Age-Related Fluorescence in Rat Lung Collagen

M. J. Bellmunt, M. Portero, R. Pamplona, M. Muntaner, and J. Prat

1Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina, Universitat de Lleida, c/ Rovira Roure, 44 (Hospital Provincial), E-25198 Lleida, Spain, 2Departament de Fisiologia i Biologia Cel·lular, Facultat de Medicina, Universitat Autònoma de Barcelona

Abstract. Mechanical lung properties are impaired with age. In other organs an age-related increase in collagen-linked fluorescence, attributable to advanced glycation endproducts (AGE), or other nonenzymatic reactions such as those related to lipid peroxidation derivatives has been described. Moreover, oxidative processes accelerate some of these reactions. In several tissues, these AGE products have been found to be responsible for protein cross-linking and lack of elasticity. We have evaluated the fluorescence levels of lung collagen in rats aged from 1 to 25 months at two distinct wavelengths: the standard AGE fluorescence (Exc 370 nm/Em 440 nm) and the pentosidine fluorescence (Exc 335 nm/Em 395 nm). In pulmonary tissue, fluorescence at both 370/440 nm ($p < 0.05$) and 335/395 nm ($p < 0.001$) increases with age. However, a relative stabilization of values is seen in the 25 months group that could be related to the kinetics of fluorescent products in vivo. So, as observed in other tissues, AGE products may increase in pulmonary tissues with time. This may explain the age-associated decline in pulmonary compliance.

Key words: Rat lung—Fluorescence—Collagen—Aging—Glycation.

Introduction

Functional studies of the volume-tension ratio demonstrate that lung elasticity decreases with age [19, 36]. In lung strips, as in other tissues, the ratio tension-length increases with age.

As demonstrated in other collagenous structures, this age-related process may be due to the accumulation of glucose-derived collagen bonds that are...
formed in connective tissue via the Maillard reaction (Advanced Glycation Endproducts, AGE). As Maillard established in 1912, glucose reacts nonenzymatically with the free amino groups of proteins (the glycation reaction). The very complex pathways subsequent to this reaction were extensively studied in food and agricultural sciences.

Since 1976, when Bunn [5] found that the initial steps of the Maillard reaction occur in human tissues, an effort has been made to detect and quantify the early and advanced glycation products in different human specimens. As the glycation reaction is time- and glucose-dependent, the focus of this research is to explain age- and diabetes-induced pathological changes, as well as to solve if (or which) diabetic and aging complications may be explained by the Maillard reaction [6, 22, 24, 26].

Chemical studies have lead to the discovery of the structure of some AGE, such as pentosidine or pyrraline. Some AGE products are fluorescent, and for this reason, they have usually been evaluated by measuring fluorescence levels [1, 9, 15, 20, 28, 30–32]. AGE products are synthesized in stable protein matrices by nonenzymatic polymerization of initial glucose-protein adducts with sugars and their derivatives [16]. These reactions lead to the formation of protein cross-linking structures. Moreover, oxygen radicals seem to play an important role in this process, at least in the formation of fluorescent polymers [13]. Thus, we suspected that lung structures must be rich in fluorescent products. On the other hand, there is no evidence supporting a cross-linking role for the lipid peroxidation-derived fluorescent products.

Functional studies have shown that AGE products impair several protein functions, mainly by altering its elasticity and other properties. Several authors assume that this impairment is among the basis of diabetic complications [4] and age-related changes. Due to their long, mean life and their relative abundance in tissues typically altered in aged and diabetic patients, glycation of collagenous proteins has been studied in several tissues, in human or in rat, such as intervertebral disk, skin, aorta, and tail tendon [1, 15, 25, 28]. In most cases, important dysfunction has been demonstrated, at both molecular [3] and organic [18] levels. Moreover, these changes were found to be age-related [30] and increased in diabetics [9].

We have studied the occurrence of fluorescence in pulmonary collagen at two typical wavelengths and the relationship between this fluorescence and age in healthy rats.

Materials and Methods

All chemicals were of reagent grade. Collagenase type Ia was purchased from Sigma, St. Louis, MO, USA.

Animal Surgery and Tissue Extraction

Twenty fresh lungs of Sprague-Dawley rats aged 1, 7, 13, 19, and 25 months (4 lungs per time period) were obtained by standard surgical methods after decapitation of the animals under ether anaesthesia.