Reduced collagen biosynthesis in oral mucosa of beige (Chediak-Higashi) mice

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Summary. Oral mucosa of beige (Chediak-Higashi) mice had decreased levels of collagen synthesis and prolyl hydroxylase activity compared with normal animals. No significant difference was observed in non-collagen protein synthesis between the two groups. These results suggest that decreased collagen biosynthesis in oral tissues may be partially involved in the increased incidence of periodontal disease in the Chediak-Higashi syndrome.

Key words. Collagen synthesis; prolyl hydroxylase; oral mucosa; Chediak–Higashi mouse

The Chediak-Higashi (CH) syndrome is a genetic disorder of man[2] and certain animals[3-6], including the beige mouse. It is charcterized by oculocutaneous albinism, photophobia, and recurrent infections. The major oral manifestation of this syndrome is a severe periodontal disease. In humans and animals, severe periodontitis leading to tooth loss occurs in individuals affected by the CH syndrome[7-10]. However, the relationship between periodontal disease and CH syndrome is not known at present.

Collagen is a major component of the extracellular matrix of oral tissues, and the disorganization of collagen is an important feature in the progress of periodontal disease[11-14]. It is accepted that collagen destruction may result from an imbalance in the relative rates of its production and degradation. In this study, we compared collagen biosynthesis in oral mucosa between normal and beige (CH) mice.

Materials and methods. Beige mice (C57BL/6J bg/bg) and the heterozygous littermates of male mice were obtained from a breeding colony maintained at the National Institute of Health. At the age of three months, animals were lightly anesthetized with ether and killed by exsanguination. Oral mucosa containing gingival tissues was immediately excised from the palatal regions. For the measurement of collagen synthesis, the excised tissues were immediately transferred to α-minimum essential medium (α-MEM) and kept for about 30 min at room temperature in order to disperse microorganisms. The medium was then removed, and the tissues were diced into 1–2 mm cubes and labeled for 5 h with 10 μCi of L-[3,4-^{3}H] proline in 2 ml of α-MEM containing 50 μg each of ascorbic acid and β-aminopropionitrile. After incubation at 37 °C for 5 h in a moist chamber containing 95% air and 5% CO_2, the medium was removed by centrifugation (5000 × g for 10 min), and the residual tissues were homogenized in 1 ml of 0.2 N NaOH with a glass homogenizer. Proteins in the homogenates were precipitated by adding 0.2 ml of 50% trichloroacetic acid (TCA) containing 5% tannic acid, washed three times with 10% TCA containing 1% tannic acid, and then twice with ice-cold acetone. The pellet was dissolved in 1 ml of 0.05 N NaOH, and collagenase-digestible and non-collagen proteins in the solution were measured according to the procedure of Peterkofsky and Diegelman[15]. For the measurement of prolyl hydroxylase activity, the tissues were homogenized in a glass homogenizer with 4 volumes of 0.05 M Tris-HCl buffer, pH 7.6, containing 0.1 mM dithiothreitol. The homogenates were centrifuged at 15,000 × g for 30 min, and the resulting supernatants were used for enzyme assay and protein determination. Prolyl hydroxylase activity was measured by a tritium release assay using L-[3,4-^{3}H] proline-labeled unhydroxylated collagen as substrate, as described by Peterkofsky and DiBlasio[16]. Protein content was determined by the method of Lowry et al.[17].

Results and discussion. As shown in table 1, non-collagen protein synthesis in the oral mucosa was almost equal in normal and CH animals. On the other hand, collagen synthesis was significantly lower in CH animals than in normal animals ($p < 0.01$). Thus, the ratio of collagen synthesis to protein synthesis was markedly reduced in the CH animals. Additionally, the activity of prolyl hydroxylase in the oral mucosa was also significantly lower in CH animals ($p < 0.05$) (table 2). This enzyme catalyses the hydroxylation of specific peptidyl proline in the polypeptide collagen precursors and is used as an indicator of collagen synthesis, because the enzyme activity changes in parallel with the amont of collagen formed[18-20]. Therefore, our results suggest that the decreased rate of collagen biosynthesis in the oral mucosa of CH mice is due to the inhibition of hydroxylation of collagen precursors.

Generally, the CH syndrome is well known as a functional and morphological disorder of leucocytes. Since it is known that oral bacteria are important in the pathogenesis of inflammatory periodontal disease, leucocyte defects including impaired chemotaxis and low bactericidal activity[22] may be closely involved in the etiology of periodontal disease in the CH syndrome. However, the disorder is not be limited to leucocytes, but might be more generalized, involving other cell types[5]. Oliver et al.[21] have reported that defective cyclic GMP could be related to the formation of giant granules in fibroblasts from CH mice, suggesting a defect in microtubule assembly in these cells. Fibroblasts are well known as a site of collagen formation in connective tissues. The results presented here suggest that reduced collagen biosynthesis in oral tissues in the CH state may be involved in the increased incidence and severity of periodontal disease in this disorder.

Table 1. Collagen and non-collagen protein synthesis in palatal mucosa of normal and CH mice

	Non-collagen protein ($\times 10^{-5}$ dpm/h/mg protein)	Collagen ($\times 10^{-3}$ dpm/h/mg protein)	Ratio of collagen to total protein (%)
Normal mice	1.84 ± 0.54	1.31 ± 0.03	0.71 ± 0.03
CH mice	1.86 ± 0.77	0.98 ± 0.04**	0.52 ± 0.01**

Each value shows the mean ± SE of 5 animals; ** $p < 0.01$ vs normal control.

Table 2. Prolyl hydroxylase activity in palatal mucosa of normal and CH mice

	Activity (dpm/h/mg tissue)	(dpm/h/mg protein)
Normal mice	122 ± 13	3560 ± 370
CH mice	88 ± 4*	2450 ± 114*

Each value shows the mean ± SE of 5 animals; * $p < 0.05$ vs normal control.

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Adenosine uptake in pyrimidine 5′-nucleotidase deficient human erythrocytes via a high affinity transport system

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Summary. Using a pulse-labeling technique, ^{14}C-adenosine uptake into pyrimidine 5′-nucleotidase (P5N) deficient erythrocytes (RBC) was found to be impaired. The Lineweaver-Burk plot showed K_m values of 2.0×10^{-3} mM and 0.2×10^{-3} mM for normal RBC and P5N deficient RBC, respectively. These results indicate that P5N is one of regulators of the adenosine transport system and/or is associated with adenosine carrier protein.
Key words. Erythrocytes; pyrimidine-5′-nucleotidase; ^{14}C-adenosine uptake.

Valentine et al.[1] have described a chronic hereditary hemolytic anemia with a deficiency of pyrimidine 5′-nucleotidase (P5N) and with an accumulation of glutathione and total nucleotides. However, the mechanism(s) of the accumulation of total nucleotides is still unclear, even though some characteristics of variant P5N in human RBC were demonstrated[2-7]. The uptake and transport of adenosine have been studied in various cell types[8-19], revealing some of the uptake mechanisms. Even though adenosine can be phosphorylated by adenosine kinase and can be deaminated by adenosine deaminase, its transport was not inhibited by the addition of Erythro-9-(2-hydroxy-3-nonyl) adenine, a potent adenosine deaminase inhibitor, to neuroblastoma cells deficient in adenosine kinase[10]. In cultured P388 murine leukemia cells, however, adenosine deaminase and nucleoside phosphorylase were found to have no role in adenosine transport; the adenosine deaminase inhibitor 2-deoxycoformycin (2dCF), which markedly inhibits adenosine phosphorylation and its deamination, had no effect on adenosine transport[14]. On the other hand, compared to normal fibroblasts, cultured nucleoside phosphorylase deficient cells could incorporate ony 2% and 4% of ^{14}C-inosine and ^{3}H-guanosine, respecitively[20].

All of these various mechanisms of nucleoside transport suggest important differences between the variant enzyme and the normal enzyme with or without the presence of inhibitor in the adenosine transport system. On the other hand, adenosine deaminase is associated with the nucleoside transport system in the human RBC membrane[21]. Kraupp et al.[12] have suggested the existence of an 'adenosine carrier protein' in the erythrocyte membrane, associated with P5N. If such a protein is present, it might be very valuable to investigate the molecular basis of adenosine uptake by this 'carrier protein' in RBC where variant P5N is present, in order to clarify the relationship between the 'carrier protein' and the enzyme.

Materials and methods. P5N deficient RBC. Leukocytes and platelet-rich plasma were removed by centrifugation from heparinized blood drawn from patients of two families[22-24] with P5N deficient hemolytic anemia. RBC were washed three times with saline and suspended in 10 mM-Tris HCl-0.15 M-NaCl (pH 7.4) at a cell concentration of 10^7/ml. All experiments were performed within 2–4 h after drawing the blood and within a period of 1–3 h after washing with saline. Reticulocytes comprised 0.2% of the normal RBC and 7.2% of the P5N deficient RBC. The RBC suspensions from a patient with hereditary spherocytosis whose reticulocytes were from 0.9 to 5.0% were also used as a control.

^{14}C-adenosine uptake to RBC. In ^{14}C-adenosine uptake studies[18], the RBC suspension was warmed to 37 °C for 5 min, and ^{14}C-adenosine (7.5 n mole) was then added to the suspension and rapidly mixed. After incubation at 37 °C for various time intervals, 0.17 ml of RBC suspension was layered into an Eppendorf microfuge tube containing 0.04 ml of 10% perchloric acid as the bottom layer, and 0.17 ml of layer of mixed oil (corn oil:n-butyl phthalate, 3:10). The tube was centrifuged at 15,000 rpm for 30 sec using an Eppendorf microfuge. Cellular material (15 ml) which passed through the oil layer and into the acid layer was removed and the radioactivity was determined in a scintillation counter.

(8-^{14}C) Adenosine was purchased from Amersham, all nucleotides were from Sigma Chemical Co., St. Louis, Mo., and all other chemicals were from Wako Pure Chemical Co., Japan.

Results. The time course of adenosine uptake by RBC is shown in figure 1. The maximal peak of the uptake was from 7 to 9 min in normal human erythrocytes. On the other hand, adenosine uptake was reduced in P5N deficient RBC with a shift of the maximal peak to the left and an asymmetrical curve. The total adenosine uptake of P5N deficient RBC was 31.7% that of normal RBC. These results indicate that the adenosine carrier system(s) is affected by P5N and the enzyme may be one of the regulator(s) of the adenosine transport system. Figure 2 represents the adenosine uptake of normal and P5N deficient RBC; both cell groups showed hyperbolic curves. The