Acta Neuropathol (1992) 83: 440 – 444

Acta Neuropathologica

Colchicine myopathy in a case of familial mediterranean fever: immunohistochemical and ultrastructural study of accumulated tubulin-immunoreactive material

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Received August 27, 1991/Revised, accepted November 7, 1991

Summary. Severe colchicine myopathy occurred in a 24-year-old patient treated with colchicine because of familial mediterranean fever complicated by renal amyloidosis. In addition to prominent autophagic vacuoles containing heterogeneous osmiophilic material and pleomorphous bodies, cytoplasmic deposits of finely granular material were detected that have not been noted in previous cases of colchicine myopathy. This granular material was immunoreactive for antibodies to tubulin, α-tubulin, and β-tubulin. These observations substantiate the suggestion that alterations of the microtubular network represent the initial step in the pathogenesis of colchicine myopathy.

Key words: Colchicine – Myopathy – Tubulin – Microtubules – Familial mediterranean fever

Colchicine is a drug with an antimitotic property that prevents polymerization of tubulin into microtubules [9]. The substance is known to cause a characteristic myopathy in animals [10], and recently several cases of colchicine myopathy with characteristic morphological features have been described in man [9, 12]. The exact molecular sequence of events in the pathogenesis of colchicine myopathy has not been eludicated. Alterations of the microtubular network, however, are regarded as the pathogenetic basis of colchicine myopathy. However, microtubules are only rarely observed in adult skeletal muscle fibers [5, 7, 16]. We report a case of familial mediterranean fever and renal amyloidosis treated with colchicine who subsequently developed colchicine myopathy. For the first time a striking accumulation of large amounts of granular substances immunoreactive for tubulins was observed in skeletal muscle fibers that appear to represent the missing link in the pathogenesis of colchicine myopathy.

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Case report

In a 24-year-old female patient with a history of recurrent episodes of fever, abdominal pain, and synovitis, familial mediteranean fever had been diagnosed 2 years before admission. Colchicine had been given for about 6 months at a daily dose of 2 × 0.5 mg to prevent progression of amyloidosis which is known to develop in familial mediterranean fever. The patient presented with myalgia, weakness, vomiting, and diarrhea. Diffuse alopecia was thought to be caused by ketokonazol that had been given because of soor infection of the gastrointestinal tract. Neurological examination revealed proximal muscle weakness. Deep tendon reflexes were absent. Laboratory findings included an increased creatine kinase level of 900 μmol/l and myoglobinuria with a myoglobin level of 1600 μg/l. Electromyography of the peroneal and anterior tibial muscle showed motor unit potentials which were very small in amplitude, polyphasic and long in duration. In addition, abnormal spontaneous activity was observed in the anterior tibial muscle. Nerve conduction velocities were in the normal range. After reduction of the colchicine dose, the patient's strength became normal and renal function improved following administration of increased amounts of fluids [further clinical details will be reported separately (J. Stefanidis and H.-G. Sieberth, in preparation)].

Material and methods

The biopsy of the peroneus brevis muscle was processed for light microscopic examination according to standard procedures [14] (paraffin sections: H&E, elastica van Gieson; cryostat sections: mATPase at pH 9.4, 4.6 and 4.2; H&E, NADH, trichrome, methylene blue, PAS, oil red). Muscle tissue for semithin and ultrathin sections was fixed in 6% glutaraldehyde with 0.1 M phosphate buffer, pH 7.4. The sural nerve biopsy was fixed in 3.9% glutaraldehyde with 0.1 M phosphate buffer. The biopsies were both postfixed in 2% phosphate-buffered osmium tetroxide and processed for epoxy resin embedding. Semithin sections were stained with paraphenylenediamine and toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a Philips EM 400T electron microscope.

Immunohistochemistry was performed on 1-μm semithin sections of muscle and nerve tissue [15]. In short, sections were pretreated with sodium methoxide (10%) and a 0.3% solution of hydrogen peroxide. After incubation with normal bovine serum albumin diluted in Tris buffer, pH 7.4, 1:30 for 30 min, the sections were incubated overnight with antibodies to α-tubulin 1:100, β-tubulin 1:10, tubulin 1:10 (Sigma, Deisenhofen, FRG), desmin 1:50, vimentin 1:10 (Dako, Hamburg, FRG), vinculin 1:50, and

α-actinin 1:50 (ICN, Meckenheim, FRG). The primary antibodies were then visualized by using the peroxidase-antiperoxidase method. Antibodies were diluted in Tris-buffered saline and 2.5 % bovine serum albumin. A sural nerve biopsy served as a positive control for the immunoreaction against tubulin, α-tubulin, and β-tubulin. Negative controls were obtained by ommission of the primary antibody.

Results

Muscle biopsy

There was an increased variability of fiber size. Considerable numbers of atrophic fibers, occasionally in small groups, were observed. Histochemical staining revealed involvement of both fiber types. Small clusters of type 1 and type 2 fibers were occasionally seen. Vacuoles of varying size (Fig. 1a) occurred predominantly in large muscle fibers. The number of vacuoles varied in different fascicles to some extent, but 30 %–50 % of the fibers showed this change. Some of the vacuoles stained positively with Gomori's trichrome stain. About 10 % of the vacuolated or non-vacuolated fibers showed various stages of necrosis, some of which were undergoing phagocytosis. Small groups of basophilic fibers were also detected.

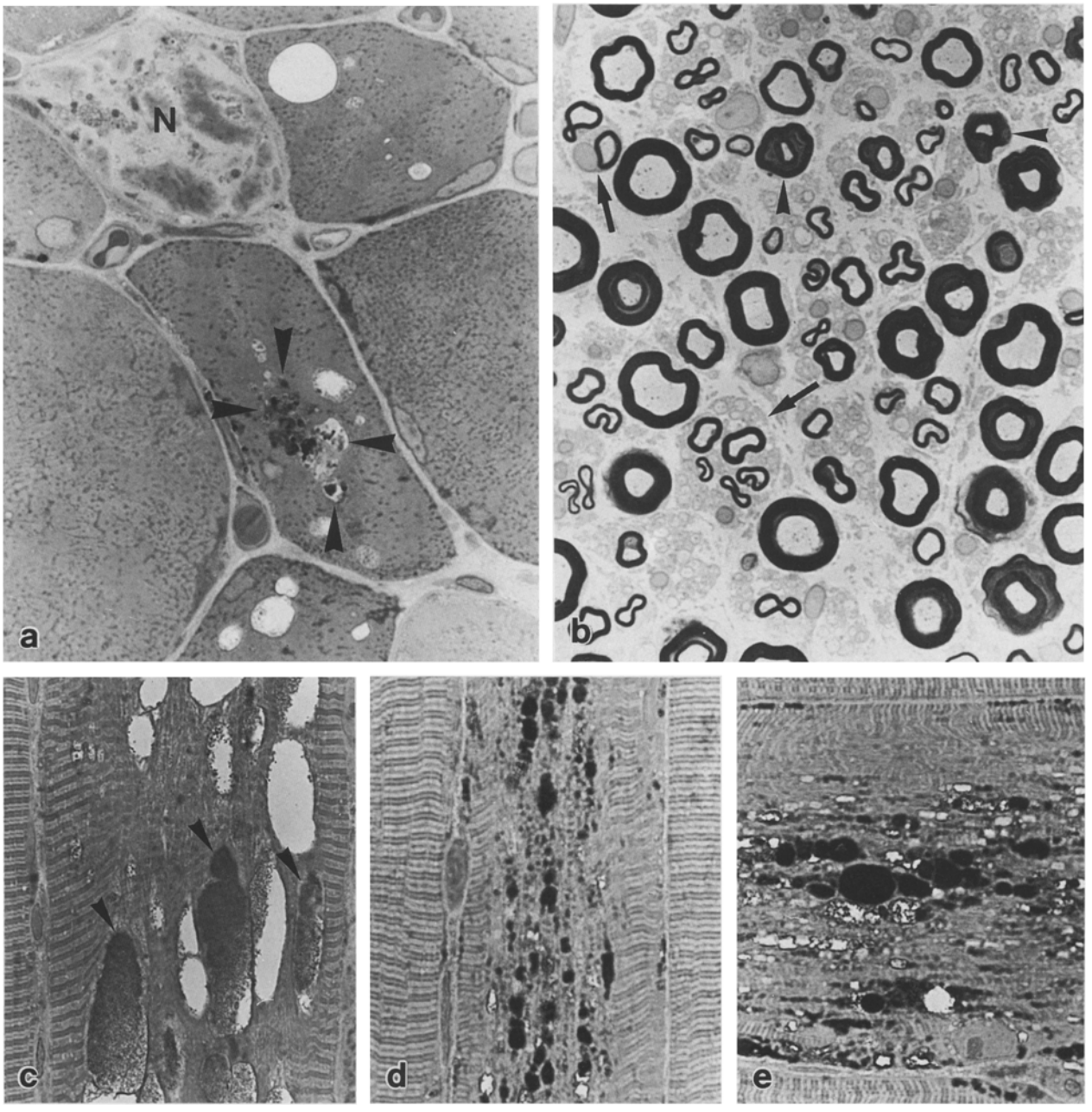

Fig. 1. **a** Semithin section showing necrotic muscle fiber (*N*) and vacuoles with granular content (*arrowheads*); paraphenylenediamine, × 1,200. **b** Reduction in the number of myelinated nerve fibers in addition to groups of regenerating nerve fibers (*arrows*) and atrophic axons (*arrowheads*) in the sural nerve; toluidine blue, × 1,300. **c** Faint staining of granular material in autophagic vacuoles (*arrowheads*) immunoreactive with antibodies to β-tubulin, × 750. **d, e** Intermyofibrillar granular material of varying size immunoreactive for tubulin; **d** × 975; **e** × 875

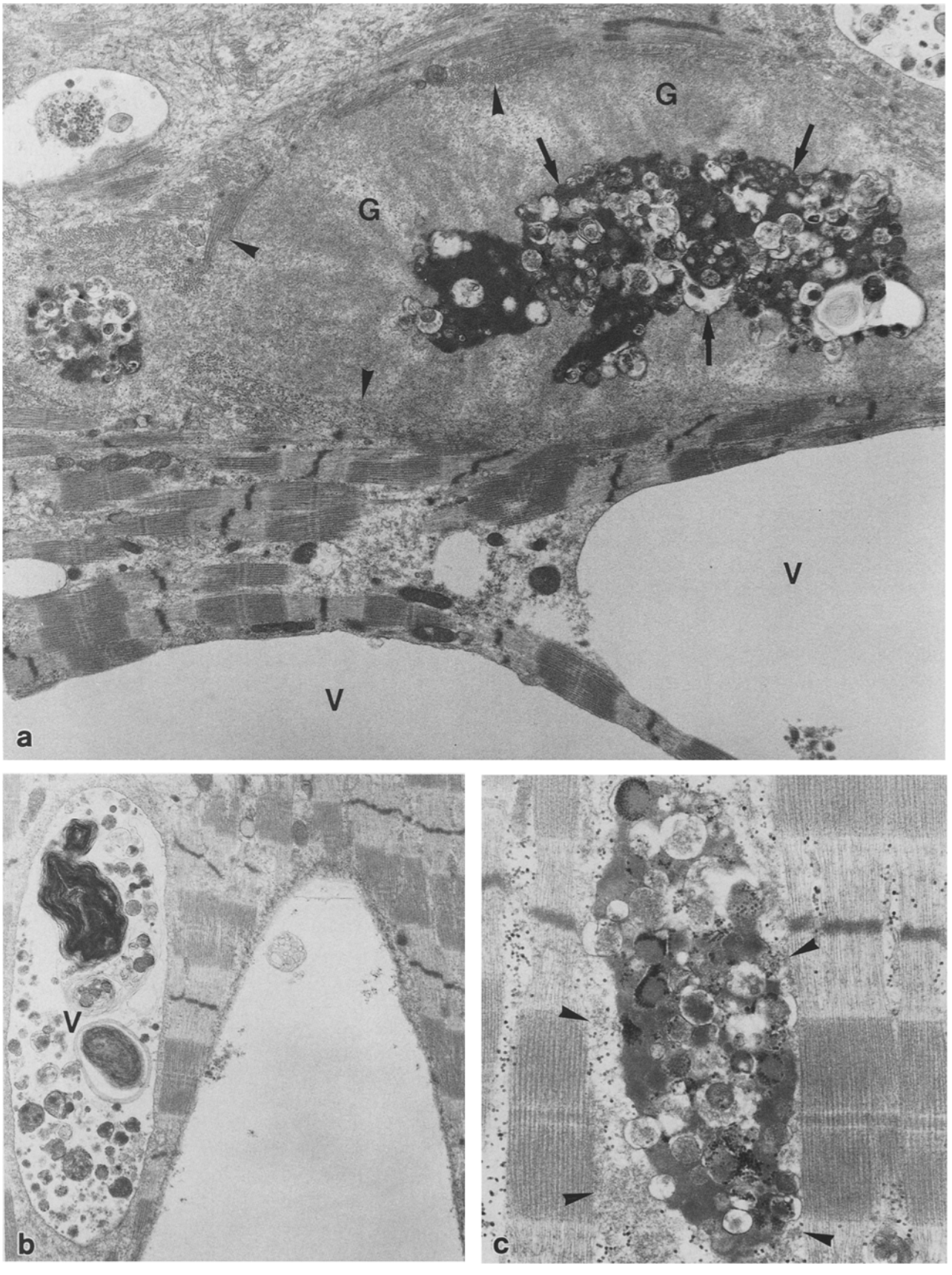

Fig. 2. **a** Electron micrograph showing large vacuoles (*V*) and heterogeneous membranous material (*arrows*) surrounded by a large accumulation of finely granular material (*G*). Fragmented and disorientated myofibrils (*arrowheads*) are also seen; × 10,800. **b** Electron micrograph showing intermyofibrillar vacuoles one of which (*V*) contains heterogeneous membranous and granular bodies; × 11,000. **c** Intermyofibrillar heterogenous deposit surrounded by finely granular substances (*arrowheads*); × 9,600

Immunocytochemical examination of the muscle biopsy revealed accumulation of tubulin-reactive material in many muscle fibers (Fig. 1d, e). In longitudinal sections, tubulin-immunoreactive deposits were located mainly between myofibrils and at the site of disintegrating myofibrils. The immunoreactive material had a granular appearance forming rounded or longitudinal structures with a diameter between 1 μm and 35 μm. Prominent accumulation of granular material was also observed in some small or large vacuoles of degenerating fibers. However, staining of the nuclei or myofibrils in a normal or overcontracted state was not observed. The distribution of tubulin immunoreactivity within single muscle fibers was uneven. Whereas in some areas large numbers of granular substances were observed, other parts showed only a few and small or no immunoreactive particles.

The distribution of the tubulin subunit α-tubulin in the muscle biopsy corresponded to the distribution of tubulin. Staining for α-tubulin was also observed in some autophagic vacuoles. There was less intense immunoreactivity for β-tubulin (Fig. 1c) than for tubulin, although the distribution of β-tubulin was similar to that of α-tubulin and tubulin. In a few muscle fibers granular substances appeared to be immunoreactive for desmin; however, staining was not always discernable from nonspecific background staining. No immunoreactivity of granular substances for α-actinin, vinculin, or vimentin was observed, although vimentin immunoreactivity was apparent in perivascular cells and some small perinuclear granules stained with vinculin antibodies.

By electron microscopy, the vacuoles were identified as autophagic vacuoles containing pleomorphous osmiophilic material (Fig. 2a, b), including typical spheromembranous bodies similar to those described by Markand and D'Agostino [10]. In addition, prominent cytoplasmic deposits of an unusual, finely granular material were seen (Fig. 2a). Disorganization of myofibrils (Fig. 2a) was associated with various degree of myofibrillar loss. Focal Z-band streaming and occasional target fibers were also present (not illustrated). Furthermore, honeycomb-like structures due to proliferation of the T system were observed.

Nerve biopsy

Light microscopic examination of the sural nerve biopsy (Fig. 1b) revealed moderate loss of myelinated nerve fibers, clusters of small myelinated axons, and rare axonal degeneration.

The axonal cytoplasm of the sural nerve displayed a faint immunoreactivity for tubulin, α-tubulin and β-tubulin. The staining was more intense in nodal and paranodal regions than in internodal areas. No abnormal deposits of tubulin were detected in the sural nerve biopsy.

By electron microscopy, the sural nerve biopsy showed abnormal lysosomal structures with membranous inclusions in the cytoplasm of Schwann cells. Vesicular disintegration of isolated myelin sheaths was also observed.

Discussion

Accumulation of an unusual, finely granular material in addition to well-known morphological features of colchicine myopathy were detected in the muscle biopsy of a 24-year-old patient with mediterranean fever, proximal weakness, rhabdomyolysis, and acute renal failure. The patient was treated with colchicine to prevent progression of amyloidosis which is known to occur in familial mediterranean fever. The granular material was immunoreactive for tubulin, α-tubulin, and β-tubulin, and to a certain extent also for desmin (cf. [15]), but not for vinculin and vimentin.

The diagnosis of colchicine myopathy was corroborated by the characteristic spheromembranous bodies that have been found in skeletal muscle after administration of colchicine in rats [8] and man [9]. Previous ultrastructural examination of muscle biopsies in colchicine myopathy revealed heterogenous membranous debris within autophagolysosomes, and occasional perinuclear aggregates of 'filamentous material' [9, 12] that, however, had not been illustrated. Patients usually present with proximal weakness and elevation of serum creatine kinase [9]. In the present patient, the morphological features and the development of proximal muscle weakness associated with a serum creatine kinase level of 1000 U/l during treatment with colchicine are, therefore, consistent with the diagnosis of colchicine myopathy. Impaired renal function has been shown to represent a risk factor for the development of colchicine myopathy because of its insufficient elimination [9]. Renal insufficiency and amyloidosis, regarded as the most serious complication of familial mediterranean fever, had both been diagnosed in the present patient. Thus, an increased risk for the development of colchicine myopathy was established.

The neurological symptoms, however, could not be explained as a consequence of familial mediterranean fever alone, although myalgia and contractions have been described [3, 4, 13]. In addition to muscle fiber alterations, colchicine induces an axonal type of neuropathy with loss of large myelinated axons, degenerating axons, and clusters of regenerating axons [9]. The present nerve biopsy, in fact, showed changes consistent with the diagnosis of a mild axonal type of neuropathy. Myopathy, however, was far more severe than neuropathy so that classification of the biopsy changes as neuromyopathy would be somewhat inadequate.

Little information about muscle alterations in familial mediterranean fever is available. Amyloid deposits have been described in the interstitial matrix of various tissues [1]. Increased amounts of endomysial and perimysial collagen fibrils have also been noted [13]. Both of these features were not apparent in the present patient's muscle biopsy, although renal amyloidosis had been diagnosed.

The pharmacological action of colchicine is based on its binding to tubulin which prevents the polymerization of tubulin into microtubules. Colchicine myopathy is also ascribed to alterations of the microtubular network [8, 9]. Microtubules are believed to participate in the

transport of autophagic vacuoles and in the linkage of autophagic vacuoles with primary lysosomes [6]. Therefore, it has been assumed that the accumulation of autophagic vacuoles in colchicine myopathy is due to alterations of the microtubular network [9]. However, in normal skeletal muscle of adult mammals microtubules are only rarely observed [5, 7, 16]. By contrast, microtubules obviously play an important role during the differentiation of myogenic cells. Microtubular function is regarded as essential for the parallel, longitudinal orientation of myofilaments in myogenic cells [2, 11]. Therefore disorientation of myofibrils as observed in the present muscle biopsy is also suggested to be related to disruption of the microtubular network.

In addition to the accumulation of autophagic vacuoles and the disorientation of myofibrils, accumulation of finely granular material was observed in the present muscle biopsy. Whereas accumulation of autophagic vacuoles has been described before and was supposed to be induced by a disturbance of the microtubular transport of these organelles, the granular material, documented in the present study, has not previously been noted, and its nature and the mechanism of its accumulation has not been elucidated. Since colchicine prevents the polymerization of tubulin into microtubules it is tempting to speculate that the granular material consists of abnormally aggregated tubulin. In fact, the large aggregates of tubulin-immunoreactive granular material seen in the sarcoplasm and within the autophagic vacuoles of the present patient's muscle biopsy support this hypothesis. Disruption of the microtubular network with accumulation of unstructured tubulins might, therefore, play an important role in the pathogenesis of colchicine myopathy.

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