Protective effect of Tβ4 on central nervous system tissues and its developmental prospects

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Abstract
Tissue repair and regeneration in the central nervous system (CNS) remains a serious medical problem. CNS diseases such as traumatic and neurological brain injuries have a high mortality and disability rate, thereby bringing a considerable amount of economic burden to society and families. How to treat traumatic and neurological brain injuries has always been a serious issue faced by neurosurgeons. The global incidence of traumatic and neurological brain injuries has gradually increased and become a global challenge. Thymosin β4 (Tβ4) is the main G-actin variant molecule in eukaryotic cells. During the development of the CNS, Tβ4 regulates neurogenesis, tangential expansion, tissue growth, and cerebral hemisphere folding. In addition, Tβ4 has anti-apoptotic and anti-inflammatory properties. It promotes angiogenesis, wound healing, stem/progenitor cell differentiation, and other characteristics of cell migration and survival, providing a scientific basis for the repair and regeneration of injured nerve tissue. This review provides evidence to support the role of Tβ4 in the protection and repair of nervous tissue in CNS diseases, especially with the potential to control brain inflammatory processes, and thus open up new therapeutic applications for a series of neurodegenerative diseases.

Keywords
Alzheimer’s disease, astrocyte, central nervous system, inflammation, microglia, neurons, oligodendrocyte, thymosin beta4

Introduction
Thymosin was successfully extracted from fetal bovine thymus for the first time in 1966 by Goldstein et al.1 It was originally considered to be a thymic hormone with immunological effects on lymphocytes. It was later identified as a G-actin-binding protein, which is required for cell movement and organogenesis.2,3 According to their isolectric points, they are divided into three subfamilies of α, β, and γ. The β subfamily (Tβs) is distributed in all eukaryotic cells except red blood cells. Tβs is a water-soluble small molecule peptide composed of 40–44 amino acid residues. The chemical structure is highly conserved. It can specifically bind to G-actin to regulate the polymerization of actin and affect angiogenesis to varying degrees4,5 and induce apoptosis.4,6 So far, there are 15 types of Tβs found. In humans, there are three main forms of Tβs, namely Tβ4, Tβ10, and Tβ15. Among them, Tβ4 is the most widely distributed

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and is the most abundant, accounting for 70%–80% of the total amount of Tβ4.1,7,8

Tβ4 is a highly conserved and highly expressed 43 amino acid polypeptide with multiple intracellular and extracellular functions. In recent years, the biological function of Tβ4 has received much attention, as it plays an important role in many physiological and pathological activities. Studies show that Tβ4 is closely associated with wound healing,9 angiogenesis,10 tumor metastasis,11 cell apoptosis,12,13 corneal repair,14 and myocardial repair.15 It is also involved in anti-inflammatory and neurodegenerative processes,16 especially important for the occurrence and repair of the nervous system.17–23 At present, synthetic Tβ4 has been widely used in research to explore its mechanism of action in different physiological and pathological activities. Studies have found that Tβ4 messenger RNA (mRNA) is widely expressed in mammalian brains such as the hippocampus, dentate gyrus, cerebral cortex, amygdala, and some microglia.17,24 It also participates in the differentiation and development of the nervous system after birth,8 such as synapse generation, neuronal migration, axonal growth, and dendritic plasticity changes, suggesting that it could have neuroprotection, promote axonogenesis and synapse formation properties.25

Tβ4 has great potential to promote CNS plasticity and nerve cell regeneration and appears to be a good candidate as a neural repair agent.26 It can cause neurological recovery in a variety of neurological diseases.27 Studies have shown that Tβ4 can target multiple nerve cells (including neurons, oligodendrocytes, and microglia) in animal models of nerve injury and can also provide neuroprotection, immunosuppression, and nerve repair such as myelin sheath regeneration, synapse formation, and axonal growth (Figure 1).28–33

Tβ4, a polypeptide, inhibits inflammation,34 regenerates saxon,35,36 helps development of nervous system,37 and promotes glial cell differentiation.26 Therefore, its medicinal value in the treatment of neurological diseases cannot be ignored (Figure 2). There are a large number of Tβ4 in the brain, the molecular mechanism of action in CNS is unclear, and participation in the different signaling pathways is poorly understood.38 However, with the in-depth study of the role and mechanism of Tβ4 in neural repair, Tβ4 could become another important target for new treatment of neurological diseases (especially a series of neurodegenerative diseases such as Alzheimer’s disease (AD)).39,40

**Protective effect of Tβ4 on neuronal damage**

Tβ4 plays a key role in many cellular processes, including mobility, axon pathfinding, neurite formation, proliferation, and neuron survival.41,42 Sabara et al.43 reported the neuroprotective and neurorepairing effects of Tβ4 on injured nerve cells and the interaction between thymus and thymosin and the nervous and endocrine systems.

Exogenous Tβ4 treatment can increase the survival rate of neurons, such as Tβ4 or normal saline given by intraperitoneal injection 3 min or 5 days after spinal cord injury (SCI) in rats. All behaviors were evaluated, and the number of surviving neurons and oligodendrocytes in Tβ4-treated animals increased significantly.23 In addition, in the animal model of traumatic brain injury (TBI), early treatment with intraperitoneal injection of Tβ4 (6 h after injury) reduces the volume of cortical lesions and the loss of hippocampus cells and improves functional recovery, indicating that it is possible to have a neuroprotective effect.42 Treatment with Tβ4 significantly reduces apoptosis of neural progenitor cells due to oxyglucose deprivation.43 Wirsching et al.44 analyzed the expression of Tβ4 in chicken (Gallus domesticus) developing cones and overexpressed and knocked down Tβ4 during egg retrovirus transduction and plasmid electroporation. The results indicate the effect of Tβ4 on neural stem cell and/or progenitor cell populations. This indicates that Tβ4 has a greater effect on neural stem cell and/or progenitor cell populations. Choi et al.45 reported that Tβ4 is involved in the control of programmed cell death (PCD) of chicken embryo.

![Figure 1. Mechanism of thymosin β4 protection and repair of CNS.](image-url)
motor neurons (MNs). Tβ4 can significantly reduce the death of chicken embryo MNs caused by staurosporine (protein kinase C inhibitor). It is suggested that Tβ4-derived peptides can be used for anti-apoptotic treatment of neuropathology related to neuronal apoptosis. Popoli et al.\textsuperscript{46} also reported that Tβ4 could reduce the toxic effect of glutamate on primary cultured cortical neurons, significantly reduce neuronal apoptosis, and protect neurons in rat models of excitatory amino acid damage induced by kainic acid. Morris et al.\textsuperscript{47} found that Tβ4 could promote the migration and differentiation of neural stem cells from the subventricular zone (SVZ) to ischemic lesions, and reduce brain function damage after cerebral ischemia in adult rats, suggesting that Tβ4 may achieve these effects by stimulating neuron migration and inducing the expression of extracellular matrix. According to the latest report, based on Tβ4 potential anti-inflammatory molecules and neuroprotective and myelin regeneration molecules and their mechanism of action, it is conceivable to use Tα1 (thymosin-α1) and Tβ4 alone in multiple sclerosis (MS) or with other approved possible application of drug combination therapy.\textsuperscript{48}

**Protective effect of Tβ4 on glial cell injury**

Glial cells are interstitial cells of the nervous system. They support and provide nutrition to the neurons. They also participate in the transduction and transmission of information in the brain, regulate the secretion and uptake of neurotransmitters, and maintain the environmental balance in the brain. Glial cells mainly include astrocytes, oligodendrocytes, and microglia.

**Protective effect of Tβ4 on astrocyte injury**

Activation of astrocytes is often found in related studies such as neurodegenerative changes after chronic cerebral ischemia and neuronal damage in the hippocampus.\textsuperscript{49}

Studies have found that the expression of astrocyte markers S100B (S100 calcium binding
protein B) and glial fibrillary acidic protein (GFAP) in the hippocampus of rats with chronic cerebral ischemia are increased, but glutamate intake is reduced resulting in cognitive impairment. The analysis showed that there is a positive correlation between the two, suggesting that astrocytes may be involved in neurodegenerative changes and cognitive dysfunction after chronic cerebral ischemia. However, Yang et al.\textsuperscript{50} found that T\textbeta 4 can reduce the neurotoxicity of ethanol to astrocytes, and has a significant protective effect, suggesting that T\textbeta 4 can protect astrocytes and reduce their damage by inhibiting apoptosis.

**Protective effect on oligodendrocyte damage**

Oligodendrocytes are the myelinating cells of CNS. Their damage mainly affects the production of myelin, impairs the synthesis of myelin sheath, and eventually leads to pathological changes such as white matter defects and volume reduction. Signal transmission between the brain and the subcortical center causes long-term neurobehavioral defects, which can clinically manifest as a decline in somatomotor function, visual spatial skills, association ability, and psychomotor function. Thus, oligodendrocytes are closely related to white matter damage.\textsuperscript{51} Clinical studies have shown that cerebral white matter is vulnerable to ischemia. After chronic cerebral ischemia, certain degree of oligodendrocytes damage, DNA breakage, activation of caspase apoptotic pathway, and apoptosis-related proteins caspase-3 (caspase protease 3), TNF-\textalpha (tumor necrosis factor-alpha), the expression of Bax (BCL2-associated X Protein, B-cell lymphoma/leukemia-2 gene (Bcl-2)), and so on were further increased, which further aggravated the damage of white matter and the apoptosis of oligodendrocytes. Therefore, it is necessary to protect oligodendrocytes. According to clinical data, T\textbeta 4 has significant advantages in neuroprotection and myelin regeneration, and has good safety, tolerance, and effectiveness, indicating that it is an excellent candidate for the treatment of demyelinating diseases.\textsuperscript{52–54} Santra et al.\textsuperscript{29} said that T\textbeta 4 promotes oligodendrocyte differentiation and inhibition of the Toll-like receptor (TLR) pro-inflammatory pathway by up-regulating microRNA-146a, suggesting that T\textbeta 4 has development prospects for the treatment of nerve damage. T\textbeta 4 can achieve its anti-inflammatory and immunoregulatory properties by inhibiting the activation of nuclear factor-\textkappa B (NF-\textkappa B) to protect oligodendrocytes from damage and death.\textsuperscript{25,55,56} Zhang et al.\textsuperscript{25} found that oligodendrocytes in the brain of experimental animals with MS model were significantly increased after T\textbeta 4 administration, and the neurological function recovered well in the short term and long term. The pathology of MS is characterized by irregular demyelination and glial hyperplasia in the white matter of the central nervous system (CNS). Therefore, the above results suggest that oligodendrocytes may promote the formation of myelin sheaths, which is helpful for myelin regeneration. Some studies have suggested that the protective effect of T\textbeta 4 is related to the promotion of oligodendrocyte differentiation and myelination,\textsuperscript{36} and it has a protective effect on white matter damage.

**Protective effect of T\textbeta 4 on microglia**

Imai et al.\textsuperscript{57} have shown that injection of exogenous microglia can increase the expression of brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor in ischemic hippocampus and induce neurotrophin dependence on damaged neurons. This shows that the administration of exogenous microglia may be a potential candidate for therapy in the CNS repair after transient global ischemia. We know that immunization with amyloid-beta (A\textbeta) peptides is a treatment for AD. Takata et al.\textsuperscript{58} said that we can increase the clearance of A\textbeta by transplanting exogenous microglia. Microglial activation promotes apoptosis of neurons and aggravates white matter damage by activating superoxide free radicals, hydroxyl free radicals, NO (nitric oxide) and other reactive oxygen species (ROS), and pro-inflammatory response factors such as TNF-\textalpha. Farkas et al.\textsuperscript{59} found that microglia can cause delayed nerve damage by producing pro-inflammatory factors, on one hand, and participate in the removal of necrotic tissue and promote nerve regeneration, on the other hand. Some studies have found that T\textbeta 4 is involved in microglial activation. Dong et al.\textsuperscript{60} found that the expression of T\textbeta 4 in hippocampal microglial cells blocked by olfactory nerve transmission was up-regulated, suggesting that T\textbeta 4 participates in the process of microglial activation; Zhou et al.\textsuperscript{61} used T\textbeta 4 to treat microglial cells in hypoxic brain injury model mice and found that T\textbeta 4 can inhibit inflammatory mediators such as TNF-\textalpha, the secretion of
interleukin-1β (IL-1β), and NO plays an anti-inflammatory role and inhibits the activation of the unfavorable pro-inflammatory phenotype feedback loop of microglia in vitro. In addition, Zhang et al. found that AcSDKP (N-acetyl-serine-aspartyl-lysyl-proline) can significantly reduce fibrin accumulation and microglial/macrophage activation in the brain. Dong et al. reported the progressive aggregation of Tβ4-positive activated microglia in the brains of patients with Huntington’s disease (HD). Therefore, we speculated that Tβ4 may be involved in the activation of microglia after chronic cerebral ischemia and promote the clearance, which is also conducive to the recovery of neural function.

Protective effect of Tβ4 on nerve tissue through anti-inflammatory process

Tβ4 is a major G-actin segregation molecule with anti-inflammatory effects and the mechanism of action is complex (Figure 3). Tβ4 has been used to treat various neurological diseases. Increasing evidence suggests that modulation of thymosin has anti-inflammatory potential in inflammation and autoimmune diseases. For example, Tβ4 treatment could improve functional recovery after experimental autoimmune encephalomyelitis (EAE) by reducing inflammatory infiltration and stimulating oligodendrocytes production. Sosne et al. first proposed a possible mechanism for Tβ4 to exert its anti-inflammatory properties by inhibiting NF-κB activity and cell function.

The activity of Tβ4 directly affects the repair and regeneration after injury. It plays an anti-inflammatory role by regulating the polymerized G-actin sequence and plays an important role in inhibiting tissue damage and fibrosis. Researches have shown that Tβ4 can effectively prevent the increase of ethanol-induced inflammatory mediators. It can significantly inhibit IL-1β and macrophage inflammatory proteins (MIP-1, MIP-1 by down-regulating TNF-α and interfering with NF-κB pathway), mononuclear chemokine protein (MCP-2), and other inflammatory factors gene transcription and expression, indicating that Tβ4 can reduce the number of inflammatory cells, down-regulate the expression of inflammatory chemokines and cytokines, and prevent new tissues from being inflammatory damage. In addition, Tβ4 significantly increased the expression of miR-146a in microglial cells and significantly inhibited the secretion of pro-inflammatory mediators. At the same time, Tβ4 was introduced as a key regulator of miR-146a and TLR signals, which can further regulate the secretion of pro-inflammatory cytokines and thus NF-κB factor. Moreover, Tβ4 can also reduce ROS, increase antioxidant proteins, and protect cells from damage factors released from damaged tissues.

According to the literature, when Tβ4 exerts a variety of physiological and pathological processes, and its high mRNA expression in specific cells, it is found that the concentration of metal ions such as calcium, iron, zinc, and copper in this cell will also increase, indicating that Tβ4 metal coordination (Tβ4 is a small molecule peptide with a metal-binding site) is the driving force for Tβ4 cell translocation and actin binding. Therefore, the function of Tβ4 can be recognized by studying the metal coordination mechanism of Tβ4, which is of great significance for Tβ4 to play its important role in disease treatment. It is well documented...
that excessive free redox active metals such as copper and iron can generate ROS and inflammation, which can cause neurodegeneration. Brain tissue is quite sensitive to ROS. ROS can destroy biomolecules and cause them to fold incorrectly to produce inclusions. Morris et al.39 have found that in neurodegenerative diseases, such as AD, Parkinson’s disease (PD), HD, and amyotrophic lateral sclerosis (ALS), there are many such inclusion bodies. Tβ4 is widely involved in the formation and maintenance of the nervous system. For example, Tβ4 plays an important role in the development of CNS, differentiation of progenitor cells, tangential expansion, neurogenesis, and neurite growth.48,70–72 Tβ4 can effectively promote the recovery of neurological diseases, mainly through the plasticity and neurovascular remodeling of the central (CNS) and peripheral nervous system (PNS).73 Since Tβ4 has good effects on vascular regeneration, neurogenesis, and so on, even if Tβ4 is used for 24 h or more after injury, the neural function can be significantly improved.74,75 In the brains of patients with AD and HD, Tβ4 is found to be elevated in reactive microglia.76 Reactive glial hyperplasia is the primary protective mechanism when nerve tissue is damaged, so elevated Tβ4 may have anti-inflammatory and repair functions in microglia.77 Tβ4 inhibits pro-inflammatory TLR signaling in vitro by activating microglia.37 It reverses increased pro-inflammatory factor concentration27 and reduces expression of inflammatory mediators and secretion of pro-inflammatory factors (TNF-a, IL-1B, and NO).78 Studies have shown that intracellular calcium ions are involved in the inflammatory process,79 and the removal of extracellular calcium ions can delay the increase of intracellular calcium ions before cell death.80 It is hoped that Tβ4 can prevent the relationship between calcium and other ions, thereby preventing inflammatory processes and cell death.

**Protective effect of Tβ4 on cerebral vascular regeneration**

Tβ4 is widely distributed in the nervous system and runs through the entire developmental process of the nervous system.81 Vartiainen et al.82 found in a rat model of focal cerebral ischemia that Tβ4 is a nerve repair molecule with many repair functions related to cell proliferation, migration, angiogenesis, and axon remodeling.26,83,84 Treatment of neurological damage, disorders, and diseases with Tβ4 can increase neurovascular plasticity, such as neurogenesis, neurite and axon growth, angiogenesis, and oligodendrocyte formation, which can significantly improve functional and behavioral outcomes.22 In the late period after the onset of nervous tissue damage or chronic nervous system symptoms, Tβ4 provides the environment by repairing neurovascular remodeling to repair damaged neural tissue.85 Tβ4 mRNA expression was found in the rat hippocampal ischemia model at 12 h after ischemia,82 and the expression was highest on the seventh day and was distributed throughout the infarcted lesions. However, the study did not elaborate on the effect of increased Tβ4 mRNA on the nervous system and the mechanism by which it increased. Later studies have shown that the expression of Tβ4 in the hippocampus area of rat cerebral ischemia is also significantly increased,86 and it is also highly expressed in brain tissues of HD76 and AD.87 The reason for the high expression of Tβ4 in many diseases of the nervous system may be that it is widely distributed and involved in many biological effects of the nervous system. Current research has shown that Tβ4 is involved in neuronal apoptosis,80 axonal growth and repair,46,51 and cerebral vascular regeneration.88

Morris et al.47 found that intraperitoneal injection of exogenous Tβ4 (6 mg/kg, four times every 3 days) 24 h after focal cerebral ischemia–reperfusion in rats, and that Tβ4 can significantly promote neurological recovery and axon repair in cerebral ischemia and neural stem cells differentiation, can significantly increase blood vessel density in the striatum area and ischemic penumbra (about 1.5 times the blood vessel density in the control group). Xiong et al.34 intraperitoneally injected exogenous Tβ4 into rats with brain injury in the same way. Compared with the control group, the blood vessel density increased significantly in the injured cerebral cortex and hippocampus. It has been reported that Tβ4 cleavage fragment (AcSDKP) is an effective peptide that promotes angiogenesis.89 It can promote endothelial cell migration and secrete active matrix metalloproteinase (MMP-1), indicating that Tβ4 and AcSDKP have overlapping functional regions.

The mechanism of Tβ4-promoting angiogenesis is still in deep research, but it can be summarized as follows: (1) the combination of Tβ4 and G-actin increases endothelial cell proliferation and
adhesion and promotes endothelial cell migration.90 (2) Induction of increased secretion of pro-angiogenic factors such as vascular endothelial growth factor (VEGF)91–94 and matrix metalloproteinases (MMPs).95 (3) Activates pro-angiogenic pathways, such as the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway. Studies suggest that Tβ4 protects acute and chronic ischemic embryonic endothelial progenitor cells through the PI3K/Akt pathway96 and it can also up-regulate p-Akt (Phosphorylated Akt) expression in myocardial infarction rats, activate downstream factors, and promote vascular regeneration.65,66 (4) Inhibits apoptosis and aging. Tβ4 can activate PI3K/Akt signaling pathway97 and regulate the phosphorylation of downstream key apoptotic proteins such as Bad (BCL2-Associated D Protein) and GSK-3β (Glycogen synthase kinase 3β) of the pro-apoptotic factor Bcl family and plays an anti-apoptotic effect. In addition, Tβ4 can inhibit serum-induced endothelial progenitor cell apoptosis by activating integrin-linked kinase (ILK) and downstream Akt and has a dose–effect relationship. The mechanism may be that Tβ4 up-regulated the expression of anti-apoptotic protein Bcl-2, while inhibiting the expression of pro-apoptotic protein Bax, leading to an increase in the Bcl-2/Bax ratio, stabilizing the mitochondrial membrane transmembrane potential, and reducing the release of endothelial progenitor cell pigment C.9,10 Tβ4 can also activate downstream endothelial nitric oxide synthase via PI3K/Akt pathway to slow the senescence of endothelial progenitor cells.95 Other studies have shown that Tβ4 can inhibit inflammation and provide a good environment for vascular regeneration.56

Conclusion and future development prospects

Many previously published studies indicate that Tβ4, a new type of multifunctional bioactive molecule, plays a crucial role in neuroprotection and nerve repair in the treatment of traumatic and neurological brain injuries. Tβ4 may be a promising therapeutic approach to reduce neuroinflammation and reduce symptoms associated with the pathogenesis of progressive psychosis and neurodegenerative diseases. However, research to determine their effects on the developing and injured brain is still in its infancy, and the potential role and molecular mechanism of Tβ4 in CNS function is still lacking, especially whether and how Tβ4 affects psychosis and direct pharmacological studies on the impaired behavior of neurodegenerative diseases. Furthermore, detailed preclinical studies are needed to specifically evaluate the therapeutic potential of Tβ4 in animal models of these conditions and determine its potential mechanism of action.

As shown in the preclinical model, the most important activity of Tβ4 may be its actin-binding properties to promote the migration and differentiation of neural stem/progenitor cells in the injured area, thereby promoting brain repair or regeneration processes, and can be used as a potential nerve repair. It has therapeutic potential for various nervous system injuries and neurodegenerative diseases (such as AD). This has great social significance for the high incidence of AD caused by the increasingly serious global aging. However, there is still a significant translation gap between preclinical data and clinical application. First, it is necessary to verify nerve repair and regeneration activity in human neurons or brain tissue, preferably coupled with changes in intracellular signaling pathways known to promote nerve regeneration and survival. Second, the optimal therapeutic dose or method remains to be determined. However, even in the preclinical model, the absorption and distribution of Tβ4 after various dosing regimens are unknown. Finally, the development of translated biomarkers that provide pharmacodynamic evidence of Tβ4 activity in brain tissue or nerve cells will facilitate dose selection.

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