PROTEASES AS A FACTOR IN THE PATHOGENESIS OF INFLAMMATION IN TISSUES OF THE PERIODONTIUM

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Periodontitis is an inflammatory disease of the periodontium, characterized by a lesion of all its elements, destruction of the gingivodental junction and progressive destruction of the bone of the alveolar processes. Under these circumstances, there is a disturbance of the metabolism of the main components of the connective-tissue matrix — collagen, proteoglycans, and glycoproteins [1, 4, 5, 8]. The source of the proteinases involved in the development of the process may evidently be both cells of the periodontal tissues and enzymes of the blood cells and, in particular, polymorphonuclear leukocytes and mononuclear cells present in the inflammatory focus [7, 8].

Recently, attention has been paid by research workers to the study of the contents of the periodontal pocket, in view of evidence that quantitative and qualitative changes in the gingival fluid (GF) can take place before the appearance of clinically visible changes in the periodontal tissues [7].

Despite a few investigations to determine collagenolytic activity (CA), problems concerned with the regulation of the level of this activity have not been solved. The aim of the present investigation was a comparative study of CA in GF of patients with gingivitis and periodontitis at different stages of the disease, and also to study the regulation of CA.

EXPERIMENTAL METHOD

Patients aged from 18 to 50 years old were studied. Group 1 comprised 16 patients with gingivitis, group 2 — five patients with mild periodontitis, and groups 3 and 4 — patients with moderately severe and severe forms of periodontitis (12 and four patients, respectively). The diagnosis was made on the basis of a clinical and roentgenologic investigation (the periodontal index was determined and orthopantomography was carried out).

GF was sampled by the method of Brill and Crasse [6] and proteins was estimated by Lowry's method [11].

The substrate used to determine CA [13] was type I collagen from rat skin, acetylated with ^{14}C-acetic anhydride, with specific radioactivity of $(1.5\text{-}2)\cdot10^6$ cpm/mg protein. Relative CA was expressed in micrograms ^{14}C-collagen, hydrolyzed during 1 h, and calculated per milligram protein of GF. As inhibitors of proteolytic activity we used EDTA, phenylmethylsulfonyl fluoride (PMSF), and *p*-chloromercuribenzoate (PCMB) (from "Serva," West Germany) — specific inhibitors of the three main classes of neutral proteinases: metal-dependent, serine, and thiol proteinases, respectively. Some of these reagents, EDTA for example, may be inhibitors of metal-dependent proteinases and activators of thiol proteinases. The inhibitors were used in a final concentration of 10^{-3} M. The action of trypsin CA was studied by the method in [10].

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TABLE 1. Effect of Dilution of GF in Vitro on Its CA in Patients with Gingivitis and Periodontitis in Different Stages of the Disease (M ± m)

Group of patients	Without dilution		Diluted twofold		Diluted fourfold	
	protein, μg	CA, μg collagen/mg protein	protein, μg	CA, μg collagen/mg protein	protein, μg	CA, μg collagen/mg protein
1						
Subgroup A (n=9)	20,0±1,9	43,1±5,1	10,0±0,8	65,5±7,8	5,0±0,4	85,3±9,1
Subgroup B (n=7)	22,8±2,3	42,6±7,8	11,4±1,1	47,1±8,8	5,7±0,9	81,0±6,9
2	36,0±6,4	47,9±9,9	18,0±3,2	82,4±14,9	8,8±1,6	110,7±27,8
3	63,5±9,6	53,1±7,4	31,8±5,4	59,3±9,1	15,9±2,7	117,0±12,5
4	66,2±8,4	154,4±62,8	33,1±4,2	159,6±46,8	16,6±2,2	196,0±47,2

Legend. Here and in Tables 2 and 3, n denotes number of patients.

TABLE 2. Action of Trypsin on CA and GF in Patients with Inflammatory Disease (periodontitis) (M ± m)

Group of patients	Inhibition	Activation	No change
	percent relative to initial level		
1	—	54,2±7,7 (n=9)	0 (n=7)
2	38,3 (n=1)	47,1±6,5 (n=3)	0 (n=1)
3	21,1±2,2 (n=2)	26,0±7,1 (n=5)	0 (n=5)
4	63,1±6,4 (n=2)	—	0 (n=2)

TABLE 3. Action of EDTA, PMSF, and PCMB on CA in GF in Patients with Inflammatory Diseases of the Periodontal Tissues (in % relative to initial level, M ± m)

Group of patients	EDTA, 10^{-3} M		PMSF, 10^{-3} M		PCMB, 10^{-3} M	
	inhibition	activation	inhibition	activation	inhibition	activation
1	30,7±5,6 (n=4)	140,0±33,4 (n=12)	4,0 (n=1)	200,0±39,3 (n=15)	40,0 (n=1)	193,0±49,1 (n=15)
2	33,3±6,0 (n=3)	25,0±6,0 (n=2)	24,5±2,0 (n=2)	72,0±4,0 (n=3)	—	39,4±6,1 (n=5)
3	49,6±8,4 (n=6)	23,0±8,3 (n=6)	25,8±4,3 (n=6)	57,7±14,4 (n=10)	18,5±3,2 (n=2)	72,6±15,2 (n=10)
4	83,1±12,1 (n=2)	—	69,1±9,8 (n=2)	190,0±32,4 (n=2)	58,8±10,1 (n=2)	254,4±40,2 (n=2)

EXPERIMENTAL RESULTS

The data in Table 1 show that relative CA, depending on the degree of activity of the pathological focus, was increased, and in a severe form of periodontitis, when destructive processes of the periodontal tissues were most marked, CA rose sharply compared with activity in other forms of the disease. The value of CA is known to be connected not only with the presence of the active form of the enzyme, but also with the presence of inhibitors and the latent form of the enzyme [9], which under certain conditions can change into the active form. Thus, the latent form of the enzyme and endogenous inhibitors may behave as regulators of CA.

It is an interesting fact that on dilution of GF, the relative CA in samples from some patients was increased, evidently due to a decrease in concentration of the inhibitors. For instance, in patients with gingivitis, with comparable protein concentrations in the samples, CA in GF in subgroup A (Table 1) increased on dilution more than in subgroup B. It can be tentatively suggested that in patients with subgroup A the content of inhibitors was less than in patients of

subgroup B, and they could most likely be placed in a risk group, and their gingivitis could most probably change into periodontitis. In patients with a moderately severe form of periodontitis the picture was similar to that in patients with a mild form, with comparable protein concentrations in the sample, with respect both to relative CA and to the increase in activity on dilution. In the severe form, a sharp increase in CA was observed, and with corresponding protein concentrations dilution of the GF led to an increase of only 20% in activity. This indicates that the increase in CA in patients with severe periodontitis evidently took place through exhaustion of inhibitors in GF.

The increase in CA can also be connected with activation of the latent form of collagenase, which under conditions in vitro can take place through the action of trypsin. Table 2 shows that with the development of the pathological process the quantity of the inactive form of collagenase decreases, whereas in group 4, in patients with the severe form of periodontitis, none can be found. It is evident that with intensification of inflammation the inactive form of collagenase is converted into the active form, due to which collagen destruction is intensified.

Investigation of the action of inhibitors EDTA, PMSF, and PCMB on CA in GF showed (Table 3) that all the principal types of neutral proteinases are present in GF. Certain differences were found in the range of enzymes in different groups of patients. The data presented show that proteolytic enzymes are an essential factor in the pathogenesis of inflammation in the periodontal tissues.

Inhibition of CA by EDTA indicates that GF contains metal-dependent collagenolytic enzymes, notably collagenase, as is confirmed by data in the literature [2, 3, 12, 14].

At the present time serum proteinase inhibitors, namely trasylol, contrykal, and pantrypin [4, 5] are used to treat a developing inflammatory condition, whereas the use of inhibitors of different types of proteolytic enzymes is essential.

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