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A histological study of the deep fascia of the upper limb

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SUMMARY

Post-mortem specimens taken from the antebrachial and brachial fasciae of 20 upper limbs were studied by histological and immunohistochemical staining in order to evaluate collagen fibre bundle arrangement, the presence of elastic fibres, and the density of innervation in deep muscular fascia. The study demonstrated that the fasciae are formed of numerous layers of undulating collagen fibre bundles. In each layer, the bundles are parallel to each other, whereas adjacent layers show different orientations. Each layer is separated from the adjacent one by a thin layer of adipose tissue, like plywood. Many elastic fibres and a variety of both free and encapsulated nerve endings, especially Ruffini and Pacini corpuscles, are also present, suggesting a proprioceptive capacity of the deep fascia.

Thanks to the undulating collagen fibre bundles and elastic fibres, the fasciae can adapt to stretching, but this is only possible within certain limits, beyond which nerve terminations are activated by stretching. This mechanism allows a sort of "gate control" on the normal activation of intrafascial receptors. The capacity of the various collagen layers to slide over each other may be altered in cases of over-use syndrome, trauma or surgery. In such cases, the amortising mechanism of the fascia on the nervous terminations is lost, causing incorrect paradoxical activation of nerve receptors within the fascia, resulting in the propagation of a nociceptive signal even in situations of normal physiological stretch. At the same time, the layered collagen fibres allow transmission of tension according to the various lines of force. This structure of the muscular fascia guarantees perceptive and directional continuity along a particular myokinetic chain, acting like a transmission belt between two adjacent joints and also between synergic muscle groups.

INTRODUCTION

In recent years, the deep muscular fascia has attracted increasing interest and is currently indicated in the pathogenesis of a wide variety of conditions. Bednar et al. (1995) found inflammation and micro-calcification of the thoracolumbar fascia in patients with chronic lumbalgia, suggesting the role of the fascia in the aetiology of lower back pain. In the transverse fascia of patients with inguinal hernia, Pans et al. (2001), Rodrigues et al. (2002) and Rosch et al. (2003) verified the presence of a web of disorganised collagen fibres and an increase in vascularisation in comparison to healthy control subjects. It has been suggested that the fascia is implicated in the regulation of posture (Palmieri et al., 1986), muscular biomechanics (Gerlach and Lierse, 1990), peripheral motor coordination (Stecco, 2004) and proprioception (Stecco et al., 2006). For some authors (Rolf, 1997; Paoletti, 1998), the fascia has the capacity to adapt to physical stress, and manual therapies can influence its tone, viscosity or structure.

Despite growing interest in the fascia, a comprehensive anatomical and histological description of this tissue is still lacking. The deep fascia of muscles is described in most texts as a lamina of dense connective tissue surrounding muscles (e.g. Moore and Agur, 2001), with the sole function of an inert structural support (Kuslick et al., 1991). Some authors (e.g. Yahia et al., 1993) have expressed the need for a histological study of the fascia.

Hence, the aim of this study was to clarify the histological structure of the deep muscular fascia of the upper limb, with particular reference to its content and arrangement of collagen and elastic fibres and types of innervation.

MATERIALS AND METHODS

The study was performed on 20 upper limbs (12 right, 8 left) from 13 subjects (10 males, 3 females; mean age 79.9 years) on the basis of research approved by the Normal Anatomy Institute of the René Descartes University in Paris. No limbs showed any evidence of traumatic lesions or pathologies, and had not been embalmed or frozen prior to examination. For each limb, 4 different samples of the same size (1 x 1.5 cm) of the middle thirds of the anterior brachial fascia and of the anterior antebrachial fascia. The samples were mounted on cardboard to avoid deformation artefacts.

All specimens were immediately preserved in formaldehyde 4% in phosphate buffer saline (PBS) 0.1 M, pH 7.0, embedded in paraffin, and then cut into 10-αm thick sections, which were stained with hematoxylin and eosin (H.E.), azan-Mallory, Weigert's Van Gieson stain for elastic fibres and silver impregnation. Anti-S100 immunohistochemistry was also performed. The intrafascial vascular network was also analysed.

Immunohistochemical method: Five-αm thick sections were treated with H_2O_2 0.15% for 15 minutes in order to inhibit endogenous peroxidase activity. After washing in PBS, the sections were incubated with normal goat serum 1:100 for 30 minutes and then with polyclonal antibodies raised against S100 (DAKO, Italy) for nervous tissues, diluted 1:500 in PBS at 37°C in a humid chamber for 60 minutes. Repeated washings were performed and the sections were then incubated with secondary antibody (goat anti-rabbit IgG peroxidase-coniugated antibodies DAKO) 1:50 for 30 minutes. Lastly, the reaction was enhanced with 3,3'-diaminobenzidine (DAB substrate tablets, Sigma, 0.1% v/v H_2O_2). The preparations, contrasted with hematoxylin, were dehydrated and mounted on Canadian balsam (BDH, Italy). Negative controls were obtained by omitting the primary antibody. All preparations were observed under a Leica DM 4500B microscope.

RESULTS

The deep fasciae of the arm and forearm presented analogous histological characteristics. Both fasciae had a mean thickness ranging from 100 to 200 αm, and were formed of multiple layers of collagen fibre bundles. They were of variable size, showed an undulating course, and were parallel to each other. Fibroblasts, at times exhibiting star-shaped cytoplasmic elongations, were arranged between the collagen fibre bundles and parallel to them. The alignment of the bundles differed from layer to layer (Fig.1a). Each layer was separated from the adjacent one by a thin layer of adipocytes. At some points, these laminae were packaged and connected with the underlying epimysium, without interposing adipose tissue (Fig.1b). Numerous elastic fibres were also evidenced by van Gieson stain. They appeared as short, branched fibres, not arranged in bundles, arranged between the collagen bundles and the various layers in a less orderly manner, to form an irregular mesh (Fig. 1c). Numerous vessels also followed rather tortuous paths through the collagen layers of the muscular fascia.

Some small nerve branches were highlighted with silver impregnation, and, with anti-S100 immunohistochemistry, nerve fibres were found in all specimens of the deep fascia. Although they were particularly numerous around vessels, they were also distributed throughout the fibrous components of the fascia. Some nerve fibres were connected to collagen fibres, others were surrounded by loose connective tissue. In some specimens, Ruffini, Pacini and rare Golgi-Mazzoni corpuscles were also highlighted (Fig. 1d).

DISCUSSION

From a histological viewpoint, some authors (Geneser, 1986; Standring et al., 2005) consider the deep muscular fascia as consisting of dense, regular, connective

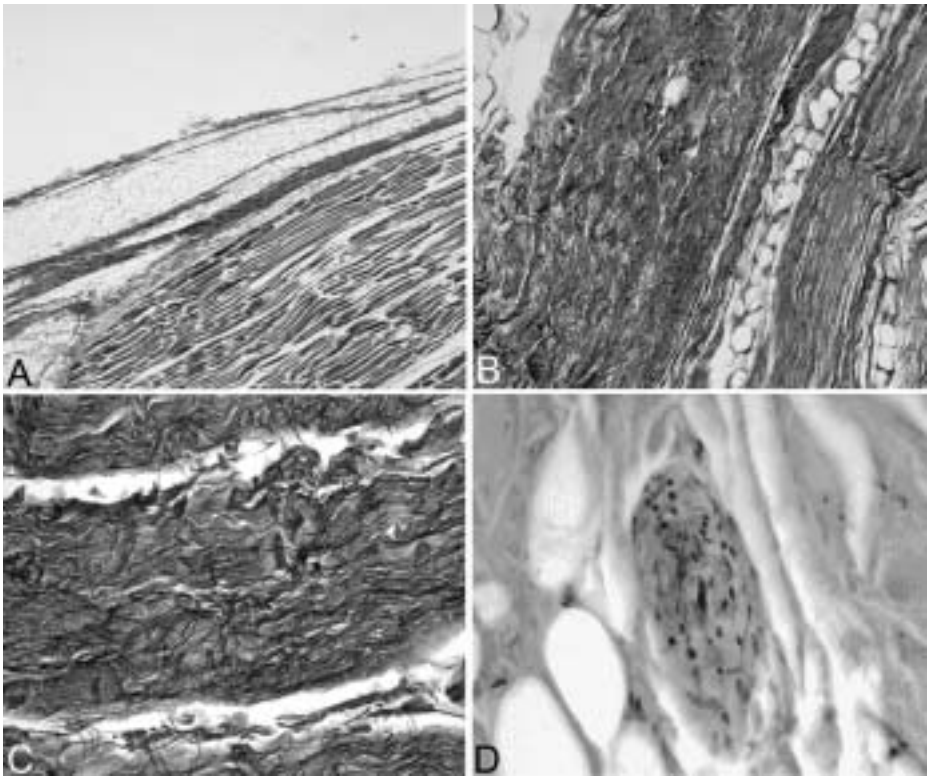

Fig. 1 — a) Layered arrangement of deep muscular fascia and its connections with epimysium (azan-Mallory stain, magnification 5x).
b) Undulating collagen fibres interspersed with fusiform fibrocytes. Layered arrangement is clearly evident, layers being separated from the next by adipose tissue. In each layer, collagen fibres are parallel, but their direction varies from one layer to the next. Numerous small vessels are also visible between bundles of collagen fibres (azan-Mallory stain, magnification 10x).
c) Note numerous fine elastic fibres with differing orientation, interspersed with collagen fibres (van Geison stain, magnification 20x).
d) Ruffini corpuscle immersed in loose connective tissue, between two bundles of collagen fibres (anti-S100 stain, magnification 40x).

tissue similar to aponeurosis, characterised by extremely well-ordered, parallel bundles of inelastic collagen fibres, although for Standring et al. (2005) the fascia is sometimes also irregular loose connective tissue. Conversely, Gerlach and Lierse (1990) described the muscular fascia of the lower limb as composed of intertwined bundles of collagen fibres. For other authors (Bogduk and Macintosh, 1984; Martini et al., 2004), it is formed of numerous laminae of dense connective tissue in which collagen fibres may be aligned in several directions, whereas for yet others (e.g. Fawcett, 1986), the laminae are difficult to distinguish because the collagen fibres often pass from one lamina to the adjacent one.

Our results show that the fascia is essentially composed of numerous layers of parallel, undulating collagen fibre bundles, intermingled with many elastic fibres. Thin layers of adipocytes separate adjacent layers, allowing single layers to slide over

each other. The alignment of the collagen fibres varies from layer to layer. As a whole, this type of structure may be compared with that of plywood, as in the descriptions by Bogduk and Macintosh (1984) and Martini et al. (2004). Instead, the significant quantity of elastic fibres found means that the description of Geneser (1986) is not in agreement with ours. In addition, the deep muscular fascia of the upper limb cannot be considered as loose, areolar, connective tissue (Standring et al., 2005), due to its significant collagen component.

The structural organisation of the fascia allows strong resistance to traction, even when it is exercised in different directions, due to the differing orientations of the collagen fibres in the layers. At the same time, the fascia can adapt to stretching, thanks to the elastic fibres together with the undulating arrangement of the collagen fibres. Once traction stops the same elastic fibres probably allow the fascia to return to its resting state.

In 1899, Testut wrote that "although the fasciae are extremely pliable, at the same time, they are very resistant and almost inextensible". The histological structure described in our study represents the anatomical basis of this apparent functional contradiction.

The presence of many free and encapsulated nerve terminations, particularly Ruffini and Pacini corpuscles, indicates that the deep muscular fascia probably plays a proprioceptive role. The capsules of these corpuscles are connected to the collagen fibres that surround them and are therefore probably also subjected to stretching (Stecco, 2006). Instead, the larger nerve fibres are often surrounded by loose connective tissue, which preserves the nerve from the traction to which the fascia is subjected. Adaptation of the fascia is only possible within certain limits, beyond which the nerve terminations are activated by stretching. This mechanism allows a sort of "gate control" on normal activation of the intrafascial receptors. Further studies will be necessary to reveal the presence of fibres of the autonomic nervous system and to determine whether such innervation pertains to the vessel walls or if is specifically related to the muscular fascia.

The capacity of the various collagen layers to slide over each other may be altered in cases of over-use syndrome, trauma or surgery. In such cases, the amortising mechanism of the fascia on the nervous terminations is lost, causing incorrect paradoxical activation of nerve receptors within the fascia, resulting in the propagation of a nociceptive signal even in situations of normal physiological stretch.

Paoletti (2002) and Stecco (2004) hypothesise that the deep fascia between two joints guarantees perceptive and directional continuity along a specific myokinetic chain, acting as a transmission belt between two adjacent joints and also between synergic muscle groups. The layered arrangement of the collagen fibres of the deep muscular fascia, allowing the transmission of traction, is the anatomical basis to this hypothesis.

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