Pathologie Biologie 63 (2015) 24–31

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Review

A genetic anomaly of oriented collagen biosynthesis and cross-linking: Keratoconus

Une anomalie génétique de la biosynthèse orientée du collagène et de son pontage : le kératocone

J.L. Bourges [a,b], A.M. Robert [b,c], L. Robert [b,*,c]

[a] *Centre d'ophtalmologie HUPC, hôpital Hôtel-Dieu, Assistance publique–Hôpitaux de Paris, 1, place du Parvis-Notre-Dame, 75181 Paris cedex 04, France*
[b] *Faculté de médicine Paris Descartes, université Sorbonne Paris Cité, 15, rue de l'École-de-Médecine, 75006 Paris, France*
[c] *Laboratoire de recherche ophtalmologique, hôpital Hôtel-Dieu, Assistance publique–Hôpitaux de Paris, 1, place du Parvis-Notre-Dame, 75181 Paris cedex 04, France*

ARTICLE INFO

Article history:
Received 23 June 2014
Accepted 20 October 2014
Available online 4 November 2014

Keywords:
Keratoconus
Cross-linking
Riboflavin-UV-mediation
CXL
Collagen
Cornea

Mots clés :
Kératocône
Pontage
Stimulation par riboflavine et UV
CXL
Collagène
Cornée

ABSTRACT

Oriented collagen biosynthesis is one of the major mechanisms involved in tissue and organ formation during development. Corneal biogenesis is one example. Defects in this process lead to anomalies in tissue structure and function. The transparency of cornea and its achievement are a good example as well as its pathological modifications. Keratoconus is one example of this type of pathologies, involving also inappropriate cross-linking of collagen fibers. Among the tentatives to correct this anomaly, the riboflavin-potentiated UV-cross-linking (CXL) of keratoconus corneas appears clinically satisfactory, although none of the experiments and clinical results published prove effective cross-linking. The published results are reviewed in this article.

RÉSUMÉ

La biosynthèse orientée des fibres de collagène est un des mécanismes de la biogenèse des tissus et organes et en particulier de la cornée. Des anomalies génétiques de ce processus sont à l'origine de pathologies variées, tel le keratocône. La faiblesse et fragilité de ces cornées résultent de l'insuffisance du pontage des fibres de collagène. Le traitement par UV et riboflavine (CXL) tente d'y remédier. Une critique de cette littérature et du procédé est présentée dans cet article.

1. Introduction

Keratoconus is a corneal disease that progressively induces conic deformation of the cornea, as a result of stromal thinning associated with biomechanical impairment. This disease is known to begin rather early, about teenage, and develops progressively to spontaneously stabilize by the end of the third decade. An original method was introduced some years ago for the treatment of this disease, which consists of stabilizing the corneal shape by riboflavin-potentiated UVA-irradiation (CXL) in order to increase cross-linking of collagen in the corneal stroma [1]. It is now widely accepted that CXL is able to stop or to slow down the evolution of keratoconus. Still the pertinent literature describes no direct biochemical proofs in favour of this mechanism of increased cross-linking. It seems therefore important to evaluate critically the literature of CXL to better understand the corneal modifications induced by this procedure. We will first shortly summarize the

Abbreviations: Col, collagen; col I, collagen type I and so on (There are about 29 to 30 different collagen types, only in vertebrates); LOX, lysyl oxidase; LXL, lysyloxidase-like proteins; ROS, reactive oxygen species, comprising free radicals as superoxide or hydrogen peroxide, etc.; ECM, extracellular matrix; MR, Maillard reaction; AGE, advanced glycation endproducts; GAG, glycosaminoglycan; PG, proteoglycan; Hyal, hyaluronan; CS4, chondroitin-4-sulfate; CS6, chondroitin-6-sulfate; KS, keratan sulfate; DS, dermatan sulfate; CXL, riboflavin-potentiated cross-linking of corneal collagens.

* Corresponding author.
E-mail address: lrobert5@orange.fr (L. Robert).

http://dx.doi.org/10.1016/j.patbio.2014.10.004
0369-8114/

physiological mechanisms of collagen biogenesis and cross-linking, as well as some of their pathological alterations, and finally we will propose a succinct review of the possible mechanisms of CXL action.

2. Normal collagens of the intercellular matrix

2.1. Physiological cross-linking of collagen

Collagens are the major protein-constituents of animal bodies, all through phylogenesis from sponges to humans. About one third of all proteins of the body are collagens. Genes coding for the different α-chains (3 for each molecule) can be different or identical, resulting in a variety of collagen types [2]. Several of these collagen types are present in the human cornea and constitute its transparent and resistant matrix [3]. The mechanical resistance of the corneal stroma is dependent on the vectorial synthesis of collagen fibers during development [4]. It also depends on their interactions with glycosaminoglycans (GAG-s) as hyaluronan (HA) and proteoglycans (PG-s) carrying a variety of GAG-chains such as chondroïtin-4-sulfate (C4S), chondroitin-6-sulfate (C6S), keratan sulfate (KS), and dermatan sulfate (DS). These interactions, covalent cross-linking of collagens and non-covalent interactions between collagen fibers and PG-s and GAG-s are the major mechanisms of corneal matrix biogenesis.

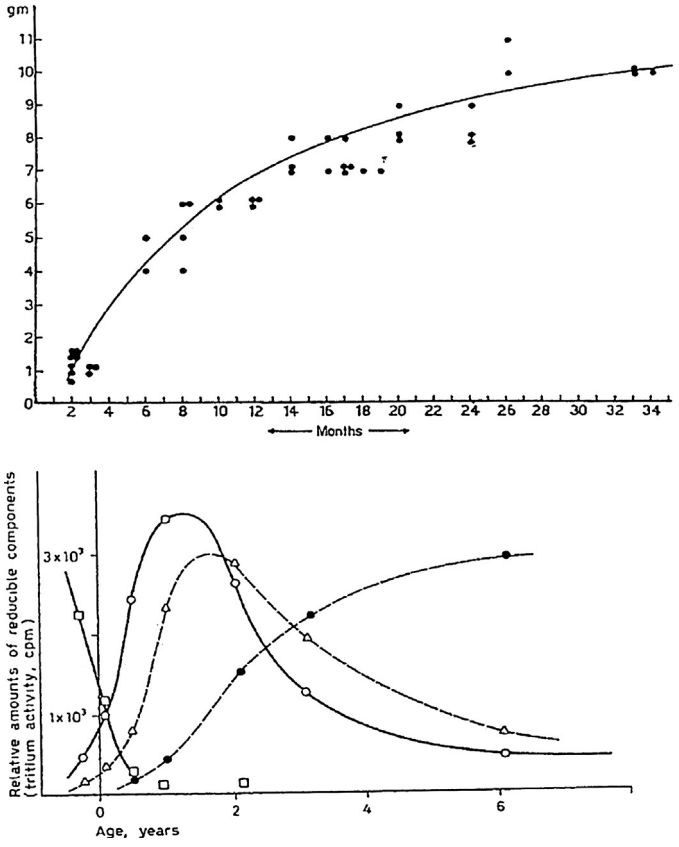

Fig. 1. Age-dependent variation of collagen cross-linking. Lower curve: physiological cross-linking catalyzed by lysyloxydase (LOX) after Bailey and Robbins [5]. Notice the successive increase and decrease of cross-linking amino-acids. The continuous curve (●–●) designated "hexitol lysines" represents the Maillard reaction mediated cross-linking, similar to the upper curve.
From Verzar's experiments showing the age-dependent cross-linking of rat tail tendon collagen, attributed to the Maillard reaction [20–22].

2.2. Covalent collagen cross-linking

Covalent cross-linking is a long and complex process summarized by Allen Bailey in 1973 [5]. As shown of Fig. 1 (lower part), several LOX-catalysed cross-link-amino-acids are formed sequentially, mostly from modified lysine residues. Some of these increase early during development and decrease later to be replaced by more stable compounds (Fig. 1). These are the only physiological covalent cross-linking reactions catalysed by LOX-type enzymes. Reaction details vary from tissue to tissue and were more or less completely described during the 7th and 8th decades of the last century. In the cornea, different types of collagen can variably cross-link with each other to form fibers. Most fibers comprise collagen types I, III and V. Quantitatively, type I is the most important constituent. The ratio of constituents changes with tissue-specificity, with age and diseases. Cornea is a special tissue insofar as it is rich in type VI collagen, the structure of which is very different compared to the above-mentioned fibrous collagens [2,6]. The precise relation of collagen VI to the above-mentioned fibrous collagens is not yet definitively established. As however this type of collagen makes up about 20% of total corneal collagens, there is no doubt that it must play an important role. At the tissue level; collagen fibers are synthesized spatially oriented according to a vectorial mechanism, investigated and described when the different types of collagens were not yet known [4]. Some types of collagen, such as type VII, are involved in the fastening of the stroma to the epithelial and endothelial basement membranes (Bowman's and Descemet membranes), and participate also in the anchoring of the basement membranes to the matrix [7,8].

2.3. Non-covalent cross-linking by interaction with PG-s and GAG-s

As important as the covalent cross-links are the non-covalent cross-links between PG-s, GAG-s and collagen fibers. These interactions were shown by John Scott in Manchester, GB to play a crucial role in the spacing of collagen fibers and their orientation [9]. PG-s are macromolecules containing a central protein with variable number of GAG-chains attached to it (Fig. 2). As shown by electron microscopy (TEM), the GAG-chains are associated with the collagen fibers, their length determining the space between the fibers (Fig. 3) [6,7]. Scott also described the exact site of association of PG-GAG-chains and the protein core with collagen fibers [9–12]. During fibrillogenesis, collagen molecules are aligned with a small shift to the next molecule. These shifts are shown as alternating white and dark bands by TEM (Figs. 4 and 5). Scott could identify the exact site on the collagen fiber where DS- and KS-chains interact as shown on Figs. 4 and 5. Although non-covalent cross-links are weaker than covalent bonds, their number per collagen fiber is so high (Figs. 4 and 5) that they represent a solid, tight linkage between collagen fibers mediated by PG-s and GAG-s. This means that both covalent and non-covalent cross-linking of collagen fibers participate in the functional biomechanics of the cornea.

3. Collagens are constantly turned over in the cornea

Cornea is not a static tissue. Its macromolecular constituents are constantly renewed, mainly by the keratocytes, phenotypically highly specialized fibroblast-like cells, located and interconnected in the corneal stroma. These cells follow a program of extracellular matrix (ECM) biosynthesis and renew continuously matrix constituents. They are responsible for the oriented (vectorial) biosynthesis of collagen fibers in plywood-type of sheets with rotating orientation, taking place essentially during embryonic development [4]. This is suggested also by the close contact of