Hyaluronan within fascia in the etiology of myofascial pain

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Abstract The layers of loose connective tissue within deep fasciae were studied with particular emphasis on the histochemical distribution of hyaluronan (HA). Samples of deep fascia together with the underlying muscles were taken from neck, abdomen and thigh from three fresh non-embalmed cadavers. Samples were stained with hematoxylin–eosin, Azan-Mallory, Alcian blue and a biotinylated HA-binding protein specific for HA. An ultrasound study was also performed on 22 voluntary subjects to analyze the thickness of these deep fasciae and their sublayers. The deep fascia presented a layer of HA between fascia and the muscle and within the loose connective tissue that divided different fibrous sublayers of the deep fascia. A layer of fibroblast-like cells that stained prominently with Alcian blue stain was observed. It was postulated that these are cells specialized for the biosynthesis of the HA-rich matrix. These cells we have termed “fasciacytes”, and may represent a new class of cells not previously recognized. The ultrasound study highlighted a mean thickness of 1.88 mm of the fascia lata, 1.68 mm of the rectus sheath, and 1.73 mm of the sternocleidomastoid fascia. The HA within the deep fascia facilitates the free sliding of two adjacent fibrous fascial layers, thus promoting the normal function associated with the deep fascia. If the HA assumes a more packed conformation, or more generally, if the loose connective tissue inside the fascia alters its density, the behavior of the entire deep fascia and the underlying muscle would be compromised. This, we predict, may be the basis of the common phenomenon known as “myofascial pain.”

Keywords Hyaluronic acid · Fascia · Sliding system · Loose connective tissue · Fasciacyte · Myofascial pain

Introduction

The etiology of myofascial pain is not certain. Some suggest a central origin of it, others a peripheral one. There is evidence of central nervous system sensitization and of hyperalgesia and temporal summation of pain in a specific area. Another hypothesis would suggest a facilitated processing of pain messages in the central nervous system, perhaps manifested by neural reorganization in the brain, brainstem, and spinal cord [29]. The peripheral theory suggests, in contrast, that myofascial pain is due to an alteration of innervations or of nerve stimulation of muscles or of fascia. This theory is based to the hypothesis that the fascia could be considered as a proprioceptive organ, and that it could be altered by trauma, overuse and surgery. It is well demonstrated that fascia is rich in innervations [8, 20, 29], including proprioceptors [20], as well as abundant vascular and lymphatic channels [2, 3]. Besides, recent work [1, 10] demonstrated that the deep fascia is a multi-layered structure formed by two to three layers of densely packed collagen fibers, together with a few scattered elastic
fibers. A layer of loose connective tissue was present between these fibrous layers.

The aim of the present study was to closely examine these layers of loose connective tissue found within deep fascia, and in particular to evaluate the distribution of hyaluronan (HA) in association with these structures.

HA is a high molecular weight glycosaminoglycan polymer of the extracellular matrix (ECM). It is composed of disaccharides of alternating glucuronic acid and N-acetylg glucosamine connected by β1–3 and β1–4 glycosidic bonds, respectively. It has a wide variety of physiological functions in the mammalian body; as a shock absorber (synovial fluid), a space-filling agent providing tissue tur- gor (the aqueous fluids of the anterior and posterior chambers of the eye), as a protective agent to prevent vascular compression (Wharton’s jelly of the umbilical cord), as a lubricant, and as a protective shield (the cumulus mass surrounding the ovum) [14, 27]. HA is ubiquitous, but is particularly abundant in soft connective tissues, in tissues undergoing rapid growth and development, in fertilization, during embryogenesis, whenever repair and regeneration are occurring, during cell migration, cancer initiation and malignant progression [5–7, 11, 14, 17, 19, 21–25].

Previous studies demonstrated that HA occurs between deep fascia and muscle, and within muscle [9, 13, 15]. It is presumed that HA facilitates smooth gliding between these two structures. The occurrence of HA present additionally within the loose connective tissue of fascia would support the autonomy in sliding of the two to three adjacent fibrous layers, thus guaranteeing the normal function of deep fascia.

Materials and methods

Histochemical study

Three fresh non-embalmed cadavers (3 men, mean age 71 years) were studied. Samples of the deep fascia together with underlying muscle were taken from three different regions: the sternal ending of the sternocleidomastoid, the rectus abdominis and the rectus femoralis muscles. Samples were paraffin-embedded and 10 µm thick sections were obtained and stained with hematoxylin–eosin (H&E), Azan–Mallory, Alcian blue, and a biotinylated HA-binding protein that has high specificity for HA. The latter, commercially available, is derived from a tryptic digest of bovine nasal cartilage, being the amino-terminal peptide of the proteoglycan aggrecan. The isolated peptide is then biotinylated so that it is able to function in an anti-HA antibody-like reaction. Biotin–streptavidin peroxidase is employed as the color reaction. HA identity was confirmed by preliminary digestion of samples with hyaluronidase, using an enzyme derived from Streptomyces hyalo-lyticus (Sigma, USA). All preparations were observed under a DM4500-B light microscope (Leica Microsystems, Wetzlar, Germany) and recorded in full color (24 bit) by a digital camera (DFC 480, Leica Microsystems).

Clinical study

Ultrasound studies were performed on 22 voluntary subjects (7 M, 15 F; age range 27–52 years, mean age 37.5 years), without history of pathological conditions or lesions of the trunk and thigh. This portion of the study was approved by the Ethics Committee of the Department of Anatomy and Physiology, University of Padova, Italy. The thickness of whole fascia and of its sublayers (both fibrous and loose connective tissue) was recorded by an ultrasound machine with middle frequency (10 MHz) and linear array. We elected to evaluate the sternal ending of the sternocleidomastoid, the rectus abdominis and the rectus femoralis muscles, as these provided the optimal quality of data. In addition, these three muscles are fusiform and are therefore easier to localize and to analyze. We used as anatomic landmarks 2 cm laterally to umbilicus for the rectus abdominis muscle and the proximal quarter of the rectus femoralis for the fascia lata, and the distal quarter of the sternocleidomastoid muscle for the neck. We positioned the transductor longitudinally in the neck and femoral region and transversally on the abdomen in the center of the muscle belly, corresponding to the highest thickness of the muscle belly. We measured the thickness of the entire deep fascia and of the various sublayers of dense and loose connective tissues. Mean values and standard deviations of these measurements were calculated.

Results

Histological study

The microscopic evaluation confirmed previous studies [1, 20] in which we demonstrate that deep fascia is formed by two to three layers of parallel collagen fiber bundles, densely packed, divided by a thin layer of loose connective tissue.

Staining with the Alcian blue (Fig. 1a) documented a layer of HA between fascia and muscle. Confirmation of the HA nature of the Alcian blue staining was achieved with the highly specific HA-binding peptide (Fig. 1b). Prominent deposition of HA was observed. A loss of such staining was observed when a preliminary digestion of the tissue sections was performed with the bacterial hyaluronidase. At greater enlargement, Alcian blue staining documented bands of HA also within the deep fascia.
Fig. 1  

(a) Hyaluronan in loose connective tissue inside and under the deep fascia (Alcian blue ×12.5).  
(b) Hyaluronan (brown color) within the fascia lata as demonstrated with the HA-binding peptide (×400).  
(c) Hyaluronan within the fascia lata (Alcian blue ×400). Three different layers of dense connective tissue inside the deep fascia are positive in the loose connective tissue.  
(d) Hyaluronan associated with muscle perimysium (Alcian blue ×400)

(Fig. 1c), and in particular inside the loose connective tissue separating the fibrous sublayer of the fascia. Further analysis of HA-staining using Alcian blue documented bands of HA between muscle fibers (Fig. 1d). This is consistent with an HA distribution enveloping muscle bundles, as well as in the perimysium and endomysium of the muscles.

In a number of samples, in the fascia adjacent to muscle, and in apposition to it (Fig. 2), a layer of fibroblast-like cells was observed that stained intensely with the Alcian blue. With hematoxylin and eosin (H&E) staining, prominent nuclei were observed. It is postulated that these are specialized cells that are the biosynthetic source of the observed HA-rich matrix.

**Clinical study**

The deep fascia was easily evaluable with the ultrasound in all the analyzed regions. It appeared as a linear hyperechoic layer. The thickness of the fascia lata, rectus sheath, and sternocleidomastoid fascia is recorded in Table 1. In addition, the fascial sublayers are easily recognizable in the fascia lata (Figs. 3, 4a) and rectus sheath (Fig. 4b), in particular the dense collagen layers within the fascia are represented by the white layers, while the layers of loose connective tissue are seen as the black layers. The sternal ending of the sternocleidomastoid did not consistently demonstrate the sublayers of the dense connective tissues and the loose connective tissues. This was observed in only 2 of the 22 subjects.

An idealized demonstration of the localization of the HA in deep fascia, loose connective tissue, and muscle, including epimysium and perimysium, is shown in Fig. 5.

**Discussion**

This study has highlighted a prominent layer of loose connective tissue rich in HA between deep fascia and underlying skeletal muscle and less prominent layers within the deep fascia (Fig. 5). These layers are well evaluable both with histology and ultrasound, except for the HA layer under the deep fascia that is difficult to
analyze with ultrasonography, because there is little variation in echogenicity between loose connective tissue and the muscle fibers. This is in marked contrast with the HA layer inside the deep fascia. The latter is rather easy to analyze with ultrasound, for there is a great difference in ecogenicity between the dense and loose connective tissues. Our data suggest that there are no major variations in the thickness of fascial layers between patients, nor are there major differences in histology. This supports the concept that ultrasonography may be a good method for studying the deep fascia and its conformation in different regions. The data suggest that the full thickness of the fascia is proportional to the number of sublayers of which it is composed. For this reason the deep fascia over the sternocleidomastoid is only half of that observed in the other muscles. In comparison to the others, it did not show sublayers of loose and dense tissue, with the exception of two of the subjects.

Future research will be necessary to study the different thicknesses of the loose connective tissue layer inside the deep fascia and whether the increase or decrease of thickness could be correlated with dysfunction of the normal sliding of the deep fascia over the underlying muscle.

In the entire vertebrate body, the loose connective tissue cushions and separates different structures from each other. It is known that this tissue is an important reservoir of water and ions for surrounding tissues. It may also function as a reservoir to accumulate and remove various degradation products and toxic substances. The subsequent variations in the concentrations of water, ions or other

| Table 1 | Mean thickness of the deep fascia in the different evaluated regions of the body and of its sublayers, as seen with the ultrasound |
|------------------|------------------|------------------|------------------|------------------|
| Thickness ± standard deviation (mm) |
| Fascia | Superficial fibrous layer | Superficial LCT layer | Middle fibrous layer | Deep LCT layer |
| SCM: sternal head |
| sx 1.11 ± 0.40 | 0.42 ± 0.15 | 0.18 ± 0.10 | 0.46 ± 0.11 | 0.17 ± 0.10 | 0.46 ± 0.18 |
| dx 1.07 ± 0.38 | 0.54 ± 0.10 | 0.16 ± 0.10 | 0.41 ± 0.13 | 0.20 ± 0.13 | 0.40 ± 0.22 |
| Rectus abdominis |
| sx 1.66 ± 0.39 | 0.54 ± 0.16 | 0.15 ± 0.16 | 0.38 ± 0.12 | 0.19 ± 0.13 | 0.40 ± 0.10 |
| dx 1.69 ± 0.50 | 0.55 ± 0.18 | 0.17 ± 0.18 | 0.38 ± 0.14 | 0.21 ± 0.11 | 0.43 ± 0.15 |
| Rectus femoralis |
| sx 1.88 ± 0.38 | 0.54 ± 0.16 | 0.15 ± 0.16 | 0.38 ± 0.12 | 0.19 ± 0.13 | 0.40 ± 0.10 |
| dx 1.87 ± 0.48 | 0.55 ± 0.18 | 0.17 ± 0.18 | 0.38 ± 0.14 | 0.21 ± 0.11 | 0.43 ± 0.15 |

$LCT$ loose connective tissue

Fig. 3 Evaluation of rectus femoralis muscle and fascia lata with ultrasound. It is evident that the three layers of dense connective tissue forming the fascia lata, are divided by two layers of loose connective tissue.

Fig. 4 Measures of the deep fasciae and their sublayers with ultrasound. a Rectus femoralis and fascia lata with multiple layers of dense connective tissue. b Rectus abdominis and rectus sheath with the different layers of dense connective tissue that compose the deep fascia.
substances could alter the biomechanical proprieties of the loose connective tissue and thereby the sliding functions of the different fascial layers. A fundamental element of the loose connective tissue is the HA, and its concentration determines, together with temperature and other physical parameters, the density of the matrix. The prominent role of HA in the sliding function between fascia and muscle and between the different fascia sublayers is postulated. The role of HA in providing a substance for the smooth gliding between surfaces between the different motor units within muscle has been described by McCombe et al. [13]. This suggests that the layer of HA between the fascia and the muscle bundles and its widespread presence in the perimysium and endomysium [15] could provide planes of potential movement, and appears to function as a lubricant. In the studies by Piehl-Aulin et al. [15], in addition to the histolocalization of HA between fascia and muscle, perivascular and perineural connective tissue also stained positively for HA. In those studies, quantitative measures of HA were also performed, and the effect of exercise examined. The retention of HA after exercise, combined with an endomysial location supports the concept that HA not only lubricates but also facilitates movements between muscle fibers [15]. Changes of HA function can occur with changing concentrations and aggregation properties in response to van der Waal and hydrophobic forces, in particular with increasing concentrations, HA chains begin to entangle, conferring distinctive hydrodynamic properties on HA solutions, with a dramatic increase in viscosity [12]. The hydrodynamics of HA solutions may provide the viscoelasticity needed for skeletal muscle movement. Inter-chain interactions are reversible, with disaggregation occurring with an increase in temperature and by alkalinization [12, 18].

We have observed in a number of samples fibroblast-like cells aligned on the inferior surface of fascia (Fig. 2). We term these modified fibroblast-like cells associated with fascia “fasciacytes,” and suggest this is a new class of cells, not previously appreciated. Furthermore, we suggest that these cells may be related to other fibroblast-like cells in the vertebrate body with a specialized function of HA synthesis and secretion. Indeed similar cells were previously described in other tissues: the synoviocytes on the inner surface of joint capsules [4], and the hyalocytes in the eye [16]. These fibroblast-like cells are actually of monocyte/macrophage lineage, as ascertained by surface marker studies.

The myofibroblast is another of the fibroblast-like cells that actively secretes HA, as well as other ECM components [27]. The myofibroblast, active in wound repair and associated with the scarring and contractures associated with the pathology of that process, is not of monocyte/macrophage origin, but is actually a modified fibroblast. Until further studies are performed, it cannot be established whether the “fasciacyte” is of monocyte/macrophage or of fibroblast origin, whether it resembles more closely the synoviocyte and hyalocyte, or the myofibroblast.

The physical–chemical properties of HA are modulated by temperature, chemical elements and pressure. If the HA assumes a more packed conformation, or more generally if the loose connective tissue inside the fascia alters its density, the behavior of the whole deep fascia and of the underlying muscle could be compromised. Besides, there is much evidence [23, 28] that the activation of the receptors is strongly dependent on the viscoelasticity of the surrounding tissue. So, it is known that the HA is one of the most important elements that determines the viscoelasticity of a tissue. Its important presence inside the fascia could permit us to suppose that its alteration could modify the activation of the receptors inside the fascia. This could be the origin of the common phenomenon of myofascial pain.

Conflict of interest The authors declare that they have no conflict of interest.

References

