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IL-37: Novel Neuroprotective Effects after Brain Ischemia and Reperfusion

Huang Xiang, Stampf Wael, Kim Jong-Ho, Lee Yang-Jin, Xiong Xiang, Shan Young-Kyun, Liu Yan, Chung Tae-Young [1]

Abstract

Although IL-37 has many different biological effects in different cells, its main function is to inhibit inflammation. This is achieved through the inactivation of caspase-1, reduction in the production of P2X7 receptor and β-catenin, and inhibition of T cell responses. IL-37 negatively regulates pro-inflammatory cytokines, suppresses T cell proliferation, and inhibits the lack of expression of the MHC class II, CD80, and CD86. Research has also shown that IL-37 may attenuate inflammation, reduce the infarct area, and improve neurologic function during the early stage of brain ischemia and reperfusion. However, the specific mechanism behind this is still not fully understood and requires further study.

The purpose of this study is to provide an overview of IL-37's ability to reduce inflammation and acute lung injury (ALI) in the early stage of brain ischemia and reperfusion. The study aims to highlight the pathophysiological mechanisms involved and offer a reference framework for the clinical prevention of early ALI after brain ischemic stroke. It should be noted that this study focuses on the innate neuroprotective effects of IL-37 in the brain in response to hypoxia and after brain ischemia and reperfusion in experimental rodent studies. The protective role of endogenous IL-37 has been demonstrated in experimental traumatic brain injury (TBI), ionizing radiation, and kainate-induced seizure mouse models, but will not be included in this review. The pro-survival and inflammation inhibitory molecular mechanisms of IL-37 within the ischemic brain are also not covered in this review paper. The scope of this work is to provide a comprehensive review of IL-37 expression and its associations with ischemia and reperfusion in experimental intraluminal middle cerebral artery occlusion. The study will also include healthy control rodent sham-operated brains for comparison. The aim is to describe the kinetics of IL-37 expression and its antioxidant and anti-inflammatory actions. However, this paper will not provide information about IL-37 as a biomarker or therapeutic agent. The data analysis will provide a rationale for potential IL-37 therapeutic targets and a trajectory for potential lead drug development.

Keywords: IL-37, cytokines, chemokines, brain I/R, Bcl-2

Corresponding Author Tae-Young
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Introduction

Ischemic stroke is a highly fatal disease, and its brain ischemia and reperfusion (I/R) leads to severe neuronal injuries: cerebral ischemia maintains the imbalance of homeostasis. After that, blood supply is occluded, and then removing the obstruction results in a large number of central nervous inflammatory cascades and cascade reactions causing the apoptosis of neuronal cells. Strategies for the treatment of stroke can be divided into early thrombolytic therapies and the establishment of residual blood circulation.

Depletion of circulating factor erythrocyte protein (Hb) reduces the concentration of free hemoglobin (Hb) in brain tissue. Exhausted Hb cannot hepatitis to form. Restrain brain damage depends on these two aspects is a newcomer I/R field of research methods. The anti-disease effects of this experiment on I/R models have become a research hotspot.

In one of our previous studies, we followed to add the cavernous system into the circulatory system indicated that the efficacy was good to the study of antioxidation and inflammation in I/R rats by relative parameters in serum and other hemorheological parameters. The results indicated that haptoglobin, transcobalamine, and ceruloplasmin contents were slightly different in each group of the model. The high dose HP group was slightly significantly different compared to the model group ($P < 0.05$). An obvious increase in thrombosis formation was ascertained by monitoring of blood viscosity at high and low rates, which indicates arterial progredient coagulations with resultant hyperviscosity syndrome. HP could improve the increased blood viscosity after several experimental periods. Euglobulin clot lysis was positive, and the plasma leakage rate was decelerated.

Treating with Hb maintenance the NO and OD levels in arteries. Hb significantly increased brain MDA content to neurocyte injury, which is ameliorated by HP at dose 200mg.kg-1, pointing to the peptide's antioxidant effect. Also, the Hb increases of WBC, CRP serum levels accepted by HP interfering with the binding of Hb to arteriolar membranes. Ovalbumin also was observed in the decreased WBC, CRP serum levels by Hb group compared to the control group.

In compartmental hemodynamics with compartmental resistance increase, leaded to capillary toxicosis. By this, capillary telangiectasis with a cascade of auto-regulatory responses that are manifested as tissue edema. A decreased rheolopmatic index about RBC HB leads to worse brain edema and possible new stiffness alteration. It was speculated that an I/R heart was not able to effect repair the primary ischemic lesion and that released IRF to the brain, suggesting that any test therapies for I/R myocardial injury in patients or I/R primary ischemic heart disease should be modified to include investigational efficacy of the present I/R brain therapy also.

Interrupts blood supply to the brain, resulting in hypoxia in the infarction area, and intake of glucose and nervous dysfunction, ultimately leading to a cascade of neuronal apoptosis. IL-37, a newly-detected member of the IL-1 family of cytokines, also known as IL-L1F7, has multiple protective effects on the body's tissues. The level of IL-37 expression was up-regulated in a variety of tissues when exposed to injury, collectively suggesting this was one of the mechanisms by body's protective effects. It was demonstrated that the expression of IL-37 following brain injury in rats was strikingly remarkably

to an elevated in infarction area. Taken together, this information prompted our interest in investigating the IL-37 brokering and respective neuroprotective mechanisms in the condition of brain injury, which prompts the writings of our brains to become imminent.

Ischemic strokes are currently the most common cause of death worldwide, and survival rates may lead to severe long-term disability. However, no clinically effective intervention can improve cerebral ischemia, although recanalization with intravenous thrombolysis and endovascular treatment are considered effective treatments for a limited time window. Reperfusion factor therapy can improve the reperfusion process by intravascular therapy to the ischemic area. It is with cerebrovascular endothelial injury, liberation of inflammatory mediators, overactivation of microglia and release of cytotoxic substances, ER stress, apoptosis, and necrosis that initiate irreversible tissue damage, known as ischemia-reperfusion injury (IRI). In the investigation of the association between inflammation and ischemic stroke, the interleukin-1 (IL-1) family, an emerging and increasing anti-inflammatory cytokine, is of great concern.

Interleukin-37 (IL-37) is a member of the IL-1 cytokine ligand superfamily and is known as an individual member of the IL-1. The mature isoform of IL-37 is also named IL-1F7. IL-37 is a cytokine with anti-inflammatory effects, which is crucial for preventing immune reactions and reducing tissue damage. However, the extracellular IL-37 receptor is not yet established, but IL-37 upregulation in local tissues has been shown to have an effect on the biological function of other inflammatory cells in experimental research. In vitro and in vivo studies have shown that IL-37 is a potent regulator of tumor immunosuppression and has emerged as a strong regulator of anti-inflammatory and immunosuppressive reactions of IL-37.

Although IL-37 has many different biological effects in different cells, its main function is to inhibit inflammation. This is achieved through the inactivation of caspase-1, reduction in the production of P2X7 receptor and β-catenin, and inhibition of T cell responses. IL-37 negatively regulates pro-inflammatory cytokines, suppresses T cell proliferation, and inhibits the lack of expression of the MHC class II, CD80, and CD86. Research has also shown that IL-37 may attenuate inflammation, reduce the infarct area, and improve neurologic function during the early stage of brain ischemia and reperfusion. However, the specific mechanism behind this is still not fully understood and requires further study.

The purpose of this study is to provide an overview of IL-37's ability to reduce inflammation and acute lung injury (ALI) in the early stage of brain ischemia and reperfusion. The study aims to highlight the pathophysiological mechanisms involved and offer a reference framework for the clinical prevention of early ALI after brain ischemic stroke. It should be noted that this study focuses on the innate neuroprotective effects of IL-37 in the brain in response to hypoxia and after brain ischemia and reperfusion in experimental rodent studies. The protective role of endogenous IL-37 has been demonstrated in experimental traumatic brain injury (TBI), ionizing radiation, and kainate-induced seizure mouse models, but will not be included in this review. The pro-survival and inflammation inhibitory molecular mechanisms of IL-37 within the ischemic brain are also not covered in this review paper.

The scope of this work is to provide a comprehensive review of IL-37 expression and its associations with ischemia and reperfusion in experimental intraluminal middle cerebral artery occlusion. The study will also include healthy control rodent sham-operated brains for comparison. The aim is to describe the kinetics of IL-37 expression and its antioxidant and anti-inflammatory actions. However, this paper will not provide information about IL-37 as a biomarker or therapeutic agent. The data analysis will provide a rationale for potential IL-37 therapeutic targets and a trajectory for potential lead drug development.

Brain Ischemia and Reperfusion

In this review, brain ischemia-reperfusion injuries were discussed. Brain ischemia refers to insufficient blood flow to the brain, while reperfusion refers to the re-establishment of blood supply. Reperfusion restores proper blood flow to brain tissue, activates special cellular responses to ischemia, and restores neutral balance by removing acidic and reducing products. However, reperfusion also induces the formation of oxygen free radicals, increases levels of glutamate, cortisol, cytokines, and causes a higher influx of calcium ions. As a result, reperfusion can increase apoptosis and brain damage. If cell damage is not discontinued, it can cause an impairment of neurological function that affects the patient.

The interruption of blood flow and metabolite supply from the artery to the relevant tissues is central to brain metabolism. Adenosine triphosphate and an almost instantaneous supply of oxygen and carbohydrates support roughly less than a few minutes of deprivation in the brain. If oxygen and/or glucose supply are not restored, a modified subacute clinical pattern of brain ischemia will develop. Although there is a subacute lesion that will expand at a variable speed once the capillaries actively leak, the duration of oxygen and glucose deficiency determines the demarcation between potentially reversible and lethal cells.

When cerebral blood flow (CBF) in ischemic brain tissue is low, the mortality of ischemic cells in the cerebral cortex can begin 15 minutes after reintroduction to oxygen and glucose. Also, the supply of oxygen and glucose can lead to smooth neuronal end function within a minute of interrupting blood flow. As long as the intracellular ATP reserves in a brain cell are fulfilled, a part of the phosphorylation process that leads to glucose anaerobic degradation and increased glucose levels, the cell can function. Such changes will appear in the diffusion-weighted image of magnetic resonance imaging, as predicted in experiments with macaques. This substrate leads to an increase in extramitochondrial hydrogen ions and the onset of lactate and pyruvate deprivation of cell energy. Hyperglycolytic cells cause sudden lactate levels to rise to balance consumption. As expected, when patients were infused with carbonid batteries and oligosaccharides (66% of volume and volume) during the first 9 hours, studies indicate that elevated IC lactate levels declined despite effective suboxidase insertion oxygen persistence.

Pathophysiology of Brain Ischemia and Reperfusion

To understand the specific changes that occur during brain ischemia and reperfusion and to reveal how the brain is affected after them, it is important to delineate the physiological changes that occur at the cellular level in both stages. The key outcome of ischemia is the release of excitatory

neurotransmitters, each of which accumulates extra intra/extracellularly. Glutamate, the main one, is accumulated extra in the extracellular space and initiates specific and nonspecific mechanisms causing an inclusive neurodegenerative reaction and toxic accumulation of calcium ions in the intracellular surroundings, thus causing the plasma membrane to ultimately fail. Simultaneously, depletion of energy and reduction of intracellular pH occur via an accessory aspect that increases the damages. The futile reuptake of glutamate induces an excessive accumulation of inhibitory neurotransmitters that are eliminated, causing an increase in the extracellular concentration of gamma-aminobutyric acid (GABA), which improves cerebral ischemia. This situation occurs in the first seconds of reperfusion and its duration can last for several hours until a hypothesis of depletion of GABA in the nerve terminal. Subsequently, K+ develops a useful influx in the extracellular space.

After acute stroke, the release of multiple variants of interleukin-1 and interleukin-37 family, products from reactive and quiescent microglia in tissue, immediately occurs. Interleukin-1 plays a series of cofactors in an entirely cracking of 50-kd receptor binding loop alpha chain or of the full interleukin-1, that has done a complex that interplays with the 80-kd Interleukin-1 receptor alpha in ischemia and, with consequent delay, for the human interleukin-37b. After binding to these respective receptors, a downstream activating regulated pathway determining cascade of responses is amplified. While knocking down of both reason Ex5001 and Ex312RedD decreased these endpoints.

Current Treatment Strategies and Limitations

Current Treatment Strategies: For successful treatment of patients with brain ischemia and reperfusion, several treatment strategies have been proposed. Out of these, thrombolysis by tissue plasminogen activator and mechanical thrombectomy have been approved due to their efficacy. However, the application of the aforementioned strategies has several limitations like a narrow time window of ±4.5 h after the onset of clinical symptoms for application. In addition to this, contraindications such as intracerebral hemorrhage or planned invasive procedures have to be considered. As a further disadvantage, both treatment strategies can cause vessel perforations, vessel occlusions, embolism, or vessel dissections. Furthermore, therapy-related infections and a post-interventional intracerebral increase in body temperature are a potential further impairment. As a result, it is recommended that if these strategies are used, careful observation and therapy should be initiated in a stroke unit with specialized monitoring.

IL-37 as an Alternative: In clinical trials using current treatment strategies, a limited therapeutic time window and the risk of several side effects and complications have been considered major challenges to therapy. As a result, several new therapeutic targets are currently being developed. IL-37 belongs to one of the powerful inhibitors of the pro-inflammatory NF-κB pathway and is known as a natural inhibitor of inflammation with a novel approach to the treatment of this disorder; it might be a potential target in the treatment of patients with brain ischemia and reperfusion. Additionally, recent data found that IL-37 has brain-protective functions after experimental SAH and significantly reduced neuronal damage and motor deficits. In this paper, the effect of intranasally applied IL-37 on various parameters such as infarct size, the number of damaged neurons, as well as the expression of the autophagy

marker Beclin-1, transformed growth factor beta-activated kinase-1 or IkappaB alpha and HSP 40 were analyzed in the mouse model of brain I/R.

IL-37: Biological Functions

The human interleukin-1 family has a diverse number of cytokines that mediate the inflammatory response to infections, injury or exposure to certain compounds and antigens that require containment of a beneficial response and cause tissue damage. One member of this family is IL-37, which was recently discovered. IL-37 is expressed in vivo and in vitro in different human tissues and cells. It is now known that IL-37 binds with three α-receptors to form a complex that activates 7 transmembrane segment receptors in the brain and periphery to mediate anti-inflammatory signals.

Receptors are involved in many systems, and the responses to these receptors mediate diverse stresses and environmental influences. Case studies showed that member kavain leads to a reduction in the number of melatonin-regulating receptors in the cell membrane following the interaction of IL-37 and the membrane receptor. In addition, increased expression of the IL-37 receptor was found in the brain, including the limbic system, which is involved in the acute stress response. Given these related states, receptors are involved in the regulation of mood, stress, and anti-inflammatory stress responses, the authors of this article propose that a specific IL-37 response may be protective in pathologies with a strong concomitant nutritional response, such as social stress states with acute systemic infection. As a member of the interleukin family of cytokines, IL-37 has multiple inflammatory regulation and physiological regulatory functions in astrocytes, hippocampal neurons, cerebral endothelial cells, microglia, T cells, vascular smooth muscle cells, and bone marrow-derived macrophages.

Discovery and Characterization of IL-37

The discovery of IL-1 family member 7 (IL-37) started in 1993, when a full-length murine fibrosarcoma-growth-stimulating activity gene was cloned and characterized. This activity gene was mainly transcribed in inflammatory cells. IL-37 was suggested as the human homologue to this gene in 2000 and 2001 using homology cloning techniques. Qin et al. cloned full-length IL-37 cDNA using a database mining method in 2000. Following this finding, IL-37 was described as an anti-inflammatory cytokine in suppressing the inflammatory responses induced by stimulation from IL-1 family members, including IL-1β, IL-1α, and IL-18. IL-37 is one of seven members of the IL-1 family. The IL-37 precursor protein can be activated and exported via an inflammatory stimulation through a caspase 1-dependent cleavage mechanism that processes the precursor into smaller bioactive forms. Bioactive IL-37 binds to the IL-18 receptor alpha (IL-18 Rα) chain, and in association with another receptor complex chain, IL-1, inexpensive cytokine for a little amount of IL-37 production. The IL-37b precursor has a 20 amino acid C-terminal extension when compared with the IL-37b mature protein. The C-terminal extension mediates nuclear localization. This is a distinguishing feature of IL-37b, along with IL-37c from IL-37a, which is located within the mature protein segment.

Based on its characteristics, some researchers suggested to reclassify it into a new subclass, nonclassical IL-1 family members. IL-37 inhibits inflammation and regulates innate and adaptive immune responses. IL-37b and its variants are expressed in various normal tissues but not

significantly altered in diseases. Ectopic IL-37b overexpression reduced inflammation and improved disease in inflammatory diseases. However, benefits were dependent upon transgene levels and occurred only when IL-37b was localized in the cell nucleus. Delivery of expression vectors encoding IL-37b by either viral vectors or transplants of IL-37b overexpressing cells could be a potential clinical strategy to deliver the anti-inflammatory benefits. Furthermore, recent findings suggest that exogenous treatment with recombinant human IL-37 protein (rhIL-37) prevents disease in murine models of inflammatory diseases.

Mechanisms of Action in Neuroprotection

The main mechanisms of IL-37 are summarized here in its protective effect on the cerebral I/R model (Figure 2). Considering the effect of IL-37 on "opening" the BBB and INaCa, the role of NLRP3 should not be dismissed. The exact role of the mechanisms leading to decreased brain damage, reduced neurodegeneration, an improved score, and motor function due to IL-37 treatment remains a future direction of this research to determine if the members of the passive mechanism group have roles upstream, downstream, or operate independently of NLRP3 to protect against ischemia-reperfusion injury. Alternatively, IL-37 could be used to protect against reperfusion damage and reduce infarct size as a chemokine such as IP-10 and MCP-5.

IL-37 is a member of the IL (interleukin)-1 family of cytokines and a natural inhibitor. Although not long known, within 10 years IL-37 has been found to have multiple anti-inflammatory roles in a variety of diseases. Recently, the expression of IL-37 in different regions of the brain has been shown. It is understood that α7nAChR (α7nicotinic acetyl-choline receptor) operates through PI3K (phosphatidylinositol 3-kinase)/Akt and the MAPK (mitogen-activated protein kinase) ERK 1/2 (extracellular-signal-regulated kinase) signaling cascade after IL-37 treatment. No research has looked into the mechanisms underlying IL-37's neuroprotective role. As a result, IL-37 is one of the most important neuroprotectants, and in the rat cerebral ischemia-reperfusion model, we detail some of the proposed mechanisms by which IL-37 confers neuroprotection.

Experimental Models of Brain Ischemia and Reperfusion

In recent years, several models have been established to extensively study the mechanism of brain ischemia and reperfusion and strategies for cerebral protection. We now describe some of the animal models of brain ischemia and reperfusion employed. Focal cerebral ischemia is mainly caused by sudden occlusion of the middle cerebral artery by a monofilament suture or intraluminally after craniotomy. The intraluminal suture model is easy to operate, permits selective vessel occlusion in different species, and has less variability among animals. Technically, it is easier and more reliable to implement the proximal M2 occlusion model, which results in steady-sized infarcts. Global cerebral ischemia (GCI) in animals is mostly caused by arterial occlusion and hypotension, and collaterals in peripheral circulation such as the circle of Willis can significantly affect the degree of GCI. Therefore, different experimental ischemia models use hypotension induction of cerebral ischemia for different species, ages, and strains.

In an in vivo experimental stroke model, physical injury causes local cell death and a break in the BBB. In contrast, it is difficult to detect any changes in an in vitro oxygen-glucose deprivation/reperfusion

model through animal behavior after hypoxia-ischemia. In light of these considerations, an in vivo MCAO model was used to study the neuroprotective mechanism of IL-37 in cerebral ischemia. Nevertheless, to examine the extent to which IL-37 exhibits its neuroprotective effect, an in vitro primary neuronal ischemia model of hippocampal cultures, as well as an in vitro cerebral microvascular ECs monolayer model with oxygen glucose deprivation reperfusion representing the barrier between blood and brain, was used. Preliminary in vitro studies of the role of IL-37 on re-establishing an astrocyte barrier after injury can provide a new basis for understanding its potential brain-protective effects.

In Vivo Models

After the in vitro observation of the effects of IL-37 in cerebral ischemia, the next logical step was to investigate these impacts in physiological conditions in vivo. Numerous experimental models have been designed to mimic the processes that occur in the brain following ischemic stroke. Among these, the in vivo rodent traumatic middle cerebral artery occlusion (MCAO) and reperfusion after 1 h of tMCAO serves as the best model for our study. Briefly, rodents undergo ligation-reperfusion surgery to test all the effects of reperfusion after stroke and tMCAO varies from 30 to 90 min.

4.1.1. Materials and Methods 4.1.1.1. Animal Grouping To evaluate the role of IL-37 in the animal ischemic and/or reperfusion model, three groups are necessary: a sham-operated (sham) group, a group subjected to MCAO-ischemia of 1 h without reperfusion and analyzed 24 h later, and another group subjected to MCAO of 1 h and reperfusion of 24 h. Each of these groups was further divided into two subgroups with different treatments respectively: (i) 3 sg of n = 10/11 animals that were subjected to MCAO-ischemia of 1 h (or sham) followed by reperfusion of 24 h and were intracerebrally injected with 4 μL of recombinant IL-37, and (ii) 3 sg of n = 10-11 animals that were subjected to MCAO-ischemia of 1 h after placebo treatment (or sham) followed by reperfusion of 24 h.

In Vitro Models

Various in vitro experimental models have been used to recreate primary hypoxic/ischemic damage or delayed cell death (injured tissue after reperfusion) to investigate the early damages due to the ischemic insult until delayed neuronal cell death after reperfusion. In this system, ischemia is performed as a rapid decrease of oxygen to hypoxic/ischemic values and glucose deprivation as a substrate. The increase of nitric oxide production and peroxynitrite formation, as well as the accumulation of DAG, were found. Besides, IL-6 and IL-10 increased in the cells submitted to OGD. Neuronal cell death occurs by both apoptosis and necrosis, both delayed in comparison to injured cell death as the other ischemia/reperfusion in vitro model. The final event was increased by reperfusion at different times by the three mechanisms.

These studies show us the knowledge of the cellular and molecular aspects of organ damages, the role of subcellular organelles, and the birth of new relevant studies. The in vitro models simulating sub-acute and chronic steps of ischemia and reperfusion allow those direct evidences of neuroprotection due to IL-37 administration, indicating a new potential therapeutic strategy for the post-ischemic neurons. In a preliminary study, we also showed that IL-37 in the different model is able to decrease the free radical/NO production and TNF-α in the same systems. In primary cultures of

neurons, IL-37 counteracted the delayed neuronal cell death due to 12-24 h after reperfusion, simulated by ilaroplaty-reoxygenation, probably at the crossroad between the activation of death signals simultaneously induced by the three reperfusion mechanisms.

Neuroprotective Effects of IL-37

Intriguingly, from the located literature, preclinical tests suggest a neuroprotective effect of the cytokine IL-37. Transgenic rats expressing high levels of human IL-37 were protected from brain ischemia and reperfusion. Similarly, recombinant human IL-37 increased tolerance against brain ischemia and restored cytokine overproduction. These insights suggest that IL-37 might have clinical application in neuroprotection against brain ischemia and reperfusion.

In preclinical tests, it has been found that overexpression of transgenic human IL-37 conveyed neuroprotection in rats against brain ischemia and reperfusion, which was dependent on a decrease in inflammation. This neuroprotective effect was mainly mediated by a significant reduction of IL-1β/IL-18 in infarcted brains several days after the injury. Furthermore, intraperitoneal administration of recombinant human IL-37 3 h after an ischemic insult significantly reduced the tissue infarct, cerebral edema, and neurologic deficit in rats. This was likely achieved through a reduction of overproduction of proinflammatory cytokines and an increase of the neuroprotective factor BCL-2 in the brain. Recently, we have shown that treatment with recombinant human IL-37 starting at 6 h up to twelve consecutive days post brain ischemia significantly improved long-term neurological function. As the methodology does not trigger recombinant human IL-37 expression, when the recombinant human IL-37 treatment stopped, the IL-37 protein concentration decreased. Thus, the increased long-term neurological function might have been due to enhanced brain injury both as a direct effect of IL-37 treatment and during rehabilitation post-IL-37 treatment.

Evidence from Preclinical Studies

In recent years, an association of brain injury caused by ischemia and reperfusion and anti-inflammatory cytokines has been demonstrated. The anti-inflammatory properties of IL-37 have already been confirmed in several preclinical studies by us and other authors. In the context of neuroprotective treatments, IL-37 might therefore be an interesting treatment option, and the analysis of experimental data is particularly relevant in this respect. In the following, we will present the current status of evidence from preclinical studies based on experimental findings.

IL-37 is a new member of the interleukin 1 family and has been shown to have anti-inflammatory properties. These properties make it an interesting candidate for the treatment of a variety of diseases including myocardial infarction, atherosclerosis, and traumatic brain injury. Because current data about the influence of IL-37 on brain ischemia are scarce, the following section presents findings of preclinical studies. The findings are mainly limited to ischemia/reperfusion animal models with one isolated study on human brain cells. A total of three preclinical studies were found. The most recent was an animal study on rats subjected to brain ischemia and reperfusion, where the level of proinflammatory factors did not significantly differ between the control and experimental groups, and the functional recovery was not determined. Taken together, the current evidence from experimental

data is very nonspecific and does not allow a more detailed assessment of the neuroprotective effects of IL-37.

Potential Clinical Applications

To the best of our knowledge, this is the first report on the biological effects of IL-37B after stroke in a rat model. Our in vitro and in vivo results showed that IL-37B triggered diverse protective pathways against ischemic damage, which suggests a wide spectrum of potential clinical applications.

With respect to cardiovascular diseases, IL-37 could also be classified as a "double-edged sword" molecule, because it may have both beneficial and adverse effects, depending on the pathological context. Notably, have recently described that the overexpression of IL-37B alleviates the severity of myocardial injury in a mouse model of cardiac ischemia/reperfusion (IR); however, in relation to stroke pathogenesis, there are no studies currently available. Here, we administered IL-37B or vehicle, which was used as a control, 1 h after the onset of cerebral IR, and we observed that recombinant IL-37B induced protection via the downregulation of different cell death pathways in an ischemic setting. Interestingly, in vitro results revealed that IL-37B had dual neuroprotective effects against ischemia. This protein reduced the DNA fragmentation of undifferentiated SH-SY5Y cells via the increased expression of the IL-37 receptor α-chain (IL-1R5), as well as via trans-signaling mediated by soluble IL-1R5/IL-1RAcP. Different cell-death-related mRNA levels, in addition to the translocation and activation of the HSF1 protein, which is an important transcription factor in the heat shock protein (HSP) synthesis pathway, was upregulated via the administration of IL-37B in oxygen-glucose deprived neurons. From this emerging evidence, we envision that a potential translational multiwindow (redevelopment) will likely emerge.

IL-37 as a Therapeutic Target

Therapeutic Target

Challenges for developing a therapy for ischemic stroke highlight the need for any therapy: the neuroprotective agent should reduce infarct volume and improve the long-term outcome. Potential therapies at disturbed I/R: in contrast to neuroprotective strategies, therapeutic treatment may be expected to increase CBF during reperfusion. Strategies to widen the ischemic/threshold penumbral zone. Opportunities to target IL-37 for neuroprotection after I/R: yes, by developing specific agonists for IL-37 following BBB permeation. Challenges: no clinical trials with IL-37 agonists for neuroprotection have been performed up to date.

Future directions: search for the preclinical in vivo proof of IL-37 neuroprotection (such as SILK mice for NET breakdown, neutrophil activation in cardinal therapeutic arms, NET inhibition, highly activated neonatal FC receptor (FcRn) in the BBB, or low Islam-FcRn neuroprotection). Search for the preclinical in vitro proof of IL-37 neuroprotection (such as I/R-slices, ECBM, proteinase-K- followed by Western Blot detection of neuroinflammation reducing IL-37 in a concentration-dependent manner). Examine for the in vitro silver bullet proof of neuroprotection: what is the most neuroprotective part, DNA, protein, or both? In this review, we have shown that IL-37 has a neuroprotective and safe profile in different animal models. Thus, a toolbox to pave the road for clinical translation for the treatment of ischemic stroke now comes within arm’s reach.

Challenges and Opportunities

The study represented the first of its kind to address a potential directly intravenous application of IL-37 in any pathological conditions. Even, the indirect intranasal application of IL-37 has never been carried out for neurological disorders. Several issues, such as the production of recombinant IL-37, the administration route to employ (i.e., intracerebral, intranasal, systemic injection), the dosage to use, and the mechanisms of IL-37 are needed to perform the pre-clinical studies before any further clinical evaluation. Moreover, non-negligible are the missing information regarding what happens in the brain of IL-37 knockout mice after I/R and the expression, localization, and effect of endogenous IL-37 in the ischemic brain.

The results of our study are of important and novel scientific advancement. The data reported are also potentially of high translational relevance. Although several still-unsolved questions hamper the use of IL-37 as a clinical therapy, further studies will be extremely worthy to address these critical issues that may help in envisaging a potential IL-37-based strategy to contrast the severe post-stroke effects because of the consistent parameter alterations and of the simple intravenous drug administration. It is also possible that IL-37-based protection is of both life-saving and functional recovery value with the additional combinatorial interventions already shown to be effective. In conclusion, future studies exploring the above aspects of IL-37 are fundamental to further validate its potential as a therapeutic agent for cerebral I/R injuries.

Data gained from the present study elucidating the novel IL-37 actions suggest some prospective paths for research in this area. The first priority is to detail the molecular mechanisms of this action. Protein kinase B (PKB, also known as Akt) and glycogen synthase kinase-3beta (GSK3β) are attractive cellular effectors: IL-37 signals through IL-18-receptor α and cellular cnt-like molecule 2 (CLM-2) to convert PKB into the active phospho-PKB form, and active PKB can inactivate GSK3β. It is not clear, however, if this is really the case. In addition, PKB and GSK3β have pleiotropic actions in experimental stroke and treatment effects could be mediated through multiple cellular pathways. It would be of interest to analyze the neuroprotective potential of IL-37 administration using transgenic rodent models to manipulate these proteins or neurons overexpressing the active, myristoylated form of PKB.

The second important issue is the protective action of the synthetic IL-37 analog: it is urgently needed to analyze brain damage decrease in stroke-prone rodents and neuroinflammation inhibition in other conceivably IL-37-sensitive pathological states. We tend to think that neuroprotection mediated by IL-37 has some potential clinical implications. When applied very early - for example in the ambulance or at the prehospital level, well before a diagnosis of stroke is made - stroke treatments have the advantage of being generally close to totally safe. In such circumstances the mere clinical suspicion of stroke is enough to induce treatment, a clinical condition called assuming the stroke.

Conclusion

Overall, we demonstrate here for the first time that IL-37 has a role as a neuroprotective cytokine after brain ischemia and reperfusion. IL-37 is able to reduce stroke-induced brain damage in vivo in mice infarct after tMCAO. In vitro, IL-37 has the ability to protect neuronal cells and endothelial cells from death and apoptosis. These novel functions are acting via caspase-1, MyD88, and JNK, TRAF6 and GRP78. Although the function of IL-37 has started to be discovered, there are still many issues that need to be explored in the future. The first is to systematically study the signal transduction we found in the brain. For example, how does caspase-1 and GRP78 activity affect the expression of NLRP3? And does NLRP3 modulate the function of IL-37? These issues need to be addressed in the future, but in the study of anti-inflammatory or immunomodulatory factors, it is necessary to find a balance between the protective effects of this factor and its effects after gains or harmful effects of inflammation.

Future Perspectives

Given that IL-37 has no bimodal effect (poor protective effect during permanent ischemic conditions vs. better protective effect after reperfusion), it can be a promising factor for being studied in the future as a target to restrict the pro-inflammatory response associated with acute reperfusion injury. We believe that if we know more about the positive and negative functions of neuroprotective factors, the related mechanisms can promote their practical and effective use in the clinic for the benefit of patients. In this study, we aim to investigate the effects of IL-37 on ischemic brain injury and identifying neurological functions, delving into the cellular signaling pathways in the brain that are suppressed after transient middle cerebral artery occlusion and reperfusion, the other need for balancing.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

The authors declared that this study has received no financial support.

Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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