Comparative Evaluation of the Depth of Collagen and Hyaluronic Acid Hydrolysis *In Vitro* by Collagenase and Hyaluronidase Preparations

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The molecular weights of collagen and hyaluronic acid solutions after their incubation with collagenase and hyaluronidase were evaluated by capillary viscosimetry. The results indicate high amylolytic activity of collagenase and the absence of proteolytic activity in hyaluronidase in an *in vitro* system.

Key Words: *collagen; hyaluronic acid; viscosimetry; characteristic viscosity; collagenase*

The content of humor is reduced in keloid and hypertrophic cicatrices in comparison with normal skin tissues [2,3]. It is also known that hyaluronic acid providing optimal water metabolism in tissues and eventually rendering the turgor, elasticity, young and healthy appearance to the skin, is one of the main components of water-binding extracellular matrix. By its chemical structure hyaluronic acid is a polysaccharide, consisting of long chain molecules with the mean molecular weight of 200-500 kDa and even more than 1000 kDa.

Enzyme drugs of the collagenase and hyaluronidase groups are used for the treatment of pathological cicatrices of the skin. These drugs provide hydrolysis of pathological collagen and hyaluronic acid and recovery of normal composition and structure of extracellular matrix. On the other hand, it is not quite clear to what degree collagenases, hydrolyzing the peptide bonds, cross-react with polysaccharides (glycosaminoglycans), for example, with hyaluronic acid.

Fermencol contains a group of 9 collagenases obtained from hydrobionts, providing hydrolysis of peptide bonds not only between certain amino acids of collagen threads, such as Gly-Leu and Gly-Ile (similarly as bacterial collagenases), but between any other amino acids. In addition, it is only wise to suggest that hydrolytic activity of hydrobiont collagenases will manifest not only towards collagen, but also towards polysaccharides.

We compared the hydrolytic activities of enzyme drugs fermencol (collagenase) and lidase (hyaluronidase) towards hyaluronic acid and collagen *in vitro*.

MATERIALS AND METHODS

The mean molecular weight of the substrate (collagen and hyaluronic acid) after its incubation with fermencol and lidase served as the criterion of hydrolysis completeness (depth). As our interest was focused on the mean molecular weight, but not on the detailed distribution of molecular weights of the enzyme treatment product, capillary viscosimetry was selected as a method for evaluation of the molecular weight of high molecular-weight compound (HMC) in aqueous solution.

Evaluation of molecular weight of a dissolved substance involves measurement of characteristic viscosity of HMC solution. Characteristic viscosity

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TABLE 1. Molecular Weights of Collagen and Hyaluronic Acid after Their Incubation with Collagenase and Hyaluronidase Preparations of Different Concentrations at Different Temperatures and Duration of Exposure (*M*±*m*)

Enzyme preparation	Collagen, kDa	Hyaluronic acid, kDa
Intact HMC solution	320±20	750±40
Fermencol		
0.01 mg/ml (24 h at 22°C)	142±20	130±20
0.1 mg/ml (24 h at 37°C)	5.7±1.2	3±1.0
Lidase		
0.4 mg/ml (24 h at 22°C)	320±20	480±30
0.4 mg/ml (96 h at 22°C)	300±20	44±10

is proportional to molecular weight of high molecular weight substance in solution and is related to the dynamic viscosity of HMC solution and solvent by a formula:

$$[\eta]=\lim(\eta-\eta_0)/(\eta_0\times c) \text{ at } c\to 0,$$

where $[\eta]$ is characteristic viscosity, n solution viscosity, η_0 solvent viscosity, and c mass concentration of HMC in solution. In turn, characteristic viscosity is related to the molecular weight and shape of molecules by proportion:

$$[\eta]=A\times M^{\alpha},$$

where A is proportionality coefficient, M molecular weight, and α exponent depending on the molecule shape and size. For rigid spherical molecules $\alpha=0$, for hydrophilic molecules completely permeable for water $\alpha=1$, and for HMC molecules completely impermeable for water $\alpha=0.5$ [3,4].

RESULTS

Concentrated aqueous solution of hyaluronic acid potassium salt (Fluka, BioChemika) and collagen (Sigma; Collagen from calf skin) with the initial concentration of 10 mg/ml was diluted (by a series of double dilutions) to 2^8 solution. Dynamic viscosity of HMC solutions was measured on a VK-4 capillary viscosimeter and, by plotting a series of characteristic viscosities $[\eta]_1$, $[\eta]_2$, $\downarrow[\eta]_3$.....$\sqrt{[\eta]_8}$ of successive dilutions, the succession limit was estimated: $\lim(\eta-\eta_0)/(\eta_0\times c)$ at the limiting dilution, when $c\to 0$. Based on the data on the molecular weight, known from the manufacturer's certificate, coefficient A was estimated: $A=[\eta]/M^{\alpha}$, where M is molecular weight (kDa). Using calibration measurements of collagen and hyaluronic acid viscosities of known molecular weights, the values for collagen and hyaluronic acid were determined: $\alpha=0.78$ for hyaluronic acid solution, $\alpha=0.5$ for collagen solution.

The initial collagen and hyaluronic acid solutions (5 ml) of 10 mg/ml concentration were incubated in a thermostat at 22 and 37°C with different concentrations of collagenase (fermencol) or hyaluronidase (lidase). After incubation the characteristic viscosity $[\eta]$ of solution was evaluated and the molecular weight of HMC was estimated.

The results of evaluation of mean molecular weights of dissolved collagen and hyaluronic acid after their incubation with collagenase (fermencol) and hyaluronidase (lidase) are presented in Table 1.

Evaluation of the mean molecular weight by viscosimetry demonstrated high hydrolytic activity of collagenase towards glycosaminoglycans — even higher than towards collagen. Amylolytic activity of collagenase is higher than that of hyaluronidase; hyaluronidase has virtually no collagenolytic activity. These preliminary data prompt clinical studies of the efficiency of collagenase drugs in conservative therapy of hypertrophic and keloid cicatrices. Clinical efficiency of collagenases will be presumably higher than the clinical results of traditional anticicatricial hyaluronidase drugs (lidase and ronidase).

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