SERUM MODULATES COLLAGEN TYPES IN HUMAN GINGIVA FIBROBLASTS

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1. Introduction

Collagen is the most abundant structural protein of connective tissues. At least four different collagen types have been described so far. The tissue content and ratio of collagens are altered in pathologic states such as scleroderma and atherosclerosis, as well as during normal growth [1–3]. However, very little is known about the factors which influence the rates of synthesis and degradation of collagen or the molecular species produced. Serum factors have been known to regulate cell function by effecting various cellular processes such as cell movement, viability and proliferation [4], and recently they have been implicated in the pathogenesis of certain fibrotic lesions such as atherosclerosis [5]. We have studied the effect of serum on collagen synthesis by human gingiva fibroblasts and we report that serum enhances type I collagen several-fold; however, the type III collagen is increased only slightly, and as a result, its proportion decreases with increasing serum concentration.

2. Materials and methods

Fibroblasts were obtained from biopsies of normal, interproximal gingiva and maintained in Dulbecco-Vogt medium containing 10% complement-inactivated fetal-calf serum, as previously described [6]. Cells between 8 and 15 doublings were used. Confluent cells were preincubated for 1 h in serum-free medium lacking the amino acid to be labeled, but containing 50 μg/ml each of ascorbic acid and β-aminopropionitrile. Then the medium was replaced with fresh medium containing β-aminopropionitrile and ascorbic acid, and the appropriate amount of complement-inactivated fetal calf serum. After adding 2.5 μCi/ml of [G-^{3}H]proline and/or 1.0 μCi/ml of [U-^{14}C]lysine, the cells were incubated for 24 h at 37°C. The media were then separated and dialyzed against 0.5 M CH_3COOH. The dialyzed media were combined with the cells and digested with 1.0 mg/ml of pepsin for 6 h at 15°C [6]. The collagens were precipitated from the pepsin digest by 5.0 M NaCl at neutral pH and separated by CM-cellulose chromatography at 42°C [6]. Types I and III collagens were quantitated by totalling the radioactivity under the appropriate peaks indicated in fig.1.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of type III chains, without or with reduction and alkylation, was done on 5% gels as described elsewhere [6].

For amino-acid analysis, the samples after labeling were dialyzed at 4°C against water to remove unincorporated amino acids, lyophilized and then hydrolyzed in constant boiling HCl at 108°C. Radioactive amino acids were separated on a Beckman model 120C amino acid analyzer equipped with a stream-splitting device. Fractions were collected and radioactivity of fractions was measured by counting aliquots in Aquasol [6].

3. Results

Figure 1 shows a typical CM-cellulose chromatogram of the pepsin-digested collagenous proteins synthesized by human gingival fibroblasts. The position of elution of α1- and α2-chains is indicated. In addition, a peak eluting at the position of α1 [III]-chains was present. In this system, β12-chains also

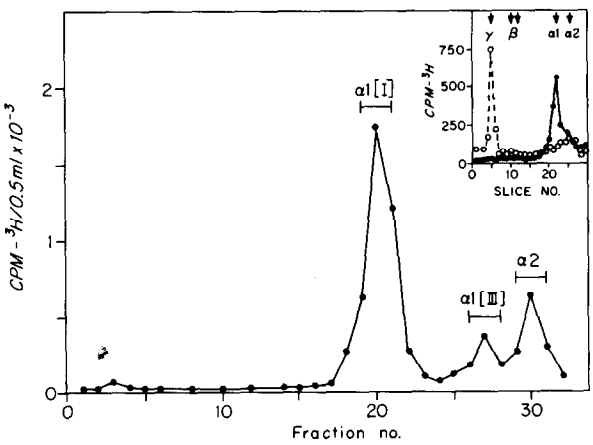

Fig.1. CM-cellulose chromatography of pepsin-digested collagenous proteins synthesized by human-gingiva fibroblasts. The column was previously standardized with human-fetal-skin type I and III collagens. Fractions of 6.9 ml were collected and the radioactivity of 0.5 ml portions is represented. The radioactivity of the α1[I], α2 and α1[III] peaks, respectively, was 3501, 1179 and 712 cpm. Type I collagen was quantitated by totalling the radioactivity of the α1[I] and α2 peaks. The type III peak accounted for 13.2% of the total. Inset. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of type III peak before (o- - - -o) and after (•——•) reduction and alkylation. 6219 cpm of the untreated material and 5978 cpm of the reduced material were loaded. Radioactivity of each 1.0 mm slice is shown.

elute at the position of type III chains; however, the cultures would not be expected to synthesize β12-chains because the labeling was carried out in the presence of β-aminopropionitrile, an inhibitor of cross-linking. The peak was identified as type III collagen by demonstrating that: (a) it eluted with known α1[III]-chains in CM-cellulose chromatograms (fig.1) [6]; (b) it had a molecular weight of 300 000, corresponding to that of γ-chains, but after reduction and alkylation it migrated with α1 chains (mol. wt 100 000) (fig.1-inset); and (c) it was precipitated at neutral pH by 1.5 M NaCl [6]. The type III collagen accounted for 10 to 13% of the total collagen in several experiments.

Inclusion of serum in the labelling medium caused a 4- and 7-fold increase in the incorporation of lysine and proline respectively (table 1). Lysine incorporation was maximal in 5% serum, but at higher concentrations it slightly decreased. The proline incorporation increased with serum concentration, reaching a maximum at 20% serum.

In the presence of serum, the Hyp/Pro and Hyl/Lys ratios were decreased to half, indicating a reduction in the proportion of collagens synthesized (table 1). However, the total quantity of type I and III collagens increased. A three-fold enhancement of type I collagen occured at 20% serum (fig.2). Type III collagen increased to a maximum of 1.5-fold at 10% serum but at higher serum concentrations it decreased.

Table 1
Effect of fetal-calf serum on protein and collagen synthesis by human gingiva fibroblasts

% Serum in labeling medium	Incorporation[a], cpm × 10^{-6}		Hyp/Pro[b]	Hyl/Lys[c]
	proline	lysine		
0	0.2	2.2	0.19	0.04
5	0.6	8.6	0.08	0.02
10	1.1	8.3	0.09	0.02
20	1.4	7.6	0.09	0.02
100	0.6	1.7	0.08	0.01

[a] Nondialyzable radioactivity per culture, labelled with [G-^{3}H]proline and [U-^{14}C] lysine. The values were obtained after hydrolysis and amino-acid analysis and include the respective hydroxyamino acids.

[b] Cpm hydroxyproline/cpm proline; the ratios for type I and III collagens are 0.73 and 1.19 respectively, calculated from [3]

[c] Cpm hydroxylysine/cpm lysine; the ratios for type I and III collagens are 0.22 and 0.17 respectively, calculated from [3]

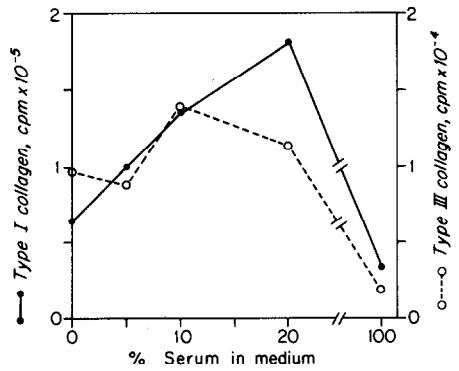

Fig.2. Effect of serum on synthesis of type I (●——●) and III (○- - -○) collagens by human gingiva fibroblasts. The collagens were quantitated from CM-cellulose chromatograms by totalling the radioactivity of α1[I] + α2 or type III peaks, respectively.

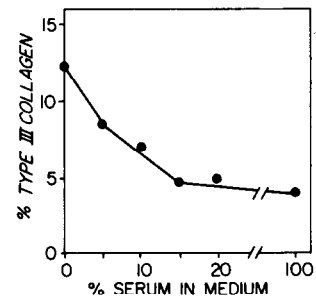

Fig.3. Effect of serum on the proportion of type III collagen synthesized by human-gingiva fibroblasts.

More interestingly, the proportion of type III collagen diminished with serum concentration and reached lowest values at greater than 15%. In a typical (separate) experiment, type III molecules comprised 12% of the total in the absence of serum, whereas at 100% serum they accounted for only 4%.

Both incorporation and collagen synthesis were minimal in the presence of 100% serum.

4. Discussion

We have observed that fetal-calf serum stimulates protein and collagen synthesis by human-gingiva fibroblasts. However, serum lowers the Hyp/Pro and Hyl/Lys ratios, thereby indicating that the proportion of collagen synthesized is decreased. Our results are in agreement with those of Manner [7] who noted greater levels of synthetic activity and a reduction in the proportion of collagen synthesis in human-foreskin fibroblasts in the presence of serum. However, whereas the reduction in the proportion of collagen synthesis occured only in foreskin fibroblasts that had undergone 50–55 cell divisions and not in younger cells, we have noted this phenomenon in young gingiva fibroblasts (<15 cell divisions).

Recently, environmental factors have been reported to influence the collagen types in cells that normally synthesize type II collagen. Thus, in cultures of mouse-embryo hind-limb-bud mesodermal cells, omission of serum from the medium favors the synthesis of type I collagen [8]. Chondrocytes exposed to 5-bromo-2′-deoxyuridine or chick-embryo extract synthesize a mixture of type I and $\alpha 1[I]_3$-collagens [9,10]. No such effects have been demonstrated for the fibroblasts so far. We have shown that serum affects the synthesis of type I and III collagens differently in human gingiva fibroblasts. Synthesis of type I collagen is enhanced threefold, whereas that of type III is stimulated to only 1.5-fold. Maximum stimulation of type III collagen occurs at 10% serum and at higher concentrations it is inhibited. Because of this, the proportion of type III collagen decreases with serum concentration and reaches minimum values above 15% serum. To our knowledge, this is the first demonstration that the synthesis of type I and III collagens by fibroblasts can be modulated in culture by an environmental factor.

Such modulation of fibroblast activity by serum may be important in normal physiological processes such as differentiation, wound healing and aging, and in pathological processes such as atherogenesis, scleroderma and fibrosis. For example, in human aortic media, type III collagen constitutes 70% of the total whereas in the intimal plaque, which is constantly exposed to blood, it is present only as a minor constituent (35% of the total) [2].

One other aspect of the effect of serum on the protein and collagen synthesis deserves comment. When the synthetic activities of cells from various sources are compared, the ability of serum to modify synthetic activities must be taken into consideration, because, in most experiments the cells are labelled in

the absence or presence of serum. Such factors may explain the differences in collagen synthetic activities reported for cells from normal and scleroderma tissues [1,11].

Acknowledgements

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