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Biological effects of hyaluronan in connective tissues, eye, skin, venous wall. Role in aging

Effets biologiques de l'hyaluronane dans les tissus conjonctifs, l'œil, la peau, la paroi veineuse. Rôle dans le vieillissement

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ABSTRACT

Hyaluronan, as most macromolecules of the extracellular matrix, are produced by the differentiated mesenchymal cells. These cells produce also enzymes degrading hyaluronan. This results in the presence of several hyaluronan pools of different molecular weights, all capable of interacting with surrounding cells, mediated by hyaluronan binding proteins and receptors. These interactions modulate cell phenotype and produce a variety of effects conditioning the specific functions of tissues. We shall discuss here several examples studied in our laboratory, concerning skin, cornea and the venous wall. Some of these actions might even be harmful, and could play an important role in aging of connective tissues with loss of function. Some of these age-dependent modifications mediated by hyaluronan will be reviewed and commented, especially the upregulation of matrix degrading enzymes as MMP-2 and MMP-9. We shall also mention some of our experiments for finding molecules capable of counteracting the harmful effects mediated by hyaluronan.

RÉSUMÉ

L'hyaluronane, comme la plupart des macromolécules de la matrice extracellulaire, est produite par les cellules mésenchymateuses. Ces mêmes cellules produisent aussi des enzymes capables de la dégrader. Ainsi, l'hyaluronane est présente à des masses moléculaires distinctes dans la matrice intercellulaire. Toutes ces formes de ce polysaccharide sont capables d'interagir avec les cellules qui la produisent, interactions médiées par des récepteurs. Ces interactions produisent des effets variés, qui jouent des rôles importants dans les fonctions spécifiques de ces tissus. Nous allons discuter quelques exemples étudiés dans notre laboratoire, la cornée, la peau et la paroi veineuse. Certaines de ces réactions peuvent être nuisibles et contribuer au cours du vieillissement à des pertes de fonctions. Nous allons décrire certaines de ces réactions et, en particulier, l'augmentation de l'expression de protéases pouvant dégrader la matrice extracellulaire, en particulier les MMP-2 et MMP-9. Nous allons mentionner aussi certaines de nos études conduites dans le but de trouver des molécules pouvant freiner ces actions nuisibles médiées par l'hyaluronane.

1. Abbreviations

GAG glycosaminoglycans (*glycosaminoglycannes*)
HA hyaluronan (*hyaluronane*)
GF growth factor (*facteur de croissance*)
C4S chondroitine-4-sulphate (*chondroitine-4-sulfate*)
C6S chondroitine-6-sulphate (*chondroitine-6-sulfate*
DS dermatan sulphate (*dermatane sulfate*)
HS heparan sulphate (*heparane sulfate*)

2. Introduction

HA is the most ubiquitous polysaccharide (glycosaminoglycan) in vertebrate connective tissues. Its local production by differentiated mesenchymal cells was demonstrated by several authors since the first description of its chemical structure by Karl Meyer [1]. Its huge literature was summarised by several authors [2–5]. Several of its main properties are related to its high molecular

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weight and its capacity to retain a high amount of water in its interstices. This is particularly the case for the native polysaccharide produced at a molecular weight of several times 10^6 Da. It has to be reminded, however, that the same cells which produce HA do also release hyaluronidases degrading the polysaccharide to lower molecular weight oligosaccharides [6]. As a result of the regulation of its biosynthesis and degradation, HA in cell cultures, tissue explant cultures or extracted from native tissues is present at several, relatively well-defined molecular weight classes, as shown for instance by the experiments realised by Tammi et al. (see Fig. 1, p. 17 in [2]). Skin contains about half of the total amount of HA of the human body, estimated to about 5 g [2]. A number of authors published estimates of human skin HA with however a wide range of values, between less than 200 to more than 600 μg/g wet weight (see Table 1 of [2]). Most reports agree about an age-dependent decrease of HA-content of the skin [7–10]. These results have to be evaluated with some caution because of the large differences in HA-content at different sites of the skin. The age-dependent decrease of HA concentration appears to vary also according to the anatomical site of the skin. The strongest decrease concerns the mesenchymal condensations below growing hair bulbs, as shown by Underhill [11] using histochemical methods. There is little doubt that the different methods used by different laboratories to isolate and determine HA from tissues might well be at the origin of the different results reported. This is true also for the age-dependent variations of HA-content of the skin of different species. Miyamoto and Nagase [12] described the age-dependent variation of HA molecular weight extracted from rat skin. The results reported showed a three-phase variation of average molecular weight with age: 39×10^4 in fetuses and up to the age of one week, 55×10^4 up to 26 weeks, and 87×10^4 at 52 weeks of age. Shimada and Matsumura [13] studied the molecular weight of HA in rabbit skin and found values of $1{,}6 \times 10^5$ to $1{,}3 \times 10^6$. The age-dependent variation of HA in human skin was studied by Meyer and Stern [14]. They found no significant variation of the HA content with age from 28 weeks to 88 years. The values found in fetal and adult skin and senescent skin did not show significant variation. There was however an age-dependent decrease of extractability of HA from the skin samples. The HA content of the papain-digests of the TritonX-100 and guanidine-extracted skin samples showed progressive increase with age, from 1,0 μg/mg protein extract from fetal skin to 2,7 μg/mg protein in adult, and 3,4 μg/mg protein in senile skin extracts [14]. Apparently most of this increase occurs between the fetal and adult stages. The relative constancy of total HA in human skin at increasing ages as reported by these authors [14] does not exclude variations of HA distribution in the different skin layers. With increasing age the HA content of the epidermis is decreasing as shown by histochemistry [14]. There is a concomitant increase in the upper, papillary dermis and at the dermo-epidermal basement membrane. One should however take in account the age-dependent loss of skin tissue, estimated by morphometry on biopsies from the sun-protected upper-inner arm, to represent on the average a loss of 7% / decade of the original (extrapolated to 0 age) skin thickness [15]. This tissue-loss of about 50% of the original HA-content of total skin tissue at 70 years of age is important, even at constant HA concentration, with however large individual variations [15]. The loss of HA with loss of skin tissue is not really compensated by the above calculated age-dependent constancy of HA, as reported in [14], because skin tissue loss is equivalent to HA loss also. The increasing fixation of HA to proteins which doubles between the fetal and adult (or senile) stages, according to Meyer and Stern [14] might well be the result of a free radical mediated reaction, cleavage of the ring structure of uronic acid and/or of hexosamine and formation of covalent bonds between the remaining HA-chain, formation of aldehyde groups reacting with amino-groups (ε-amine of lysine) on proteins. Other possibilities are also available.

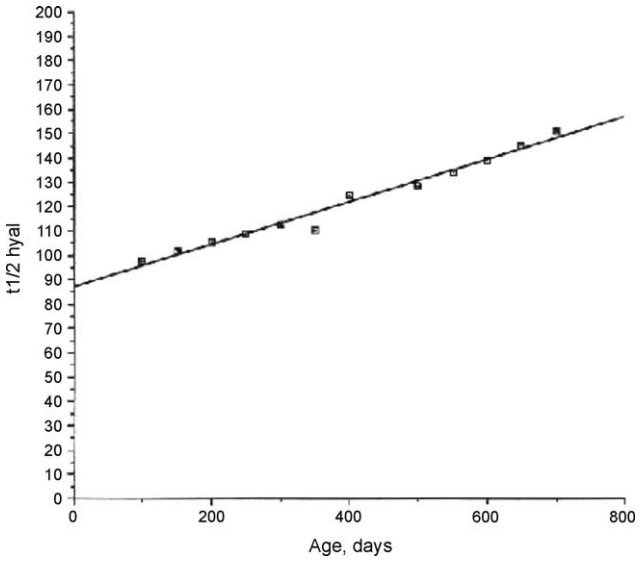

Fig. 1. Half-life of hyaluronan in skin of mice as a function of age. Abscissa: age in days; ordinates: calculated half-life.Figure 1 drawn from Table 2 [16].

It appears that even the data of [14] do not exclude quantitative age-dependent loss in HA-content of the skin.

Another important argument for major age-dependent modifications of skin-HA can be found in the publication of Sobel [16]. Table 2 of this note presents half-life values of mouse-skin HA. These data were used to construct Fig. 1, which clearly shows an age-dependent increase in half-life of HA in this tissue. This finding, if validated for human skin also, is in line with data accumulated since the original publications of the Gershons [17] showing an age-dependent decrease of the turnover of cellular and extracellular proteins. Such processes might well be differentially regulated in the successive layers of the epidermis and dermis and deserve more studies to be confirmed.

Unfortunately less data are available for the age-dependent modifications of HA in other tissues, such as cornea or the vessel-wall. Author of new, reliable methods for HA determination, Ulla Laurent [18] published values for bovine cornea, found in the range of 1,0 to 1,6 μg/g wet tissue. The same author, Ulla Laurent, determined the HA-content of the aqueous humour of human eye, using the above-mentioned radio-immuno-essay [19]. The value reported for 47 samples was $1{,}14 \pm 0{,}46$ μg/g, comparable to the above value.

These data are comparable to those found for bovine cornea and might support an equilibrium between the HA content of the cornea and the aqueous humour.

Several authors studied the ultrastructural distribution of HA in the cornea, as well as of its main receptor, CD44. Eggli and Graber [20] found in rat cornea HA localised to epithelial and endothelial plasma membranes, both at the intra- and extracellular aspects of the membranes. Similar localisation was found on stromal keratocytes. Basal epithelial cells showed a faint labelling, whereas wing- and superficial layers were intensely stained. Endothelial cells were labelled essentially at the apical and lateral membranes. Asari et al. [21] carried out similar studies on rabbit cornea, including, besides HA and CD44, also labelling chondroitine-sulfates. Using unfixed sections, these authors found similar localisation as the preceding authors, mainly on cell-plasma membranes and only a faint labelling in the extracellular stroma. Chondroitine-sulfates (CS) were intensely stained in the epithelial layer and the stroma and weakly at the endothelial layer. CD44 was