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Iduronic Acid-Rich Proteoglycans and Post-burn Scar Maturation: Isolation and Characterization

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Remodeling of human burn tissue involving loss of dermis results in different types of scarring. Iduronic acid-rich proteoglycans (PGs) are important macromolecules present in skin. Close association of these molecules with collagen suggests that PGs influence collagen fibril formation. A recent study found that the core protein of PG interacts with collagen fibrils (R. Fleischmajer *et al., J. Struct. Biol.* 106 (1991) 82–90). Changes in PG structure when compared to normal skin have been reported by us (*J. Invest. Dermatol.* 84 (1985) 527-531). To understand the role of PGs in scarring, PGs from normal and hypertrophic scars at two different stages of maturation after burn injury (i.e. 2–5 yrs: changing, unstable and immature and 5+ yrs: unchanging, stable and mature) have been isolated (with 4M guanidinium chloride) and characterized. The extracts were fractionated by ion exchange chromatography, followed by ethanol precipitation. The size of PGs decreased with maturation of the scars. The Mr values of PG protein cores of the mature hypertrophic scars (5+) and immature hypertrophic scars (2–5 yrs) were 22.6 and 25 kDa, respectively. The Mr values of mature normal scars and immature normal scars were 19 and 21 kDa, respectively. The iduronic acid and sulfate contents of PGs from both types of scar increased during the maturation phase. The amino-terminal amino acid sequences of all the PGs were similar to those of normal human skin or bone PGII (Decorin), i.e. (Asp-Glu-Ala-B-Gly-Pro-Glu-Val-Pro-Asp-Arg). PGs had the sulfate group mainly attached at C-4 of a *N*-acetylgalactosamine residue. This study suggests that alteration in PG structure occurs during the process of scar maturation (supported by research grant No. 15857 from Shriners Hospitals for Crippled Children of America).

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Nonreducing Termini of Aging Dermatan Sulfate

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The nonreducing ends of DS_{18} and DS_{28}, the major dermatan sulfates of adult human skin, change with aging. The effect of senescence on the nonreducing termini of these heteropolysaccharides, which were purified from a glycosaminoglycan pool by sequential precipitation with 18% and 28% ethanol, was investigated with the exoglycosidase enzymes, α-L-iduronidase β-glucuronidase and β-*N*-acetylhexosaminidase. The monosaccharides released by the action of these enzymes were quantified, respectively, by the orcinol, carbazole and Morgan-Elson methods in a microplate reader.

Though no sugar was removed from the nonreducing termini of dermatan sulfate (DS) with β-*N*-acetylhexosaminidase, under a variety of experimental conditions, uronic acid was present at these positions in both DS_{18} and DS_{28} of the ages investigated.

DS	Uronic acid, nmol/mg DS (L-IdUA)	(D-GlcUA)	Age (Years)
DS_{18}	27.7	–	33
	14.3	171.1	46
	11.3	113.1	76
DS_{28}	25.0	162.3	33
	18.3	56.2	46
	24.5	17.2	59

The data show that DS_{28} contains unsubstituted L-IdUA and D-GlcUA at its nonreducing termini at 33 years of age, while DS_{18} has L-IdUA. With senescence, these sugars may be replaced by different ones, or modified with groups which inhibit the exoglycosidase enzymes that specifically remove them. Thus, by age 76 years, L-IdUA and D-GlcUA at the nonreducing ends of DS_{18} drop by 59.2% and 34.0%, respectively. By 59 years of age, DS_{28} loses 89.4% of its D-GlcUA located at the nonreducing termini, while L-IdUA remains practically unchanged. The results suggest that GalNAc is either absent from the nonreducing ends of DS_{18} and DS_{28} of the ages examined, or modified with moieties that interfere with the action of β-*N*-acetylhexosaminidase. Furthermore, these findings confirm the polydispersity of DS.

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Comparison of Glycoprotein Synthesis by Aortic Explants from Control and Cholesterol-Fed Rabbits

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In vitro aorta explants culture model was adopted to follow structural glycoprotein synthesis on the basis of incorporated labelled precursors ^{35}S-sulfate [100 μCi] or ^{14}C-mannose [50 μCi].

The entire aortas were quickly removed from control and cholesterol-fed rabbits, aortas stripped of all extraneous tissues, cut into 2–3 mm segments and incubated as described previously.

Proteoglycans and other structural glycoproteins were released from the connective tissue fibrous proteins by extraction with 4 M Gdn-HCl and 0.5% CHAPS (a), by elastase digested (b), and finally the undigested residue of the tissue was hydrolyzed by 0.1 M NaOH, then was adjusted to pH 6.4 and digested by papain (c).

Proteoglycans from culture medium were precipitated of CPC in 0.03 M NaCl to a final concentration of 1% CPC. The ^{35}S-activity or 14C-activity were determined in a LKB Scintillation Spectrophotometer Model 1211 Racbete.

The present study demonstrated that the increase of glycoprotein synthesis and sulfation of proteoglycans secreted into experimental culture medium in comparison to control. However, incorporation of $^{35}SO_4$-sulfate as well as ^{14}C-mannose into the certain fractions from aortic tissue (e.g. after elastase digesting) was lessened in comparison to control group.