Structural and Functional Peculiarities of Mast Cells in Undifferentiated Connective Tissue Dysplasia

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We performed an immunomorphological study of mast cells from undamaged skin in women with phenotypical evidence of undifferentiated connective tissue dysplasia syndrome, patients of cosmetological clinics. It was found that the numerical density of mast cells containing chymase granules in this condition 1.7-fold surpassed the corresponding parameter in patients without signs of connective tissue dysplasia syndrome, which probably was a result of compensatory and adaptive reaction aimed at activation of the synthesis of the connective tissue extracellular matrix components. It was hypothesized that increased content of chymase-positive mast cells in the skin of patients with connective tissue dysplasia syndrome contributed to the formation of associated arterial hypertension.

Key Words: *mast cells; skin; chymase; tryptase; connective tissue dysplasia*

Elevated concentrations of hydroxyproline and glycosaminoglycans in the blood and/or urine are detected in many patients with phenotypical evidence of undifferentiated connective tissue dysplasia syndrome (CTD) [4,8], which is believed to reflect enhanced degradation of some extracellular matrix (EM) components of the connective tissue due to their hereditary defectiveness. These processes in CTD are thought to be compensated to a different extent by activation of the synthesis of EM components aimed at the maintenance of tissue homeostasis [6]. At the same time, the main regulatory factors determining the above peculiarities of the connective tissue metabolism in CTD are poorly studied. Mast cells attract much attention in this respect. There are two main phenotypes of these cells differing by the content of protease granules: connective tissue mast cells secreting tryptase and chymase and mucosal mast cells secreting tryptase only [1,2]. It is known that chymase from mast cells is a potent activator of fibrogenesis [11]. The highest levels of chymase activity were detected in mast cells of human skin, esophagus, stomach, and uterus (listed in descending order) [12]. On the contrary, mast cell tryptase activates procollagenases and cleaves fibronectin, *i.e.* promotes degradation of collagen and other structural EM components, but its effect on the connective tissue metabolism is less pronounced [2]. Thus, mast cells can largely modulate the connective tissue metabolism under normal and pathological conditions, but their structural and functional peculiarities in CTD remain little studied.

Here we studied structural and functional peculiarities of mast cells in individuals with phenotypical signs of CTD.

MATERIALS AND METHODS

We examined 28 women aged 28-40 years (mean age 32.4±2.9 years) undergoing blepharoplasty surgery in cosmetological clinics. Phenotypical signs of CTD (hyperelasticity of the skin, joint hypermobility, spine and thorax deforminies, thumb sign, wrist sign, vascular fragility, varicose veins, and telangiectasias) were detected during examination using common methods [6,7]. The patients were divided into 2 groups: 12 patients with phenotypical signs of CTD (study group)

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and 16 patients without these signs (reference group). Only patients with 5 or more phenotypical signs of CTD were included in the study group [7]. The groups were comparable by age.

In patients of both groups, immunomorphological analysis of eyelid skin removed during surgery was performed (all patients gave informed consent for participation in the study). Immunohistochemical visualization of mast cells in the skin was performed on deparaffinized serial sections dehydrated in ascending alcohols followed by antigen demasking in microwave oven. Markers of mast cells tryptase (MCT) and chymase (MCC) were detected (Novocastra). For visualization, Envision detection system (Dako) was used. The time of incubation with antibodies was 50 min at room temperature. The positive reaction to proteases appeared as brown staining of cell cytoplasm. Morphometry of chymase-positive and tryptase-positive mast cells was performed by measuring their size (μ) and numerical density (per 1 mm^2 skin); the relative number of cells in the state of exocytotic degranulation was determined (by the presence of secretory granules outside the cell cytoplasm).

The data were processed by methods of variation statistics. Significance of differences was evaluated using Student *t* test. The differences were significant at $p<0.05$.

RESULTS

Tryptase-positive cells in the skin of patients of both groups were primarily located in the papillary dermal layer; somewhere they formed perivascular clusters consisting of 2-4 cells. Only solitary cells were revealed in the reticular dermal layer. Only few tryptase-positive cells (2.6 and 2.2% in the study and reference groups, respectively) were in the state of exocytotic degranulation: spherical positively stained granules were seen in the extracelular space near the mast cell. This was probably explained by the fact that we studied undamaged eyelid skin without visible pathological changes. It is known that exocytotic (anaphylactic) degranulation of mast cells can be induced by immunological (IgE reception) and nonimmune mechanisms (substance P, somatostatin, vasoactive intestinal peptide, neurotensin) [2] and is observed in allergic inflammation, when the relative count of mast cells in this state in the inflammatory infiltrate increases to 90% [6]. Another variant of mast cell degranulation (gradual, mediated by microvesicular transport) provides primarily regulation of physiological processes in the connective tissue, but it can also occur in pathological states; it consists in gradual release of small amounts of the granule content into the extracellular space without fusion of these granules with the plasma membrane or its destruction [2] (this is why this variant of degranulation cannot be detected by immunomorphological methods).

The numerical density of tryptase-positive mast cells in the skin and their size were similar in patients of the study and reference groups (Table 1).

Chymase-positive cells in the skin of patients of both groups were scattered primarily in the papillary dermal layer; no morphological signs of exocytotic cell degranulation were observed (Table 2).

The size of chymase-positive cells was similar in patients of the study and reference groups (Table 2), but in patients with phenotypical signs of CTD their numerical densities surpassed the corresponding parameter in the reference group by 1.7 times. In the ref-

TABLE 1. Morphometric Parameters of Tryptase-Positive Mast Cells in the Skin of Patients with Phenotypical Signs of Nondifferentiated CTD Syndrome (*M*±*m*)

Group	Numerical density of mast cells per 1 mm^2 skin	Cell size, μ	Relative number of cells undergoing exocytotic degranulation, %
Study group (*N*=12)	87.49±7.70	18.29±1.60	2.6±0.7
Reference group (*N*=16)	78.11±8.10	18.78±1.20	2.2±0.5

TABLE 2. Morphometric Parameters of Chymase-Positive Mast Cells in the Skin of Patients with Phenotypical Signs of Undifferentiated CTD Syndrome (*M*±*m*)

Group	Numerical density of mast cells per 1 mm^2 skin	Cell size, μ	Relative number of cells undergoing exocytotic degranulation, %
Study group (*N*=12)	102.81±9.20*	14.88±1.40	0.10±0.06
Reference group (*N*=16)	59.94±5.60*	15.12±1.30	0.08±0.02

Note. Significant differences between the analyzed parameter in groups *$p<0.05$.

erence group, the tryptase- to chymase-positive mast cells in the skin was 1.3, which agrees with published data [2], while in patients of the reference group this parameter was 0.85.

Chymase can activate the production of transforming growth factor (TGF-β) by fibroblasts [11]. TGF-β is a potent activator of collagen and fibronectin synthesis by fibroblasts [14]; in contrast to basic TGF, it inhibits metalloproteinases (MMP), *e.g.* MMP-1, MMP-2, and MMP-9, which leads to activation of plastic processes in EM [9]. Some investigators believe that chymase directly stimulates TGF-β synthesis by fibroblasts [11], while others consider that this effect is mediated by angiotensin II [13]. It is known that chymase is a key tissue enzyme of the so-called alternative pathway of angiotensin II formation from angiotensin I without participation of angiotensin-converting enzyme [10]. In light of this, chymase under physiological conditions can modulate perfusion in EM and act as a pathogenetic factor determining inefficiency of angiotensin-converting enzyme inhibitors during therapy of arterial hypertension in some patients [10]. The increase in numerical density of chymase-positive mast cells in the skin of individuals with phenotypical manifestations of CTD is probably a compensatory and adaptive reaction aimed at activation of the synthesis of collagen fibers and non-fiber structures in EM under conditions of their accelerated degradation providing, among other things, adequate microcirculation conditions essential for active metabolic processes in EM in CTD. It is also known that in some patients CTD is associated with arterial hypertension syndrome; the pathogenetic mechanisms of this syndrome remain poorly studied [3]. It cannot be excluded that activation of the alternative pathway of angiotensin II formation due to increased content of chymase in CTD is an important pathogenetic mechanism of arterial hypertension in these patients responsible for previously reported therapeutic inefficiency of angiotensin-converting enzyme inhibitors [3].

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