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Original article

Pharmacology of skin aging. Stimulation of glycosaminoglycan biosynthesis by L-fucose and fucose-rich polysaccharides, effect of in vitro aging of fibroblasts

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Abstract

The effect of L-fucose and fucose-rich polysaccharides (FROP-s [Biomed. Pharmacother., 2003; 57: 187–94]) was investigated, using human skin fibroblast cultures at several passages. The cells were incubated with radioactive glucosamine for 24 h, followed by the determination of individual glycosaminoglycans (GAG-s) by selective hydrolysis using specific enzymes. The effect of L-fucose and of FROP-3 [Biomed. Pharmacother., in 2003; 57: 187–94], both at 1 and 10 μg/ml, added to the culture medium, was investigated. L-Fucose stimulated the incorporation of the tracer in heparan sulfates by fibroblasts at the ninth passage by 20%. FROP-3 stimulated incorporation in keratan sulfates by 45% by fibroblasts of the fifth passage. This effect was identical at both concentrations tested. For fibroblasts at the ninth passage FROP-3 stimulated incorporation in dermatan sulfate. This effect was dose dependent of the order of +67% at 1 μg/ml and +128% at 10 μg/ml. Incorporation in hyaluronan was also stimulated by about +27%. These stimulations of GAG-biosynthesis might play a role in the increase of total skin thickness of hairless rats treated with L-fucose, as well as in several other favorable results recorded for FROP-3 such as the increased hydration (resistance to pressure) and elasticity of human skin (Robert C, Robert AM, Robert L).

Keywords: L-Fucose; Fucose-rich polysaccharide; Matrix biosynthesis; Glycosaminoglycan; Hyaluronan; Keratan sulfate; Dermatan sulfate; Heparan sulfate

1. Introduction

Proteoglycans (PG-s) and glycosaminoglycans (GAG-s) play important biological roles in tissue structure and function [1,12]. A large number of proteins were identified, which are modified by the fixation of GAG chains in variable numbers, from one to more than a hundred, according to the protein involved, the tissue considered, with variations as a function of age and pathologies [1,6,8,12]. The selective biosynthesis of some PG-s and GAG-s is a characteristic of the state of differentiation of cells in specific tissues. Some PG-s and GAG-s were shown to be involved in the regulation of collagen fiber deposition, orientation and the regulation of fiber size [9,10]. We have shown previously that L-fucose and fucose-rich polysaccharides (FROP-s) influence several of the cell and tissue reactions, which are known to undergo age-dependent modifications. Such effects are the down regulation of MMP-2 and MMP-9 biosynthesis and activation [3]. Other effects recorded for L-fucose and FROP-3 were the improved collagen fibrillogenesis [6] and an increase of elastin biosynthesis in vivo, in L-fucose treated hairless rat skin, and in vitro by adding L-fucose or FROP-3 to human skin fibroblast cultures [7]. It was, therefore, indicated to explore the effect of L-fucose and FROP-3 on GAG-biosynthesis by human skin fibroblasts. These experiments will be described.

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Table 1
Effect of FROP-3 on GAG biosynthesis by fibroblasts at the ninth passage. Concentration of FROP-3 is given in μg/ml, the stimulation of the biosynthesis as percentage of the control value. Statistical significance: * $P < 0.01$; ** $P < 0.001$

GAG studied	Concentration of FROP-3 (μg/ml)	Percentage stimulation and percentage of total GAG-s	Concentration of FROP-3 (μg/ml)	Percentage stimulation and percentage of total GAG-s	*P*
Keratan sulfate (KS)	1	+44	10	+45	*
KS % of total GAG-s		+56		+53	*
Dermatan sulfate (DS)	1	+67	10	+128	**
DS % of total GAG-s		+72		+87	**

2. Materials and methods

2.1. Cells and culture conditions

Fibroblasts were obtained from the skin of an 8-year-old boy (otoplasty) and cultured in DMEM–Glutamax medium with 10% fetal calf serum (FCS) and 1% penicillin, streptomycin and fungizone, as described previously [2,5]. The cells were synchronized by keeping them for 3 h in a serum free medium, followed by adding complete medium with or without L-fucose or FROP-3 at 1 or 10 μg/ml, as indicated in Section 3. Then the tracer, [^{3}H]-glucosamine HCl (Amersham), was added. and incubation continued for 2 h in a 5% CO_2/air thermostat at 37 °C. Fibroblasts at several passages were used as indicated in Section 3. After incubation the medium and cells were separated, excess label eliminated by dialysis in presence of excess unlabelled glucosamine and gel filtration on a G25 Sephadex column and, for the cell layer, by repeated washings with PBS containing 1% glucosamine.

2.2. Separation of GAG-s

Aliquots of the concentrated culture media (by lyophylization) and the cell layer resuspended by sonication in PBS, were incubated with specific endo-glycosidases, as previously described [4]. The degraded GAG-s were recovered and radioactivity determined as described [2,4]. Hyaluronidase from *Streptomyces*, chondroitinase ABC and AC from *Proteus vulgaris*, dermatanase from *Flavobacterium*, heparinase, keratanase from *Pseudomonas* were used, all from Sigma. Heparan sulfate was chemically degraded, using nitric acid as described by Shievley and Conrad [11]. All other details of these procedure were identical to those described in our previous experiments [2–5–7].

2.3. Chemicals

L-Fucose was from Sigma; the preparation of FROP-3 was described [5]. All other chemicals were of the best grade available.

Statistical evaluation was done with the distribution free Mann and Whitney *U*-test.

3. Results

L-Fucose stimulated incorporation in heparan sulfate at the ninth passage of cells. This stimulation was of the order +20% at the lower concentration used, 1 μg/ml, and did not increase further at the higher concentration used, 10 μg/ml. At a later passage, the 29th, L-Fucose stimulated incorporation in heparan sulfate by +155% at 10 μg/ml and in keratin by +53% at 10 μg/ml.

Table 1 shows the effect of FROP-3 on the biosynthesis of GAG-s by human skin fibroblasts. The results were expressed as cpm per 10^6 cells, and given in percentage variation produced by L-fucose or FROP-3 as compared to the controls. It can be seen that the effect of FROP-3 was more pronounced than that of L-fucose. The stimulation of GAG-biosynthesis depended also on the passage number of the cells. For cells at the ninth passage FROP-3 stimulated by about 44% incorporation in keratan sulfate. This effect was about the same at both concentrations used. For cells at the ninth passage, stimulation by FROP-3 of glucosamine incorporation was stronger and dose-dependent in dermatan sulfate, 67% at the lower concentration, and 128% at the higher concentration. Hyaluronan biosynthesis was also stimulated by about 27% as well as total glycoconjugate biosynthesis by about 22%.

FROP-3 increased incorporation in total glycoconjugates (+44% at 1 μg/ml) and in chondroitine sulfates ABC (+41% at 1 μg/ml).

At even later passages, the 27th passage, close to the arrest of cell-proliferation, no more stimulation could be seen, incorporation in heparan sulfate was slightly inhibited, both by L-fucose and FROP-3 (not shown).

Another way to compare results is to represent the distribution of label in total GAG-s and calculate the percentage of label in individual GAG-s related to this sum. Fig. 1 shows this percentage distribution for the control cultures with no

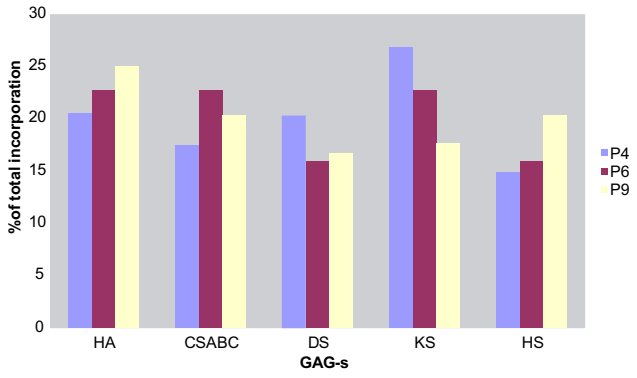

Fig. 1. Distribution of label in the separated GAG fractions in the successive passages of keratocytes. Averages of six parallel experiments.

additions, at the fourth, sixth and ninth passages. Hyaluronan is a major component and represents more than 20% of total label incorporated in GAG-s. Chondroitine sulfates are second with about 20%, followed by heparan and keratan sulfates.

Significant modifications were produced in this distribution by FROP-3 at the ninth passage by increasing label in dermatan sulfate from 13% in the control cultures to 22% and 24% with 1 and 10 μg/ml of the polysaccharide, respectively. At the 13th passage and at 10 μg/ml FROP-3 significantly increased the percentage of hyaluronan, from 56% to 61%, chondroitine sulfates A, B and C from 54% to 62%, and of dermatan sulfate from 8% to 20%. No other significant modifications were seen at other passages.

4. Discussion

The reported results show that both L-fucose and FROP-3 stimulated GAG-biosynthesis. The fucose-rich polysaccharide, FROP-3 was more efficient than L-fucose. The stimulation was dependent on passage number and differed also between the monosaccharide and the polysaccharide. Of particular interest is the effect of FROP-3 on dermatan sulfate biosynthesis. This GAG is one of those involved in the regulation of collagen fibrillogenesis [9,10]. The stimulation of heparan sulfate biosynthesis by L-fucose may be involved in the stimulation of cell proliferation, found both for L-fucose and for FROP-3 [5]. Of particular interest also is the stimulation by FROP-3 of total glycoconjugate biosynthesis. This may be related to the rapid intranuclear penetration of this polysaccharide when added to fibroblast cell cultures [5]. We hypothesized that at this site the polysaccharide might interfere with the regulation of gene-expression. GAG and glycoconjugate biosynthesis is dependent on the coordinate regulation of a number of enzyme systems responsible for the synthesis of the nucleotide–sugar precursors and specific glycosyl- and sulfo-transferases involved in total glycoconjugate and GAG-biosynthesis. As, however, the cells undergo more population doublings and get closer to proliferation arrest (the Hayflick-limit), no more stimulation could be seen, neither with L-fucose, nor with FROP-3. Instead, a slight inhibition, of the order of 20–30% could be observed both for heparan sulfate and for keratan sulfate. There is little doubt that the observed effects may be involved in some of the previously observed effects of L-fucose and of FROP-3 on the human skin in vivo (Robert C, Robert AM, Robert L, in press in J. Cosmethol Dermatol), and on fibroblasts in vitro [3,5–7]. The stimulating effect is, however, dependent on the population-doublings the cells underwent before the experiments and is no more observed at very late passages.

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