EXPERT OPINION

Advances in the basic and clinical applications of thymosin β_4

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Introduction: Thymosin β_4 ($T\beta_4$), a multifunctional peptide, has been used successfully in several clinical trials involving tissue repair and regeneration. The review will first update the current information on the common underlying cellular cascades and pathways that are basic to $T\beta_4$'s regenerative activity and second, on the current and potential uses of this protein in the clinic.
Areas covered: Significant advances in our understanding of the actions of $T\beta_4$ have occurred in directing stem cell maturation and in regeneration and repair of injuries. Many of its activities directly affect the repair cascade following injury. Using PubMed, we summarize the discovery and isolation of $T\beta_4$ as well as the studies on tissue repair, which have provided the scientific foundation for ongoing and projected trials in the treatment of eye injuries, dermal wounds, repair of the heart following myocardial infarction and healing of the brain following stroke, trauma or neurological diseases.
Expert opinion: Based on its multifunctional activities during tissue regeneration in various animal studies, $T\beta_4$ has the potential for new clinical applications such kidney and liver disease, as well as repair of spinal cord, bone and ligament damage. In addition, it may be useful in the treatment of a wide range of other applications, including the consequences of aging and viral infections.

Keywords: angiogenesis, apoptosis, endothelial progenitor cells, fibrosis, inflammation, ischemic injury, migration, regeneration, scar formation, thymosin β4, tissue repair

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1. Introduction: thymosin fraction 5 and the discovery of thymosin β4

Thymosin β_4 ($T\beta_4$) was first identified as a biologically active component of thymosin fraction 5 (TF5), in 1981 [1]. The yield of $T\beta_4$ from TF5 is ~ 1%. TF5 also contains a family of other biologically active heat-stable peptides that modulate T-cell responses and stimulate the maturation of T-cells [2]. Several of these peptides, including Thymosin α_1 [3], Thymosin β_{10} [4] and Thymosin β_{15} [5], have been studied extensively in both the lab and clinic. The biologically active thymosin peptides found in TF5 belong to the family of biological response modifiers (BRMs), which are now known to regulate a large number of immune responses and also participate in the repair and regeneration of tissues following injury.

In 1974, TF5 was the first thymic preparation to be used clinically in the treatment of children with DiGeorge Syndrome and with a number of life-threatening primary immune deficiency diseases. After going through a university-based (University of Texas Medical Branch, Galveston, TX) scale-up and requisite preclinical toxicology and safety studies, in 1974 the FDA allowed a New Drug Application in the USA to use TF5 to treat children with life-threatening primary immunodeficiency diseases. In April 1974, the first clinical trial with TF5 began under the clinical direction of Arthur Ammann, the Director of Pediatric Immunology at the University of California San Francisco Medical School. Dr. Amman's very first patient treated with

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Article highlights.

- Thymosin β_4 ($T\beta_4$) is one of the active components of a thymus gland extract.
- $T\beta_4$ has multiple active sites that regulate important activities for tissue repair and regeneration, including anti-inflammation, cytoprotection, anti-apoptosis, cell migration, promotion of stem cell recruitment and differentiation, increased laminin 5 synthesis and reduced scar formation.
- $T\beta_4$ has a very strong safety profile both as a topical agent and with an injectable formulation in Phase I clinical trials.
- In animal models, $T\beta_4$ was effective in repairing the eye, skin, nervous system, cardiovascular system, and so on.
- In human Phase II trials, it was safe, well tolerated, and showed efficacy in dry eye and in dermal wound patients.

This box summarizes key points contained in the article.

TF5 was a 5 year old girl with DiGeorge Syndrome, presenting with a body weight of 26 lbs, with extremely low numbers of T-cells, and with overwhelming infections. In a landmark paper published in 1975 in the *New England Journal of Medicine*, the results of the use of TF5 to treat children with a variety of PIDs were first reported [6]. The patient was identified after lab bench *in vitro* incubation with TF5 of the patient's white blood cells increased the number of T-cell rosettes from 15 to 48%. After starting therapy with TF5, T-cell rosettes increased to 50% by 2 weeks, and by 3 weeks to 55%. In addition, many of the patient's infections cleared and her symptoms improved, including development of a positive response to delayed hypersensitivity skin tests, weight gain, and clinical improvement. The dramatic recovery of the patient received significant coverage in the medical as well as in the lay press worldwide and heightened scientific and public interest in the use of biological response modifier (BRM) in clinical medicine.

2. Early translational studies with TF5 to treat other life-threatening diseases and injuries

Since the early pre-clinical and clinical studies with TF5, in patients with PIDs [6], cancer [7,8], infectious diseases [9], and autoimmune diseases [10], the major biochemical focus of research with TF5 has been in purifying and characterizing the biologically active components in TF5 responsible for the clinical effects observed and in translating these studies from the lab bench to the clinic. In these initial studies, TF5 was found to modulate a number of T-cell mediated responses, reduce infections, and improve clinical responses.

3. $T\beta_4$: activities and mechanisms of action

One of the major active components in TF5 is $T\beta_4$. As shown in **Figure 1**, $T\beta_4$ is acetylated at the N-terminal serine position. It is a peptide of 43 amino acids and is the first of the synthesized β-thymosins to reach the clinic [1,11]. Many of $T\beta_4$'s pleotrophic biological activities are now known to be defined by its active sites. **Table 1** summarizes the biological activities and defined mechanisms of action of $T\beta_4$. Its activities directly affect the repair and regeneration cascade following injury. Reducing inflammation is important in preventing tissue damage and fibrosis. $T\beta_4$ down-regulates NFκB [11,12] and reduces the levels of a large number of inflammatory chemokines and cytokines [12-15]. Furthermore, ROS (reactive oxygen species) are also reduced with $T\beta_4$ treatment [16]. $T\beta_4$ decreases apoptosis and protects cells from damaging agents released from the injured tissue. $T\beta_4$ also decreases the infiltration of scar-forming cells, the myofibroblasts [17]. Clearly the promotion of cell migration by $T\beta_4$ is important in tissue regeneration and likely involves the ability of $T\beta_4$ to bind actin. For example, keratinocyte migration is important for dermal wounds, and corneal epithelial cell migration is important for corneal repair. $T\beta_4$ also increases the quality of the repaired skin and eye outer layers by increasing laminin-5 synthesis, which is important in cell–cell and cell–substratum contacts [18]. Thus, the healed tissue is stronger and loss of fluid and entry of bacteria are prevented. $T\beta_4$ increases angiogenesis, an important early part of tissue repair involving re-oxygenating tissues (most wounds are hypoxic) and waste removal [19]. Endothelial progenitor cells (EPCs) are recruited by $T\beta_4$ and their release of trophic factors, including VEGF, and differentiation is enhanced by $T\beta_4$ in the wound [19]. Early studies demonstrated a role in angiogenesis using *in vitro* and *in vivo* angiogenesis assays [19]. The increase in angiogenesis also accelerates subsequent steps in repair, such as the deposition of collagen [20,21]. As illustrated in **Figure 2**, the known and newly emerging clinical applications of $T\beta_4$ are expanding. $T\beta_4$'s pleiotropic biological activities on wound healing and repair and have provided the scientific foundation for ongoing and projected human trials. Indications for treatment include dermal wounds [22-25], eye injuries [11,25], including severe dry eye [26,27] and neurotropic keratitis [28], and repair of the heart following a heart attack [29-31]. Ongoing animal studies presented elsewhere in these proceedings indicate that $T\beta_4$ may also be useful in treating brain injuries following stroke [32], trauma [33] or neurological diseases, such as multiple sclerosis [34] as well as peripheral neuropathies [35].

4. $T\beta_4$: clinical application in the treatment of pressure and venous stasis ulcers and epidermolysis bullosa

No agent has yet been identified that has more than marginal activity in patients with chronic wounds in humans. In the absence of an effective drug, simple cleansing, debriding and good wound care is still the primary treatment available. However, such good wound care provides only a slow and in many cases modest healing. Molecular

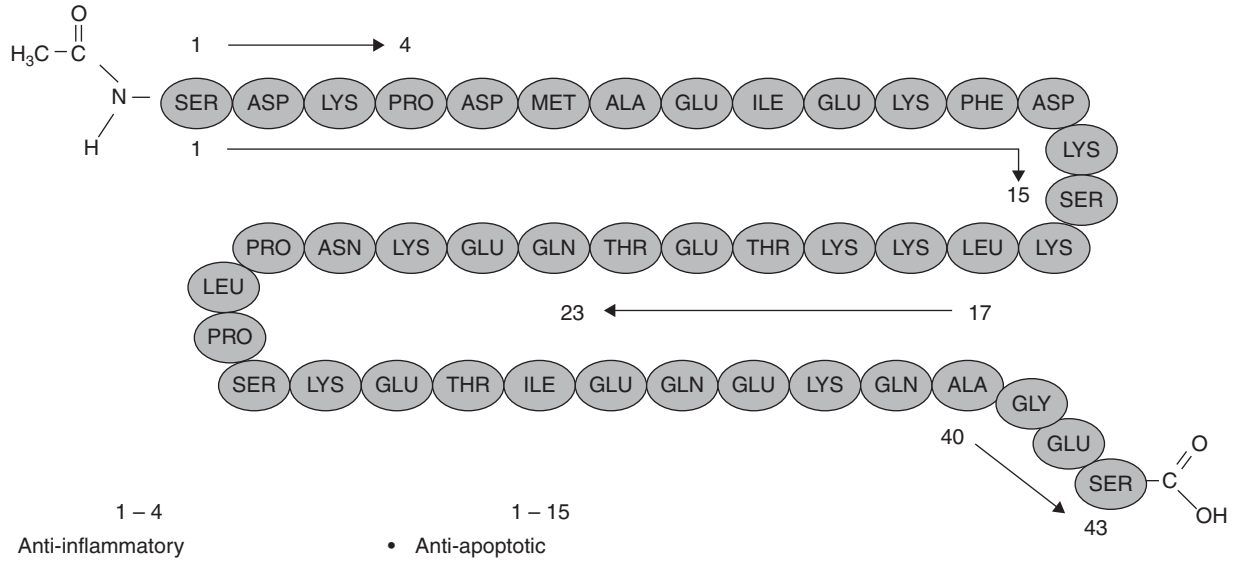

Figure 1. Biological activities defined by active sites. The sequence of thymosin β4 is shown with the location of active sites indicated. There is also a lot of the activities found in various fragments.
The figure is adapted from data presented in [43] but has additional new activities.

Table 1. Biological activities and defined mechanism(s) of action of thymosin β_4.

Activity	Mechanism
Reduces senescence of endothelial progenitor cells	Modulation of P13K/AKT/eNos signaling
Reduces inflammation	Down-regulates inflammatory mediators Inhibits activation of NFκβ
Reduces inflammation	Modulates active polymerization sequences G-actin
Promotes cells from injury	Reduces ROS/increases antioxidant proteins
Promotes angiogenesis	Increases VEGF synthesis
Promotes stem cell recruitment/differentiation	Increases cell migration/maturation
Reduces apoptosis	Increases anti-apoptotic enzymes/decreases Bax/Bcl2 ratios
Accelerates laminin-5 synthesis	Activates gene for laminin-5 synthesis
Reduces scar formation/better organized collagen fibrils	Reduces infiltration of myofibroblasts

analysis of chronic wounds indicates that there is no shortage of growth factors in the wound site, but rather an overabundance of inflammatory cytokines and chemokines due to the dysregulation of the normal cycle of inflammation, proliferation, and remodeling that must occur if a wound is to heal.

As summarized in **Table 2**, a Phase I safety study using a gel formulation of $T\beta_4$ (RGN-137) was completed in 2009. In this study of 15 health volunteers, $T\beta_4$ was found to be safe and well tolerated. Following this trial, two multi-center trials in 144 patients with stage III and IV pressure ulcers were carried out. Early Phase II multicenter, double-blind, placebo-controlled trials evaluating the safety, tolerability and wound-healing effectiveness of $T\beta_4$ have been conducted in the USA and in Europe in patients with stage III/IV pressure ulcers [36] and venous stasis ulcers [37] using three escalating doses of $T\beta_4$ applied once daily for up to 84 days (**Table 2**). In both studies, it was observed that the middle

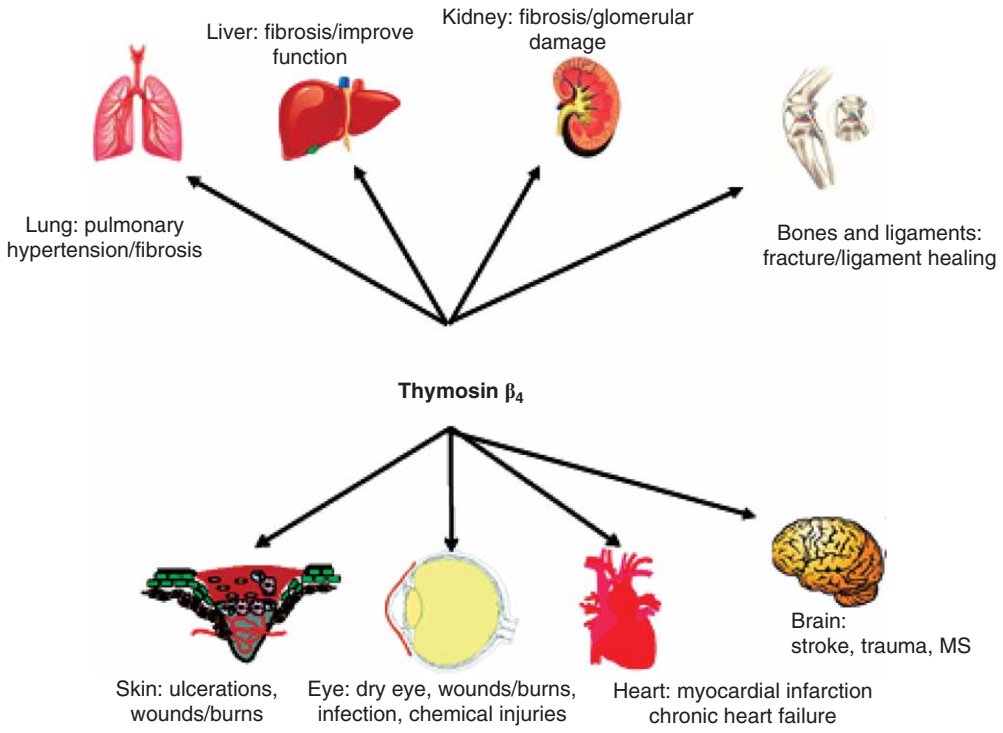

Figure 2. Known and newly emerging clinical applications of thymosin β4. Shown schematically are various organs and the injuries that have been found to be repaired or affected by thymosin β4.
The figure is adapted from [27] but has additional new indications.

Table 2. Results of thymosin β_4 dermal clinical trials.

Phase I dermal safety-completed 2009
15 healthy volunteers, 4 doses
Safe and well-tolerated
Phase II stage III and IV pressure ulcers-completed 2011
72-patient trial at 19 US sites
Randomized, double-blind, dose-escalation (3 doses + placebo), 84-day treatment
Safe and well-tolerated
Trend toward increased rate of healing at 0.02% dose median time to healing 22 vs 57 days
Phase II venous stasis ulcers-completed 2011
72-patient trial at 10 European sites
Randomized, double-blind, dose-escalation (three doses and placebo), 84-day treatment
Safe and well-tolerated
Statistically significant increased rate of healing at 0.03% dose, median time to healing39 vs 71 days
Phase II epidermolysis bullosa - partially funded by FDA under Orphan Drug
30-patients treated at 12 US sites, completed 2013
Randomized, double-blind, dose-escalation (three doses and placebo), 56-day treatment
Safe and well-tolerated
Trend toward increased rate of healing at 0.03% dose (mid dose), at day 14 wound size decreased by 57 vs 30% (p = 0.0149)

dose of $T\beta_4$ accelerated the rate of wound closure. The observation that the drug was safely tolerated and that the mid-dose (0.02/0.03%) accelerated the rate of wound closure has provided a promising framework for the development of future trials in dealing with chronic wounds. A recently completed trial of $T\beta_4$ in patients with Epidermolysis Bullosa (EB) has found similar effects and at the same dose (0.03%) of $T\beta_4$ in accelerating the early closure of wounds [38]. Furthermore, there were no adverse events in the EB patients who represent a very fragile population, and many patients in the study were children, thus further demonstrating the safety profile of this peptide in humans.

Table 3. Results of thymosin β4 ocular clinical trials.

Phase II neurotrophic keratitis-completed 2009
9 patients with neurotrophic keratitis who had lesions that had not healed in 6 weeks prior to trial
Physician sponsored 28 day trial
Safe and well tolerated
Statistically significant healing/improvement
56 day follow-up showed lasting effect
Dunn, Archiv. Opthalmol, 2010
Phase II moderate dry eye trial-completed 2012
72 patients in 28 day trial
Safe and well tolerated
Statistically significant sign (size of lesion) and symptom (patient comfort) improvements in patients
Pending ocular trials
Phase III for neurotrophic keratitis (approved for orphan drug use by FDA) in USA in 2015
Phase II for dry eye to be conducted in China, Investigational New Drug application, submitted
Phase II or III for severe dry eye to be conducted in Korea, Investigational New Drug application, in preparation

5. Tβ4: clinical application in the treatment and repair of dry eye and neurotropic keratitis

There is only one approved treatment available in the United States for patients with dry eye. It requires 6 months of treatment and is effective in only a small percentage of patients. Therefore, there is an unmet need for a treatment that is rapid and has efficacy. To date, several compassionate Phase II trials of Tβ4 (RGN-259) have been carried out in patients with diabetic victrectomy [11], dry eye [26,27], and neurotropic keratitis [28]. The results from these trials have been promising and are summarized in **Table 3**. The patients experienced no adverse events and had significant improvement in both the signs (area of lesion size reduced) and the symptoms (less pain or irritation) of the disease. The patients with long standing eye injuries responded well to this treatment also with no adverse events. Plans are now underway to initiate late stage clinical trials in patients with dry eye and neurotropic keratitis to confirm and extend current observations.

6. Potential clinical applications

The ability of Tβ4 to reduce scarring [29,30] points to a number of additional activities in treating autoimmune and inflammatory diseases, including liver fibrosis [39], kidney glomerular disease [40], and a number of other metabolic disorders associated with the aging process. Of particular interest with regard to the potential role of Tβ4 in aging is the observation that the levels of Tβ4 in human tears and in saliva decrease significantly with age [41] and that *in vitro* addition of Tβ4 in preliminary studies can reduce the senescence of EPCs in a concentration-dependent manner [42]. In this later study, it was also observed that Tβ4 increased telomerase activity and the expression of telomerase reverse transcriptase mRNA in EPCs. Given the important role of telomerase in maintaining the length of the telomeres and its known association with the aging process, the use of Tβ4 as a novel therapeutic approach to deal with a number of age-associated diseases, including diseases associated with increased cellular senescence, such as dry eye, atherosclerosis, and heart disease, are emerging as new potential clinical opportunities and are currently under investigation.

7. Conclusion

The advances in our understanding of the roles of the Tβ4 in health and diseases have enabled us to move its application rapidly into the clinic. This protein has multiple functions and specific functional sites that are important in the tissue repair and regeneration cascade. In early clinical trials, Tβ4 has shown clinical activities in accelerating the healing of chronic wounds and in the treatment of severe dry eye and neurotropic keratitis. The gene for Tβ4 is known to be up-regulated in cells and in tissues following tissue injury and is known to be essential during normal cellular activities and growth. Thus, Tβ4 is a natural endogenous repair factor that is normally activated during development and in tissue injury. Based upon positive results observed in several animal models following injury and trauma and the completion of Phase I clinical trials with an intravenous formulation of Tβ4, clinical trials are in the planning stage in patients following an acute myocardial infarction, and in patients with brain injuries and pathologies, such as trauma, stroke and MS. One or more of these trials are expected to begin in 2015 with the goal of confirming and extending the early clinical studies in patients with neurotropic keratitis and severe dry eye.

8. Expert opinion

Now, almost 35 years after the original discovery of Tβ4, advances in genomics, proteomics, and gene therapy are rapidly amplifying our understanding of the important roles of Tβ4 in both health and disease and its future potential. The

promising results of early clinical trials in patients with moderate to severe dry eye and with neurotropic keratitis have set the stage for Phase II and Phase III trials, which are in the planning stage. The availability of synthetic $T\beta_4$ has significantly accelerated animal experimentation in the field and is helping researchers to consider a number of new and novel clinical applications following ischemic injuries and trauma. These advances have also raised numerous new questions and identified some weaknesses in our understanding of this protein, such as the characterization of the $T\beta_4$ receptors, the signaling events in the cell that modulate $T\beta_4$ gene activation, the function of $T\beta_4$ in the nucleus, and the specific function of the two other β-thymosins ($T\beta_{10}$, $T\beta_{15}$) found in human cells. The high safety profile observed in Phase I clinical trials of topical and injectable formulations and in Phase II clinical trials in the skin and eye has also led to the anticipation that $T\beta_4$ will be safe and efficacious in a number of other indications. Since the cascade of tissue healing and regeneration involves common pathways in various disorders and injuries, we speculate that $T\beta_4$ will likely have wide uses in the clinic in the near future. From a clinical perspective, advances in the field suggest that given the ability to increase telomerase activity, $T\beta_4$ may be useful in the treatment of a number of difficult to treat life-threatening chronic diseases of aging. In addition, the anti-inflammatory properties of $T\beta_4$ raise new opportunities to utilize this property to treat septic shock as shown in an animal model [13] and potentially the consequences of a number of serious viral diseases such as Ebola that can induce end-organ failure and sepsis resulting from a severe cytokine storm and a dysregulated immune response to infection.

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Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Low TL, Hu SK, Goldstein AL. Complete amino acid sequence of bovine thymosin beta4: a thymic hormone that induces terminal deoxynucleotidyl transferase activity in thymocyte populations. Proc Natl Acad Sci USA 1981;78:1162-6

•• **This paper defines the 43 amino acid sequence of thymosin β4 ($T\beta_4$).**

2. Hooper JA, McDaniel MC, Thurman GB, et al. Purification and properties of bovine thymosin. Ann N Y Acad Sci 1975;249:125-44

3. Goldstein AL, Goldstein AL. From lab to bedside: emerging clinical applications of thymosin alpha 1. Expert Opin Biol Ther 2009;9(5):593-608

4. Erickson-Viitanen S, Ruggieri S, Natalini P, et al. Thymosin beta10, a new analog of thymosin beta4 in mammalian tissues. Arch Biochem Biophys 1983;225:407-13

5. Baynard J, Hutchinson LM, Zetter BR. Thymosin beta-NB is the human isoform of rat thymosin beta15. Ann N Y Acad Sci 2007;1112:286-96

6. Wara DW, Goldstein AL, Doyle NE, et al. Thymosin activity in patients with cellular immunodeficiency. N Engl J Med 1975;292(2):70-4

7. Schafer LA, Goldstein AL, Gutterman JU, et al. In vitro and in vivo studies with thymosin in cancer patients. Ann N Y Acad Sci 1976;277:609-20

8. Schulof RS, Lloyd MJ, Ueno WM, et al. Phase II trial of thymosin fraction 5 in advanced renal cancer. J Biol Response Mod 1984;3(2):151-9

9. Schulof RS, Simon GL, Sztein MB, et al. Phase I/II trial of thymosin fraction 5 and thymosin alpha one in HTLV-III seropositive subjects. J Biol Response Mod 1986;5(5):429-43

10. Lavastida MT, Goldstein AL, Daniels JC. Thymosin administration in autoimmune disorders. Thymus 1981;2(4-5):287-95

11. Sosne G, Qiu P, Christopherson PL, et al. Thymosin beta 4 suppression of corneal NFkappaB: a potential anti-inflammatory pathway. Exp Eye Res 2007;84:663-9

12. Qui P, Wheater MK, Sosne G. Thymosin beta 4 inhibits TNF-alpha-induced NF-kappaB activation, IL-8 expression, and the sensitizing effects by its partners PINCH-1 and ILK. FASEB J 2011;25:1815-26

•• **This paper defines the molecular mechanisms of how Tβ4 reduces inflammation.**

13. Badamchian M, Fagarasan MO, Danner RL, et al. Thymosin beta4 reduces lethality and down-regulates inflammatory mediators in endotoxin-induced septic shock. Int Immunopharmacol 2003;3:1225-33

• **This paper demonstrates that $T\beta_4$ can rescue animals from sepsis.**

14. Sosne G, Szliter EA, Barrett R, et al. Thymosin beta 4 promotes corneal wound healing and decreases

inflammation in vivo following alkali injury. Exp Eye Res 2002;74:293-9

15. Young JD, Lawrence AJ, Mac Lean AG, et al. Thymosin beta 4 sulfoxide is an anti-inflammatory agent generated by monocytes in the presence of glucocorticoids. Nat Med 1999;5:1424-7
16. Wei C, Kumar S, Kim IK, et al. Thymosin beta 4 protects cardiomyocytes from oxidative stress by targeting anti-oxidative enzymes and anti-apoptotic genes. PLoS One 2012;7(8):e42586
17. Ehrlich HP, Hazard SW. Thymosin β4 affecting the cytoskeleton organization of the myofibroblasts. Ann N Y Acad Sci 2012;1269:74-8
18. Sosne G, Xu L, Prach L, et al. Thymosin beta 4 stimulates laminin-5 production independent of TGF-beta. Exp Cell Res 2004;293(1):175-83

• **This paper shows that laminin-5 which is important in cell–cell and cell–matrix contacts is upregulated by Tβ4.**

19. Melinda KM, Goldstein AL, Kleinman HK. Thymosin beta4 stimulates directional migration of human umbilical vein endothelial cells. FASEB J 1997;11:474-81
20. Malinda KM, Sidhu GS, Mani H, et al. Thymosin beta4 accelerates wound healing. J Invest Dermatol 1999;113(3):364-8

•• **This was the first paper to show that thymosin β4 promoted dermal healing.**

21. Ehrlich HP, Hazard SW III. Thymosin beta4 enhances repair by organizing connective tissue and preventing the appearance of myofibroblasts. Ann N Y Acad Sci 2010;1194:118-24

•• **This paper shows that Tβ4 reduces scarring by inhibiting myofibroblast appearance in regenerating tissues.**

22. Treadwell T, Kleinman HK, Crockford D, et al. The regenerative peptide thymosin β4 accelerates the rate of dermal healing in preclinical animal models and in patients. Ann N Y Acad Sci 2012;1270:37-44

• **This paper presents the first results of the clinical trials in the skin showing some efficacy of Tβ4 in stasis and pressure ulcers.**

23. Crockford D, Turjman N, Allan C, et al. Thymosin beta4: structure, function, and biological properties supporting current and future applications. Ann N Y Acad Sci 2010;1194:179-89
24. Kim S, Kwon J. Thymosin beta4 improves dermal burn wound healing via downregulation of receptor of advanced glycation end products in db/db mice. Biochim Biophys Acta 2014;1840(12):3452-9
25. Sosne G, Qiu P, Kurpakus-Wheater M. Thymosin beta-4 and the eye: I can see clearly now the pain is gone. Ann N Y Acad Sci 2007;1112:114-22
26. Sosne G, Qiu P, Ousler GW 3rd, et al. Thymosin β4: a potential novel dry eye therapy. Ann N Y Acad Sci 2012;1270:45-50
27. Goldstein AL, Hannappel E, Sosne G, et al. Thymosin β4: a multi-functional regenerative peptide. Basic properties and clinical applications. Expert Opin Biol Ther 2012;12(1):37-51
28. Dunn SP, Heidemann DG, Chow CY, et al. Treatment of chronic nonhealing neurotrophic corneal epithelial defects with thymosin beta4. Ann N Y Acad Sci 2010;1194:199-206
29. Srivastava S, Srivarava D, Olson EN, et al. Thymosin beta4 and cardiac repair. Ann N Y Acad Sci 2010;1194:87-96
30. Rossdeutsch A, Smart N, Riley PR. Thymosin beta4 and Ac-SDKP: tools to mend a broken heart. J Mol Med 2008;86:29-35
31. Postrach J, Schmidt M, Thormann M, et al. Adeno-associated viral vector 2.9 thymosin β4 application attenuates rejection after heart transplantation: results of a preclinical study in the pig. Transplantation 2014;98(8):835-43
32. Morris DC, Cui Y, Cheung WL, et al. A dose-response study of thymosin β4 for the treatment of acute stroke. J Neurol Sci 2014;345(1-2):61-7
33. Xiong Y, Mahmood A, Meng Y, et al. Treatment of traumatic brain injury with thymosin beta4 in rats. J Neurosurg 2011;114:102-15
34. Zhang J, Zhang ZG, Morris D, et al. Neurological functional recovery after thymosin beta 4 trreatment in mice with experimental auto encephalomyelitis. Neuroscience 2009;164:1887-93

•• **This paper shows the potential based on animal models of Tβ4 in the treatment of multiple sclerosis.**

35. Wang L, Chopp M, Szalad A, et al. Thymosin β4 promotes the recovery of peripheral neuropathy in type II diabetic mice. Neurobiol Dis 2012;48(3):546-55

•• **Using an animal model of peripheral neuropathy, the authors found that Tβ4 promoted functional recovery of the nerve damage.**

36. Treadwell T, Kleinman HK, Crockford D, et al. The regenerative peptide thymosin β4 accelerates the rate of dermal healing in preclinical animal models and in patients. Ann N Y Acad Sci 2012;1270:37-44
37. Guarnera G, DeRosa A, Camerini R. The effect of thymosin treatment of venous ulcers. Ann N Y Acad Sci 2010;1194:207-12
38. Fine JD. Epidermolysis bullosa: a genetic disease of altered cell adhesion and wound healing, and the possible clinical utility of topically applied thymosin beta4. Ann N Y Acad Sci 2007;1112:396-406
39. Reyes-Gordillo K, Shah R, Arellanes-Robledo J, et al. Protective effects of thymosin β4 on carbon tetrachloride-induced acute hepatotoxicity in rats. Ann N Y Acad Sci 2012;1269:61-8
40. Zuo Y, Chun B, Potthoff SA, et al. Thymosin β4 and its degradation product, Ac-SDKP, are novel reparative factors in renal fibrosis. Kidney Int 2013;84(6):1166-75
41. Badamchian M, Damavandy AA, Damavandy H, et al. Identification and quantification of thymosin beta4 in human saliva and tears. Ann N Y Acad Sci 2007;1112:458-65
42. Li J, Yu L, Zhao Y, et al. Thymosin β4 reduces senescence of endothelial progenitor cells via the P13K/Akt/eNOS signal transduction pathway. Mol Med Rep 2013;7(2):598-602
43. Sosne G, Qui P, Goldstein AL, et al. Biological activities of thymosin beta 4 defined by active sites in short peptide sequences. FASEB J 2010;24(7):2144-51

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