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Cell Biology International 29 (2005) 458–471

Cell Biology International

www.elsevier.com/locate/cellbi

Ultrastructural, immunohistochemical and biochemical analysis of glycosaminoglycans and proteoglycans in the mouse pubic symphysis during pregnancy

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Received 18 November 2003; revised 20 November 2004; accepted 30 November 2004

Abstract

During pregnancy, an interpubic ligament is formed in the mouse pubic symphysis. In late stages, this ligament undergoes "relaxation" to allow proper delivery, which is expected on the 19th day. Proteoglycans and hyaluronic acid play an important role in the remodeling of the extracellular matrix in these tissues. Glycosaminoglycans and proteoglycans were studied by electron microscopic, immunohistochemical and biochemical methods in samples of mouse pubic symphysis from the 12th to 18th day of pregnancy.

At the ultrastructural level, using cuprolinic blue and enzymatic digestion by chondroitin lyases, two types of proteoglycan filaments were observed in the fibrocartilage on the 12th day, as well as in D15, D17 and D18 pubic ligaments. The only sulfated glycosaminoglycan in these filaments was chondroitin sulfate, as shown by chondroitin lyase treatment. Their electrophoretic mobility, before and after enzymatic degradation, corroborated this inference. The ratio of chondroitin sulfate/dry weight of symphysis showed two phases of increase: between D12 and D15, and between D17 and D18. We suggest that the first corresponds mainly to an increase in decorin when the ligament is formed, and the second to versican, during "relaxation". Versican and hyaluronic acid, working as water holding molecules would be responsible for the hydration of the ligament at the end of pregnancy, allowing an increase in resiliency. The presence of hyaluronic acid was confirmed by labeling with HA-probe in the perichondrium, fibrocartilage and ligament. The role of collagen fibers as physical restrictors of the complete expansion of glycosaminoglycans and hyaluronic acid in tissue is discussed.

Keywords: Proteoglycan; Hyaluronic acid; Pubic symphysis; Pregnancy

1. Introduction

In some species, proper delivery is dependent on the enlargement of the pubic symphysis – necessary to allow the passage of fetuses through the osseous birth canal (Talmage, 1947a; Gamble et al., 1986; Sherwood, 1994) – besides the concomitant effacement and dilation of the uterine cervix. The pubic symphysis, which is a very thin joint, undergoes significant changes during pregnancy in these species, including transformations in its osseous and cartilaginous components to ensure that

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doi:10.1016/j.cellbi.2004.11.025

it is lengthened at the time of delivery (Ruth, 1937; Hall, 1947; Frieden and Hisaw, 1951; Crelin and Newton, 1969; Schwabe et al., 1978). The pubic symphysis in mice is composed by the medial ends of the pubic bones, which are capped with hyaline cartilage, united by an interpubic disk of fibrocartilage in the middle; a typical perichondrium surrounds the symphysis dorsally and ventrally (as well as cranially and caudally). By the 12th day of pregnancy (D12) this fibrocartilage has been slightly enlarged but its histoarchitecture is almost unchanged. On day 15, however, a typical ligament is present in the central portion of the joint and a sheath of dense connective tissue, corresponding to the perichondrium, still surrounds the whole symphysis. This ligament persists until the end of pregnancy (Hall, 1947; Talmage, 1947a; Sherwood, 1994; Pinheiro et al., 2004), when it goes through various transformations that prepare the structure for delivery. All these adaptations are under the influence of steroid hormones and relaxin (Perl and Catchpole, 1950; Manning et al., 1965; Steinetz et al., 1965).

The above-mentioned process involves dramatic changes in the extracellular matrix components, which assume different arrangements (Crelin and Newton, 1969; Culav et al., 1999). It is well known that proteoglycans and collagen fibers are closely associated in tissues and that their amounts, types and distribution, together with elastic system fibers, water and minerals, are responsible for the structural organization and, thus, for the biomechanical properties of each specific connective tissue. The distinct mechanical characteristics of connective tissues, such as their ability to resist tension and compression, greatly depend on the type, proportion, and arrangement of the proteoglycans and the collagen fibers (Parry and Craig, 1988; Fleischmajer et al., 1991; Huijing, 1999; Culav et al., 1999; Neame et al., 2000). Hence, the composition and spatial arrangements of these two classes of extracellular matrix macromolecules are evidently important.

Various studies regarding collagen in the pubic symphysis have been published (Hall, 1947; Talmage, 1947b; Storey, 1957; Samuel et al., 1980; Zhao et al., 2000; Ortega et al., 2001; Pinheiro et al., 2004). However, there is still very little information concerning the influence of proteoglycans and glycosaminoglycans (GAG) on the adaptations observed in the pubic symphysis. Storey (1957) and Viell and Struck (1987) analyzed changes in the proteoglycans of the pubic symphyses of virgin mice after administration of exogenous relaxin. To our knowledge, there are no reports on the proteoglycan and glycosaminoglycan components of mouse pubic symphysis in different physiological hormonal conditions.

Thus, the aim of the present study is to describe the fundamental changes in proteoglycans and glycosaminoglycans that occur in the interpubic tissues (fibrocartilage and ligament) in pregnant animals from the 12th to the 18th day of pregnancy (D12, D15, D17 and D18). Proteoglycans were identified and localized ultrastructurally and immunohistochemically in pubic symphyses. The glycosaminoglycan side chains of these proteoglycans were characterized biochemically. The association of the ultrastructural and immunohistochemical findings allowed the determination not only of the type of proteoglycan present but also its specific localization and some of the associations with other components in situ. The results help to elucidate some of the correlations between collagen and proteoglycans in the adaptations that occur in the symphysis during pregnancy.

2. Materials and methods

2.1. Animals

Virgin female Swiss mice, 3 months old and over 25 g of body weight (Center for Animal Care of State University of Campinas, SP, Brazil), were housed in a room maintained at 20 ± 3 °C, with controlled light (lights on from 06:00 to 18:00 h). Pellet laboratory chow and water were available ad libitum.

Mating opportunity was provided and the day on which a vaginal "plug" was found was considered day 1 of pregnancy (D1). Delivery could be expected on the 19th day of pregnancy. Pubic symphyses were obtained from pregnant mice on the following days: D12 ($n = 42$), D15 ($n = 42$), D17 ($n = 42$), D18 ($n = 42$). A fifth group was composed of normal cycling animals in estrus ($n = 37$).

All animal work was conducted in accordance with the Guide for the Care and Use of Laboratory Animals issued by NIH/USA and approved by the local ethics committee (CEP No 0925/02). Whole pubic symphyses were excised and immediately immersed in the appropriate fixative depending on the method of analysis. For biochemical studies, fresh samples were stored at −70 °C.

2.2. Ultrastructural cytochemistry

2.2.1. Cuprolinic blue treatment

Pubic symphyses for ultrastructural analysis were treated with cuprolinic blue, which stains all the proteoglycans in tissues. Specimens were fixed overnight at room temperature with 2.5% (w/v) glutaraldehyde in 0.025 M sodium acetate buffer (pH 5.6) containing 0.30 M $MgCl_2$ and 0.2% (w/v) cuprolinic blue (BDH Chemicals Ltd., Poole, England) (Scott, 1980). After this treatment, samples were washed (3 × 10 min) in the same buffer solution, immersed in 1% aqueous sodium tungstate for 30 min, dehydrated in ascending