國科會專題研究計劃成果報告

題目:肩部旋轉肌腱隨年齡其分子結構之改變:一種在老鼠分子生物學之研究

Age-related Changes in Macromolecular Composition of Rotator Cuff Tendon: A Molecular Level Study in Rat

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中文摘要

肩部旋轉肌之細胞外間質包含了多種蛋白質及多醣類， 它們由局部之細胞分泌聚合成網狀結構， 其與細胞表面及其細胞外間質相互之間相互影響. 主要的兩種細胞外間質包括:一爲葡萄糖胺聚合醣，它與蛋白質共價聯結成蛋白多醣的形式; 另一爲纖維蛋白質，其依功能包括結構型蛋白(例如膠原蛋白及彈性素)及黏結型蛋白(例如纖維結合素及板素). 葡萄糖胺聚合醣及蛋白多醣在結締組織中會形成一種多水凝膠物質，它們因此而可抵抗肌腱外來之壓迫應力; 相對地膠原纖維， 尤其是其分子內及分子間之交叉聯結體更提供了肌腱抵抗外來之牽拉應力之力量.從本研究可以達到一些肩部旋轉肌腱隨年齡其分子結構改變之定性及初步定量成果， 葡萄糖胺聚合醣在十二個月時呈現下降趨勢， 而膠原蛋白及纖維結合素在十二個月時都還維持在高值. 這意味著隨年歲之變老對抗著壓迫應力的能力比對抗牽拉應力的能力差. 肩部旋轉肌病變是產生肩關節疼痛及功能不良的一個主要來源， 正意味著或許是能對抗著壓迫及牽拉應力的細胞外間質發生改變而導致病變. 因爲旋轉肌在是肩部一個包圍骨頭的特殊結構， 所以研究此一特殊解剖位置的肌腱內之細胞外間質成份的改變將有助於旋轉肌病變成因的瞭解.

Abstract

The two main classes of extracellular macromolecules that make up the matrix in rotator cuff tendon are glycosamionglycans (GAGs), which are usually found covalently linked to protein in the form of proteoglycans (PG), and 2) fibrous proteins of two functional types: mainly structural (for example, collagen and elastin) and mainly adhesive (for example, fibronectin and laminin). In our study, GAG increased gradually from age 3-week to 6-month, then decreased a little bit at the age of 12-month. It might be needed in maturation and decreasing in the degeneration stage of life. Conversely, collagen type I and III significantly increased from age 3-week to 6-month, then maintained at maturation level. GAG and (PG) molecules resist compressive forces on the matrix and the collagen fibers provide tensile strength. If GAG is decreasing in late life which is shown in our result, it will result in degenerative disease; on the other hand, the collagen content remained high in late life, the tensile resisting problem is not serious at the age of 12 months.

Rotator cuff develops at the location where tendons wrap around bone and are subjected to transverse compressive loading, in addition to logitudinal tension. In our study, the decreasing GAG with decreasing resisting compressive strength might be the main cause. The other macromolecule such as collagen type I, III and fibronectin still remained athe maturation level at the late life. The macromolecular composition characterictics of extracellular matrix of the tendon in this study is crucial to identify the pathoetiology of rotator cuff tear.

計劃緣由與目的

Rotator cuff pathology is a significant source of shoulder pain and dysfunction. Cuff pathology is especially prevalent in individuals over 40 years of age, suggesting that normal aging process may play an important role in pathology (11, 12) . However, the condition also appears in younger individuals, especially those who subject their ritator cuff tendons to repetitive eccentric loading as in overhead sports, or direct truma as in contact sports; this suggests a extrinsic and mechanical (compressive or tensile) component to the pathoetiology. Rotator cuff develops at the location where tendons wrap around bone and are subjected to transverse compressive loading, in addition to logitudinal tension.The macromolecular composition characterictics of extracellular matrix of the tendon in this region is crucial to identify the pathoetiology. This study of rotator cuff tendons from Sprague-Dawley rat is undertaken to determine the changes of macromolecular composition of extracellular matrix with age. Our hypothesis is that the ability to resist the compression and tensile stress will decline with age due to the changes of macromolecular composition of extracellular matrix. A more precise understanding of the macromolecular composition of the rotator cuff tendon is of great importance to clarify the pathological process in this region.

材料及方法

A total of 30 (60 shoulders) healthy male rats of Spraque-Dawley strain are used. They will be raised from the age of 3-week. The animals are sacrificed with CO_2 narcosis at the age of 3-week (n=6), 3-month (n=6), 6-month (n=6), 9-month (n=6) and 12-month (n=6). The upper limbs are exarticulated at the scapulothoracic joint, and the skin is removed. the limbs are kept 24 hrs in 2% glutaraldehyde buffered with cacodylate at pH 7.4. The rotator cuff tendon is removed and postfixs for additional 48hrs. Finally, the tendons are cut longitudinally and transversally.

1. Histochemical analysis (light microscopy)- localization for GAGs:

The cryostst serial sections are stained with HE, PAS (also after salivary amylase digestion), Alcian Blue, and Alcian Blue-PAS stainings (Alcian Blue 8GX at pH 0.5 and 2.5). Adjacent sections are processed with critical electrolyte concentration (CEC) with 0.15M, 0.30M, 0.60M, 0.80M, 1.00M, 1.20M MgCl2 and stained with Alcian Blue (at pH 0.5 and 2.5) as described by Scott and Dorling (13, 14, 15) for the demonstration of the individual GAGs.

2 Immunohistochemical analysis- localization for collagen and fibronectin:

Collagen:

Five-micrometer sections are cut from the paraffin-embedded specimens of the rotator cuff tendon. After digestion with 0.1% trypsin (2hr at 37°C) the sections are stained with collagen antibodies (1:100 dilution for the anti-type I collagen and 1: 500 dilution for the anti-type III collagen) by the peroxidase-antiperoxidase method (24). The color reactions are produced with 0.03% 3,3-diaminobenzidine tetrachloride. The analysis of type I and type III collagen include appropriate control sections without the antibodies of these collagens.

Fibronectin:

Immunoperoxidase staining (PAP) (Antiserum to Fibronectin, Behringwerke AG, Marburg, Germany) for fibronectin is performed according to Burns et al.(25) The tendon

samples are fixed in buffered 6 percent formalin solution (pH7.4), embeded in paraffin, and cut in 5-um logitudinal and transverseal sections. Enzyme pretreatment of the sections are performed as described by Burns et al. Finally, the sections are counterstained with hematoxylin.

結果

1. Histochemical analysis (light microscopy)- localization for GAGs: (Fig.1)

GAG (12m)
HE
4X

GAG (12m)
Alcian Blue
4X

2 Immunohistochemical analysis- localization for collagen and fibronectin: (Fig.2)

collagen I. (12m)

4X

fibronectin (12m)

4X

3.Quantitative ratio of each macromolecule in tissue specimen: (Fig.3)

1).Collagen type I and III significantly increased from age 3-week to 6-month, then maintained at 38%.
2). Fibronectin also increased gradually from age 3-week to 6-month, then reached to another peak level at the age of 12-month.
3). GAG also increased gradually from age 3-week to 6-month, then decreased a little bit at the age of 12-month.
4). At each particular age, fibronectin is the least content except at the age of 12-month; on the other hand, collagen type I is the highest content at each evaluation age.

討論

The extracellular matrix is composed of a variety of versatile proteins and polysaccharides that are secreted locally and assembled into an organized meshwork in close association with the surface of the cell and the interaction of the macromlecules themselves. The two main classes of extracellular macromolecules that make up the matrix are 1) polysaccharide chains of the class called glycosamionglycans (GAGs), which are usually found covalently linked to protein in the form of proteoglycans, and 2) fibrous proteins of two functional types: mainly structural (for example, collagen and elastin) and mainly adhesive (for example, fibronectin and laminin). In our study, GAG increased gradually from age 3-week to 6-month, then decreased a little bit at the age of 12-month. It might be needed in maturation and decreasing in the degeneration stage of life. Conversely, collagen type I and III significantly increased from age 3-week to 6-month, then maintained at 38%. GAG and proteoglycan (PG) molecules in connective tissue form a highly hydrated, gel-like "ground substance", which resists compressive forces on the matrix and the collagen fibers (especially the intramolecular and intermolecular cross-links) provide tensile strength. If GAG is decreasing in late life which is shown in our result, it will result in degenerative disease; on the other hand, the collagen content remained in late life, the tensile resisting problem is not serious at the age of 12 months.

Rotator cuff pathology is a significant source of shoulder pain and dysfunction. Cuff pathology is especially prevalent in individuals over 40 years of age, suggesting that normal aging process may play an important role in pathology. However, the condition also appears in younger individuals, especially those who subject their rotator cuff tendons to repetitive eccentric loading as in overhead sports, or direct truma as in contact sports; this suggests a extrinsic and mechanical (compressive or tensile) component to the pathoetiology. Rotator cuff develops at the location where tendons wrap around bone and are subjected to transverse compressive loading, in addition to logitudinal tension. In our study, the decreasing GAG with decreasing resisting compressive strength might be the main cause. The other macromolecule such as collagen type I, III and fibronectin still remained athe maturation level at the late life. The macromolecular composition characterictics of extracellular matrix of the tendon in this study is crucial to identify the pathoetiology of rotator cuff tear.

計劃成果自評

雖然從本研究計劃可以達到一些肩部旋轉肌腱隨年齡其分子結構改變之定性成果，本研究計劃也從電腦軟體作相對定量分析，其結果還須下學年度之研究計劃之定量分析再確定，其價值會更高.

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	CO I	CO Ⅲ	Fribronectin	GAG(Alcian-Blue)	GAG(PAS)
3-week	0.179	0.086	0.114	0.123	0.106
3-month	0.247	0.216	0.166	0.202	0.205
6-month	0.436	0.393	0.225	0.308	0.313
9-month	0.405	0.38	0.171	0.321	0.323
12-month	0.381	0.383	0.347	0.236	0.271

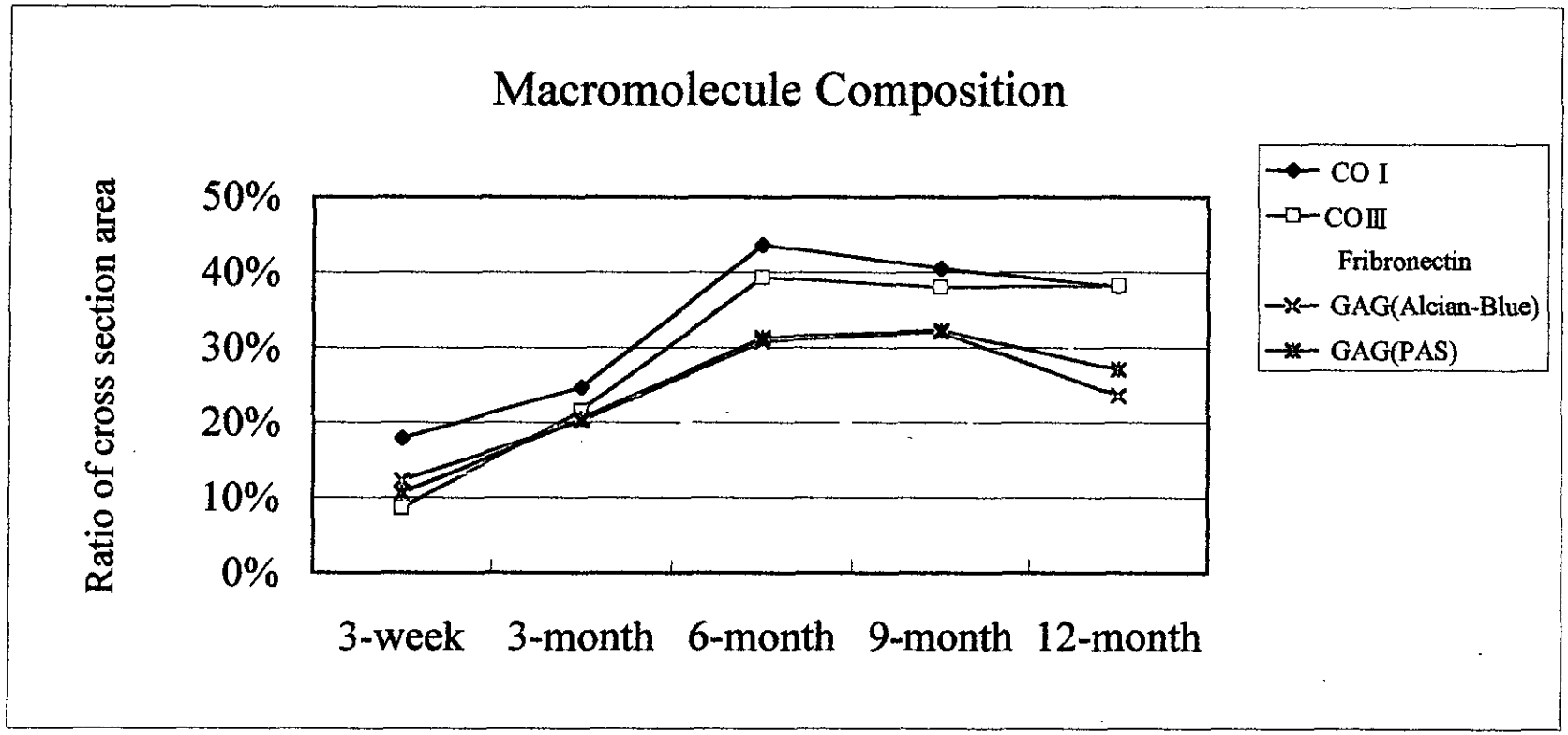

Fig. 3