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Effectiveness study of the recombinant enzymes pbserum HIGH in the treatment of pathological scars: a pilot study

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ABSTRACT

Introduction: Pathological scars, despite the achievements of modern medicine, are still a problem. Its prevalence can reach up to 50% in emergency surgeries. These scars can lead to physical complications, including impaired mobility, altered sensation, and discoloration, and may even cause pain. In this study, we explore the possibilities of using the combined drug of recombinant collagenase and lyase enzymes, and high molecular weight hyaluronic acid (HMWHA) pbserum HIGH in the treatment of pathological scars.
Methods: patients of the main group received a course of intra-cicatricial injections of the drug, treatment results were assessed clinically, according to the Vancouver Scar Scale (VSS) and Observer Scar Assessment Scale (POSAS) scales, the results were compared morphologically with standard scars treatment methods (biopsies were taken before and after treatment).
Results: Clinically, patients of the main group received a pronounced positive transformation of scar tissue in 6 weeks, statistical processing of data confirms the reliability of changes, morphological studies prove the normotrophic nature of the changes in the scars (including comparison with the control group).
Conclusions: Remedy of recombinant collagenase and lyase enzymes in combination with HMWHA pbserum HIGH in the form of the course of intra-cicatricial injections is a safe and effective method of treating pathological scars.

Keywords: Collagenase, Hyaluronic acid, Lyase, Programed scar remodeling, Recombinant enzymes, Scars

Introduction

The treatment and prevention of pathological scarring remains a pressing issue in all areas of medicine. Epidemiological studies estimate that the prevalence of hypertrophic scars ranges from 32 to 72% (1), whereas keloids affect between 4.5 and 16% of the general population (2), with a higher prevalence among individuals of African, Asian, or Hispanic descent. The condition affects both sexes equally, with the highest incidence occurring during the second and third decades of life (3,4) and in 8-67 % of burn convalescents (5). The etiopathogenesis of pathological scarring is well-studied, but clinical dissatisfaction with the treatment outcomes persists. Scar tissue can only be completely removed through surgical treatment, which involves scar excision and the replacement of the wound defect with a healthy skin flap. However, this is not always possible due to the scar's location, area, and type. Patients may also not be ready for surgery (e.g., young children). Therefore, in most cases, the specialist's objective is to make the scars as invisible as possible on the patient's body, both visually and subjectively. Today, various physiotherapy protocols and instrumental techniques, as well as steroid hormone injections, are actively used (6-12).

Previous scientific, clinical, and morphological studies of the effectiveness of injectable scar treatment methods have contributed to the development of the Programed Pathological Scar Remodeling protocol by the authors of this article (8,13,14). The main concept of the method is to inject several groups of drugs that normalise the functional characteristics of the extracellular matrix in scar tissue, leading to its remodeling (not destruction!) and the formation of more normotrophic tissue. When this method is combined with continuous elastic compression, patients achieve satisfactory results in a relatively short period of time (8,13,14). This protocol is especially important for preventing pathological scarring in patients undergoing surgical treatment for scarring pathology, as well as in the field of reconstructive plastic surgery in general, when the drugs specified in the protocol are injected during the immediate post-operative period (14,15). This approach aims to optimise conditions in the extracellular matrix of the surgical wound for the formation of a normotrophic post-operative scar (14,15).

The subject of this study was to investigate the mechanisms and results of the targeted action of recombinant collagenase and lyase enzymes and high molecular weight hyaluronic acid (HMWHA) in pbserum HA 1.5 HIGH (Proteos

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Biotech, S.L., Spain) on the formation or transformation of scar tissue via intra-cicatricial injection. The drug was chosen based on the widely studied mechanisms of action of collagenase. Recombinant collagenases target abnormal collagen accumulation, breaking it down enzymatically. These enzymes cleave triple-helical collagen at multiple sites, aiding in ECM degradation. Approved previously for Dupuytren's and Peyronie's diseases and the Edematous-fibrosclerotic panniculopathy, also called cellulitis (16,17). Across these clinical applications, the enzyme has consistently shown a favorable safety profile and encouraging therapeutic outcomes (16,17). Another interesting enzyme, lyase (a type of hyaluronidase, a depolymerizer), which provides conditions for collagenase activity and removes degradation products; promotes the reduction in the viscosity of biological fluids, increasing vascular permeability and accessibility of other active compounds at the application site, promotes fluid drainage from the ECM—thus reducing edema—and modulates the inflammatory response (18). Lipase function is to catalyze the reversible hydrolysis of triglycerides from adipocytes embedded in fibrotic tissue into glycerol and free fatty acids, enhancing mechanical pliability. In humans, lipase activity is regulated by hormonal factors (such as insulin), dietary habits, and physical activity, influencing both lipogenesis and lipolysis (17,19). High molecular weight (>400 kDa) hyaluronic acid (HMWHA) is more than just a volumizing molecule. It hydrates tissues, facilitates enzyme diffusion across the extracellular matrix, and acts as a physical barrier that helps modulate inflammatory responses. It also acts as a mechanical barrier (anti-adhesive gel), facilitating debridement in hypertrophic scars. Moreover, hyaluronic acid is not just a passive vehicle; it's an active player in the wound healing cascade. It inhibits the upregulation of TGF-β, a key driver of fibrosis. Unlike low-molecular-weight HA, HMWHA decreases fibroblast hyperproliferation (20), been considered as an ECM modulator (21,22).

The technology of recombinant enzyme production minimises the risk of immunological incompatibility, i.e., allergic reactions, while maintaining enzyme properties and activity identical to human characteristics.

We define two objectives: (i) to evaluate the drug's clinical effectiveness at various stages of scar tissue formation (including comparison with standard treatment); (ii) to examine the morphological changes in tissues (including comparison with standard treatment).

Materials and methods

In 2016-2017, the Burn Centre in Kharkiv City treated 15 patients with pathological scars of varying duration, including women and men aged 18-54. These patients were the main study group and received the presented scar treatment protocol (Table 1). The scar aetiology in the main group was 11 post-burn patients, four post-operatives. The results of the treatment were compared with a control group of 15 patients from previous studies on the treatment of scars conducted by the author (8,13,14). Patients in both groups had scars of two types: atrophic and hypertrophic (Table 1). The study has been performed in accordance with the Declaration of Helsinki and approved by an ethics committee.

All the patients in the main group received intra-cicatricial injections at 2-week intervals for a total of 6 sessions. A unit of Pbserum HA 1.5 HIGH (Proteos Biotech, S.L., Spain) was applied in each session. It consisted of a vial containing a cocktail of recombinant enzymes—collagenase, lipase, and lyase—reconstituted with 2 mL of HMWHA and 3-10 mL of 0.9% sodium chloride (NaCl) solution, depending on the scarred area. Additionally, 0.5 mL of a 2% lidocaine solution was added. Injections of 0.5 cc per point were administered into the scar tissue using a 27G needle, employing either a retrograde linear or multipuncture technique. The patients completed the POSAS table before each session and at the end of the treatment course to subjectively assess the characteristics of their scars, including pain and itching, thickness, difference from surrounding tissues, and colour. At the same time, a doctor who was not involved in patient treatment completed the Vancouver Scar Scale (VSS) tables to objectively assess scar tissue characteristics, such as autonomic response, pliability, height above the skin level, and pigmentation, before each session and at the end of the treatment course. In addition, in patients with hypertrophic scars, a biopsy was performed to the full depth of the scar in a specific area before the start of the treatment course, and the same area was re-sampled 6 months later at the end of treatment. The microscopic specimens were stained with hematoxylin-eosin, Van Gieson's picrofuchsin (for collagen), Einarson's gallocyanine-chrome alum (for total nucleic acids), and the PAS reaction was carried out. Microscopy was done with an Axiostar-plus microscope (Zeiss, Germany). Photocontrol was carried out at all stages of treatment.

TABLE 1 - Patient groups

Diagnosis	Main group, people (%)	Control group, people (%)
Atrophic scars	4	5
Hypertrophic scars	11	10
Total	15	15

Treatment outcomes were assessed using PSOAS for subjective observation results; objective VSS before treatment, before each procedure, and after treatment; morphological examination of hypertrophic scar biopsy specimens before and after treatment; comparative morphological examination of scar tissue biopsy specimens from patients in the control group and patients receiving standard treatment.

All results were statistically analyzed using the Statistica (StatSoft, Inc.) software.

Results

The clinical outcomes of the patients in the control group were used retrospectively, as the author's previous works demonstrated a significant difference in the timing and outcomes of treatment in favour of the Programmed Pathological Scar Remodeling protocol (8, 13-15); biopsy specimens for this morphological study were obtained in a remote period of time. The statistical analysis of the data for both scales revealed a statistically significant difference before and after treatment (Tables 2 and 3).

TABLE 2 - Treatment outcomes according to the VSS scale: changes in %

Types of scars	Autonomic response, %	Pigmentation, %	Pliability, %	Scar height, %
Hypertrophic scars	–81.3 ± 3.12	–38.7 ± 0.84	–19.9 ± 0.38	–60.3 ± 2.1
Atrophic scars	+78.8 ± 2.49	+62.1 ± 1.29	+38.6 ± 0.95	+78.9 ± 2.93

TABLE 3 - Treatment outcomes according to the PSOAS scale: changes in %

Types of scars	Pain, %	Itching, %	Pigmentation, %	Pliability, %	Relief, %
Hypertrophic scars	63.4 ± 2.12	68.2 ± 1.27	58.3 ± 0.76	58.7 ± 1.15	70.1 ± 1.84
Atrophic scars	50 .9 ± 1.94	71.2 ± 1.44	39.1 ± 0.36	54.5 ± 1.08	62.5 ± 0.97

All patients showed clinical improvement after the first treatment session, with more pronounced outcomes at the end of the treatment course. Importantly, the changes intensified within a few months after completion of the treatment course. In patients with hypertrophic scars, softening of the scars, reduction in the height of the scars above the healthy skin, lightening, decrease in epidermal peeling, and decrease in the intensity of the autonomic response were observed, and the scar tissue could be pinched. Subjectively, there was a decrease in pain and itching; the tissues became softer, more elastic, and lighter (Figs 1 and 2). In patients with atrophic scars, volume filling and smoothing in height compared to healthy skin were observed, as well as a decrease in the brightness of the vascular pattern and a change in the colour of the scar tissue to more flesh-like. In general, the scars became less visible (Fig. 3). No local or systemic adverse effects of the drug were observed during the treatment. During this time, the patients received no additional anti-scarring therapy.

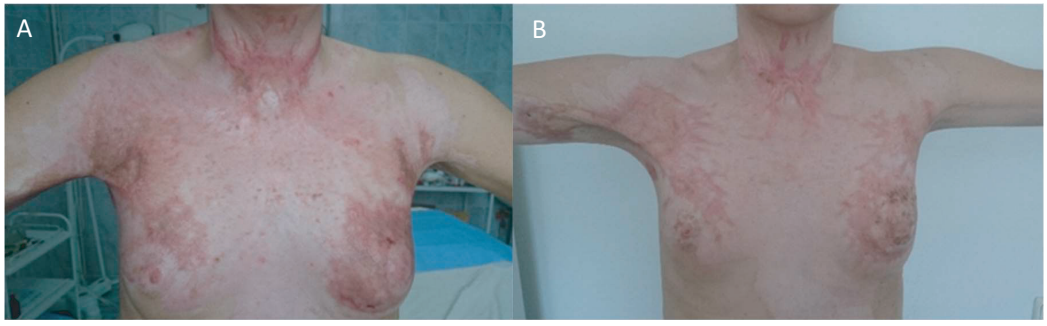

FIGURE 1 - Patient 18 years old, post-burn hypertrophic scars, main group. A) before treatment. B) after treatment.

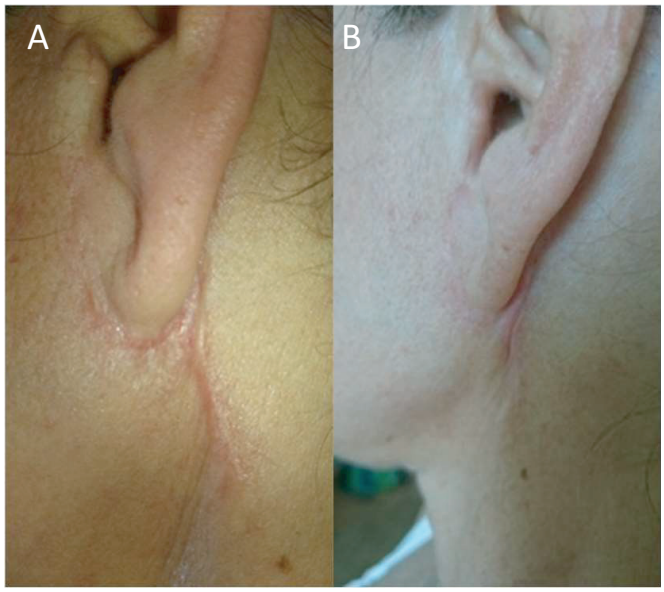

FIGURE 2 - Patient 54 years old, post-operative hypertrophic scars on the face, main group. A) before treatment. B) after treatment.

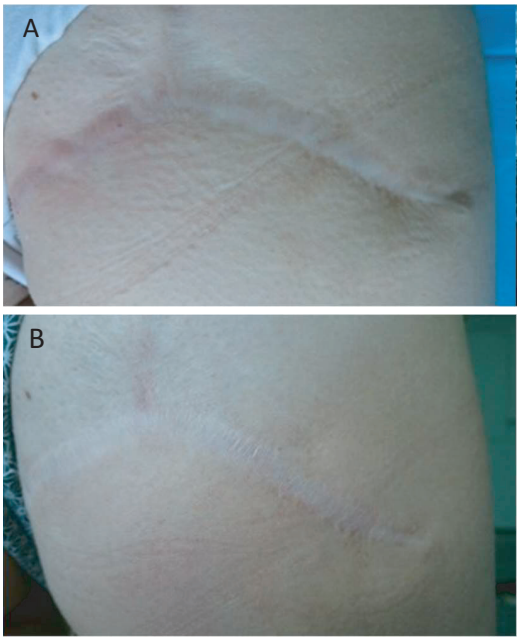

FIGURE 3 - Patient 22 years old, post-operative atrophic scars on the right buttock, main group. A) before treatment. B) 2 months after treatment.

Morphological examination

In patients with hypertrophic post-burn scars in the main group, numerous fibroblasts with very high morphological and functional activity were observed under the microscope before treatment, both in the depth of the scar and in the subepidermal layer. The optical density of fibroblast nuclei when stained by Einarson is 0.187 + 0.009 conventional units of optical density. Collagen was typically represented by thin, tortuous fibers, but in some cases, its compaction, similar to homogenization, was already visible. The presence of inflammatory infiltrate foci (lymphocytes, macrophages) in the scar tissue is noteworthy, with a decrease in collagen fiber density in these areas. The epidermis was thick, lacked a basement membrane, and had hyperproliferation of epidermocytes in the basal and lower prickle cell layers. Patients in the main and control groups showed a decrease in fibroblasts and an increase in fibrocytes in the scar depth 6 months or more after injury, indicating a slowdown in protein synthesis processes. However, the situation was reversed in the subepidermal layer. During standard treatment, a very large amount of collagen was accumulated in the upper layers of the scar tissue, which was so densely packed that it appeared homogeneous. Clinically, this is manifested as a dense, inelastic, and uneven scar. Active fibroblasts were also present, as were small numbers of diffusely distributed macrophages and lymphocytes. At the same time, the depth of the scar is dominated by fibrocytes, which are inactive in the function of protein synthesis, and this is also combined with the dissociated arrangement of collagen fibers. The optical density of fibroblast cells when stained according to Einarson is 0.142 + 0.006 conventional units of optical density - that is, the nuclei of fibroblasts are more euchromic. The proposed treatment reduced the number of fibroblasts and the accumulated collagen in the subepidermal layers. In addition, hyperproliferation in the basal cell layer was virtually eliminated, and the epidermis thinned, without the formation of acanthosis or papillomatosis (Figs 4A and B; Figs 5A and B).

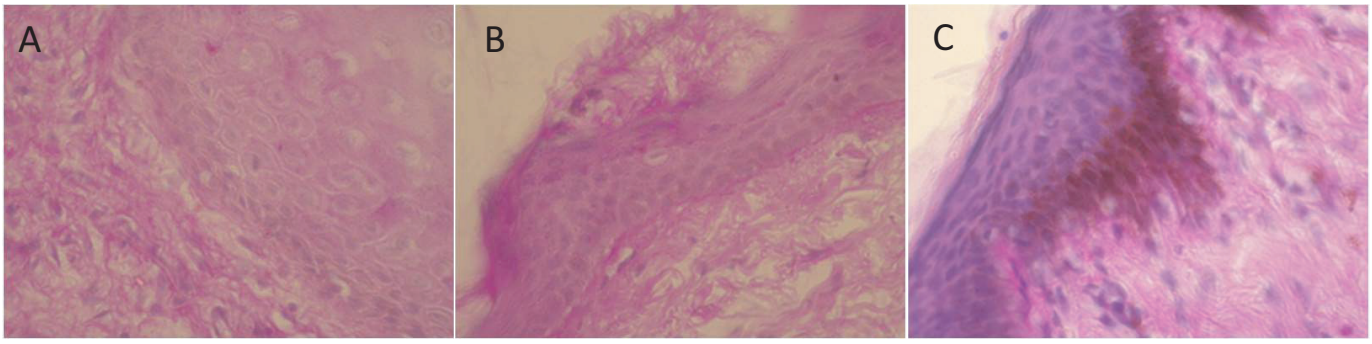

FIGURE 4 - PAS reaction, 400× magnification. A) post-burn hypertrophic scar on the torso prior to treatment, main group. An area of the epidermis with no basement membrane. B) post-burn hypertrophic scar on the torso 6 months after treatment, main group. The epidermis is thin, the keratin layer has thickened, and the basal cell layer does not show any signs of hyperproliferation. The epidermis's lower edge is smooth, and the formation of the basement membrane is visible. C) post-burn hypertrophic scar after treatment, control group. Melanin hyperproduction; absence of epidermal basement membrane.

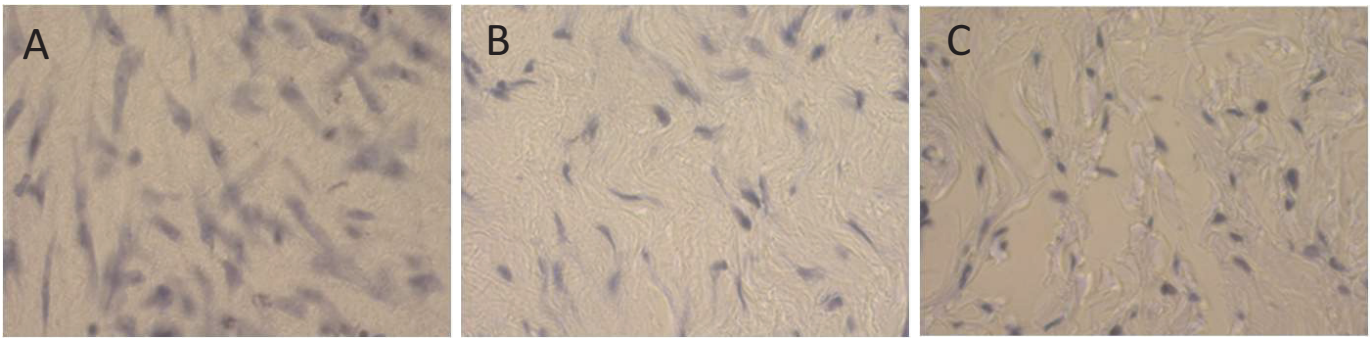

FIGURE 5 - Einarson staining, 400x magnification. A) post-burn hypertrophic scar on the torso prior to treatment, main group. The deeper layers of scar tissue contain numerous morphologically and functionally highly active fibroblasts (large volume of cytoplasm with high RNA content). B) post-burn hypertrophic scar on the torso 6 months after treatment, main group. In the scar's deeper layers, many fibroblasts lost their protein-synthesising activity and transformed into fibrocytes. C) post-burn hypertrophic scar after treatment, control group. Cellular elements of the deeper layers of the scar include both small fibroblasts with a low volume of cytoplasm and fibrocytes.

In the main group, a second biopsy specimen was taken 6 months after the first sampling, when the proposed treatment course was administered. As previously stated, the number of fibroblasts and accumulated collagen decreased not only in the depth of scars, but also in the subepidermal layers. This suggests a low level of ECM activity in terms of uncontrolled synthesis of cellular and fibrous elements. There were few lymphocytes and macrophages, indicating

that there was no inflammatory reaction associated with active pathological scarring. In addition, hyperproliferation in the basal cell layer of epidermis was virtually eliminated, the basement membrane was forming, and the epidermis thinned, with no acanthosis or papillomatosis. The formation of the basement membrane of the epidermis indicates the production of type IV collagen, the main function of which is to support tissues in the process of remodeling and regeneration (after the embryonic period). The examination of biopsy specimens from patients in the control group after standard therapy revealed the following: most microscopic specimens lacked a basement membrane, had epidermal areas with a papillomatous surface, and exhibited increased keratinization; in another part of the biopsy specimens, the epidermocytes of the basal cell layer had a vertical, sharply elongated shape due to hyperproliferation. In all cases, the epidermis produced an excessive amount of melanin. Protrusions and depressions formed on the surface of the scars (Fig. 4C, Fig. 5C). Such characteristics of the epidermal layer, particularly the basement membrane, differ significantly from those of the main group, indicating that non-physiological parameters of ECM function are preserved in the scar area in patients receiving standard therapy. The superficial subepidermal layer is uniformly fuchsinophilic, or there is significant fuchsinophilia, indicating excessive interstitial collagen accumulation with the formation of thick bundles and homogeneous areas. Cellular elements of the deeper layers of the scar include both small fibroblasts with a low volume of cytoplasm and fibrocytes. These morphological characteristics also suggest that the standard therapy does not create new, more physiological conditions in scar tissue, but, on the contrary, it preserves pathological parameters, though without pronounced activity (Fig. 5).

Discussion

The clinical outcomes of using a combined drug of recombinant collagenase, lipase, and lyase enzymes with HMWHA in the form of a course of intra-cicatricial injections demonstrated high effectiveness, as evidenced by objective and subjective observations. No complications or adverse events were observed during the treatment. A multicenter study involving 44 patients found that the enzymatic cocktail pbserum HA 1.5 High significantly reduced pruritus, pain, thickness, irregularities, and stiffness in hypertrophic, atrophic, and keloid scars from the very first application (23). The treatment showed excellent results across all evaluated parameters, with most patients experiencing noticeable improvement after just one session. The findings highlighted a clear antifibrotic effect, supporting its potential as a promising therapeutic option for scar management.

Importantly, the treatment demonstrated a strong safety profile. Adverse effects were generally mild and self-limiting, including erythema, swelling, injection site pain, and bruising, all of which responded well to standard pain relief. While 27% of patients experienced a single adverse event, 68% reported multiple symptoms. Notably, 93% of these effects were mild, and no serious adverse events were reported. Pain was the most commonly reported symptom, affecting 91% of participants, and local reactions such as edema, erythema, and bruising occurred in 75% of cases. All adverse events resolved within 48 hours, and no patient discontinued treatment due to side effects (23).

Morphological examination of the scar tissue before and after the proposed treatment revealed that all elements of hypertrophic scar tissue, as well as the epidermis that covers it, showed signs of inhibition of the processes that cause scar hypertrophy. A comparison of the morphological characteristics of the scars after treatment to those of patients in the control group demonstrated a significant difference in the quality and quantity of cellular and fibrous structures of the dermal extracellular matrix. Particularly noteworthy is the formation of the basement membrane and the normotrophic remodeling of the epidermal layers, which is clinically manifested by an improvement in the colour and microrelief of the scars—a reduction in the hyperpigmentation of hypertrophic scars and a brighter colour of atrophic scars, smoothness, and a decrease in itching and peeling. This remodeling is possible, in part, by the production of type IV collagen. It can be concluded that the proposed therapy has a direct effect on the functions and properties of the ECM in the area of the pathological scar, promoting their return to natural and physiological parameters. Structural changes in the subepidermal layers differ between the main and control groups, with cellular elements showing less pathological activity and collagen synthesis processes returning to normal. This clearly demonstrates the benefits of the presented treatment.

By directly breaking down ECM components and modulating inflammatory responses, this approach offers a rational, substrate-specific alternative to conventional therapies. When combined with HMW-HA, which supports tissue hydration and regulates inflammation, enzyme therapy not only promotes ECM remodeling but also facilitates tissue regeneration.

The use of multi-component enzymatic formulations opens new possibilities for maximizing therapeutic outcomes. Emerging clinical and experimental evidence suggests that these enzymatic cocktails may redefine the standard of care for pathological fibrotic scars. As research advances, the application of this synergistic therapy is expected to broaden into other fibrotic conditions, aligning treatment strategies more closely with the underlying pathophysiology.

As a limitation of the study, this patient series refers to patients admitted several years ago, and the therapeutic options might have changed in the meantime.

Conclusion

Therefore, the combination of recombinant collagenase, lipase, and lyase enzymes with HMWHA results in positive clinical outcomes based on the physiological remodeling of a pathological scar's ECM. More studies should be done to get more rigorous data.

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Authors' contributions

Dr. Korkunda has contributed to the clinical part, the analysis, and the writing. Gubina-Vakulyk has contributed to the morphological and immunohistochemical part. Dr. López Berroa has supported the protocol used for pbserum.

Disclosures

Conflicts of interest: Dr. Korkunda and Dr. Gubina-Vakulyk declare they do not have a conflict of interest. Dr. López-Berroa is an employee of Proteos Biotech.

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