Applications of Collagenolytic Protease Preparations from Invertebrates

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The mechanism of action of new enzyme preparations (Collagenase from hydrobionts, Polycollagenase-K, Fermenkol) was described. The structure-destroying effect of these preparations is due to biochemical properties of their active components — collagenolytic protease complexes from various hydrobiont species. These preparations can be effectively applied in medicine and cosmetology for the treatment of burns and frostbites, purulent wounds, trophic and decubitus ulcers, bone and joint inflammations, contractures, dermal scars and keloids, as well as for cell culturing and regulation of drug effects.

Key Words: *enzyme preparations; collagenolytic proteases; cell cultures; scars and keloids; regulation of drug effects*

The active agents of the most common structure-destroying enzyme preparations (trypsin, chymopsin, collagenase) are serine proteases (mainly trypsin) and in some cases collagenases (Collalysin). Both serine proteases trypsin (EC 3.4.21 [9]) and collagenase (EC 3.4.24 [9]) are highly specific proteinases [6,7].

Collagenases are specific for native collagen and hydrolyze this molecule in a certain point. Thus cleaved collagen can not be the substrate for collagenase and undergoes no further transformations. This is why collagenases are ineffective for *in vivo* degradation of natural protein complexes, especially under conditions of suppurative, inflammatory, and necrotic processes. The effect of collagenase *in vivo* can be visible only in case of optically-controlled loosening of thin layers and reticular structures (opacity of the cornea and vitreous body), since even minor loosening of dense structures considerably increases their transparency. Serine proteinases are able to hydrolyze specific substrates in many locations (i.e. to small fragments), but exhibit low activity against collagen due to their high specificity and therefore they are also ineffective for degradation of massive structures containing protein complexes. Nevertheless, the availability of serine proteases determines their rather wide utilization in medical practice [2,3].

The active agents of new enzyme preparations* are complexes of synergistically acting collagenolytic proteases extracted from various hydrobiont species. These proteases belong to different classes, but possess similar properties and differ from mammalian collagenases and serine proteases (and for this reason we refer to them as to CLPI). CLPI are non-specific enzymes, the mechanism of their action provides deep hydrolysis (to individual amino acids) of diverse polypeptide substrates (native and partly denatured collagen, elastin, casein, fibrin, hemoglobin, *etc.*). This is why they are very effective (especially in complex preparations) for degradation of all proteins. Deep hydrolysis, when intact molecules of structural proteins after repeated cleavage are still the substrate for these enzymes and undergo further transformation, provides effective destruction of reticular and massive multilayer structures, which are unavailable for other enzyme preparations [4,5].

The use of CLPI from different hydrobiont species as the active agents of medical and cosmetic prep-

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*Enzyme preparations available now are the following: "Hydrobiont Collagenase", "Polycollagenase-K", "Fermenkol" (Cosmetic collagenase); the draining wound sorbents are: "Kollasorb" and "Kolladisorb".

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arations was demonstrated in 6 our inventions. Both usual for serine proteinase and collagenases and novel applications (modulation of the rate of drug action), and even unusual (water-colour effect for tattoo paint injection) were proposed. We have previously proposed a method of obtaining tritium-labeled (in nonexchangeable positions) individual CLPI and their complexes, which preserves their physiological enzyme activity and can be applied for accurate pharmacological investigation of CLPI-containing preparations [8].

Active agent of the preparation and the preparation (application 97120340 RF November 28, 1997, MKI A61K 35/56, 38/43). We analyzed known structure-destroying enzyme preparations, their potencies and principle limitations determined by their structural and biochemical properties. CLPI are proposed as acting agents of preparation in order to overcome these limitations (improvement of the efficiency, lowering of the dosage, prevention of complications, broadening of the area of their application, and development of ready-to-use preparation with long expiration period).

Treatment method including elimination of pus and/or necrotic tissues (application 97120399 RF, November 28, 1997, MKI A61K 35/56, 38/43). Current enzymatic therapy of disorders accompanied by tissue devitalization (and probably suppuration) allows to eliminate noncollagen proteins but not to destroy tissue structure supported by native collagen, or partly hydrolyze necrotic tissues to large structural fragments. This problem consisting in low effectiveness of usual therapeutic doses can be solved by using CLPI. These complexes can be applied for the treatment of all types of wounds, burns and frostbites, trophic and decubitus ulcers and fistulas (including those infected and complicated by necrotic and suppurative processes). Examples stated in the application: infected burns of the ankle area; frostbittes of the lower extremities (foot); purulent lacerated wound of the scapular area; disseminated psoriasis; secondary open comminuted fracture of both shanks complicated by osteomyelitis in the initial stage; chemical burns of cornea and conjunctiva.

Method of treatment (for altered and inflamed tissues) (application 97120396 RF, November 28, 1997, MKI A61K 35/56, 38/43). The use of known enzyme preparations for the treatment of diseases associated with modifications of native tissue structure is often accompanied by nonspecific protein lysis in altered and adjacent tissues, so that negative consequences of the effective enzyme concentrations often exceeds their therapeutic effect. This problem can be solved by using CLPI possessing proteolytic activity and high activity against structural polypeptides, which provides sufficient effectiveness at lower doses of the preparation. Sphere of application: fungous skin and nail diseases (e.g. candidiasis); inflammatory diseases and defects of the skin, bones, and joints (psoriasis, keratosis, scars, collagenosis; fractures; bone callosities); prevention of postoperation scars; elimination of adhesions, joint and muscle contractures; inflammatory eye diseases (keratosis, conjunctivitis, iridocyclitis). Examples within the application: elimination of immature cataract; therapy of Dupuytren's hand contracture, promotion of osteosynthesis after closed comminuted fracture of both shanks; acceleration of desquamation of carious dentin; prevention of posttraumatic necrotic crust and scar formation; promotion of granulation and marginal epithelization after gunshot injury of a thigh. Positive results were also obtained when using CLPI as blood diluting agents (in particular, for prevention of thromboses after cardiac valve prosthetic appliance).

Active agent of cosmetic preparation and cosmetic preparation (variants) (patent 2141310 RF with the priority of November 28, 1997, MKI A61K 7/48, 35/56). The high effectiveness of CLPI as structure-destroying agents allows to use them in cosmetic and esthetic medicine (elimination and prevention of scars and keloids, removal of callosities and pigmented spot, wrinkle prophylaxis, treatment of problematic and fading skin, effective epilation, *etc*). Cosmetic or medical preparations can be produced in all pharmacological form: aerosol, lotion, shampoo, gel, ointment or cream (on the basis of water-lipid emulsion or polyethylene oxide), *etc*.

Patented use of CLPI from different hydrobiont species for elimination of keloids and scars is of particular interest. At present we develop a method of electrophoretic delivery of CLPI-containing preparations to these formations.

Modulator of the rate of drug effects and preparation for injection (variants) (patent 2141324 RF with the priority of November 28, 1997, MKI A61K 35/10, 35/56, 35/32, 35/37, 35/74). Usual biologically active additives modulating the rate of drug effect exhibit similar activity: they either accelerate the onset of therapeutic effect reducing the time of drug elimination from the circulation and lowering its efficiency), or prolong the therapeutic effect inhibiting diffusion of the drug in adjacent soft tissues, which also lowers its therapeutic effect and often leads to local overdose and complications. In some cases the known additives can interact with the drug unpredictably changing its properties (hyaluronic and other organic acids) or initiate severe local reaction (ethanol, adrenaline). CLPI used as a modulator partly hydrolyze interstitial collagen, induce cell disintegration in soft tissues and rapid absorption of the preparation in these tissues providing its even diffusion down to the concentration gradient in all directions from the injection point (wa-

ter-colour effect), which prolongs the therapeutic effect of the preparation and prevents its local overdose. An extraordinary application of this idea is the use of CLPI as a tattoo colour injection modulator, which allowes to reduce both the quantity of the colour per point and the linear density of puncturing without lowering colour brightness, as well as to provide background saturation of the contour.

Isolation of cell cultires (patent 2126832 RF with the priority of November 28, 1997, MKI C12N 5/00, 5/08). Trypsin and chymotrypsin used for tissue disintegration and separation of monolayer from the substrate are unable to hydrolyze interstitial collagen, while clostridial collagenase can not disintegrate other (noncollagen) proteins and always contains pathogenic and pyrogenic admixtures [1]. CLPI from cephalopod mollusks enhance disintegration of tissues and increase the yield of intact human and animal cell (hepatocytes, fibroblasts, and keratinocytes, for instance, for virus replication). The use of low active concentration of collagenolytic complex protects cell viability and their proliferative potencies after subculturing. We demonstrated high efficiency of CLPI for preparation of cardiomyocyte culture and isolation of viable bone cells.

Thus, we demonstrated that CLPI-containing preparations have a wide area of application in both medicine and cosmetology. Apart from CLPI complexes, the use of individual CLPI and their combinations selected in such a way as to potentiate the desired effect is of great importance, [5].

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