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THERAPEUTIC POTENTIAL OF TOPICAL AUTOLOGOUS ANGIOSTATIN APPLICATION IN MANAGING TUBERCULOSIS-RELATED CORNEAL INJURY: A CASE REPORT

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Ocular tuberculosis (TB) is a vision-threatening condition that frequently manifests as corneal neovascularization and stromal keratitis, which triggers a cascade of inflammatory and hypoxia-driven responses. Conventional therapeutic approaches, including corticosteroids and antimicrobial agents, often fail to halt disease progression. Here, we report a case of a 50-year-old patient diagnosed with TB-associated keratitis, unresponsive to standard treatment. The aim of the study was to evaluate the effectiveness of the alternative therapeutic strategy involving topical administration of angiostatin, a natural anti-angiogenic polypeptide derived from the autologous plasminogen. Solution of angiostatin fragment containing the first three kringle domains (K1-3) was applied in a two doses of eye drops (~15 μg per administration) five times daily for 2 months, with a cumulative exposure of approximately 4.5 mg. Treatment efficacy was monitored using both standard ophthalmologic assessments and non-invasive biochemical indicators such as the levels of hypoxia-inducible factor HIF-1α, vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP-9), fibrinogen/fibrin (Fg/Fb) and lactoferrin measured the in tear fluid across treatment time points (Day 0, 14, and 61) using Western blot analysis. The high intensity of HIF-1 α, VEGF and MMP-9 expression, Fg/Fb accumulation and the presence of low-molecular-weight fragments of lactoferrin were detected in the tear fluid prior to the treatment. Following angiostatin therapy, the patient exhibited marked regression of corneal neovascularization and restoration of corneal transparency, complemented with normalization of HIF-1α, VEGF, and MMP-9 levels, reduced Fg/Fb accumulation and the presence of intact lactoferrin in the tear fluid. The data obtained demonstrated a multifactorial mechanism of angiostatin action that extends beyond classical anti-angiogenic pathways. The convergence of clinical and molecular indicators of recovery underscores the potential of angiostatin application as a safe and effective therapeutic alternative for managing corneal complications in ocular TB, particularly in cases resistant to conventional treatment.

K e y w o r d s: ocular tuberculosis, corneal injury, angiostatin, HIF-1α, VEGF, MMP-9, lactoferrin, tear fluid.

Tuberculosis (TB) remains a significant global public health challenge, with *Mycobacterium tuberculosis* primarily targeting the lungs. However, extrapulmonary manifestations, including ocular tuberculosis, account for approximately 6% of all TB cases [1, 2]. The pathogenesis of ocular TB is influenced by the mode of infection. Direct contact with the eye can result in localized infections affecting the eyelids, sclera, conjunctiva, or cornea. Conversely, hematogenous dissemination of *M. tuberculosis* often leads to inflammation of the uveal tract, manifesting as either anterior or posterior uveitis. Additionally, hypersensitivity reactions to *M. tuberculosis* antigens can trigger immune-mediated conditions such as phlyctenular keratoconjunctivitis, characterized by an immune-allergic response to non-viable mycobacterial antigens [3].

Ocular tuberculosis encompasses a spectrum of clinical presentations, broadly categorized into forms caused by direct invasion of *M. tuberculosis* and those resulting from immune-mediated effects. Immune-mediated manifestations include scleritis,

episcleritis, interstitial and phlyctenular keratitis, uveitis, and optic neuritis [4]. Among these, inflammation and corneal neovascularization are notable complications that can significantly impair vision. Corneal neovascularization, characterized by the abnormal growth of blood vessels into the cornea, is often a response to chronic inflammation and hypoxia. This pathological process not only compromises corneal transparency but also exacerbates the disease burden in ocular TB patients [5, 6].

Angiostatins, natural polypeptides derived from the precursor protein plasminogen, known for their anti-angiogenic properties, have shown promise in inhibiting corneal neovascularization and managing ocular tuberculosis [7]. By targeting the vascular endothelial growth factor (VEGF) pathway, angiostatins may help mitigate the progression of neovascularization and preserve corneal integrity [8]. Earlier studies have demonstrated that angiostatins effectively reduce corneal neovascularization in animal models by inhibiting pro-angiogenic signaling pathways, such as VEGF, and promoting vascular regression [9, 10]. This mechanism contributes to improved corneal transparency and visual outcomes. Furthermore, angiostatins have been shown to modulate expression of the key molecular markers, including HIF-1α and MMP-9, which play crucial roles in mitigating hypoxia-induced damage and fibrosis in injured corneas [11, 12]. These findings indicate that angiostatins could represent a promising addition to the therapeutic options for ocular TB, particularly due to their demonstrated safety profile, characterized by minimal toxicity and low immunogenicity. Their ability to target both inflammatory and neovascular processes can offer a promising, multifaceted approach when integrated with existing anti-tubercular therapies. However, despite these encouraging results in animal models, there is still a lack of clinical evidence supporting their efficacy in human patients, particularly for TB-related corneal injuries.

Thus, the aim of this case report study was to evaluate the effects of topical corneal application of autologous angiostatin for the treatment of a patient with TB-induced ocular form of corneal injury.

Materials and Methods

Case presentation. A 50-year-old patient presented to the ophthalmology clinic with complaints of persistent ocular redness, a foreign body sensation in the right eye lasting over one month, and progressive visual deterioration over the past five months. Upon examination, uncorrected visual acuity in the right eye was measured at 0.08, with preserved light perception. The patient had a confirmed diagnosis of tuberculosis, complicated by secondary ocular hematogenous involvement, which was considered in the differential assessment and interpretation of ocular findings. The patient's medical history shows that he has been diagnosed with tuberculosis for more than 10 years and has been diagnosed with arterial hypertension, chronic obstructive pulmonary disease, or COPD, and nodules in the upper areas of the lungs on a chest X-ray. The patient currently received anti-tuberculosis treatment with a 4-drug regimen of ethambutol (1 g/day), isoniazid (300 mg/day), rifampicin (600 mg/day), and pyrazinamide (1.5 g/day) under the supervision of a phthisiatrician. The patient underwent a comprehensive ophthalmologic evaluation, including visometry, perimetry, tonometry, slit-lamp biomicroscopy, ophthalmoscopy, keratorefractometry, confocal corneal biomicroscopy, and anterior segment optical coherence tomography (OCT). These diagnostic procedures were performed in accordance with current clinical standards.

Slit-lamp examination showed significant conjunctival congestion, deep corneal neovascularization, and confluent disc-shaped stromal infiltrates. The intraocular pressure in the right eye was measured at 15 mm Hg, and fundus examination revealed no abnormalities within the visible area. Corneal sensation was intact bilaterally. Optical coherence tomography of the anterior segment (AS-OCT) revealed stromal thinning, leading to reduced overall corneal thickness while preserving epithelial thickness. Hyporeflective areas were observed amidst general hyperreflectivity of the corneal tissue, consistent with neovascularization. This pattern, commonly referred to in the literature as a "barcode" appearance, is indicative of neovascularization.

Despite prolonged conventional treatment, including systemic anti-tuberculosis therapy and topical ophthalmic agents (dexamethasone eye drops 4 times a day, dexpanthenol 4 times a day, cyclopentanol 1 time a day), the patient continued to suffer from persistent keratitis with no satisfactory therapeutic response. Given the refractory nature of the condition and the absence of clinical improvement, a decision was made to initiate treatment with autologous angiostatin formulated as eye drops.

The patient provided informed consent for the use of autologous biomaterial and the initiation of innovative therapeutic intervention, thereby affirming his readiness to contribute to the advancement of personalized medicine and experimental ophthalmologic care. All research procedures adhered strictly to the applicable legal and ethical standards of Ukraine, and were conducted in full compliance with the principles outlined in the Declaration of Helsinki, the European Convention on Human Rights and Biomedicine, and national ethical regulations. The study received approval from the Commission on Bioethical Expertise and Research Ethics of Bogomolets National Medical University (Protocol No. 181, dated January 29, 2024).

Angiostatin production. Briefly, plasminogen, an angiostatin's precursor protein, was isolated from 200 ml of autologous citrated blood plasma by affinity chromatography on lysine-Sepharose 4B, yielding an electrophoretically pure preparation (Fig. 1, *A*). Angiostatin K1-3 was produced through the proteolytic cleavage of plasminogen by porcine pancreas elastase (Sigma Aldrich, USA), a serine endopeptidase (3.4.21.36), as described elsewhere in details [13]. Elastase specifically cleaves plasminogen at sites that generate angiostatin fragments containing the first three kringle domains (K1-3) (Fig.1, *B*). The resulting angiostatin K1-3 fragments were separated from the reaction mixture using chromatography techniques, and SDS-PAGE was performed to ensure high purity of K1-3 preparation. A total of 5 mg of angiostatin K1-3 was isolated from the precursor protein, free from residual proteolytic activity, dissolved in sterile PBS, formulated as ophthalmic drops for treatment, and then aliquoted and stored at -20°C until use.

Treatment regime. Since the standard antituberculosis therapy proved insufficient in controlling ocular inflammation and regressing corneal neovascularization, it was decided to supplement the treatment regimen with topical application of angiostatins. This approach aimed to therapeutically inhibit neovascularization and restore corneal transparency through a pathogenetically grounded intervention. A 5 μM solution of angiostatin K1–3 dissolved in sterile PBS was applied topically as 2-drop doses (~15 μg per administration), five times daily for 2 months, resulting in a cumulative exposure of approximately 4.5 mg. Thus, a total of 200 ml of autologous citrated blood was sufficient to isolate 5 mg of angiostatin K1–3, fully covering the required therapeutic dose for the entire 61-day treatment regimen.

Tear fluid collection and sample preparation. Tear fluid samples were collected from patient with TB-induced corneal injury at the Bogomolets Medical University clinic. Samples were collected at three distinct time points: before initiation of treatment, on day 14th of therapy, and at 2 months post-treatment (final evaluation point). Prior to sampling, the patient refrained from using topical medications or eye drops for at least 2 hours to minimize external interference. Basal tears were gently aspirated from the lower conjunctival fornix using disposable glass microcapillary tubes, avoiding contact with the cor-

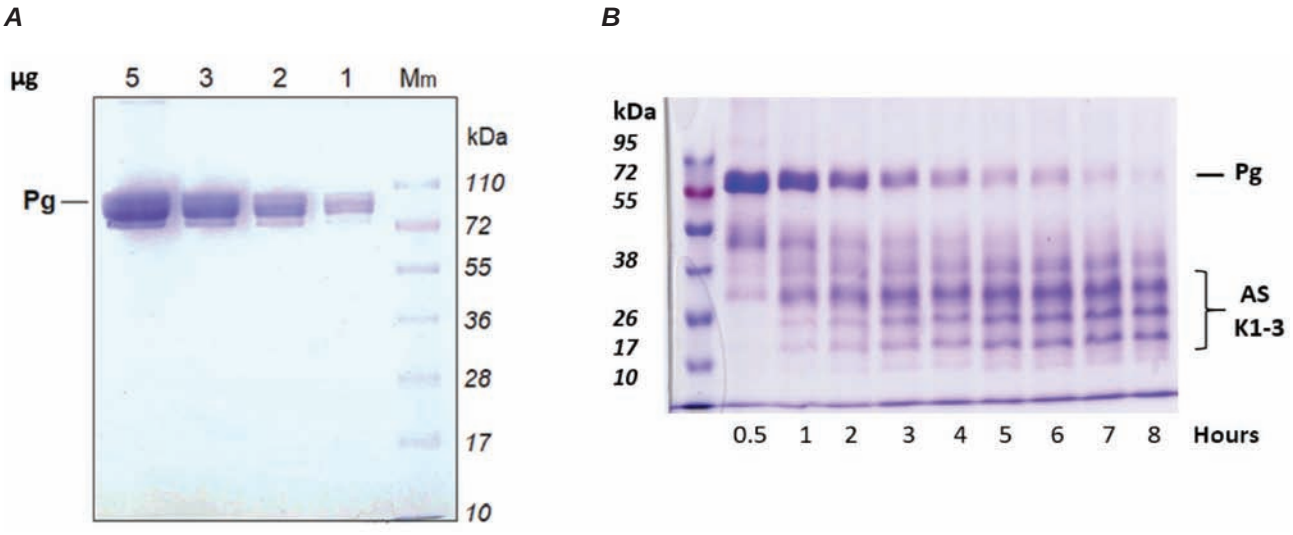

*Fig. 1. Preparation of plasminogen isolated from patient's plasma (**A**) and angiostatin production during plasminogen hydrolysis by pancreatic elastase (**B**)*

neal surface to prevent reflex tearing. A volume of 20–25 µl per eye was obtained and immediately transferred into sterile Eppendorf tubes. Samples were stored at −80°C until analysis. Total protein concentration was determined by BCA assay to standardize loading.

SDS-PAGE and Western blot analysis. An equal amount of protein (typically 50 µg) was subjected to SDS-PAGE followed by transfer to nitrocellulose membranes. After transferring, membranes were blocked for 1 h at 37 oC in phosphate-buffered saline containing 0.05% Triton X-100 (PBST) supplemented with 5% non-fat dry milk to prevent non-specific binding. Western blotting was performed using primary antibodies against selected biomarkers of hypoxia and inflammation, including rabbit anti-HIF-1α (Sigma-Aldrich, USA, cat. no. HPA001275, 1:1,000 diluted), mouse anti-VEGF (ThermoFisher Scientific, USA, cat. no. MA5-12184, 1:2,500 diluted), rabbit anti-MMP-9 (Sigma-Aldrich, USA, cat. no. AV33090, 1:1,000 diluted). Polyclonal rabbit anti-fibrinogen and anti-lactoferrin antibodies were produced in-house using electrophoretically pure antigens, following protocols described elsewhere [14]. Following blocking, membranes were incubated overnight at 4°C with the primary antibodies, diluted in PBST containing 1% milk. After primary antibody incubation, membranes were washed five times for 10 min each with PBST to remove unbound antibodies. Subsequently, membranes were incubated for 2 h 37°C with horseradish peroxidase (HRP)-conjugated secondary antibodies (goat anti-Rabbit IgG (H+L), ThermoFisher Scientific, USA, cat. no. 31460, or goat anti-mouse IgG (H+L), ThermoFisher Scientific, USA, cat. no. 31430), 1:10,000 diluted in PBST with 1% milk. Excess secondary antibodies were removed by five additional 10-minute washes in PBST. Signal detection was performed using enhanced chemiluminescence (ECL). The ECL working solution was freshly prepared by combining luminol, coumaric acid, and hydrogen peroxide in a suitable buffer (50 mM tris-HCl, pH 8.6). Membranes were exposed to X-ray films for appropriate durations to capture chemiluminescent signals. Films were then air-dried and scanned at high resolution. Densitometric analysis of the scanned films was conducted using TotalLab-120 software to quantify band intensities.

Statistical analysis. All experiments were performed in three independent biological replicates to ensure reproducibility and statistical reliability of the results. Statistical comparisons between group means were performed using one-way ANOVA followed by Tukey's post hoc test. A *P*-value of less than 0.05 was considered statistically significant.

Results

At the beginning of treatment, the patient's cornea was opaque, with an uneven optical surface, pronounced edema and stromal opacity, and signs of active inflammation (Fig. 2, *A*).

After starting therapy with angiostatin, on the 14th day of treatment, a gradual reduction in edema and partial restoration of transparency were observed, as well as a reduction in corneal opacity and, in some areas, clearing. The contours became clearer, the intensity of infiltration decreased significantly, and the edema decreased. At this stage of treatment, vascularization decreased, indicating suppression of the inflammatory process. After completing a 2-month course of treatment, the optical section of the cornea became more uniform, the surface smooth, with areas of restored transparency. Visually, the eye looks healthier, with restoration of its optical properties (at the beginning of treatment, clarity of vision was 0.02, while it became 0.1 at two weeks, and at the end of the treatment, visual acuity improved to 0.4).

Panel *B* of Fig. 2 shows fluorescein-stained images of the cornea taken under blue light, which reveal the extent of epithelial damage before and after AS-based treatment. At time point "0", the corneal surface exhibits intense green fluorescence, indicating significant epithelial defects and compromised integrity. The staining pattern is irregular and widespread, suggesting active injury or inflammation. After finishing treatment, by time point "61", the fluorescein uptake is notably reduced or nearly absent, and the corneal surface appears much clearer. This change reflects substantial healing of the epithelium and restoration of the corneal barrier function, demonstrating the effectiveness of the AS topical application in promoting recovery.

Panel *C* presents anterior segment optical coherence tomography (AS-OCT) scans of the cornea at day 0 and day 61, illustrating structural changes over the course of treatment. At day 0, the cornea appears thickened with evident stromal edema, and there are prominent hyporeflective regions suggestive of active neovascularization. These areas indicate fluid accumulation and abnormal vessel growth within the stroma. By day 61, the scans show a nota-

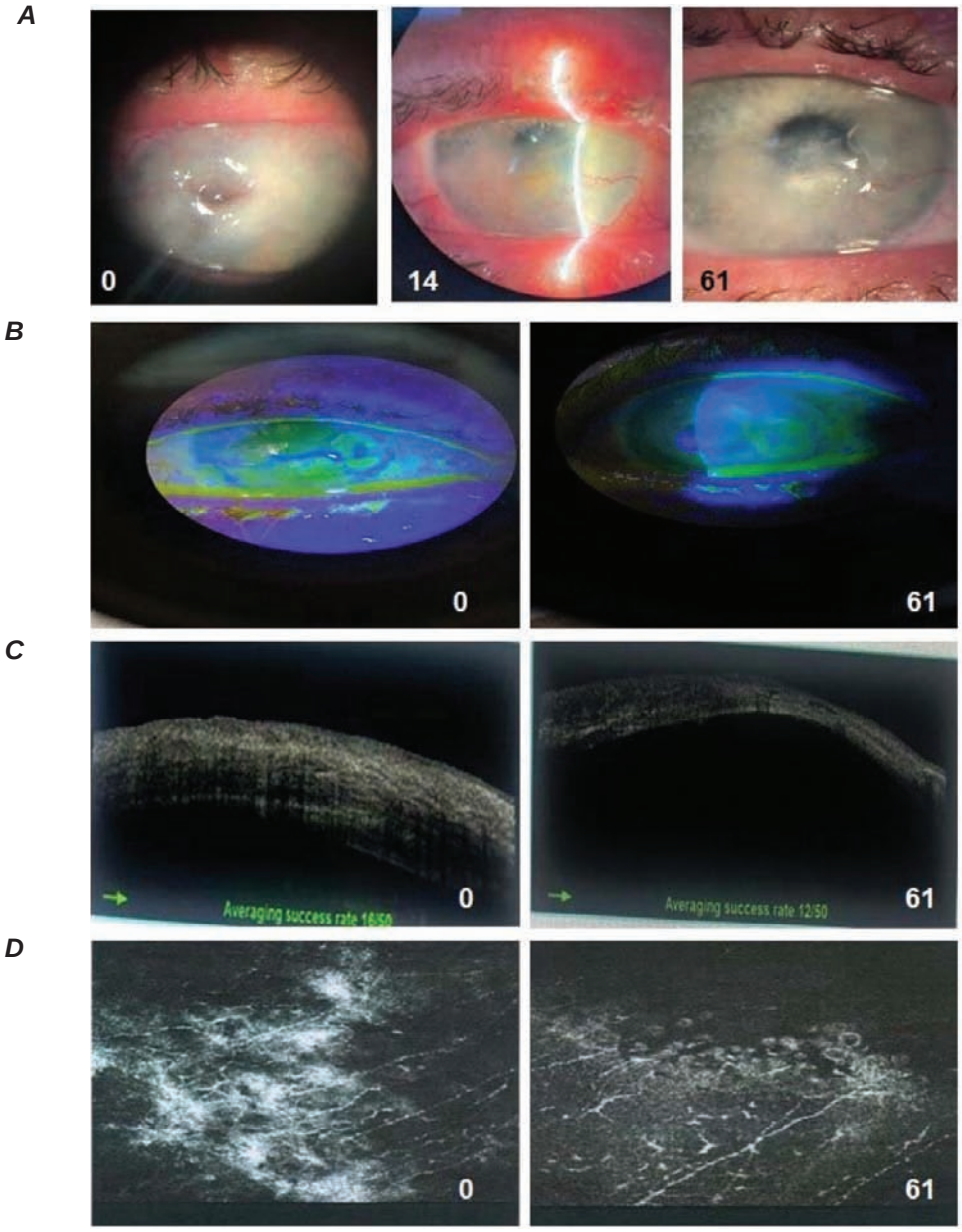

Fig. 2. Multimodal imaging of the anterior segment in a patient with stromal opacification before and after angiostatin-based topical application. ***A*** *– Slit-lamp photographs at baseline (day 0), intermediate (day 14), and final (day 61) stages, showing progressive resolution of stromal infiltration, pigmentation, and neovascularization;* ***B*** *– Fluorescein staining images at day 0 and day 61, demonstrating reduction in corneal and stromal staining;* ***C*** *– Anterior segment optical coherence tomography scans at day 0 and day 61, revealing decreased corneal edema, stromal thinning, and reduction of hyporeflective areas associated with neovascularization;* ***D*** *– Confocal microscopy of the subbasal nerve plexus at day 0 and day 61: initial disruption and loss of nerve fibers followed by partial restoration, with reduced density and twisted architecture*

ble decrease in corneal thickness, reflecting stromal thinning and resolution of edema. The hyporeflective zones are markedly reduced, indicating regression of neovascularization and restoration of corneal transparency.

Panel *D* displays high-resolution confocal microscopy images of the cornea, revealing detailed microstructural changes following treatment. The subbasal nerve plexus shows notable architectural alterations, with nerve fibers appearing thinned and

tortuous, which is indicative of stress or remodeling within the neural network. Despite these changes, both the superficial and deep epithelial layers remain intact, with no detectable abnormalities, suggesting preserved epithelial health. In the anterior stroma, a dense concentration of highly reflective linear structures is visible, likely corresponding to extracellular matrix components or fibrotic elements. At a depth of 215 μm, keratocytes are clearly visualized, maintaining their typical morphology. Deeper within the stroma, between 288 and 371 μm, multiple linear, crystal-like hyperreflective structures emerge, possibly representing deposits or remnants of prior neovascular activity. These findings collectively highlight both regenerative and residual changes in the corneal architecture over time, suggesting a positive therapeutic response with significant improvement in corneal structure and clarity.

Based on the Western blot data across treatment time points (day 0, 14, and 61), shown in Fig. 3, a semi-quantitative interpretation for each marker in tear fluid was performed. The band intensity for HIF-1α was highest at day 0, suggesting significant hypoxic stress in the untreated state. A marked decrease was observed by вay 61 by 14.3 folds compared to control ($P < 0.05$), indicating reduced hypoxia and corneal inflammation following angiostatin therapy. Both monomeric and dimeric VEGF forms show strong expression prior to treatment, consistent with active neovascular signaling. Signal intensity was substantially down-regulated at day 61 by 4.7 folds compared to control ($P < 0.05$), reflecting effective angiogenic suppression by angiostatin. MMP-9 bands diminish progressively over time, with a notable drop at day 61 by 5.6 folds compared to control ($P < 0.05$), indicating decreased matrix degradation and reduced inflammatory protease activity. This supports corneal stabilization and tissue preservation during therapy. High fibrinogen levels at a baseline reflect ongoing tissue injury and inflammation.

A visible decline by day 61 (by 4.2 folds by 4.7 folds compared to control, $P < 0.05$) suggests resolution of inflammatory signaling and vascular leakage. In addition to the full-length lactoferrin band (~78 kDa), faint lower-molecular-weight fragments, presumably representing proteolytic degradation products, are evident at day 0, reflecting heightened protease activity and epithelial turnover prior to treatment. By day 61, these fragments are markedly reduced, while the intensity of the intact lactoferrin band significantly increases by 4.6 folds compared to control, $P < 0.05$. This elevation of the full-length lactoferrin polypeptide suggests enhanced protein stability and preservation, consistent with attenuated inflammatory proteolysis and improved ocular surface integrity under angiostatin therapy. The restored presence of intact lactoferrin may further indicate a functional recovery of its antimicrobial and immunomodulatory roles.

Discussion

Ocular TB presents with a broad spectrum of clinical manifestations, which can vary depending on the mode of infection and the tissue affected. Primary ocular tuberculosis, resulting from direct infection through the skin or mucous membranes, can lead to conditions such as eyelid abscesses, conjunctival infiltration, phlyctenular keratoconjunctivitis, scleritis, and interstitial keratitis. When the infection spreads hematogenously, it often involves structures such as the uvea, retina, and optic nerve, leading to uveitis, chorioretinitis, and neuritis. The preference of *M. tuberculosis* for oxygen-rich tissues, such as the choroid, aligns with its frequent targeting of this ocular layer, characterized by high blood flow [15, 16].

Corneal involvement in TB is relatively rare but has been documented in cases of conjunctival and corneal TB and keratoconjunctivitis associated with active TB infection [17]. Peripheral ulcerative keratitis and immune stromal keratitis are observed in patients experiencing immunological reactions to *M. tuberculosis.* Phlyctenular keratoconjunctivitis is typified by a small, pink inflammatory nodule forming near the limbus that progressively migrates centrally with superficial conjunctival vessels. While the epithelium remains intact initially, its migration ultimately results in epithelial defects and, in severe cases, ulceration or erosion. Clinical symptoms such as photophobia, a foreign body sensation, redness, and lacrimation correlate with the extent of corneal damage. This condition is widely believed to stem from a hypersensitivity reaction to M. tuberculosis proteins [18].

Keratitis, particularly when associated with corneal injury or infection, triggers a cascade of inflammatory and hypoxia-driven responses. Among the most prominent molecular players in this process are hypoxia-inducible factor 1-alpha (HIF-1α), vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMPs), especially

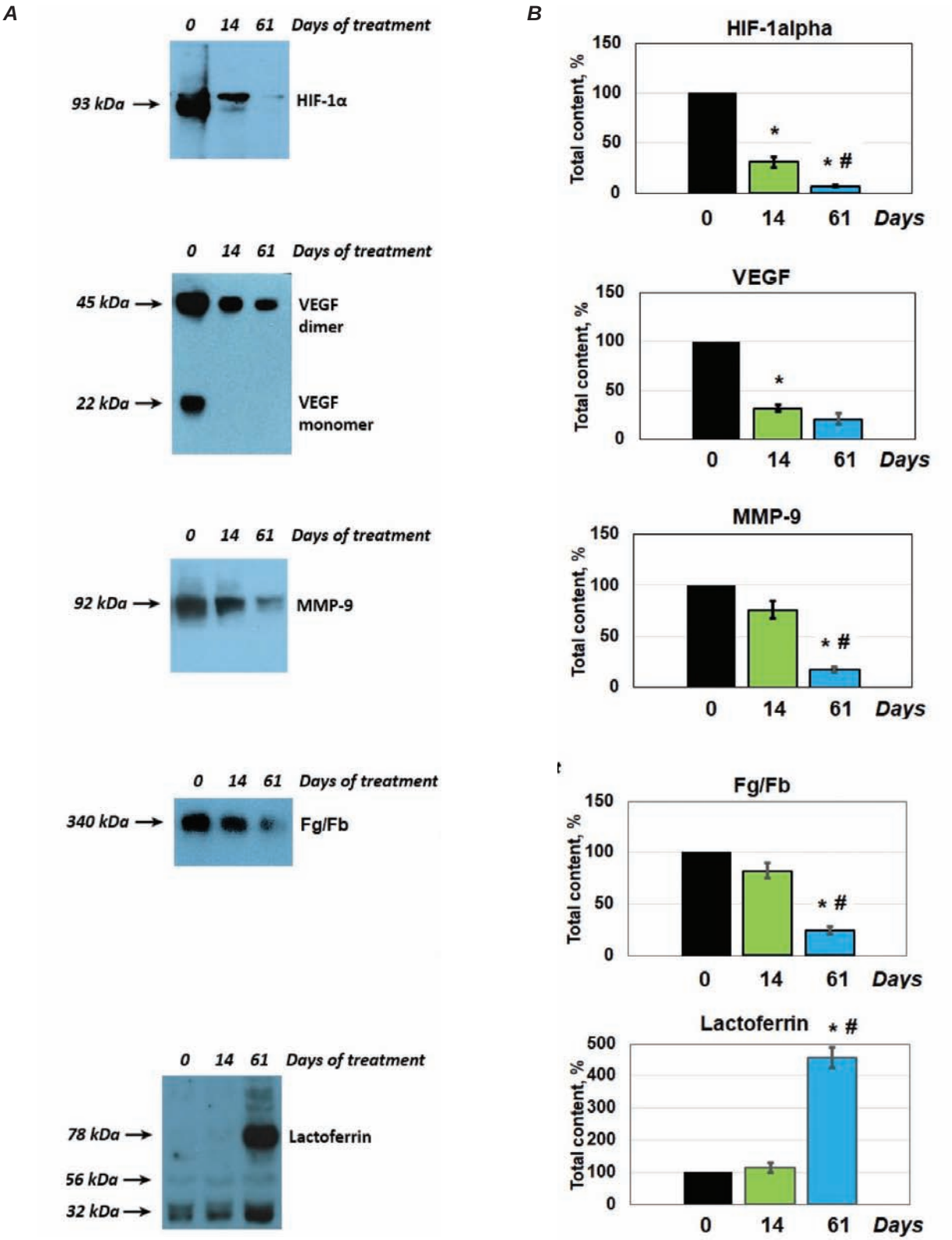

Fig. 3. Modulation of protein biomarker expression in tear fluid following angiostatin-based therapy in a patient with tuberculosis-induced corneal injury. ***A*** *– representative Western blot images (n = 3 biological replicates) illustrate changes in the levels of hypoxia- and inflammation-associated proteins, including HIF-1α, VEGF, MMP-9, fibrinogen/fibrin (Fg/Fb), and lactoferrin;* ***B*** *– results of densitometry analysis (*P < 0.05 compared to 0 day, #P < 0.05 compared to 14th day, n = 3)*

MMP-9. These proteins not only reflect the severity of corneal damage but also actively contribute to leukocyte infiltration, chronic inflammation, extracellular matrix degradation, tissue remodeling, and neovascularization [19, 20]. The therapeutic application of angiostatin in this context appears to disrupt this pathological feedback loop at several levels. The observed reduction in HIF-1α, VEGF, and MMP-9 levels in the tear fluid of keratitis patient treated with angiostatin-based eye drops reveals a multifactorial mechanism of action that extends beyond classical anti-angiogenic pathways.

One plausible mechanism involves the inhibition of neutrophil and monocyte/macrophage activity, which are among the earliest and most potent contributors to the inflammatory microenvironment in the damaged cornea. These cells not only produce reactive oxygen species and pro-inflammatory cytokines that stabilize HIF-1α, but also secrete VEGF and MMP-9, thereby amplifying tissue remodeling and neovascularization [21]. Angiostatin has been shown to suppress chemotaxis and activation of these immune cells, potentially reducing the local production of HIF-1α-stabilizing factors and dampening the transcriptional activation of downstream angiogenic mediators [22]. Moreover, angiostatin may exert direct effects on corneal epithelial and endothelial cells by modulating surface ATP synthase activity, thereby altering cellular energy metabolism and oxygen demand [23]. This metabolic shift could contribute to a reduction in hypoxia signaling, further destabilizing HIF-1α and attenuating VEGF expression. The observed upregulation of tight junction proteins such as ZO-1 in angiostatin-treated corneas supports the notion that barrier integrity is restored, limiting the extravasation of inflammatory mediators and improving tissue oxygenation [9]. Interestingly, while macrophages and neutrophils are also involved in the proteolytic generation of angiostatin from plasminogen, its exogenous administration may override endogenous regulatory loops, acting as a self-limiting brake on the very cells responsible for its production. This dual role, both as a product and inhibitor of inflammatory cell activity, positions angiostatin as a unique modulator of corneal homeostasis. The reduction of MMP-9 in tear fluid further underscores the anti-inflammatory and anti-remodeling effects of angiostatin. MMP-9 facilitates basement membrane degradation and leukocyte migration, and its suppression correlates with improved epithelial healing and reduced stromal damage [24]. Taken together, these findings suggest that angiostatin-based eye drops not only inhibit neovascularization but also recalibrate the inflammatory and hypoxic milieu of the injured cornea. From a clinical perspective, the modulation of tear fluid biomarkers offers a non-invasive window into treatment efficacy and disease progression. Angiostatin's ability to simultaneously modulate multiple pathogenic pathways, such as hypoxia signaling, immune cell activation, and extracellular matrix degradation, positions it as a promising candidate for integrated therapy in keratitis and other ocular surface disorders. Future studies should explore the temporal dynamics of these biomarkers, the dose-response relationship of angiostatin formulations, and potential synergistic effects with agents targeting oxidative stress or fibrotic remodeling.

In addition to its effects on hypoxia signaling and neovascularization, angiostatin demonstrates promising activity in modulating the proteolytic and antimicrobial landscape of the injured cornea. One notable observation is its capacity to reduce Fg/Fb accumulation, a hallmark of tissue injury and unresolved inflammation. Fibrin deposition in the corneal stroma not only impairs optical clarity but also serves as a scaffold for leukocyte infiltration and neovessel growth [6]. Angiostatin's inhibitory effects on neutrophils and macrophages, which are known as key sources of tissue factor, thrombin, and fibrin-stabilizing enzymes [25, 26], may further dampen local fibrin accumulation. This immunomodulatory action reduces the inflammatory amplification loop that sustains coagulation-like activity in the injured cornea. The result is a more controlled extracellular matrix environment, favouring epithelial regeneration and repair and restoring corneal transparency.

Synchronously, angiostatin treatment has been associated with the restoration and upregulation of lactoferrin, a multifunctional glycoprotein with potent antimicrobial, anti-inflammatory, and immunomodulatory properties. Lactoferrin is naturally abundant in healthy tear fluid and plays a critical role in maintaining ocular surface homeostasis [27, 28]. In keratitis, however, lactoferrin not only becomes depleted but also undergoes extensive proteolytic fragmentation, resulting in the accumulation of degraded, truncated forms that lack full biological activity. Western blot analysis of tear fluid collected from the injured eye of the TB patient has revealed a marked shift from native full-length lactoferrin to lower-molecular-weight degradation products, re-

flecting heightened protease activity in the inflamed microenvironment. Remarkably, angiostatin treatment not only restored total lactoferrin levels but also drastically improved the proportion of its intact, full-length form. This effect likely stems from angiostatin's ability to suppress the activity of matrix metalloproteinases and other serine proteases released by neutrophils and macrophages, cells that are both sources and targets of lactoferrin degradation. By dampening the inflammatory cell influx and inhibiting their proteolytic machinery, angiostatin may preserve the structural integrity of lactoferrin and protects it from enzymatic cleavage. In parallel, angiostatin may enhance epithelial secretory function, either directly through metabolic reprogramming or indirectly by stabilizing the epithelial barrier and reducing stress of endoplasmic reticulum, thereby promoting endogenous lactoferrin synthesis and release [10]. The restoration of native lactoferrin not only reinforces the antimicrobial shield of the ocular surface but also reactivates its full spectrum of immunomodulatory functions, including iron sequestration, cytokine regulation, and inhibition of neutrophil degranulation [29, 30]. This dual action, which includes preservation of molecular integrity and enhancement of physiological availability, positions angiostatin as a potent regulator of corneal immune balance and epithelial resilience. The shift from degraded fragments to functional full-length lactoferrin may serve as a sensitive biomarker of therapeutic efficacy and tissue recovery in keratitis treatment.

Together, the reduction of fibrin and the normalization of lactoferrin levels reflect a broader rebalancing of the corneal microenvironment under angiostatin therapy. These changes complement the suppression of HIF-1α, VEGF, and MMP-9, reinforcing the notion that angiostatin acts as a pleiotropic regulator of corneal healing. By targeting both structural and immunological components of the injury response, angiostatin-based eye drops offer a multifaceted therapeutic strategy that addresses the root causes of keratitis progression. These findings warrant further investigation into the temporal dynamics of fibrin and lactoferrin modulation, and their potential as biomarkers for treatment efficacy and long-term visual outcomes.

Once the diagnosis of ocular TB is made, systemic anti-TB therapy should be initiated immediately. Systemic treatment is successful in the vast majority of cases, with subsequent elimination of symptoms, inflammation, and often improved visual acuity. However, in some cases, particularly those involving persistent corneal opacity and progression of local inflammation, the standard treatment program does not yield satisfactory improvement in the corneal condition. In such contexts, adjunctive topical therapies may offer critical benefits. In this regard, the application of angiostatin-based eye drops has demonstrated promising results, particularly in a patient with keratitis associated with ocular TB, where conventional therapy failed to resolve local corneal pathology. For the first time, biochemical analysis of tear fluid before and after angiostatin treatment revealed a marked shift in molecular markers that correlated with clinical improvement. Specifically, levels of HIF-1α, VEGF, and MMP-9, key mediators of hypoxia, angiogenesis, and tissue remodeling, were significantly reduced following treatment. Simultaneously, the tear fluid profile showed a decrease in fibrin content and a restoration of native, full-length lactoferrin, which had previously been present only in degraded forms due to abnormally enhanced proteolytic activity.

These biochemical changes were accompanied by notable clinical improvements: regression of neovascular tufts, reduction of stromal haze, and enhanced epithelial integrity, as confirmed by slit-lamp examination and optical coherence tomography. The restoration of lactoferrin, a glycoprotein with antimicrobial and immunomodulatory functions, likely contributed to the resolution of inflammation and reestablishment of ocular surface homeostasis. The reduction in fibrin, a pro-inflammatory matrix component, further supported corneal transparency and healing. The convergence of molecular and clinical data in this patient underscores the therapeutic potential of angiostatins in ophthalmology, not only as anti-angiogenic agents, but also as modulators of immune and proteolytic balance in the injured cornea.

Conclusions. This case report presents the first documented application of autologous angiostatin eye drops in a patient with TB-induced interstitial keratitis, demonstrating both clinical and biochemical efficacy in reversing corneal neovascularization and restoring ocular surface homeostasis. The observed regression of vascular tufts, normalization of tear fluid biomarkers, including HIF-1α, VEGF, MMP-9, fibrinogen, and lactoferrin, and improvement in corneal transparency underscore angiostatin's multifaceted therapeutic potential. Beyond its anti-angiogenic properties, angiostatin appears to

recalibrate the inflammatory and proteolytic microenvironment of the injured cornea, offering a pathogenetically grounded alternative for cases refractory to conventional anti-TB and anti-inflammatory therapies. These findings lay the groundwork for broader translational exploration. Future studies should investigate the dose-response dynamics, long-term safety, and comparative efficacy of different angiostatin isoforms (e.g., K1-3 vs. K1-4.5 or K5), as well as their integration with systemic anti-TB regimens. The use of tear fluid biomarkers as non-invasive indicators of therapeutic response may further enable personalized treatment strategies. Moreover, angiostatin-based formulations warrant evaluation in other inflammatory and angiogenic ocular disorders, including herpetic keratitis, chemical burns, and post-surgical complications. Taken together, this pioneering intervention opens new avenues for biologically active eye drops capable of restoring corneal clarity and function in complex ocular pathologies.

Conflct of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ТЕРАПЕВТИЧНИЙ ПОТЕНЦІАЛ МІСЦЕВОГО ЗАСТОСУВАННЯ АВТОЛОГІЧНОГО АНГІОСТАТИНУ В ЛІКУВАННІ ТУБЕРКУЛЬОЗНОГО УРАЖЕННЯ РОГІВКИ: КЛІНІЧНИЙ ВИПАДОК

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Туберкульоз ока – рідкісне, але потенційно загрозливе для зору захворювання, що часто проявляється у вигляді неоваскуляризації рогівки та стромального кератиту, зумовлених хронічним запаленням і тривалою антигенною стимуляцією. Стандартні терапевтичні підходи, зокрема застосування кортикостероїдів і протимікробних засобів, нерідко виявляються неефективними у резистентних випадках. У роботі описано клінічний випадок 50-річного пацієнта з інтерстиціальним кератитом, асоційованим із туберкульозом, який виявився резистентним до традиційних методів лікування. У зв'язку з відсутністю клінічного покращення було обрано альтернативну терапевтичну стратегію у вигляді місцевого застосування ангіостатину, потужного антиангіогенного поліпептиду, отриманого з автологічного плазміногену. Ангіостатин було підготовлено у вигляді стерильних очних крапель, які застосовували щоденно протягом двох місяців. Ефективність лікування оцінювали за допомогою стандартних офтальмологічних методів, включаючи біомікроскопію щілинною

лампою, фотографування рогівки, вимірювання внутрішньоочного тиску та аналіз судинних змін. Крім того, у слізній рідині визначали в динаміці рівень молекулярних маркерів гіпоксії (HIF-1α, VEGF, MMP, фібриноген і лактоферин) методом вестерн-блотингу для оцінки біохімічної відповіді. Після курсу терапії ангіостатином у пацієнта спостерігалося виражене зменшення неоваскуляризації рогівки, відновлення її прозорості та нормалізація рівнів протеїнів-маркерів. Отримані результати свідчать про те, що ангіостатин може бути безпечною та ефективною альтернативою для лікування рогівкових ускладнень при туберкульозі ока, особливо у випадках, резистентних до традиційної терапії. Необхідні подальші дослідження для підтвердження клінічної доцільності ангіостатину та розширення його застосування в лікуванні патологічних станів ока, що супроводжуються запаленням і неоваскуляризацією.

Ключові слова: туберкульоз ока, ураження рогівки, неоваскуляризація рогівки, ангіостатин, контроль запалення.

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