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Glycosaminoglycan-collagen interactions *in vitro* in the hydralazine-induced collagen disease-like syndrome

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Long-term administration of hydralazine in animals results in signs and symptoms resembling those of systemic lupus erythematosus in human [1]. This disease is known as a drug-induced collagen disease-like syndrome or hydralazine syndrome and is used as an animal model of the iatrogenic collagen disease [2, 3].

In previous studies it has been found that the hydralazine syndrome is associated with abnormal metabolism of connective tissue components, including collagen [4], glycosaminoglycans (GAGs[1]) [5] and elastin [6]. On the other hand, it is known that hydralazine is a chelating [7] and complexing [8] agent and generates free oxygen radicals [9]. There are no data on the interactions of GAGs and collagen under conditions of the hydralazine syndrome. Sulphuric GAGs and proteoglycans *in vitro* under conditions similar to those *in vivo* are bound to soluble collagens by weak ionic forces [10, 11]. The hydralazine-induced disturbances of structure of the collagen-proteoglycan complexes may contribute to connective tissue abnormalities seen in animals with the hydralazine syndrome.

The presented study was designed to estimate the quantitative structural changes in GAGs and collagen isolated from the skin of rats with the hydralazine syndrome which may affect the mutual interaction *in vitro* of these molecules.

Studies were carried out on 82 male Wistar rats divided into two groups: the control group and hydralazine-treated group. Hydralazine was given *per os* in a daily dose of 25 mg/kg of body weight during 12 months [3].

GAGs (hyaluronic acid, chondroitin-4-sulphate, chondroitin-6-sulphate, dermatan sulphate, heparan sulphate and heparin) were isolated [12, 13] and determined quantitatively [14] in the skin of animals of both groups. Collagen fractions: neutral salt soluble collagen (NSC) and acid-soluble collagen (ASC) as well as type I and type III collagens were determined [15] and isolated [16, 17] from the skin of both investigated groups of rats.

Collagen fibril formation *in vitro* was measured [18, 19] in the following systems:

- GAGs isolated from the control group and collagen isolated from the control group of animals (the results of measurements were used as a reference values);
- GAGs isolated from the control group of animals and collagen isolated from the hydralazine-treated rat skin;
- GAGs isolated from the hydralazine-treated animals and collagen isolated from the control rats;
- GAGs and collagen both isolated from the hydralazine-treated animals.

The relative interaction of GAGs and collagen was calculated from the spectrophotometric determination of the so-called lag phase and growth phase of the fibril formation [18, 20].

In rats with the hydralazine syndrome, the following changes were observed:

- a decrease in total GAGs content (22.8%) resulting from a decrease in hyaluronic acid

[1]Abbreviations: ASC, acid-soluble collagen; GAGs, glycosaminoglycan; NSC, neural salt soluble collagen

(44.6%) and chondroitin-6-sulphate (21.6%), with a simultaneous increase in dermatan sulphate content (9.4%) in the skin;

- an increase in total collagen content (9.0%) resulting from an increase in NSC (76.8%) and ASC (60.9%) as well as an increase in type III collagen (17.1%) associated with a decrease in type I collagen (7.4%) in the skin.

The disturbed GAGs-collagen interaction was found in all the investigated systems containing GAGs and/or collagen from the hydralazine-treated rats.

Figure 1 shows the relative changes in fibrillogenesis of all kinds of collagens isolated from the healthy animals in the presence of GAGs obtained from the skin of hydralazine-treated rats. It was found that hyaluronic acid decreased, and dermatan sulphate increased collagen fibrillogenesis. A different pattern was in the system containing collagen isolated from the skin of hydralazine-treated rats and GAGs obtained from healthy animals (Fig. 2). Fibrillogenesis of soluble collagens (NSC and ASC) was decreased, a slight increase in type I collagen fibrillogenesis and a profound decrease in type III collagen fibrillogenesis were found. Interaction of GAGs and collagen from the rats receiving hydralazine is shown in Fig. 3. All GAGs except dermatan sulphate decreased collagen fibrillogenesis. An increase in type I collagen fibrillogenesis and a decrease in type III collagen fibrillogenesis were also found.

The obtained results suggest the occurrence of structural changes in type I and type III collagens and hyaluronic acid, dermatan sulphate and, to a lesser extent in heparin isolated from the skin of rats after a long-term treatment with hydralazine.

It is difficult to characterize the structural changes on the basis of the presented studies. Changes in collagen structure and metabolism have been reported previously in animals with the hydralazine syndrome [21]. It is suggested that after generation of free oxygen radicals partial hydrolysis of the polysaccharide chain of hyaluronic acid [9, 22] could be responsible for structural alterations in collagen, similar phenomenon may occur in heparin [23].

Enhanced collagen fibrillogenesis in the presence of dermatan sulphate following hydralazine treatment could have been caused by an increase in the amount of sulphuric groups in this glycosaminoglycan, as it had been suggested earlier by Wardas [24] and/or by an increase in iduronic acid as a result of enhanced activity of manganese-regulated hexuronic epimerase, an enzyme converting glucuronyl residues into iduronyl residues [25]. The above

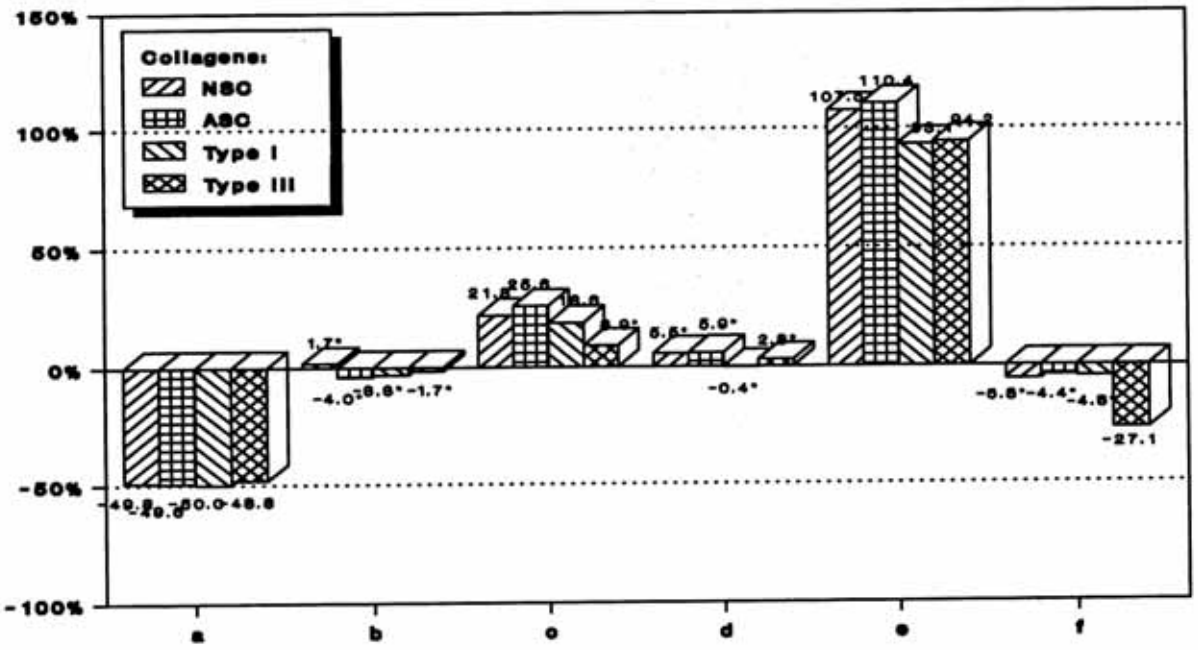

Fig. 1. Relative interaction of collagen isolated from the skin of control rats with GAGs isolated from the skin of hydralazine-treated rats.

The results are presented as percentage of the corresponding values obtained in the system of GAGs and collagen from the control group. a, Hyaluronic acid; b, heparan sulphate; c, chondroitin-4-sulphate; d, chondroitin-6-sulphate; e, dermatan sulphate; f, heparin; *differences not-statistically significant

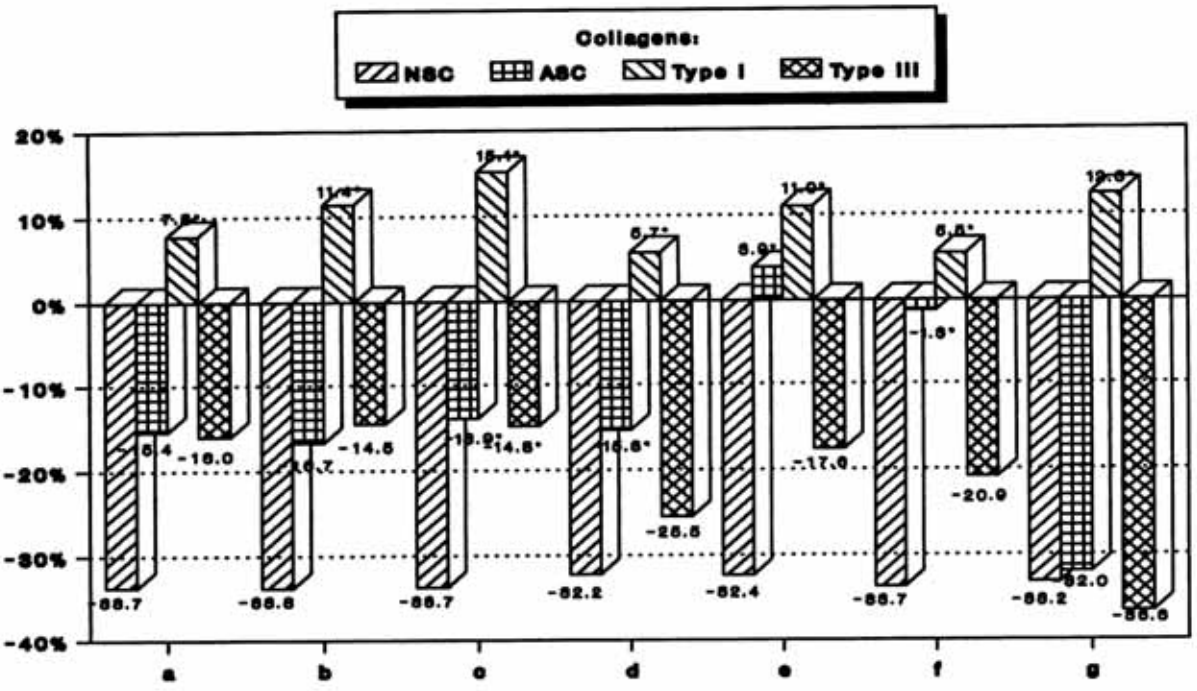

Fig. 2. Relative interaction of collagen isolated from the skin of hydralazine-treated rats with GAGs isolated from the skin of control animals.
The results are presented as percentage of the corresponding values obtained in the system of GAGs and collagen from the control group. a, Only collagen; b, hyaluronic acid; c, heparan sulphate; d, chondroitin-4-sulphate; e, chondroitin-6-sulphate; f, dermatan sulphate; g, heparin; *differences not-statistically significant

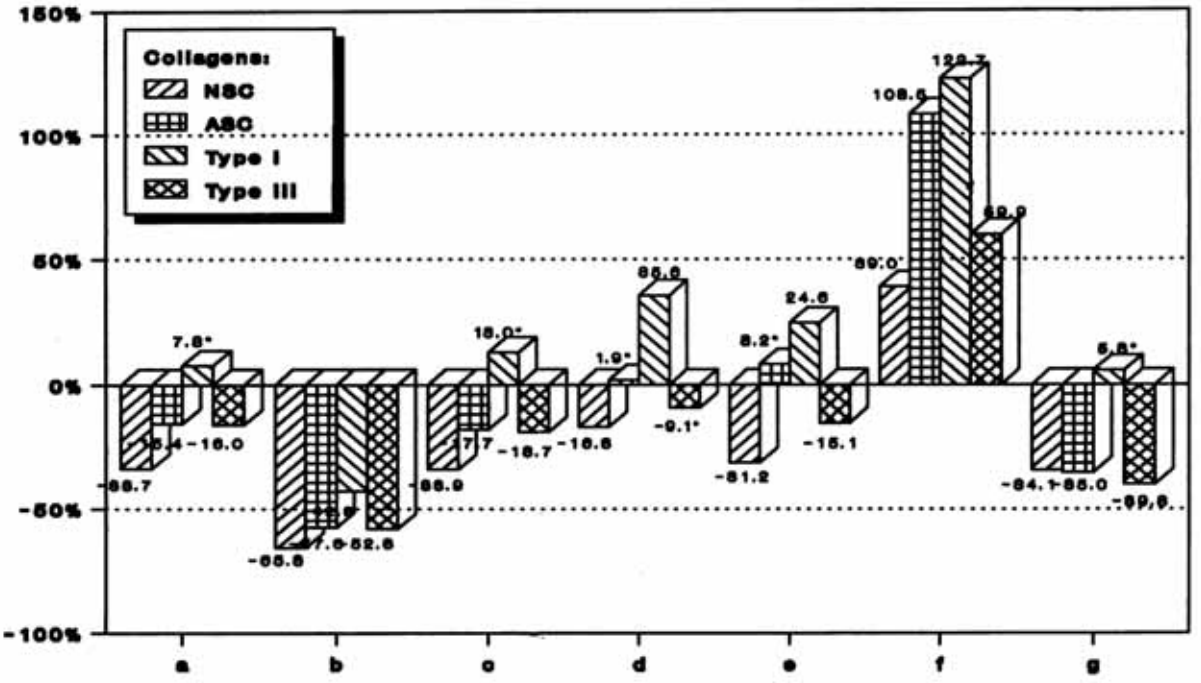

Fig. 3. Relative interaction of collagen and GAGs isolated from the skin of hydralazine-treated rats.
The results are presented as percentage of the corresponding values obtained in the system of GAGs and collagen from the control group. a, Only collagen; b, hyaluronic acid; c, heparan sulphate; d, chondroitin-4-sulphate; e, chondroitin-6-sulphate; f, dermatan sulphate; g, heparin; *differences not-statistically significant

described phenomena are suggested to increase negative charge density of polysaccharidic chains and this alteration would be responsible for altered interaction with collagen.

The obtained results point to alterations of structure of GAGs and collagen isolated from rats receiving chronically hydralazine but it is difficult to conclude wheather similar disturbances in collagen fibrillogenesis do occur *in vivo* because in such conditions proteoglycans instead of GAGs molecules interact with collagen.

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