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Thymosin beta 4 regulation of actin in sepsis

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Abstract

Introduction—Sepsis is the dysregulated host response to an infection resulting in life-threatening organ damage. Thymosin Beta 4 is an actin binding protein that inhibits the polymerization of G-actin into F-actin and improves mortality when administered intravenously to septic rats. Thymosin Beta 4 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.

Areas covered—The authors summarize the current knowledge of actin and Thymosin Beta 4 as it relates to sepsis via a comprehensive literature search.

Expert opinion—Sepsis results in measurable levels of F-actin in the circulation as well as a decreased concentration of Thymosin Beta 4. It is speculated that F-actinemia contributes to microcirculatory perturbations present in patients with sepsis by disturbing laminar flow. Given that Thymosin Beta 4 inhibits the polymerization of F-actin, it is possible that Thymosin Beta 4 decreases mortality in sepsis via the regulation of actin as well as its other anti-inflammatory properties and should be further pursued as a clinical trial in humans with sepsis.

Keywords

Actin; microcirculatory dysfunction; sepsis; thymosin beta 4

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Declaration of interest

J Belsky, E Rivers, and D Morris have made the following US patent application: Methods for treating sepsis based on biomarkers including Thymosin Beta 4, G-actin, and F-actin (patent number: US20170307609A1). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

Supplemental data for this article can be accessed here.

1. Introduction

Approximately 1.7 million adults were hospitalized with sepsis in the United States in 2014 [1] which is particularly concerning as it is the leading cause of mortality in hospitalized patients, associated with one in every two to three deaths [2]. Despite its high mortality and invasive nature within the health-care system, every large targeted pharmaceutical trial has failed [3].

Sepsis is the dysregulated host response to a pathogen resulting in life-threatening organ damage [4]. The complex interaction between the host response and infecting agent results in a pro- and anti-systemic inflammatory response syndrome (SIRS) that, when it becomes dysregulated, results in microcirculatory derangements [5], increased oxidative stress [6], vascular permeability [7], cellular hypoxia/dysoxia [8], organ dysfunction, and death.

While the pathogenesis of sepsis is highly complex and unclear, there is increasing evidence that actin may play a key role in its pathogenesis. Actin is an abundant protein present in most eukaryotic cells and participates in numerous protein–protein interactions influencing, cell morphology, muscle contraction [9], cell motility [10], and cell–cell adhesions [11]. It is a 42-kDa globular protein [12] that cycles between a monomeric (G-actin) and filamentous (F-actin) state. The intracellular pool of monomeric G-actin is complexed to and regulated by actin binding proteins (ABPs), such as TB4, which regulates the conversion of G-actin to F-actin [13]. However, when actin is released extracellularly and exceeds the regulatory actions of ABPs, it can polymerize into long-chain F-actin, resulting in deleterious effects [14].

It is known that TB4 levels are decreased in septic shock, and that F-actin is present in plasma where it is usually absent [15]; and a previous sepsis animal model has shown a mortality benefit utilizing TB4 [16]. It is therefore possible that TB4 has a therapeutic role in humans with sepsis, and a potential mechanism of action is the inhibition of formation of F-actin in the circulation. This review summarizes the current knowledge of TB4 as it relates to sepsis and encourages further studies regarding its therapeutic potential in humans with sepsis.

2. Thymosin beta 4

TB4 is an ABP that binds to G-actin into a 1:1 complex, rendering G-actin resistant to polymerization into F-actin. It is important in maintaining a sufficient intracellular volume of monomeric actin that is readily available for use if needed [17]. TB4 is a 43-amino acid protein [18] present in all cells except red blood cells. It is also present in numerous body fluids (saliva [19], tears [19], plasma [15], wound fluid [20], and cerebrospinal fluid [21]). It is located both in the cytoplasm and nucleus of the cell [22] and hypothesized that its presence in body fluids is secondary to release from damaged cells given it lacks a secretion signal [23].

Clinically, synthetic TB4 is currently being studied in human trials as an injectable (RGN-352), eye drop (RGN-259), and topical gel (RGN-137) formulation sponsored by the

biopharmaceutical company RegeneRx. The injectable formulation completed a Phase 1, randomized, placebo-controlled, single- and multiple- dose study among healthy volunteers [24]. The intravenous formulation was observed to be safe, well tolerated, and without dose-limiting toxicity or serious adverse events between a dose range of 42 and 1260 mg over 14 days. The eye drop formulation has completed Phase 2 trials in moderate-to-severe dry eyes [25,26] with a Phase 3 trial currently underway. It also has been given as a compassionate use drug for neurotrophic corneal defects [27]. The ocular benefits of TB4 in dry eyes and neurotrophic corneal defects are speculated to be in its wound healing [28], anti-inflammatory [29], anti-apoptosis, and promotion of cell migration properties [30,31].

2.1. Blood levels of thymosin beta 4 and actin in sepsis

Both animal and human studies have shown a reduction in levels of circulating TB4 in response to an infectious challenge. An LD₅₀ dosage of lipopolysaccharide (LPS), a major component of the outer membrane of gram-negative bacteria, given to rats, resulted in a reduction of TB4 in blood. In this same manuscript, a nonlethal dosage of LPS administered to healthy volunteers resulted in a decline in TB4 levels [16]. A recent retrospective study in humans compared the plasma levels of TB4, G-actin, and F-actin in healthy controls, noninfectious SIRS, and septic shock [15]. The healthy control group contained levels of TB4 and G-actin, but did not have detectable levels of F-actin (minimum assay concentration of 0.62 ng/ml). However, both G-actin and F-actin were present in vasopressor-dependent septic shock patients, while TB4 was undetectable (minimum assay concentration of 78 ng/ml). The noninfectious SIRS group also did not have detectable levels of TB4 above the lowest assay concentration and had very low, but detectable levels of F-actin (Figures 1–3). The authors conclude that free actin is released into the circulation during the inflammatory process (perhaps through cellular destruction) resulting in consumption of TB4 and the dysregulation and formation of F-actin in the circulation. It remains unknown which cells contribute significantly to the release of circulatory actin; furthermore, the relationship between circulatory actin levels and sepsis severity has not been fully characterized and to what extent various degrees of sepsis has on levels of these biomarkers.

2.2. Deleterious effects of F-actin

Given that F-actinemia is present in patients with septic shock, it is important to understand the potential deleterious effects of F-actinemia in circulation. G-actin can polymerize into its filamentous form when added to physiological buffers. However, when exposed to plasma, the ability to polymerize is regulated by an actin-scavenger system composed of various ABPs [32]. When the actin-scavenging system is overwhelmed (as seen with undetectable levels of TB4 in patients with septic shock), extracellular filamentous formation can occur. In rats exposed to increasing amounts of globular actin, the result was an increase in filamentous actin formation, endothelial injury, and microthrombi, which are also characteristics of sepsis pathology. These changes were not observed when rats were preincubated with an ABP [14]. It is possible that F-actin formation in the microvasculature contributes to microcirculatory flow disturbances through the creation of long-chain F-actin barriers that perturb laminar flow. Additionally, F-actin can activate platelets [33], may interact with fibrin as a clot is formed [34,35], and cause perturbations in clot formation and

lysis [36,37]. In another study, the addition of G-actin into plasma and its conversion to F-actin was toxic to cultured pulmonary endothelial cells [38]. It is thus hypothesized that excessive F-actin likely has pathological consequences in sepsis.

2.3. TB4 and non-actin-dependent role in sepsis

Despite the small size of TB4, at least three different active sites have been identified which regulate anti-inflammation, angiogenesis, actin binding, wound healing, and cytotoxicity protection [23]. Sepsis is hallmarked by pro- and anti-inflammatory cascades that are potentially tempered by TB4 as it reduces pro-inflammatory cytokine levels of IL-1 alpha [16] and inhibits TNF-alpha induced NF-kB activation [29]. In addition, TB4 prevents apoptosis by decreasing cytochrome c release from mitochondria, increasing bcl-2 expression, and decreasing caspase activation [31]. Rat neonatal cardiomyocytes pretreated with TB4 and then exposed to hydrogen peroxide had a reduction in intracellular reactive oxygen species (ROS) levels, upregulation of anti-oxidative enzymes, anti-inflammatory genes, and antiapoptotic enzymes [39]. TB4 also reduces pericyte loss that occurs in sepsis, decreases perivascular leak, and improves hemodynamic parameters [40]. Finally, TB4 significantly potentiates antigen presentation by macrophages [41]. These additional properties of TB4 may play a beneficial role in a non-actin-dependent manner in sepsis.

3. Conclusion

TB4 is a major regulator of the formation of F-actin and exogenous use in animals has led to mortality improvements. Given that F-actin may have deleterious effects on the micro-circulation, the modulation of F-actin may play an important role in the pathogenesis of sepsis. In addition to the actin binding properties of TB4, it also may be beneficial in patients with sepsis given its ability to decrease inflammatory mediators, decrease ROS, upregulate anti-oxidative enzymes, anti-inflammatory genes, and antiapoptotic enzymes. TB4 offers a promising diagnostic and therapeutic option for patients with sepsis given that TB4 levels are low and F-actin levels are present in patients with septic shock.

4. Expert opinion

TB4 is a major regulatory ABP that inhibits the conversion of G-actin to F-actin and has been shown to downregulate important inflammatory markers that are the hallmark of sepsis. Sepsis has proven to be a formidable foe in defeating all large pharmaceutical trials. It is likely that a new targeted therapeutic approach to sepsis such as a biomarker-driven trial is the next evolution in sepsis trials. TB4 fits this profile well given that patients can be screened for enrollment by targeting the drug to those patients with low levels of TB4 and elevated levels of F-actin. In order for this trial to be successful, a rapid and reliable point-of-care test to determine the levels of F-actin and TB4 would have to be developed or a post hoc analysis of those patients with this biomarker profile would have to be performed. This trial would not only be a unique study design but would lead to a potential targeted therapy for patients with sepsis if successful.

In addition to TB4's role as a potential therapeutic option for those with sepsis, both TB4 and F-actin may serve as an important diagnostic and risk stratification tool. Biomarker

studies are currently underway to determine if F-actin levels correlate or predict severity of sepsis in patients presenting to the emergency department with infectious symptoms. These studies could lead to earlier risk stratification of patients with sepsis and result in advancements in clinical care of those with developing disease. Interventions could be aimed toward those with declining TB4 or rising F-actin levels such as broadening of antibiotics or increasing hospital length of stays as these biomarkers could indicate that a patient with sepsis is worsening.

Further work should explore the role of the interplay between TB4 and the actin cytoskeleton on cell junctions as the formation and maturation of cell–cell contacts involves the reorganization of the actin cytoskeleton [42]. It is possible that TB4 also plays a role in endothelial integrity which is disturbed in sepsis [43] and that a potential therapeutic role could be the inhibition of capillary leak present in sepsis via its role in actin reorganization.

The role of actin and TB4 in the pathogenesis, diagnosis, and treatment of sepsis is actively being pursued. Future clinical trials, biomarker studies, and basic science work are needed to further elucidate its place in clinical medicine.

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Article highlights

- Sepsis is the dysregulated host response to a pathogen resulting in life-threatening organ damage.
- Thymosin Beta 4 is an actin binding protein that inhibits the polymerization of globular actin (G-actin) into filamentous actin (F-actin).
- Plasma F-actin is not detectable In a healthy population but is present at measurable levels in patients with septic shock.
- TB4 levels are undetectable in those with septic shock, suggesting that TB4 is consumed allowing for the unregulated polymerization of actin in the bloodstream.
- Excessive F-actin has been shown to have deleterious effects similar to those seen in sepsis.
- Animals treated with Thymosin Beta 4 and then exposed to a sepsis model resulted In decreased mortality when compared to controls suggesting that Thymosin Beta 4 may be a therapeutic option in sepsis by regulating the polymerization of actin.

This box summarized key points contained in the article.

Figure 1.

Box and whisker plot comparing median Thymosin Beta 4 levels in plasma. None of the septic shock or non-infectious SIRS group contained Thymosin Beta 4 levels above the lowest detection of the ELISA assay (78 ng/ml).

Figure 2. Box and whisker plot comparing median G-actin levels in plasma. Logarithmic scale. G-actin levels were greatest in the Non-infectious SIRS group followed by septic shock and then healthy controls, $p < 0.05$.

Figure 3. Box and whisker plot comparing median plasma levels of F-actin. Septic shock was greater than the non-infectious SIRS group, $p < 0.05$. None of the healthy control group contained F-actin levels above the lowest detection of the ELISA assay (0.62 ng/ml).