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Tubular aggregates: their association with myalgia

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SUMMARY Three thousand consecutive muscle biopsies were reviewed for the presence of tubular aggregates and their association with clinical symptomatology. Tubular aggregates were detected in 19 patients (0·6%). Twelve of these nineteen patients had severe myalgia, and the most abundant tubular aggregates were found in biopsies of patients with myalgia. Seven patients had only myalgia as their clinical symptomatology with normal physical examination. An additional five patients with tubular aggregates and myalgia had concomitant amyotrophic lateral sclerosis (2) or neuropathy (3). The high incidence of myalgia associated with tubular aggregates in our patients and the fact that tubular aggregates originate from sarcoplasmic reticulum suggest a role played by this structure in the pathogenesis of myalgia.

Tubular aggregates are visible by light microscopy within Type II muscle fibres as a mass in the subsarcolemmal region with strong NADH-tetrazolium reductase (NADH-TR) and negative succinic dehydrogenase reactions. Ultrastructurally they are closely packed, parallel, double-walled tubules which appear to be a proliferation of sarcoplasmic reticulum.[1] The diagnostic importance of tubular aggregates is uncertain because they are present in muscles of patients with a wide variety of disorders.[1–21] Recently tubular aggregates have been described in muscle biopsy specimens of patients with myalgia who are otherwise normal.[21–26] The fact that tubular aggregates originates from sarcoplasmic reticulum, which has an important role in regulation of muscle contracture and relaxation, suggests a relationship between tubular aggregates, sarcoplasmic reticulum and myalgia. To clarify this relationship, we reviewed muscle biopsy specimens for the presence of tubular aggregates and their association with clinical symptomatology.

Methods

We analysed all 3000 muscle biopsy specimens at our institution in the preceeding seven years. All were from the quadriceps or biceps muscles. For histochemical studies, tissue had been frozen with liquid nitrogen, sectioned at 10 μ in a cryostat, and stained with the following methods: Modified Gomori Trichrome (TRI), NADH-TR, adenosine triphosphatase (ATPase) at pH 9·4, succinic dehydrogenase and phosphorylase. For electon microscopy, tissue had been fixed in 1% glutaraldehyde buffered at pH 7·4 for 1½ h and then fixed in 1% osmium tetroxide for another hour. Some material was then embedded in Epon and sections 1 μ thick were cut for all blocks, stained with toluidine blue and examined under the light microscopy. Thin sections for electron microscopy were then stained with uranyl acetate and with lead citrate.

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Results

Among 3000 patients 19 (0·6%) had histochemically typical tubular aggregates; 17 patients were male and two female. With the TRI stain, the tubular aggregates appeared as bright red staining subsarcolemmal material. Tubular aggregates were seen only in the Type II fibres, were darkly stained by the NADH-TR, and unstained by ATPase and succinate dehydrogenase (fig 1). Further studies including electron microscope and epoxy resin histology were done in some cases which showed closely packed, parallel, double-walled tubules (fig 2).

Five biopsies showed less than 1% fibres affected with tubular aggregates, six between 1–10%, and eight had more than 10% fibres with tubular aggregates. This was determined after obtaining a 100× photograph of each biopsy specimen and counting the number of tubular aggregates per 200 muscle fibres.

Clinically, 12 of 19 patients had severe myalgia,

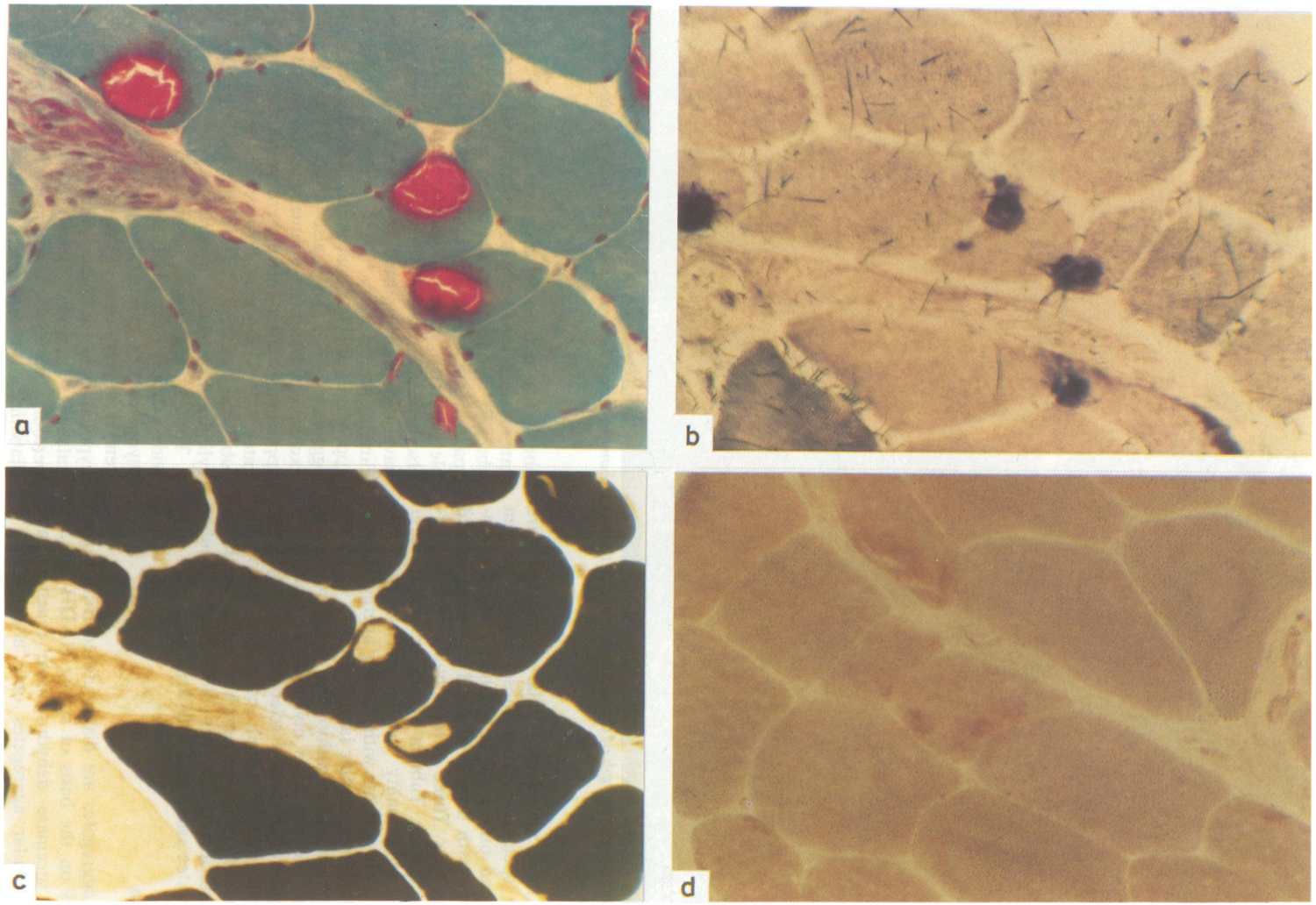

Fig 1 *Histochemical demonstration of the tubular aggregates in cross section of the quadriceps muscle. The tubular aggregates are seen as bright red staining subsarcolemmal mass with the TRI (A), darkly stained with the NADH-TR (B) and unstained with ATPase and succinic dehydrogenase (C and D). Type II muscle fibres are light in (B) and dark in (C). (×250)*

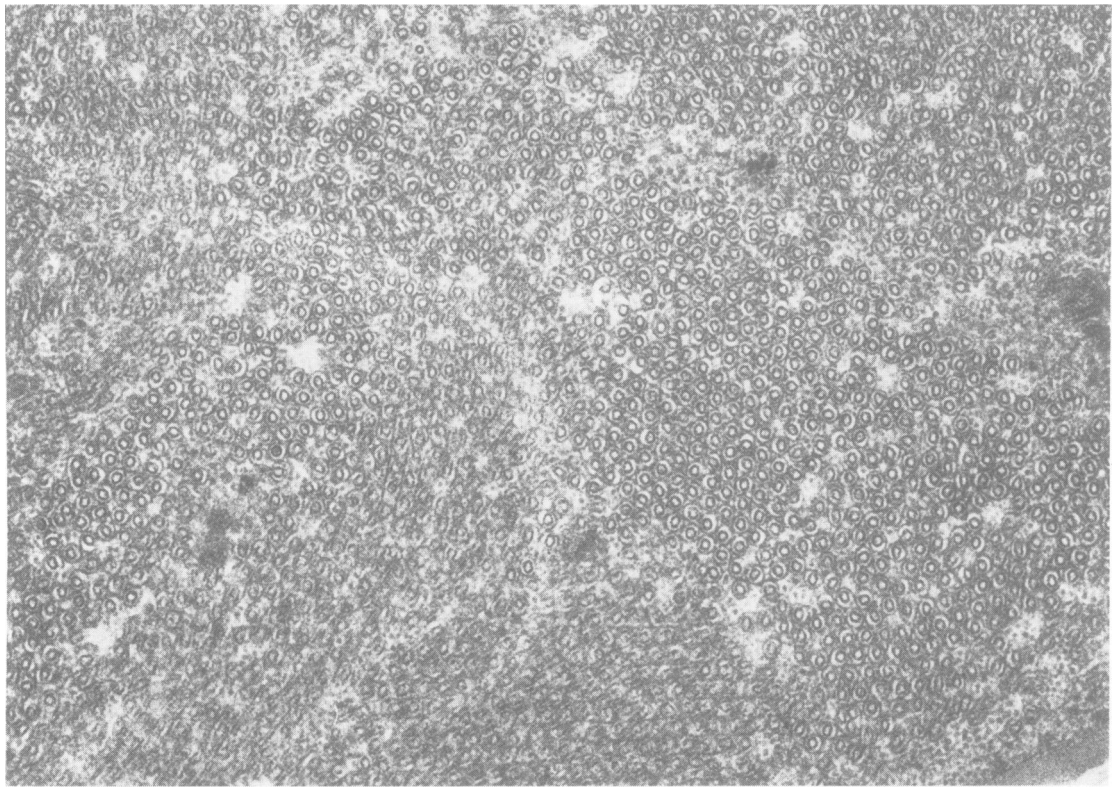

Fig 2 *Electron micrograph of the same muscle, cross section, showing closely packed, parallel and double-walled tubules. (×50,000)*

and the most abundant tubular aggregates were found in specimens from these patients. Seven patients had only myalgia as their clinical symptomatology with normal physical examination and laboratory tests. Three of them had muscle tenderness on palpation. Tubular aggregates was the predominant abnormality in the muscle of these patients. Two patients with tubular aggregates and myalgia had amyotrophic lateral sclerosis and three had peripheral neuropathy. The seven remaining cases who did not have myalgia had neuropathy (two) amyotrophic lateral sclerosis (one), systemic lupus erythematosis (one), congenital myopathy (one), and two of the patients had muscle weakness with undetermined aetiology. No patient had tubular aggregates associated with drug use.

Discussion

Tubular aggregates are distinguished from ragged red fibres on the basis of strong NADH-TR and negative succinate dehydrogenase reactions and because tubular aggregates only affect Type II fibres. The differential diagnosis of tubular aggregates include other tubular structures within muscle fibres.[12 16] Honeycomb structures are round empty spaces in an hexagonal arrangement that can resemble tubular aggregates. Tubular aggregates should also be differentiated from cylindrical spirals, which have also been reported in patients with different clinical presentations.[11 27–29] Histochemically, the appearance of cylindrical spirals is similar to tubular aggregates.[11 27 29] They also affect Type II fibres exclusively, but their unique electron microscopic appearances differentiate them from tubular aggregates.[11 27–29] They may, however, be associated with tubular aggregates.[11 27] The origin and significance of cylindrical spirals are uncertain.

Tubular aggregates have been reported in a wide variety of disorders including alcoholic myopathy,[16 17] gyrate atrophy of the eye,[2] congenital myasthenia gravis,[4–6 14] myotonia,[7 15 21] hypokalaemic periodic paralysis,[1 8 9 18–21] hyperkalaemic periodic paralysis,[1] normokalaemic periodic paralysis,[13] inflammatory myopathy,[21] diabetic amyotrophy,[3] hyperaldosteronism,[12]

prophyria cutanea tarda[1] and chronic drug exposure.[1] A similar abnormality has been found in muscles of mice after anoxia[30] and after local injection of botulinum,[31] tetanus toxin,[31] and perhexiline maleate[33] and in murine dystrophic heterozygotes.[34] Tubular aggregates have also been described in association with myalgia in otherwise normal individuals.[21-26] Twelve of 19 of our patients with tubular aggregates had severe myalgia. Seven patients had myalgia as their sole clinical symptomatology and despite extensive investigation, no definite diagnosis could be made. Like patients reported by Rosenberg *et al*,[21] myalgia was not necessarily precipitated by exercise. Three of these cases had severe muscle tenderness on palpation. Tubular aggregates was the prominent abnormality in the muscle of these patients. Five other patients with tubular aggregates and myalgia had concomitant amyotrophic lateral sclerosis or neuropathy. In these patients myalgia was one of their major complaints.

It is generally accepted that tubular aggregates originate in the sarcoplasmic reticulum, but their pathogenesis is unknown. Based on the finding of tubular aggregates in muscle biopsies of patients chronically using large amounts of analgesic-narcotic drugs and alcohol,[1 16] it has been postulated that they may form in response to exogenous or endogenous toxin. However, these reports failed to indicate why these medications were taken or why the muscle biopsies were performed,[1 16] and it may be that the reason for chronic analgesic use was for relief of myalgia.[16] It has also been suggested that tubular aggregates may represent adaptive hyperplastic sarcoplasmic reticulum derivatives to improve calcium uptake in alcoholic myopathy.[17] However, there was no history of chronic drug use or alcoholic abuse in our cases. We also doubt that this is a virus-induced lesion as postulated by Dunkle *et al*[18] and Bergman,[8 9] since no evidence of viral infection of muscle was seen. There is thus no satisfactory explanation for the formation of tubular aggregates, nor for their occurrence in a wide variety of diseases, but there may be an explanation of the high incidence of myalgia associated with them.

Sarcoplasmic reticulum has an important role in contraction and relaxation of muscle. The function of the sarcoplasmic reticulum is regulation of the calcium concentration in the vicinity of actin and myosin interaction. This regulation of calcium concentration is the control factor for turning on and off the basic contractile unit, the sarcomere. It is well known that malfunction of sarcoplasmic reticulum interferes with normal contraction and relaxation, and it may cause myalgia. The fact that tubular aggregates originates from sarcoplasmic reticulum and the high incidence of myalgia associated with tubular aggregates in our patients and previous reports of similar cases,[21-26] suggest a role played by this structure in the pathogenesis of myalgia. The relationship between tubular aggregates, sarcoplasmic reticulum and calcium metabolism may also explain why tubular aggregates are seen only in the Type II fibres. Type II fibres have considerably more sarcoplasmic reticulum than the Type I fibres.[35] In Type II fibres, the sarcoplasmic reticulum is possibly qualitatively different from that of the Type 1 fibre.[1] Also it has been shown in rabbits that white muscle fibres (Type II) have more active calcium-concentrating microsoms than red muscle fibres (Type 1).[36] These structural differences may predispose Type II fibres to tubular aggregates formation.

In spite of relationships between tubular aggregates, sarcoplasmic reticulum and myalgia, tubular aggregates are not always associated with myalgia. There may be different types of tubular aggregates,[12 16 17 37 38] but there are classic forms of tubular aggregates in patients who never complain of myalgia. Also, in our patients who had electron microscopic studies, the pattern of tubular aggregates was similar in cases with or without myalgia. Degree of muscle fibre involvement with tubular aggregates may be an important factor for appearance of myalgia since the most abundant tubular aggregates were found in our patients with myalgia.

Based on our observation and review of literatures, we suggest the association of myalgia and tubular aggregates may be important. Although myalgia is the most prominent feature in the symptomatology of a wide variety of conditions, in many patients no firm diagnosis can be made despite extensive investigation.[39] Therefore, the presence of tubular aggregates in patients complaining of mayl-gia may be a hallmark and should not be regarded as a nonspecific finding. Recognition of these combinations in time may lead to a better understanding of the significance of tubular aggregates. This relationship between tubular aggregates, sarcoplasmic reticulum and myalgia may also have important therapeutic implications, since drugs which are known to affect these structures may prove beneficial in alleviating symptoms of myalgia.

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