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Histochemical study of the blue autofluorescence of collagen in oral irritation fibroma: Effects of age of patients and of the duration of lesions

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Summary. The intensity of the autofluorescence of collagen was measured in 27 irritation fibromata of the buccal mucosa and 13 of the lip. The intensity of fluorescence correlated positively with the duration of the lesion. The fluorescence intensity also increased with the patients' age. The present observations show that in irritation fibromata of buccal and lip mucosae, the intensity of blue autofluorescence of the collagen increases with duration of the lesions and with the age of patients.

Key words: Blue autofluorescence, Collagen, Irritation fibroma, Buccal and lip mucosae

Introduction

Little attention has been given to the blue autofluorescence of collagen which can be seen in most tissues examined under ultraviolet illumination. In 15 Achilles tendons from unselected clinical autopsies (Wolman et al., 1985), the blue autofluorescence intensity of collagen was increased after the fifth decade of life. However, no correlation was found between fluorescence intensity and age in presumably ischemic tendons of amputated legs.

In the present study, the blue fluorescence in sections of irritation fibromata of the buccal mucosa and lip in relation to the patient's age and duration of the lesions was examined. Both parameters were found to play a role in the fluorescence intensity of the collagen component.

Materials and methods

Twenty-seven irritation fibroma biopsies from buccal mucosa were divided as follows: 12 males with a mean age of 48 years and 15 females with a mean age of 46 years (Table 1). The fibromata of the lip mucosa included 13 patients, 9 males with a mean age of 40 and 4 females with the mean age of 59. All biopsies were fixed in 10% formalin and embedded in paraffin.

As the study of Achilles tendons (Wolman et al., 1985) has shown that thickness of sections determines the fluorescence intensity studied by incident light microscopy, all biopsies were sectioned at 8 µm, deparaffinized and mounted unstained in a non-fluorescent mounting medium.

The intensity of blue fluorescence was determined by two methods:

1. Independent gauging by two of the authors using a Zeiss incident light fluorescent microscope with a 200 W Hg vapor lamp, excitation maximum around 365 nm and a barrier filter allowing passage of fluorescence light of wavelengths higher than 420 nm. Each observer marked fluorescence intensity with grades 1-10.

2. A similar microscope supplied with a Zeiss photomultiplier (photometer M1) and digital recording was used instead of subjective estimations of fluorescence intensity. As fading of fluorescence was rapid, adequate fields (which filled most of a small aperture) were selected under visible light, and the readings obtained in the first second of UV illumination were recorded in arbitrary units.

The measured intensity of autofluorescence of any given area of a section is obviously the product of the intensity of fluorescence of the fibers multiplied by the percentage of the field occupied by collagen. To determine the last mentioned values of the studied fields, we used Picrosirius red-staining followed by polarization microscopy of the same sections, which can selectively demonstrate the presence of collagen fibers (Constantine and Mowry, 1968; Junquiera et al., 1979; Dayan et al., 1989). The area of collagen fibers observed in microscope fields were point sampled using a square grid of 100 points. The intensity of autofluorescence of

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Table 1. Clinical data of irritation fibromata.

LOCATION	GENDER		MEAN AGE (YEARS)		DURATION OF LESION (YEARS)	
	Male	Female	Male	Female	Male	Female
Buccal mucosa	12	15	48 (9-70)	46 (19-61)	2.3 (0.5-6)	3.6 (0.5-10)
Lip mucosa	9	4	40 (10-55)	59 (52-70)	2.4 (0.5-8)	3.4 (0.5-6)

the collagens of the studied samples was defined as the relation between the digitized fluorescence value and the estimated area of the field occupied by collagen. Three different fields were measured in each sample. Data were calculated as mean ± standard error of the mean. Data significance was calculated using the Mann-Whitney U-test.

Results

The mean duration of lesions was slightly lower in males (2.3 and 2.4 years) than in females (3.4 and 3.6 years).

Data obtained subjectively by two of the authors and those obtained by photometry were comparable and only the latter will be discussed. Where full details about age of patients and duration of lesions were not available, samples were excluded.

Figure 1 shows the distribution of fluorescence intensities of collagen as a function of duration of the lesions. The figure includes data of buccal and lip mucosae irritation fibromata. It can be seen that fluorescence intensity of the lesions in both sites has a similar correlation with the duration of lesions. In lesions of duration of up to 3 years, the intensity was about 0.16 arbitrary units. However, lesions with more than 3 years duration had a significant increase of 73% ($p< 0.5$) in fluorescence intensity.

Similar results were obtained when the samples were aggregated according to age. However, the p value was less significant because of the small sample (data not shown).

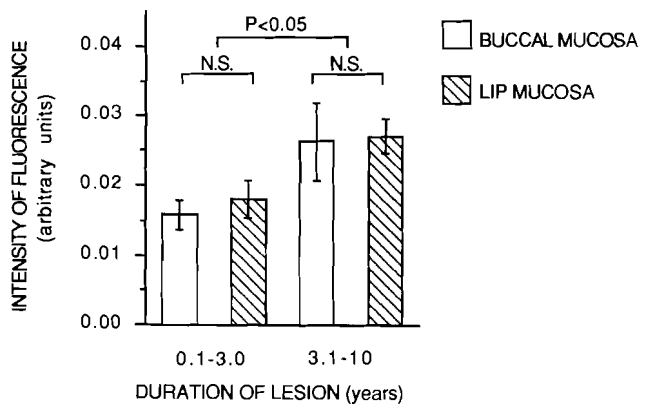

Fig. 1. Intensity of blue autofluorescence in irritation fibromata in buccal and lip mucosae in relation to the duration of lesions.

Discussion

Blue autofluorescence of collagen has probably been observed and considered unimportant by innumerable microscopists who studied tissue fluorescence. Recent studies have demonstrated that a crosslink of collagen/pyridinoline, can be quantitatively estimated by its blue fluorescence (Fujimoto et al., 1977; Fujimoto and Moriguchi, 1978). Concentrations of pyridinoline in collagens varied in different animal species and in different age groups (Moriguchi and Fujimoto, 1978; Eyre and Oguchi, 1980; Tanzer and Waite, 1982). Such compounds can be formed from oxidation products of lysine and hydroxylysine (but possibly also from other amino acids) reacting with free amino groups. Other compounds which may contribute to the blue autofluorescence of irritation fibromata originate from condensation of reducing sugars with proteins containing free amino groups (Maillard reaction products) (Monnier and Cerain, 1981).

The present observations show that in irritation fibromata of the buccal and lip mucosae, the intensity of blue fluorescence increases with the duration of the lesion as well as with the age of patients. This increase in fluorescence intensity probably indicates that the nature of the organic components responsible for the fluorescence varies with age as different types of crosslinks predominate at different phases of collagen maturation. In other words, increases in crosslinking probably involve replacement of some crosslinks by others as has been suggested by Davison (1978). Whether aging of newly-laid collagen follows a similar pattern to that of aging in tendons is an open question: the changes in fluorescence intensity in relation to duration of the lesions suggests, however, that the two processes may follow a similar pattern of changes.

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