Dynamics of Hydration of Cicatricial Tissue during Local Therapy with Collagenase Preparations

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The components of complex dielectric permeability of cicatricial tissue at the frequency of 55 GHz were studied during local therapy of cicatrices with Fermenkol (a complex of collagenolytic proteases). Electrical characteristics of tissues in the microwave range were interpreted in terms of hydration parameters (total content of water in tissues and the ratio of structured and free water). The rightfulness of interpretations is discussed on the basis of the results of measurements of cell suspension models (native blood and its fractions brought to a standard hematocrit). The results attest to a relationship between hydration of the cicatricial tissue and its morphology and function.

Key Words: *cicatrices; collagenase; hydration*

Conservative therapy of skin cicatrices is one of the most important problems of general and plastic surgery and cosmetology. Injuries or interventions are always associated with the development of cicatricial tissue (CT). Despite the attention of scientists and physicians to this problem, physicochemical mechanisms of repair processes in CT remain little studied. Many aspects of prevention and conservative treatment of pathological cicatrices of the skin require more comprehensive undertaking of their pathogenesis and necessitate improvement of methods for instrumental diagnosis and treatment of skin injuries. The shortage of noninvasive methods for objective control of the morphology and function of tissues appreciably impedes the evaluation of drug effects on CT, while histological diagnosis is not always possible. Changes in cell composition of cicatrices and the ratio of cell populations and microcirculatory bed responsible for disorders of capillary blood flow are associated with differences in water content of CT determining its histological structure and metabolic activity.

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We studied the dynamics of CT hydration parameters during therapy with Fermenkol.

MATERIALS AND METHODS

Five patients with pathological cicatrices of the skin treated with Fermenkol were examined. The "age" of cicatrices was 3-6 months in all patients; the cicatrices were located on the face and inner surface of the shin.

Fermenkol is a polyenzyme complex consisting of 9 proteases, each subunit with molecular weight of 23-36 kDa. This polyenzyme complex was isolated from the hepatopancreas of *Paralithodes camtschatica*. The patients received the preparation by electrophoresis in 0.9% NaCl in concentrations of 0.01-1.00 mg/ml. Enzyme stability was improved by adding $CaCl_2$ into solution to a concentration of 3 mmol/liter. In order to increase electrophoretic mobility of collagenase molecules, pH of the solution was brought to 5.0-5.2 by adding 0.1 N HCl. The preparation was delivered from the anode at current density of 0.05-0.15 mA/cm^2 for 20-25 min. Courses of 5-7 procedures with 1-2-day intervals were carried out.

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Fragments of hypertrophic CT with flat surface (to ensure tight contact of the entire surface of the waveguide probe, 3.4×7.2 mm) were selected for the analysis. The status of CT was evaluated visually (by standard clinical symptoms) and by EHF skin hydration parameters by EHF dielectrometry. The measurements were carried out repeatedly in the same points during a long time (up to 2 years in one female patient with cicatrices on the face).

Qualitative and quantitative parameters of hydration of the biological tissues of the skin were evaluated by EHF dielectrometry at a frequency of 55 GHz. Dielectric (DE) parameters of the blood and its fractions (plasma and erythrocyte mass) were determined. Specimens of erythrocyte mass with 80% hematocrit (volume percent of cell fraction) were selected after blood centrifugation (native blood hematocrit was 45%). Complex DE permittivity was evaluated by the waveguide method modified by authors; it was realized by measuring the modulus and argument of complex coefficient of the waveguide wave reflection at medium interface: open rectangular waveguide terminal filled with leucosapphire—studied skin surface.

According to Frenel's equations, the modulus and argument of complex coefficient of reflection from the substance interface unambiguously detect the components of complex DE permittivity of the test material. Skin hydration parameters were evaluated in accordance with Maxwell—Wagner model by the results of estimation of complex DE permittivity components. The method of tissue dielectrometry *in vivo* and estimation of skin hydration parameters were described in detail in our previous papers [3,4].

Volume percent of total water in the skin and volume percent of structured water served as skin hydration parameters. Water volume was estimated by Maxwell—Wagner formula, volume of structured water as a value proportional to the percent of DE loss in a skin site relative to tangent slope of DE loss for control water sample at a certain temperature.

The density of probing radiation energy was ≤ 5 $\mu W \times cm^2$ at the open end of the waveguide probe, which maximally reduced the impact of the test radiation for the studied biological object.

RESULTS

The tangent of DE loss in extracellular fluid (plasma) was slightly below the arbitrary DE loss, which was directly proportional to the volume percentage of water in the studied tissue (Table 1). In predominantly intracellular fluid (erythrocyte mass) the tangent of DE loss tended to surpass the value proportional to water content in the cell fraction. In native blood (erythrocyte suspension in the plasma) the measured tangent of DE loss was virtually (within the measurement error) proportional to the volume percentage of water.

These data suggest that physicochemical characteristics of water, primarily its quasicrystalline structure, vary in extra- and intracellular fluids. On the other hand, we have not grounds to assert that the structural organization of water in the cytoplasm and tissue fluid is qualitatively similar. Mature erythrocytes are specific cells containing no membrane structures with relatively large surface (endoplasmatic reticulum and mitochondria) characteristic of other cells. Presumably, keratinocytes, fibroblasts, and myocytes containing cytoplasmic membrane structures are characterized by more pronounced differences in the structural organization of intracellular, predominantly perimembrane surface-bound water.

Clinically manifest effect of treatment (decrease in the density of cicatrices) was noted 3-4 weeks after the start of therapy. The height of the cicatrices decreased later.

Changes in the parameters of tissue hydration were observed earlier and manifested by an increase in the content of total and structured water in CT (Table 2). This dynamics of water content can be explained as follows. Exposure to a complex of collagenolytic proteases leads to hydrolysis of the extracellular matrix collagen and water release. Collagen degradation is paralleled by the synthesis

TABLE 1. DE Characteristics of Blood, Erythrocyte Mass, and Plasma and Corresponding Hydration Parameters at 22°C (*M*±*m*)

Parameters	Water	Blood	Plasma	Erythrocyte mass
ε' (actual component)	12.0±0.1	12.8±0.1	12.4±0.1	12.8±0.1
ε" (presumable component)	23.2±0.1	19.7±0.1	21.4±0.1	18.8±0.1
Volume percentage of humor (p)	1.0	0.79±0.05	0.91±0.05	0.74±0.05
tgδ	1.935±0.018	1.538±0.015	1.730±0.016	1.470±0.015
Reduced DE loss ($tg\delta_{water} \times p_{object}$)	1.0	1.530±0.015	1.760±0.016	1.430±0.016

TABLE 2. DE Characteristics and CT Hydration Parameters *in Vivo* before and after Fermenkol Therapy ($M \pm m$)

Tissue	ε'	ε''	Volume percent of water content	tgδ	Structured water, %	Reduced DE loss ($tg\rho_{water} \times \rho_{object}$)
Normal facial skin (30°C)	14.6±0.2	19.1±0.2	71±1	1.30±0.03	73±1	1.25±0.03
Normal skin of the inner shin surface	15.6±0.3	18.8±0.3	68±1	1.20±0.03	68±1	1.20±0.03
Cicatrix on the face at the stage of intense growth before therapy	16.1±0.2	17.1±0.2	65±1	1.06±0.03	60±1	1.14±0.03
Cicatrix on the face after 2 courses of collagenaseelectrophoresis	16.0±0.2	18.8±0.2	67±1	1.17±0.03	66±1	1.18±0.03
Cicatrix on inner shin surface before therapy	16.5±0.2	16.5±0.2	64±1	1.00±0.03	57±1	1.12±0.03
Cicatrix on inner shin surface after 2 courses of collagenase	15.8±0.2	17.7±0.3	67±1	1.12±0.03	64±1	1.18±0.03
Water at 30°C	14.6±0.1	25.7±0.15	—	1.76±0.02	—	—

of hyaluronic acid in the extracellular space and extracellular water binding by this acid. Reduction of volume percent of extracellular matrix at the expense of collagen hydrolysis in the cicatrix is paralleled by an increase in the content of epithelial and connective tissue cells per volume unit.

Treatment of cicatrices with Fermenkol slightly increased water content in CT and tangent slope of DE loss for tissues ($p<0.05$), which we interpreted as an increase in the fraction of structured extra- and intracellular water, predominantly bound to hyaluronic acid. For example, CT hydration parameters (total and structured water) during treatment approached the values characteristic of normal skin of the corresponding location.

Dielectrometry of tissues *in situ* in the millimeter radiowave band discloses additional mechanisms of water-electrolyte balance of inter- and intracellular fluids. Noninvasive measurements taking just 10-15 sec identified the dynamics of water balance of tissues, fluid redistribution between intra- and extracellular sectors, and detected changes in the structural organization of water in tissue fluids. Contact EHF dielectrometry can be used for objective control and prediction of clinical efficiency of treatment of cicatrices.

REFERENCES

1. S. I. Aksenov, *Water and Its Role in Regulation of Biological Processes* [in Russian], Moscow (1990).
2. A. N. Kuznetsov, *Biophysics of Electromagnetic Exposure (Fundamentals of Dosimetry)* [in Russian], Moscow (1994).
3. A. N. Kuznetsov, I. I. Turkovsky, and I. A. Volkova, *Biofizika*, **46**, No. 6, 1122-126 (2001).
4. A. N. Kuznetsov, I. I. Turkovsky, V. O. Matytsin, *et al.*, *Ibid.*, **48**, No. 1, 73-75 (2003).