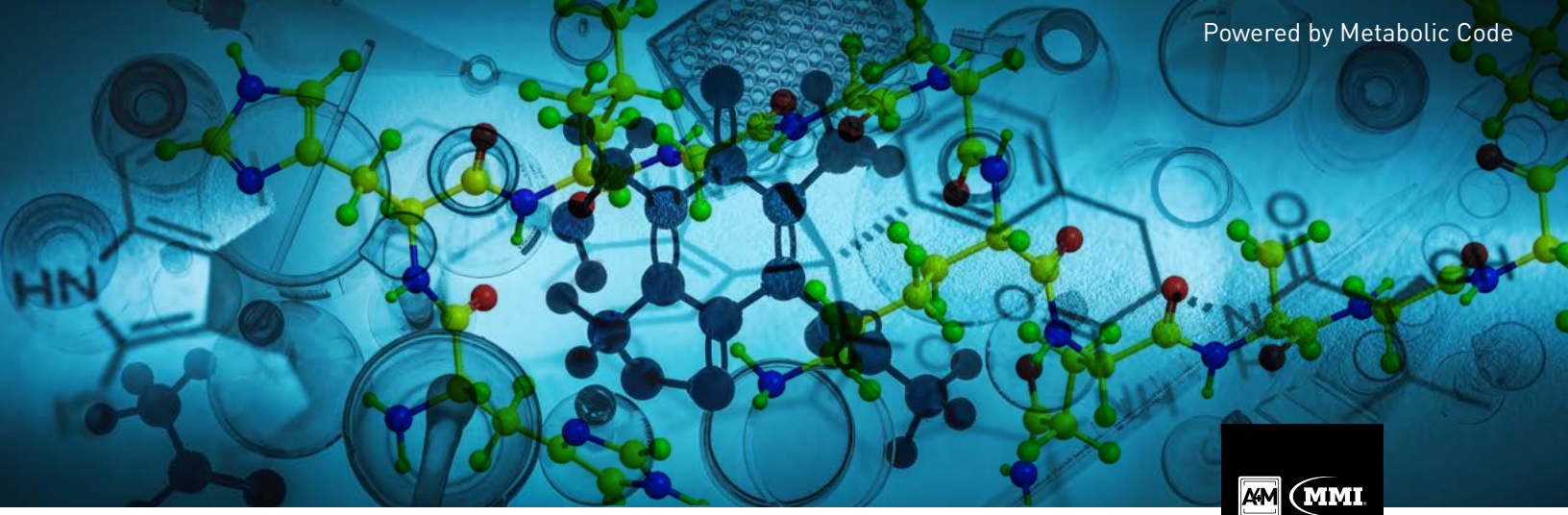


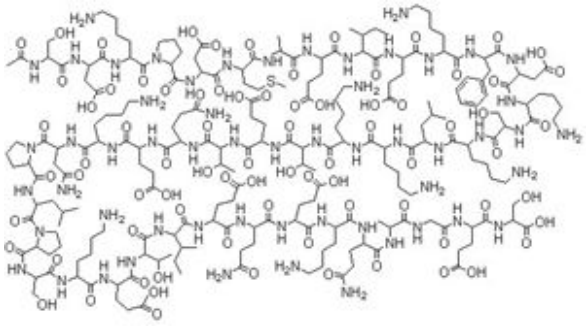
Peptide Therapy Handbook For Healthcare Professionals

Thymosin beta 4

Name(s):

- Thymosin beta 4
- Tb4
- TB-500

Sequence:



Ac-Ser-Asp-Lys-Pro-Asp-Met-Ala-Glu-Ile-Glu-Lys-Phe-A sp-Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-Glu-Lys-A sn-Pro-Leu-Pro-Ser-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gl n-Ala-Gly-Glu-Ser

Molecular Composition:

MW	4963.4408
Molecular Formula	$C_{212}H_{350}N_{56}O_{78}S$

Indications/Uses
- Sports/athletic injury
- Soft tissue repair
  - Tendon/ligament/muscle repair
- Pressure ulcers / venous stasis ulcers
- Immune support (as monotherapy or in conjunction with Thymosin alpha 1)
- Cardioprotective – especially post MI
- Brain issues if autoimmunity suspected
- Neuroinflammation – microglial inflammation
- Multiple sclerosis
- Ischemic stroke
- Spinal cord injuries
- TBI; concussion support (in conjunction with BPC 157)
- Eye disorders
  - Diabetic retinopathy
  - Dry eye disorders
  - Ocular tissue injuries including corneal wound healing and repair
  - Corneal transplants

Other Uses
- Sepsis
- Chemical burns
- Diabetes
- Corneal transplants
- NAFLD – non-alcoholic fatty liver disease
- Lung inflammation / fibrosis
- May improve hair growth

Dosage:
General Dosage:
- 300 mcg – 1 gram daily, SubQ
- Depending upon clinical presentation – less for immune, more for repair or most for both issues
- Do not dose concurrently for more than 3 months
- Cycle if needed long-term – 3 months on, 6 weeks off or 6 weeks on 6 weeks off
- Individual dosage requirements may vary based on clinical presentation

Warnings and Cautions
- Thymosin beta 4 peptide is reported safe in recommended dosages.
- Safety in pediatrics has not been established.
- As with all injections, redness and pain at the site of injection may be present.
- Do not use for more than 3 months without cycling (3 months on, 6 weeks off or 6 weeks on, 6 weeks off).
- Based on FDA recommendations and various guidance documents developed by
- the International Conference of Harmonization (ICH), 23 non-clinical studies have been performed to date that demonstrate the safety of Tb4 for its current and planned uses in man.

Summary
Thymosin beta-4 is a peptide found in most cells and tissues
- Originally isolated from calf thymus
- Main intracellular G-actin sequestering peptide
- **Up-regulates actin**
- Forms a ternary complex with actin and profilin
- Increases cells involved in healing
- Improves cell migration to site of injury
- Promotes matrix metalloproteinase expression during wound repair
- Promotes angiogenesis Cytoprotective
- Helps decrease scar tissue formation
- Improves T cells

The beta-thymosins comprise a family of structurally related, highly conserved amino acid sequences in species ranging from mammals to echinoderms. Of the 16 known family members, thymosin ß4 (Tb4), thymosin ß10 (Tb10), and thymosin ß15 (Tb15) are found in man.
Thymosin beta 4 (Tb4) is a 43 amino acid, 5 kDa polypeptide that is an important mediator of cell proliferation, migration, and differentiation. Tb4 is the most abundant member of the ß- thymosin family in mammalian tissue and is regarded as the main G-actin sequestering peptide. It is found in all tissues and cell types except red blood cells. Thymosin beta4 is angiogenic and can promote endothelial cell migration and adhesion, and angiogenesis.· TB4 also accelerates wound healing and reduces inflammation and scarring when applied in dermal wound-healing assays.·

Beta thymosins bind and sequester monomeric actin, thus preventing actin polymerization and formation of filamentous actin. Actin is a vital component of cell structure and movement. Actin is involved in many important non-muscle cellular processes including cell locomotion, chemotaxis, phagocytosis, and cytokinesis. Of the thousands of proteins present in cells, actin makes up to 10% of the total proteins in a cell, representing a major role in the genetic makeup of the cell.

Animal studies of disease and repair when using thymosin beta 4 (Tb4), the major actin-sequestering molecule in mammalian cells, have provided a base for the ongoing multicenter clinical trials for wound healing, including dermal, corneal, and cardiac.· Tb4 has of multiple biological activities, which include down-regulation of inflammatory chemokines and cytokines, and promotion of cell migration, blood vessel formation, cell survival, and stem cell maturation. Thymosin beta 4 inhibits inflammation, microbial growth, scar formation (by reducing the level of myofibroblasts), and apoptosis, and protects cells from cytotoxic damage, including glutamate neuronal toxicity. Thymosin ß4 binds to G-actin, blocks actin polymerization, and is co-released with factor XIIIa by platelets. These activities contribute to the multiple wound healing properties that have been reported in animal and human studies.

Ocular

Tb4 promotes complete and faster corneal healing than saline alone or prescription agents (doxycycline and cyclosporine) in various animal models of eye injury.[12] In human trials, Tb4 eye drops improve both the signs and symptoms of moderate to severe dry eye with effects lasting beyond the treatment period. Thymosin ß4 has also reported efficacy in three phase 2 clinical ocular trials with no evidence of any adverse events.[14] See **table 3** for a summary of corneal wound healing applications.

Table 3: Corneal Wound Healing Applications

Oral

Nonmedical application
• Chemical burns
• Patients undergoing photorefractive keratectomy (PRK)
Medical applications
• Stage 2 patients (Mackie classification) with neurotrophic keratitis
• Patients with recurrent corneal erosions
• Patients with map-dot fingerprint and/or Fuch's corneal dystrophies
• Corneal transplants
• Patients undergoing phototherapeutic keratectomy (PTK) for anterior stromal corneal dystrophies

Tb4 plays a role in suppressing the production of interleukin-8 following stimulation by tumor necrosis factor-alpha, acting as an antimicrobial, anti-inflammatory and antiapoptotic factor on gingival fibroblasts.

Cardiac

Hypoxic heart disease is a predominant cause of disability and death worldwide. Tb4 is the only known molecule to initiate organ wide activation of the embryonic coronary development program in adult mammalian hearts. Tb4 has been reported effective when used to inhibit myocardial cell death, improve

angiogenesis, have antifibrotic effects, decrease infarct size and activate endogenous cardiac progenitors. Treatment with Tb4 is reported to reduce infarct volume and preserves cardiac function in preclinical models of cardiac ischemic injury.

CNS/Brain
Tb4 is widely distributed in a majority or mammalian tissues and cell types, including those of the CNS (central nervous system). It is expressed in most neural cell types of the developing brain and in a subset of neurons and microglia. Tb4 is locally synthesized in neurons for the regulation of neurite outgrowth. Tb4 is up-regulated in various pathological conditions such as focal ischemia, Alzheimer's disease, Huntington's disease, hippocampal denervation, and kainic acid induced seizure. Its presence in the nervous system likely plays a role in neuroprotection, synaptogenesis, axon growth, cell migration, and plastic changes. If brain changes are suspected due to autoimmunity, Tb4 is a great therapeutic choice.

Sepsis
Sepsis is the dysregulated host response to an infection resulting in life-threatening organ damage. Thymosin Beta 4 is reported to improve mortality when administered intravenously to septic rats. Tb4 decreases inflammatory mediators, lowers reactive oxygen species, up-regulates anti-oxidative enzymes, anti-inflammatory genes, and anti-apoptotic enzymes making it an interesting protein to study in sepsis.

Non-Alcoholic Fatty Liver Disease - NAFLD
Studies report that Tb4 is negatively correlated with endotoxemia and could suppress proinflammatory TLR signaling and reduce inflammatory cytokines. According to the gut-liver axis theory, the effects of Tb4 could play an important role in the treatment of NAFLD. Liang et al detected Tb4 expression in the sera and tissues of patients with chronic hepatitis B combined with NAFLD, and observed that the Tb4 level was negatively correlated with inflammation and fibrosis scores, and Tb4 expression in both serum and liver tissue was negatively correlated with TNF-a expression. Tb4 plays a defensive role in the development of liver disease by inhibiting oxidative stress and proinflammatory factors. When the concentration of serum Tb4 was compared between patients with NAFLD and healthy controls, serum Tb4 levels in patients with NAFLD were significantly lower. After treatment and subsequent improvement in liver function, the concentration of Tb4 increased. Tian et al observed 83 cases of NAFLD and 80 healthy patients, and reported that Tb4 level can effectively be used as a biomarker of liver function, as increased Tb4 level indicated improved liver function, and decreased Tb4 level indicated severe liver damage. These studies indicate that Tb4 expression is related to liver function in NAFLD patients.

RegeneRx
RegeneRx Pharmaceuticals, Inc. is a publicly traded, clinical-stage, biopharmaceutical company focused on tissue protection, repair and regeneration. RegeneRx acquired the rights to Tb4 from the NIH in 1999. RegeneRx has concentrated on the development of Tb4 for tissue and organ protection, repair and regeneration. RegeneRx currently has three drug candidates in clinical development for ophthalmic, cardiac and dermal indications, three active strategic licensing agreements in China, Pan Asia (Korea, Japan, and Australia, among others) and in the U.S. and Canada. RGN-259, the Company's Tβ4-based ophthalmic drug candidate is being developed for dry eye syndrome and for the treatment of neurotrophic keratopathy (NK), both of which are being developed in the U.S and Canada through its joint venture, ReGenTree. ReGenTree has reported results of its recently completed Phase 2/3 U.S. trial in patients with dry eye syndrome and is conducting a Phase 3 clinical trial for the treatment of patients with NK, for which it has been granted orphan status by the U.S. FDA. RGN-352, the Company's Tβ4-based injectable drug candidate is a Phase 2-ready drug candidate designed to be administered systemically to prevent and restore tissue damage associated with acute events such as heart attacks, strokes, and other similar injuries. RGN-137, the Company's Tβ4-based dermal gel, is in phase 2 clinical development. See table 2 for more information on RegeneRx Tb4 Drug Candidates.

Recently, RegeneRx announced publication of a pilot clinical trial demonstrating that Tb4 was effective in the treatment of patients after an acute ST segment elevation myocardial infarction (STEMI). The trial was designed to study whether endothelial progenitor cells (EPCs or immature cells) treated with Tβ4 and transplanted into STEMI patients would improve cardiac and clinical function compared to a control

group. Ten patients with STEMI were included; they were randomized to 2 groups: a Tβ4-pre-treated EPC transplantation group (experimental group; n = 5) and an EPC transplantation group experimental group. Cardiac function was evaluated using echocardiography and emission computed tomography, as well as the 6-min walking test before and 6 months after the intervention. After 6 months, the left ventricular ejection fraction based on two different measurements improved by more than 50% ($p<0.05$), and the stroke volume, amount of blood ejected by the left ventricle, improved by approximately 50% (($p<0.05$) in the Tβ4-pre-treated group. After 6 months of follow-up, the average 6-min walking distance was improved by 14% ($p<0.01$). There were no severe complications related to the procedure in either group during the follow-up.

Table 2: RegeneRx Tb4 Drug Candidates

DISCLAIMER: Statements made are for educational purposes and have not been evaluated by the US Food and Drug Administration (FDA). They are not intended to diagnose, treat, cure, or prevent any disease. Most peptides are not regulated by the FDA. Always use a licensed Compounding Pharmacy to prepare peptide products.

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