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ENZYMATIC DETOXIFICATION OF WOUNDS: THREE GENERATIONS OF DRUGS

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Summary. *The formation of significant amounts of tissue decay products is an integral part of the pathogenesis of chronic superficial lesions of various etiologies. This significantly complicates the healing process and contributes to the chronicity and deepening of the pathological process. For detoxification ofsuch wounds, proteolytic enzymes are used widely, whose hydrolytic action helps to break down the protein components of endogenous intoxication and clean the wound. Three generations of enzymatic preparations for this purpose can be distinguished. The first one includes soluble forms of enzymes that are still quite successfully used in clinical practice. The second generation contains preparations of enzymes immobilized on insoluble fiber carriers. Immobilization allows you to bypass a number of disadvantages inherent in the first generation drugs. The development of this direction was the creation of heterophasic enzyme-containing macroporous granules embedded in a hydrophilic bactericidal gel. The significant advantages of this kind of composition allows us to talk about them as the third generation of drugs for enzymatic detoxification. This work examines the genesis of the creation of drugs of all three generations, examples of their use, characteristic advantages and disadvantages.*
Keywords: *immobilized enzymes, proteolysis, detoxification.*

Endogenous intoxication is an integral component of a wide variety of pathological processes [1]. The formation and accumulation of abnormal metabolites and tissue breakdown products causes disruption of various processes at the molecular, cellular, and tissue levels. This contributes to the deepening of the pathology and complicates the recovery of the patient [2-4]. Not the most serious complications are caused by molecules of medium mass - a large group of various molecules, among which damaged beads and their fragments occupy a prominent place [5]. Similar compounds are secondary toxins that disrupt the functioning of

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adjacent tissues and exhaust the body's defense systems. They form a nutrient environment for the development of microbial infection. The specific nature of the interaction of destabilized proteins and peptides with the cell membranes of the surrounding tissues determines the initiation of a complex cascade of unwanted activation and proteolytic processes [6]. Enzymatic fragmentation of peptide components of endogenous intoxication into non-toxic low-molecular compounds has become an effective approach to preventing these processes. Practical methods of this approach have undergone continuous development over the past 60 years [7].

The first of them is based on the use of free proteinases [8,9]. The application of free enzymes *in vulnere* in the form of solutions, tampons moistened with solutions, and even powder with a dry preparation retains its value to this day. Such drugs have a necrolytic and anti-inflammatory effect, contribute to the cleaning of the wound surface and the growth of granulations. Trypsin, chymotrypsin and their mixture are mainly used for this purpose [10]. The effect of this kind of drugs on the surrounding tissues is recognized as moderate and generally acceptable. At the same time, the propensity of soluble enzymes to autolysis and denaturation during drying significantly complicates the wound treatment process. Repeated dressings are necessary - sometimes up to twice a day, as well as permanent moistening of the bandage. This limits the use of soluble enzymes in hospital conditions [11]. The presence of blood protein inhibitors in the exudates of the surrounding tissues limits the action of trypsin and chymotrypsin. Instead, single application of these enzymes in quantities sufficient to clean the wound is practically excluded due to irritating, allergic and toxic effects. That is, for the effective cleavage of the products of necrosis, it is necessary to fulfill two opposite conditions. The deepest possible proteolysis of necrosis products without damage to healthy tissues is necessary [12]. Therefore, uncontrolled damage to surrounding tissues limits the possibilities of using proteinases of bacterial origin [3,12,13]. Awareness of the noted shortcomings led to the development of methods of proteolytic detoxification of wound surfaces with the help of immobilized enzymes.

The immobilization of enzymes on insoluble supports has been known for more than half a century. Similar drugs are used widely in biotechnology, pharmaceuticals, medicine, and biochemical research [14-16]. Enzyme immobilization methods are characterized by great diversity (Fig. 1).

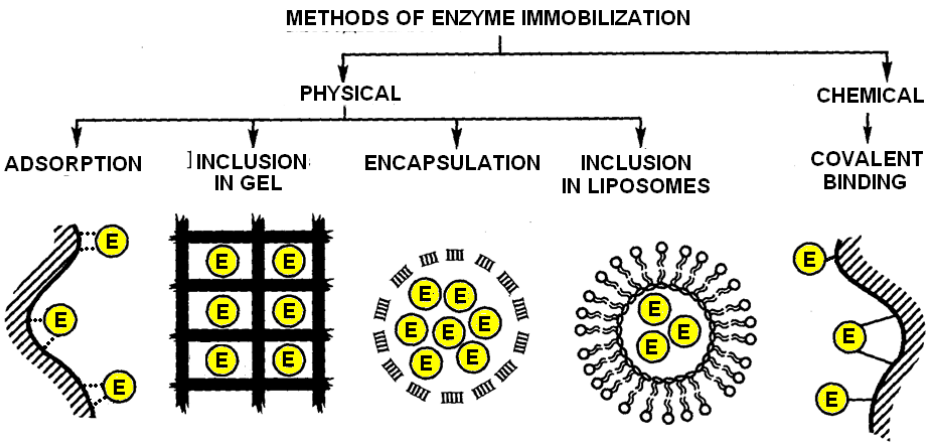

Fig. 1. Schematic representation of the leading methods of enzyme immobilization

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Physical methods are based on adsorption, encapsulation, included in a gel or in liposomes. The most widely used methods of chemical immobilization are based on covalent binding of the enzyme to an insoluble carrier [17,18]. Immobilization of proteinases relieves problems caused by autolysis and damage to healthy tissue. Therefore, immobilized proteinases are widely used to break down necrotized tissues and prevent the accumulation of endotoxins [19-21]. Typical representatives of such drugs are various forms of immobilized trypsin [22,23]. Similar drugs retain their effect for 10-15 days in contrast to 2-3 hours for free trypsin. That is, immobilization removes the restrictions caused by autolysis, but requires constant maintenance of hydration. However, immobilized trypsin not only retains its vulnerability to inactivation by protein inhibitors, but is also able to activate soluble proenzymes. This significantly limits the effectiveness of immobilized trypsin preparations. Preparations based on immobilized enzymes of bacterial origin are devoid of this drawback. They are characterized by high proteolytic activity, broad substrate specificity, and cannot be blocked by protein inhibitors [24-27]. However, the need for constant wetting of the coating limits the use of these drugs in hospital conditions.

As follows from the given data, all the considered drugs have one or another disadvantages that limit their use. Qualitative improvement of the wound healing process requires drugs that provide constant maintenance of wound hydration and enzymatic composition, deep cleavage of endotoxins without negative impact on healthy tissues, and the sufficiency of one-time application of a bandage for the entire course of treatment. The fulfillment of the first requirement was ensured by the use of immobilized enzymes in the composition of a hydrated polymer gel [28]. Such gels are widely used in medicine and cosmetology. In particular, polyethylene glycol (PEG) is included in various creams and ointments. It provides the necessary hydration of the surface and exhibits a weak bactericidal effect. Ointments based on PEG are used in the treatment of burn wounds due to the ability to absorb exudate with subsequent transfer of its peptide component to the outer layers of gauze. The use of such a gel provides constant wound hydration without additional procedures. Deep cleavage of protein endotoxins can be achieved using immobilized proteolytic enzymes of bacterial origin, characterized by high proteolytic activity and broad substrate specificity. For an insoluble matrix, it is advisable to choose a granulated microporous carrier containing the main part of enzymes in micropores that permeate the granule and are characterized by a huge internal surface (Fig. 2).

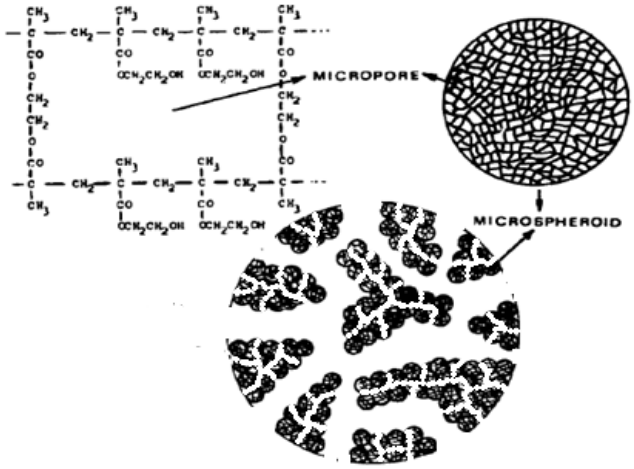

Fig. 2. The structure of a macroreticulated granular polymer gel with channels of various sizes

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All this turns each granule into a kind of proteolytic reactor. The arrival of large protein endotoxins will occur according to the classic model of a drunkard on the edge of a cliff, and the exit of small cleavage products will occur through pathways inaccessible to high molecular weight proteins and peptides. That is, the entry of large molecules and the exit of small molecules follow different streams and do not compete with each other.

In accordance with the above considerations, heterophasic composition was created based on a proteolytic complex immobilized on a granular oxyalkylacrylate carrier placed in hydrated PEG [29]. A partial test of its influence on the processes accompanying the healing of experimental burns was conducted [30]. The given data allow us to talk about the creation of a new generation of drugs for enzymatic detoxification of superficial wounds and the need for further development of this direction.

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