain, exogenous leptin combined with the OB-Rb has protective roles in the BBB. However, the functions of short-form leptin receptors remain to be fully elucidated.

4. Leptin signaling pathway

The binding of leptin to its receptors, OB-Rs, in the central and peripheral tissues results in a wide range of biological effects (31). The signal transduction pathways are mediated at the membrane surface by long functional receptors, OB-Rb (Figs. 1 and 3); these include Janus kinase (JAK)/signal transducer and activator of transcription (STAT) (32), Ras/extracellular

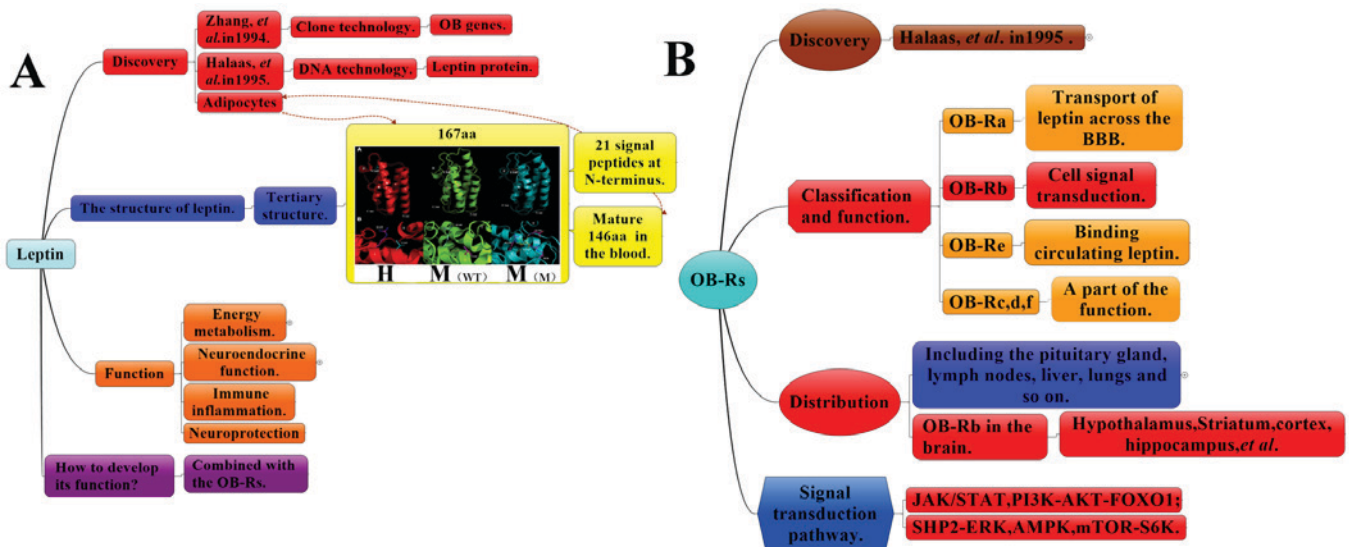


Figure 1. (A) Summary of the discovery, construction and function of leptin. Leptin is composed of 167 amino acids. Of these, 21 amino acids are released from the N-terminus in the mature leptin occurring in the blood. The tertiary structure of leptin is adapted from the National Centre for Biotechnology Information GenBank database; red indicates humans, green indicates wild-type mice and blue indicates mutant mice. (B) Summary of the discovery, construction and function of leptin receptors. BBB, blood-brain barrier; CNS, central nervous system; OB-Rs, leptin receptor; JAK2, tyrosine kinase Janus kinase 2; STAT3, signal transducer and activator of transcription 3; PI3K phosphatidylinositol-3 kinase; AMPK, adenosine monophosphate kinase; mTOR, mammalian target of rapamycin; S6K, ribosomal protein S6 kinase B1; FoxO1, forkhead box protein O1; SHP2, SH2 domain of protein tyrosine phosphatase 2; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase. H, human; M, mouse; WT, wild type; M, mutant.

signal-regulated kinase (ERK)1/2 (33), phosphoinositide-3 kinase (PI3K)/Akt/forkhead box O1 (34), SH2 domain of protein tyrosine phosphatase 2/ERK, adenosine monophosphate kinase (AMPK) and mammalian target of rapamycin/ribosomal protein S6 kinase B1 (11,35). JAK/STAT is the most important signaling pathway and has a major role in cerebral protection (36). Suppressor of cytokine signaling reportedly inhibits the JAK/STAT signaling pathway (37,38). Phosphorylation of tyrosine residues (Tyr1138) was triggered and JAK2 was activated by OB-R, thereby combining OB-Rb in cells; the phosphorylated site of OB-Rb serves as a docking site and recruits the STAT3 monomer (39). STATs are a group of cytoplasmic proteins that act as signal transducers and transcription factors and participate in normal cell - cytokine interaction and cell growth (40). STAT3, a potential transcription factor belonging to the STAT family, reaches the receptor and binds to the 705th tyrosine site via phosphorylation. In particular, STAT3 is phosphorylated by phosphorylated (p)-JAK2 (41,42). p-STAT3 behaves homotypic or heterodimeric, depending on the action of the SH2 region, and is then transferred to the cell nucleus. p-STAT3 regulates downstream target genes by interacting with DNA elements, other transcription factors or adjunct proteins. The neuroprotective mechanism of leptin and the JAK2/STAT3 signal transduction pathway are closely associated.

5. Roles of leptin

Studies have reported on the pleiotropic effects of leptin on various biological functions of the central and peripheral nervous systems. The presence of leptin receptors in various tissues suggests its pleiotropic effects on numerous biological functions (43-45). Certain arcuate nucleus (ARC) leptin receptor (OB-Rs) neurons express pro-opiomelanocortin

(POMC). Other ARC OB-Rs neurons express agouti-related protein (AGRP) along with the inhibitory neuropeptide Y (NPY) and the inhibitory neurotransmitter γ -aminobutyric acid (GABA). POMC-expressing neurons have low energy requirements, thereby promoting energy expenditure, whereas those expressing AGRP, NPY and GABA demonstrate opposite behaviors (32,33). Blood leptin levels are typically proportional to body fat levels. In particular, they reflect the organism's energy storage, with no effects on obesity, thereby revealing the pathological state of leptin resistance (46). Body weight and the hypothalamus-pituitary-gonad axis, which is closely associated with growth and weight, may be regulated by leptin. The levels of circulating leptin predict the development of heart failure in elderly individuals by modulating the influence of obesity on the increasing risk of heart failure. Indeed, the influence of leptin on the central nervous system (CNS) increases the sympathetic activity of the nerves sub-serving various tissues, including the cardiovascular organs and kidneys (33). In mice, blocking PI3K with either LY294002 or wortmannin significantly attenuated the leptin-induced increase in renal sympathetic activity. Of note, leptin promotes the switch toward type 1 T-helper (Th1) cell immune responses by increasing interferon- γ secretion, facilitating Th17 responses (47), and stimulating the release of inflammatory cytokines, including interleukin (IL)-1 β , IL-6 and IL-8, and the chemokine monocyte chemoattractant protein-1.

Although most animal studies have indicated that leptin protects the brain, human studies suggest certain contradictions. What is known for certain is that the increased circulating leptin levels may also contribute to low brain natriuretic peptide concentrations observed in heart failure patients with a high body mass index and may promote the obesity paradox of heart failure (48). In particular, the administration of leptin (14.1 ng/ml) to humans would establish a risk model in coronary

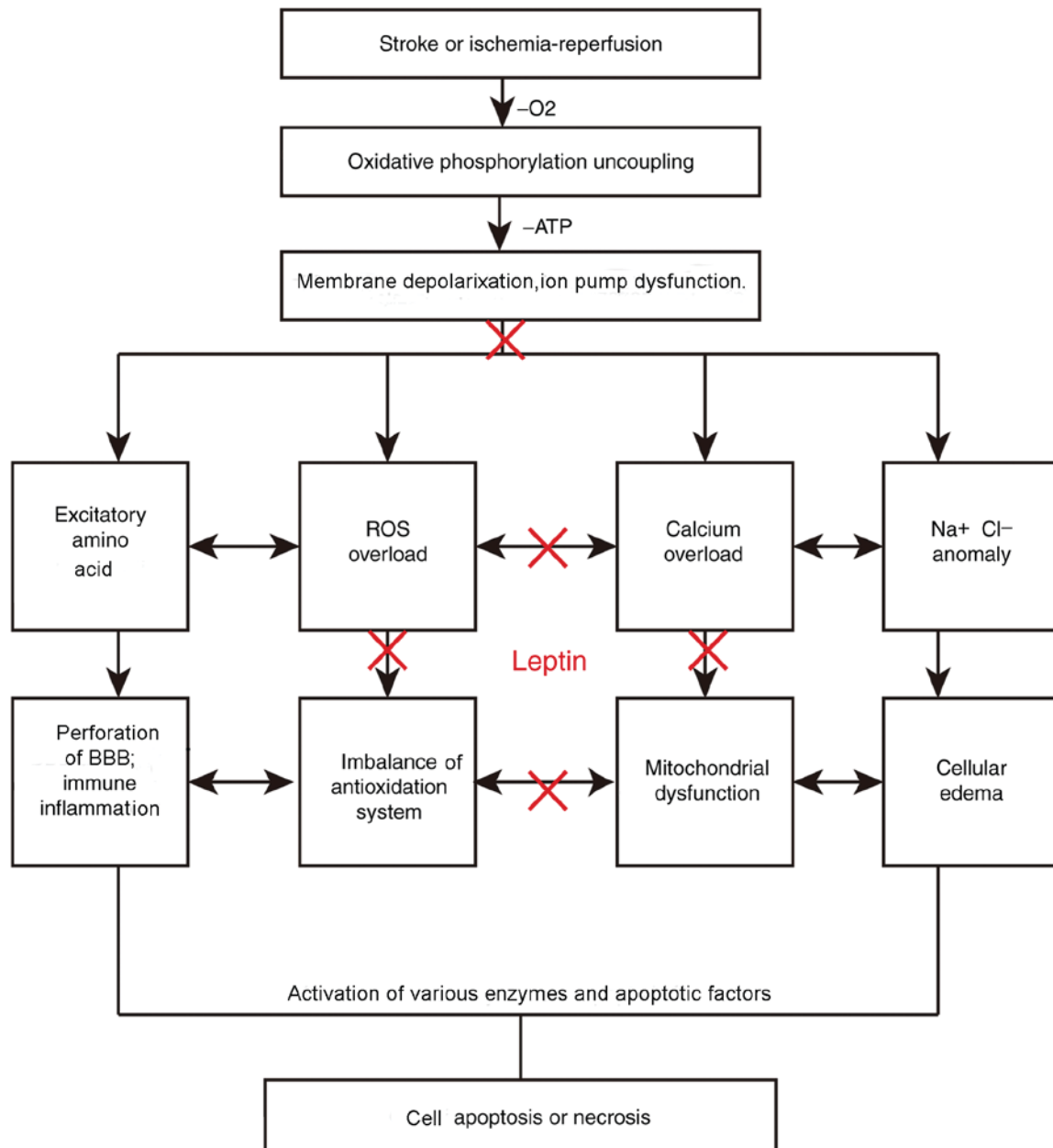


Figure 2. Mechanism of cerebral ischemia. Stroke or ischemia/reperfusion injury leads to ischemia and hypoxia and reduced energy production in brain tissue. The membrane potential and ion pump are destroyed. In the cells, numerous enzymes and apoptotic factors are activated. Subsequently, following a chain reaction, brain cells die. Leptin may cause a reduction of excitatory amino acid and ROS production by balancing the difference of ion concentration inside and outside the membrane to improve mitochondrial function. BBB, blood-brain barrier; ROS, reactive oxygen species.

artery disease. Furthermore, a prospective study involving 4,571 healthy African Americans did not identify any association between the leptin levels in the body and the risk of ischemic stroke in either men or women (49-51). A few small-scale studies evaluated leptin levels in the body during the acute phase of ischemic stroke and reported an increase in the levels (52). The patients received escalating doses of r-met-Hu-Leptin until a dose of 0.12 mg/kg/day was reached. After 18 months of r-Met-Hu-Leptin therapy, a considerable improvement in glucose homeostasis was achieved, as evidenced by the normalization of fasting blood glucose levels, lowered glycated hemoglobin and improved tolerance to an oral glucose load (7,52,53). In recent years, significant efforts have been made to clarify the neuro-protective mechanisms of leptin.

6. Protective mechanisms of leptin in the brain

The pathogenesis of cerebral ischemia/reperfusion injury has been linked to increases in the toxicity of EAAs (54), mitochondrial damage (55), free oxygen radical production, inflammatory factors and apoptosis, as well as the interaction among those factors (Fig. 2). EAAs, including glutamic and aspartic acids, are present in the mammalian CNS and most abundantly in the cerebral cortex and hippocampus, where they deliver excitatory nerve impulses (56). N-Methyl-D-aspartic acid receptors are expressed in oligodendrocytes. Of note, 40% of glutamic acid is released from synapses that are activated during ischemia (57). In general, EAAs present in the synaptic vesicles of nerve endings are released into the synaptic cleft

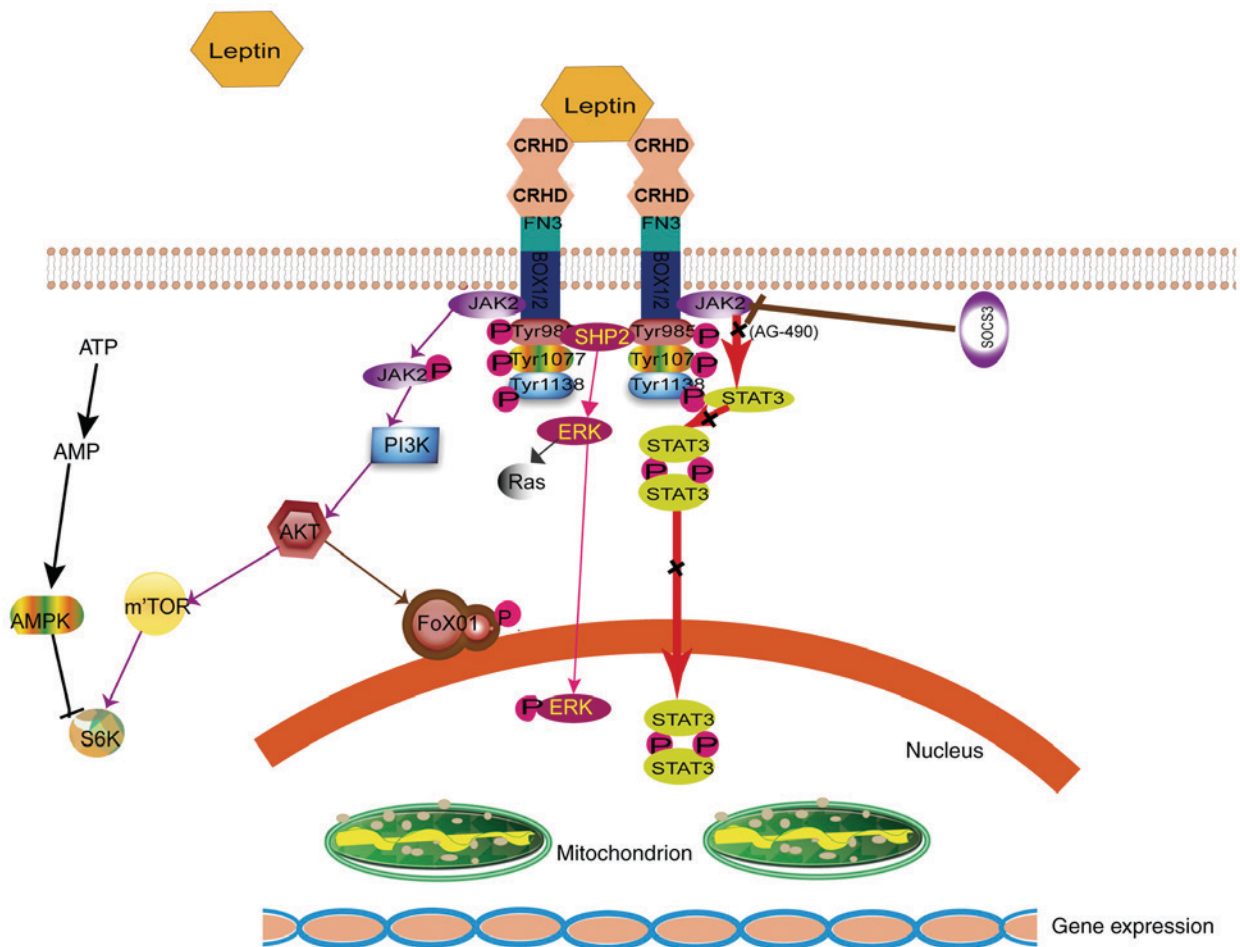


Figure 3. Leptin signaling pathways. Leptin binds with OB-Rb and activates JAK2 in box1/2 (cytoplasmic motif located in the membrane proximal region of cytokine receptors). JAK2 is phosphorylated at 3 tyrosine residues. Subsequently, downstream pathways, including JAK2/STAT3, PI3K/AKT/mTOR/S6K, AKT/FOXO1 and SHP2/ERK or Ras are activated. AMP is able to activate AMPK. Leptin has an important role in brain protection. Specifically, it activates the JAK2/STAT3 signaling pathway. However, AG-490 is capable of reversing this brain-protective effect. Finally, SOCS3 negatively regulates OB-Rb signaling. JAK2, tyrosine kinase Janus kinase 2; CRHD, cytokine receptor homology domain; FN3, membrane proximal fibronectin type III; bs; STAT3, signal transducer and activator of transcription 3; PI3K, phosphatidylinositol-3 kinase; AMP, adenosine monophosphate; mTOR, mammalian target of rapamycin; S6K, ribosomal protein S6 kinase B1; FoxO1, forkhead box protein O1; SH2, Src homology 2 domain; SHP2, SH2 domain of protein tyrosine phosphatase 2; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; AG-490, Janus kinase/signal transduction protein and transcriptional inhibitor activator; SOCS3, suppressor of cytokine signaling 3; P, phosphate; OB-Rs, leptin receptor.

acting on the corresponding receptor, transmitting nerve impulses once the nerve cell membrane is depolarized. Excessive excitatory transmitters are either hydrolyzed by proteases or re-absorbed by neurons (57). Calcium, sodium and potassium channels are activated following cerebral ischemia. Excitatory neurotransmitters are simultaneously released in the synaptic space and cannot be absorbed or used effectively. The accumulation of sodium and chloride ions causes cellular edema, thereby resulting in the accumulation of excitatory amino acids in interstitial cells, causing cellular toxicity. Dopaminergic neurons of the striatum and prefrontal cortex originate from the ventral tegmental area (VTA) of the mesencephalon.

Leptin treatment provided following transient ischemia markedly reduces neurologic deficits, cerebral infarct volumes and brain edema (58,59). Leptin treatment increased the ATP, leptin and p-Akt levels and decreased the lactate dehydrogenase levels and lactic acid/pyruvate ratio; it also alleviated histopathologic injuries, all of which were entirely reversed following treatment with the PI3K inhibitor LY294002.

Leptin inhibits the release of pre-synaptic glutamate into the VTA via JAK2 and PI3K activation. Furthermore, leptin reportedly suppresses the release of glutamate and regulates the dopamine pathway in the hippocampus to alleviate the depressive symptoms of cerebral ischemia. AG-490, a JAK2 signal transduction protein and transcriptional inhibitor activator, inhibits the effects of leptin on JAK2/PI3K signaling. (60,61) The JAK2/PI3K/Akt pathway is critical for the mediation of leptin-induced neuroprotection. These results support the use of leptin in the treatment of ischemic stroke. The effects of leptin in increasing angiogenesis and neurogenesis following brain ischemia may be partially attributed to an increase in OB-Rs, AMPK activation, transient receptor potential cation channel subfamily V member 1 expression and biosynthesis of pro-angiogenic factors (e.g., vascular endothelial growth factor). By contrast, studies assessing microsphere infusion under maximal vasodilation have reported that that leptin does not have a central role in cerebral perfusion enhancement (59,62). Systemic leptin administration for 1 week following an occlusion

surgery increased the cerebral hemodynamic reserve in a similar fashion to that caused by granulocyte-macrophage colony-stimulating factor. Leptin enhances cell growth and differentiation via the mitogen-activated protein kinase/ERK signaling pathway. Leptin infusion increases Akt and ERK1/2 phosphorylation in brain tissues. These effects were associated with upregulation of ERK1/2 phosphorylation and nitric oxide (NO) release, which promote the repair of damaged mucosa (63,64). Mitochondria are the powerhouse of cells. Ischemic or hypoxic injury to the brain terminates mitochondrial oxidative phosphorylation (65), thereby leading to the loss of mitochondrial membrane potential and decreasing the membrane permeability of ATP (66,67). Ischemia/reperfusion leads to further mitochondrial damage; in particular, it causes secondary damage with the release of cellular oxygen free radicals due to a series of chain reactions involving cell membrane damage, lysosome rupture and cell lysis. Mitochondrial biogenesis is promoted via the activation of JAK/STAT by leptin, via downstream proteins, such as proliferator-activated receptor γ a co-activator-1 α and mitochondrial transcription factor A. Such activation has been indicated to be involved in neuronal cell protection via the enhancement of anti-oxidant enzyme activities (15,47). It has been demonstrated that leptin increases superoxide dismutase (SOD) levels, stabilizes the mitochondrial membrane and alleviates endoplasmic reticulum pressure (17); this in turn inhibits the production of free radicals and ischemic injury in mice by binding to OB-Rb in the cortex, hippocampus and striatum. Inflammatory responses of leukocyte infiltration have an important role in cerebral ischemia and reperfusion. The reduction in oxidative damage to brain tissue caused by reactive oxygen represents a therapeutic approach for cerebral ischemia/reperfusion injury (1). Oxygen free radicals activate inflammatory cytokines and amplify inflammatory signals (68,69). Upregulation of adhesion molecule expression on the surface of neutrophils, reactive leukocytes and vascular endothelial cells was observed; furthermore, there was an increase in neutrophil adhesion to vascular endothelial cells (70,71).

In cerebral ischemia/reperfusion injury, inflammation caused by leukocyte infiltration and fluid has an important role; a therapeutic target is represented by oxidative damage of the brain tissue determined by reactive oxygen species (ROS) (72). In particular, the latter activates inflammatory cytokines and amplifies inflammatory signals (71,73). Hence, upregulation of adhesion molecule expression on the neutrophil surface, reactive leukocytes and vascular endothelial cells may be observed. In addition, neutrophil adhesion to vascular endothelial cells inhibited the production of NO, as determined by the aggravation of cell edema (74). Leptin has an anti-inflammatory role (75,76); it activates the nuclear factor- κ B pathway, inhibits the expression of pro-inflammatory factors [tumor necrosis factor (TNF)- α , IL-6, IL-1 β] within 7 h and increases the expression of IL-10 (74,77). Within 100 min, the human body is able to recover from hypothermia and hypotension caused by ischemia/reperfusion injury (78). Within 90-240 min, TNF- α is inhibited. This is not accompanied by changes in the levels of other cytokines. In 1981, the concept of ischemic penumbra, which refers to the reversible damage caused by the brain outside the central

area of focal ischemic necrosis, was introduced (79). While the energy supply ceases, the ionic pump in the cell remains accessible. The ischemic penumbra, an area unaffected by the stroke for a few hours from its occurrence, represents an important therapeutic target (3,80). During cerebral ischemia/reperfusion, oxidative stress leads to the breakdown of the redox balance; this is due to the accumulation of ROS and the dysfunction of important redox-sensitive protein kinases, membrane receptors and ion channels (81). ROS may damage DNA to trigger apoptosis, peroxidising the phospholipid membrane and releasing cytochrome C from the mitochondria, thereby leading to activation of pro-apoptotic caspases and apoptosis initiation (13). Leptin treatment causes the production of SOD, upregulation of the anti-apoptotic protein B-cell lymphoma-2 (Bcl-2) and downregulation of caspase-3 and Bcl-2-associated X protein expression. This stabilizes the mitochondrial membrane potential, and reduces oxidative stress and induction of apoptosis (82).

7. Conclusion and outlook

Stroke determines high morbidity, disability and mortality following cancer, and precedes cardiovascular disease (83). Leptin has an important role in decreasing excitatory neurotransmitter levels, protecting mitochondria, decreasing superoxide and free radical formation, increasing anti-inflammatory factor and -apoptotic protein levels, decreasing apoptotic protein levels and avoiding thrombolysis and the risk of cerebral hemorrhage (84). Leptin has drawn the attention of numerous experts and scholars. Several studies have indicated that exogenous leptin has a protective role in the brain (85), while also interfering with the body's energy regulation. Most studies on human subjects are relevant and provide contradictory points of view regarding leptin in this field. Regarding the association of the serum levels of leptin with stroke, the opinion of scholars is divided. By contrast, a prospective study on 4,571 healthy African Americans did not identify any association between leptin levels and ischemic stroke risk in either men or women (8,22,51,86). Only a few small studies evaluated leptin levels during the acute phase of ischemic stroke and reported increased leptin levels (7,26,50,52,87). It is currently indicated that leptin administration after stroke may be a promising treatment strategy. In particular with use of a gel, the polymer (synthetic leptin protein) may be directed to the damaged brain tissue to stimulate different functions, including the regulation of nutrition and growth, and the differentiation of neural stem cells. Based on these results, leptin may be selectively administered to the damaged parts of the brain to subsequently observe whether leptin stimulates the growth and differentiation of neural stem cells (88-90). However, further research is warranted in this regard. Leptin is a novel brain protective drug, but its application has not yet been successfully translated into the clinic. Additional research is required to implement applications developed in animal experiments as clinical treatments in humans.

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Availability of data and materials

Not applicable.

Authors' contributions

WZ, JC and DW conceived and designed the article. WZ and YJ analyzed the relevant literature. WZ wrote the manuscript and drew the figures. YJ, ZY, DW and XL made suggestions for revision. WZ, DW and JC are responsible for text layout.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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