

USAGE OF ENZYMES IN THE THERAPY OF CHRONIC
WOUNDS. PRELIMINARY RESULTS

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Abstract

We present our preliminary results of clinical application of two original enzyme-derived topical preparations, Neprolysin and Post-Neprol, on patients with chronic wounds. Neprolysin is a preparation containing a purified proteolytic enzyme possessing strong activity in the neutral pH-area. The main active component of Post-Neprol is collagen hydrolysate. The aim of the study is to assess the safety and efficacy of local enzymatic treatment of chronic wounds. For a period of 3 years, in a single institution 56 patients suffering from chronic wounds were treated. Twenty-two % of the patients were discharged with fully epithelized healed wounds. The rest (78%) of the patients were discharged with continuously healing non-infected wounds. It was found that the application of Neprolysin and Post-Neprol in complex treatment of patients with chronic wounds is safe and promises effectiveness. Control randomized clinical trials were warranted.

Key words: unhealing wounds, proteolytic enzymes, collagen hydrolysate

Introduction. Chronic wounds (ulcers) of lower extremities are non-healing wounds in the period of more than 3 months [1]. Incidence of chronic wounds in Europe is 0.18–1% [2]. Treatment of chronic wounds is complex and requires both systemic treatment of underlying pathophysiology disorders (diabetes, arterial and venous insufficiency, etc.) and topical dressing, stimulating wound healing.

The external damage of the skin integrity in relatively small degrees and of young organisms usually does not give problems. In such cases nature has ensured mighty defending and regenerative systems which in cases of destroying tissues trigger at once and blood coagulates. At next stages the wound is covered with protective layer, the microorganisms present in the wound are neutralized and the relatively slow process of healing starts. In case of injuring, the border cells “feel” that they do not contact with the inherent cells of the macroorganism or with its own tissues and this is a signal for their organized division up to

the repairment of the damaged sector. The normal cells stop dividing when touching inherent tissue or cells of the same organism. The process of tissue rebuilding is conventionally divided into three stages – 1) irritatingly-exudative stage (shortly called also cleansing stage); 2) stage of proliferation and appearance of granulative tissue (shortly granulation) and 3) maturation and epithelization stage (epithelization). Especially for a large wound, these three processes overlap each other.

Concerning the elderly patients, the picture is almost the same but all processes take place slower. In principle there are two main reasons for that – peripheral arterial or/and venous insufficiency and the fact that in these individuals many of the enzymes are glycated (glucose binds to their molecules) to a different extent and therefore the enzymes decrease or lose their activities. Besides, waste or storage substances are accumulated in the organism, which also hinders the blood flow. Other unwanted events occur as well – the immune response becomes weaker, especially in cases of diabetes mellitus, most of the organs like muscles, skin and especially the connective tissues are additionally crosslinked, thus becoming more tough or less flexible. Taking into account the mentioned processes, it becomes clear that the old methods for disinfection with iodine and its derivatives, hydrogen peroxide or potassium permanganate solutions will not only kill the present microorganisms but might additionally damage the host tissue and thus supply the next microorganisms coming from the air with more space for development. When the rate of the healing process of the wound is slower than the wound bacterial growth, the healing would never occur. Manifested wound infection leads to wound enlargement. Obviously, the strategy of healing of the wounds of elderly people and those suffering from diabetes mellitus should be different from that of young people. There are some reports of beneficial effect of topical enzymatic treatment in patients with chronic leg wounds [2, 3].

The aim of the present study is to look for new ways of curing wounds in elderly people or patients suffering from diabetes mellitus and assess the safety and efficacy of local enzymatic treatment of chronic wounds.

Materials and methods. For a period of 3 years in a single institution (Department of Surgery, Medical Institute – Ministry of Interior, Sofia) 56 patients with chronic wounds are treated after written informed consent. On Table 1 are presented the main causes of the ulcer. Male patients were 36 (64%) and female – 20 (36%). The age of the patients was in the range of 43 and 86 years (mean age 69 years). The treatment of the patients includes operative treatment (surgical debridement of the wound), systemic treatment leading to underlying disease (diabetes mellitus, chronic vascular insufficiency, etc.), deep tissue infection (in 19 pts (34%)) and wound dressing. In 6 pts (10.7%) free skin grafting was employed. Four patients (9.3%) were treated with the method of “Negative pressure wound therapy”.

The two original preparations of the Institute of Organic Chemistry at the

Bulgarian Academy of Sciences (Neprolysin and Post-Neprol) were included in complex treatment of the patients. Neprolysin [4] contains a proteolytic enzyme possessing broad specificity cleaving peptide bonds at the carboxyl group of 13 out of the 20 naturally occurring amino acids. It is produced by *Bacillus subtilis*, which is widely spread and is harmless to humans. The second preparation is Post-Neprol [4] based on enzymatically partially degraded collagen and also containing: 0.2% chlorhexidine, Zn- and Mg-ions.

The topical treatment of the wounds was made in intervals of 24 hours. Neprolysin was applied to the wounds at exudative stage with signs of necrotic tissue in the wound bed (in 34 patients (60.7%)). Post-Neprol was used at wounds in the proliferative stage (of granulation and epithelization) (in 22 patients from the beginning of the treatment and in the rest 34 patients after a period of 3–7 days).

T a b l e 1

Number of the patients treated and distribution of the causes for the wound appearance

Cause	Patients	%
Diabetic foot	32	57.2
Chronic venous insufficiency	11	19.6
Chronic arterial insufficiency	10	17.8
Thromboangiitis obliterans	1	1.8
Necrotic hyperergic vasculitis	1	1.8
Martorell hypertensive ischemic leg ulcer	1	1.8

Results. Our preliminary results show that 22% of the patients were discharged with fully epithelized healed wounds. The rest (78%) of the patients were discharged with healing non-infected wounds. There were no patients' complaints of adverse effects. The topical treatment with Neprolysin and Post-Neprol was well tolerated by the patients. All the patients and/or their relatives were educated how to change the wound dressings at home.

We did not find any adverse effect of adding Neprolysin and/or Post-Neprol in Negative pressure wound therapy.

When applying Post-Neprol in dressings after free skin grafting, there are no adverse or negative effects (Fig. 1).

Discussion. Since the partially damaged cells possess almost the same surface as that of the healthy ones, the truly healthy cells, although contacting with partially damaged cells, remain "silent" and do not divide, which delays the healing process. This shows that the wound firstly has to be cleaned from the damaged cells in order to trigger the healing process. One important condition for curing the wound is its cleansing, or debridement. Debridement could be surgical or chemical. Part of the chemical debridement is enzymatic.

Essential part of the treatment of chronic wounds is the topical treatment

and wound dressing. There is no consensus which is the best local treatment and dressing among the hundreds of commercially available dressings and preparations [5].

FRANK [6] recommends local antibiotics and antiseptics in chronic infected leg ulcers. We combine local antiseptic solution of chlorhexidine with Neprolysin. We do not combine Neprolysin with local antibiotics or Jodine-containing antiseptics. At proliferative stage, the applying of hydrocolloid dressing is beneficial [5]. We combine hydrocolloid dressings with Post-Neprol without any adverse effect.

During the 60s of the last century, one of us (Iovcho Topalov) found in the specialized literature that a preparation containing purified streptodornase and streptokinase helps substantially in the healing of wounds and suggested that such a preparation has to be elaborated and routinely used. Streptodornase is an enzyme that hydrolyzes ribonucleic acids, giving low molecular water-soluble substances, while streptokinase converts the inactive blood plasminogen into the active proteolytic enzyme plasmin. The latter hydrolyzes proteins. Plasmin cannot pass the double lipid layer of the healthy host cell membranes but passes the cell walls of the partially damaged host cells through the openings in them. After entering the partially damaged and full of microorganisms cells of the host, plasmin converts their inside proteins into water soluble peptides. In this way, the whole structure of the damaged cells is destroyed, they melt and this allows the wound to be mechanically cleaned. In some cases (thick necrotic layer) it is preferable to remove roughly the necrotic tissue surgically.

The mentioned streptodornase–streptokinase preparation was elaborated in a freeze-dried form [7, 8]. Its usage confirmed the expected good healing results but the preparation was losing fast its activity after dissolution in water and this made its application difficult. Taking into account the above-mentioned considerations about the mechanism of proteolytic action of enzymes on wounds, it was supposed that the usage of a harmless microbial proteolytic enzyme could be used as well even with better results if the enzyme possesses broad specificity. We bet on subtilisin DY which is produced by the widespread in nature and harmless microorganism *Bacillus subtilis*. After long investigations, the enzyme was obtained in a molecular-homogeneous form [9–11]. It was stabilized in solution by simultaneous action of a competitive and an uncompetitive inhibitor [12]. In this way it became stable at room temperature, losing only 10% of its activity if stored below 17 °C for a period of three years. Later it was introduced in gel and in this form it became an easily applicable preparation for curing of wounds [13, 14]. Our preparation turned to be even better than the analogic preparations of Fibrolan and Iruzol [15]. After the official approval in three hospitals in the towns of Sofia, Plovdiv and Burgas, the industrial manufacturing of the preparation Neprolysin was started in Pharmachim–Sofia plant. Unfortunately, this enterprise underwent dividing in several smaller factories and the production of Neprolysin was not continued. The need of such preparation was severe and its



A



B

Fig. 1. The chronic and nonhealing wound of the leg in 39-year-old male patient with diabetes mellitus and chronic arterial insufficiency: *A* – after treating with Neprolysin the necrotic zones have been removed; *B* – the wound after 15 days of Post-Neprol dressing and just before skin autotransplantation.



C



D

Fig. 1. The chronic and nonhealing wound of the leg in 39-year-old male patient with diabetes mellitus and chronic arterial insufficiency: *C* – 5 days after transplantation. All implants are vital and the wound is in period of granulation and epithelisation; *D* – 12 days after transplantation. Epithelization is seen in all areas of the wound

preparation was resumed in the Institute of Organic Chemistry at the Bulgarian Academy of Sciences.

At this stage another problem became important. How to accelerate the healing process? There was a lot of information that collagen in many medical and cosmetic preparations helps in rebuilding skin [16, 17]. We had some experience in preparing native solubilized collagen [18] and this helped us to make a preparation containing as main components 2% collagen, 0.3% chlorhexidin and PEG in 20% glycerol [19]. This preparation showed very good curing properties especially in the cases of Dermatitis solaris, Radiodermatitis, Endogenous exema, etc., but its manufacturing was difficult and expensive and therefore its introduction to practice was not achieved. The main obstacle was that the two high molecular components – natural collagen and polyethylenglycol, could not easily disperse in each other. Gradually, we came to another idea – to check whether instead of natural collagen another natural product – its enzymatic partial hydrolysate obtained under physiological conditions, can be used. In this preparation besides 5% enzymatically prepared partial collagen hydrolysate we also included the allowed [20] for cosmetic preparations 0.2% chlorhexidine digluconate (hibitan) – a widely used substance possessing antimicrobial action and allowed in the EU as conservant, and some other components that as a rule are deficient in the blood of the mid-statistical European. The preparation was called Post-Neprol since its usual application should be made to wounds already cleaned with Neprolysin. The purpose of applying Post-Neprol is to keep the wound sterile, supplying it with some building substances and thus giving much more time of the macroorganism to rebuild its damaged tissue. It was found that this effect was achieved even in cases in which the wound was so big that parts of bone could be seen.

In our practice, we managed to save many legs suffering from “unhealing” wounds and to “escape” the amputation. Of course, big wounds are cured for long time and the velocity of this process depends on the status of the patient. In prolonged applications of Post-Neprol, often fibrin plaques appear in the wound. This is an inherent tissue of the organism and as such it decreases the speed of dividing the cells in the wound. These plaques can be easily removed using Neprolysin. In such cases it is advisable after three wound dressings with Post-Neprol, each in 24 h, one wound dressing with Neprolysin to be made.

Recently, we have elaborated another proteolytic preparation (Keralysin) which partially degrades alpha-keratin (skin thickenings on heels, around the toe-nails, cicatrixes of old wounds, keratinoses, etc.). Keratin is profoundly crosslinked by intra- and inter-disulfide bridges and thus represents a very tough structure. This unmovable structure does not allow the active centre of proteolytic enzymes to approach the peptide bonds susceptible for hydrolysis and thus resists proteolytic hydrolysis. We introduced reductors in the proteolytic gel, which broke disulfide bridges and achieved considerable degradation of the keratin tissue.

Conclusions. Adding Neprolysin and Post-Neprol to the complex treatment of patients with chronic wounds is safe and promises effectiveness. More investigations are needed to understand the role of enzymatic preparations in the process of wound healing. Control randomized clinical trials are warranted.

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