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Immunohistochemical evidence for amyloid β in rat soleus muscle in chloroquine-induced myopathy

Kayo Tsuzuki[a,*], Ryo Fukatsu[b], Yuji Takamaru[c], Koichi Kimura[a], Masakazu Abe[d], Koji Shima[e], Nobuhiro Fujii[a], Naohiko Takahata[b]

Departments of [a]Microbiology and [b]Neuropsychiatry, Sapporo Medical University, School of Medicine, South 1, West 17, Chuo-ku, Sapporo 060, Japan [c]Department of Psychiatry, [d]First Department of Pathology and [e]Department of Neurology, Hokkaido University, School of Medicine, Sapporo 060, Japan

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Abstract

Deposition of amyloid β (Aβ) is one of the pathological hallmarks of brains affected with Alzheimer's disease (AD). The accumulation of Aβ have been observed in human myopathies with rimmed vacuoles (RVs) which might involve lysosomal function. Chloroquine, a potent lysosomotropic agent, induces muscle pathology in experimental animals similar to myopathy with RV. In this study, we demonstrate, for the first time, immunohistochemical evidence that Aβ and cathepsin D, a lysosomal enzyme, accumulate in vacuolated rat soleus muscle due to chloroquine-induced myopathy. These data indicate that lysosomes are important in the metabolism of amyloid precursor protein to generate Aβ. This experimental system seems to be useful not only to study basic mechanisms underlying RV myopathy but also to understand processing of amyloid precursor protein to Aβ in AD.

Key words: Alzheimer's disease; Amyloid β Protein; Amyloid precursor protein; Chloroquine myopathy; Rimmed vacuole; Cathepsin D

Progressive deposition of amyloid β (Aβ) is the most characteristic pathological change in the brain of the patient suffering from Alzheimer's disease (AD). Aβ, a 40-amino acid protein, is proteolytic product derived from amyloid precursor protein (APP), a large integral membrane protein [13,14,17,19]. It was generally assumed that Aβ deposition was limited to the brain. Askanas et al., however, found that rimmed vacuoles (RVs) accumulated Aβ in muscles affected with inclusion body myositis (IBM) [2–4]. There are further striking similarities between RVs in these myopathies and Alzheimer's pathology [1,3,5–7].

The endosomal/lysosomal pathway is considered by some to be involved in the generation of Aβ in AD and cells cultured with lysosomotropic agents [10,11,20]. Chloroquine, a potent lysosomotropic agent, induced RV myopathy in humans and experimental animals [15,16,22].

In this study, we present immunopathological evidence that Aβ and cathepsin D, a lysosomal enzyme, accumulate in chloroquine-induced RVs in soleus muscles.

The right hindleg of adult male Wister rats was denervated by ligating the sciatic nerve. 1 day after ligation, chloroquine, 50 mg/kg of body weight, or an equal volume of saline control was injected i.p. [15].

Pathological and immunohistochemical studies were made with innervated (left) and denervated (right) soleus muscles 0, 3, 7, 14, 21 and 28 days after the initial injection. Transverse cryostat sections were stained with hematoxyline-eosin (HE) and modified Gomori-trichrome. For electron microscopy (EM), the rats were perfused with a glutaraldehyde/paraformaldehyde fixative. Ultrathin sections were stained with uranyl acetate and lead citrate.

In chloroquine-treated rats, the muscle fibers were

*Corresponding author. Fax: (81) (11) 612–5861.

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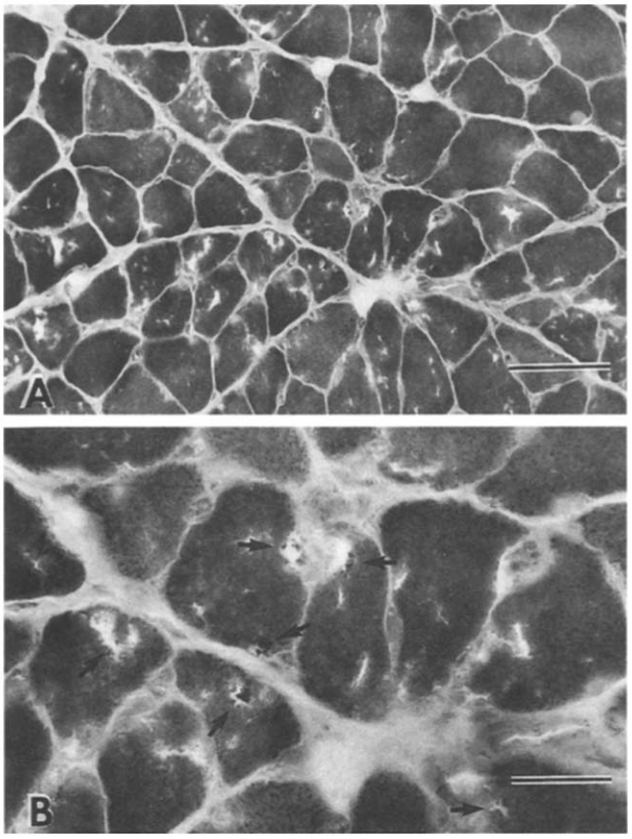

Fig. 1. Light micrographs of transverse sections of denervated chloroquine-treated rat soleus muscle on day 14 stained with modified Gomori-trichrome. Arrows indicate the vacuoles rimmed by red granular material. Bar, 50 μm in A, and 20 μm in B.

atrophic on day 7. Angulated fibers were scattered or grouped in small clusters. Vacuoles, round or irregular in shape, were located in the middle or under the surface of the affected fibers. The vacuole rims and lumens were often stained with modified Gomori-trichrome. By day 14, RVs increased in number, were conspicuous and sarcoplasm adjacent to vacuoles was stained (Fig. 1). On day 21, muscle destruction and proliferated connective tissue became more pronounced but inflammatory cell infiltrates were not seen.

By day 14, small vacuoles and cytoplasmic degeneration products were observed using EM. The small vacuoles were surrounded by irregular wavy membranes. Organella at various stages of degeneration, amorphous material, dense bodies, membranous profiles and myeloid bodies were observed. These ultrastructural changes resemble those observed in human RV myopathy [9]. The degeneration products clustered, then appeared to aggregate. Vacuoles appeared to fuse and form larger vacuoles (Fig. 2).

Immunostainings were performed using monoclonal antibodies (mcAb) raised against synthetic peptides homologous to amino acid residues 597–638 (Aβ1–42) of rat APP derived from the cDNA sequence [18]. Rabbit anti cathepsin D sera purchased from Cosmo Bio, Tokyo. Transverse paraffin sections were stained with standard streptavidin-biotin peroxidase technique following the manufactures protocol (Vectastain ABC kit; PK-4000, Vector Lab. Burlingame, CA). Deparaffinized sections were blocked for endogenous peroxidase and non-specific binding. The sections were incubated in primary antibodies diluted 1:2000 with phosphate-buffered saline (PBS) for 3 h. The sections were then sequentially incubated in biotinylated secondary antibody for 1 h, streptavidin-biotin-horseradish peroxidase for 1 h and 3'3-diaminobenzidine/H_2O_2 until reaction visualized (10–30 min).

The anti rat Aβ mcAb stained both affected and normal appearing muscle fiber diffusely though the affected muscle was stronger. Strong immunoreactivities in heterogenous structures were observed in affected soleus muscle on day 14. Vacuoles, in which strongly stained granular bodies were present, had clearly stained membranes. Other numerous irregular-sized and -shaped structures were also stained with varying intensities. These structures were identified as various cytoplasmic degradation products observed by EM. Anti cathepsin D antibody reacted in and around the vacuoles of affected muscles. Using serial sections, anti Aβ mcAb and cathepsin D antibody reacted in similar locations for certain RVs (Fig. 3). All these immunoreactions were abolished when the primary antibody was omitted or replaced by a non-immune serum.

In this study, Aβ was shown to accumulate in the vacuolated muscle fibers of chloroquine-induced myopathy in rats and to co-localize with cathepsin D. It is of great importance whether Aβ or APP accumulates in chloroquine-induced myopathy. Intensive immunopathological studies demonstrated that RVs were stained with anti-N-terminus and C-terminus of APP antibodies [2–4]. Amyloid-associated proteins, antichymotrypsin, apolipoprotein E and ubiquitin are also present in RV [1,3,5,7]. These amyloid-associated proteins have not been studied in this system.

It is a controversial matter whether or not a lysosomal/endosomal pathway is involved in Aβ generation in AD and cultured cells. APP fragments containing complete Aβ were found in lysosomal/endosomal pathways in cells cultured with lysosomal inhibitors [10,11,20]. Recent report, however, showed that lysosomotropic agents could not inhibit the secretion of Aβ in cell cultures [8]. In cells

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Fig. 3. Photomicrographs of immunostained serial sections using anti rat Aβ (A) and anti cathepsin D (B) antibodies of rat soleus muscles 14 days after chloroquine treatment. Note strong immunoreactivities in heterogenous structures in A and B. Arrows indicate vacuoles in which Aβ and cathepsin D are co-localized. Bar, 10 μm.

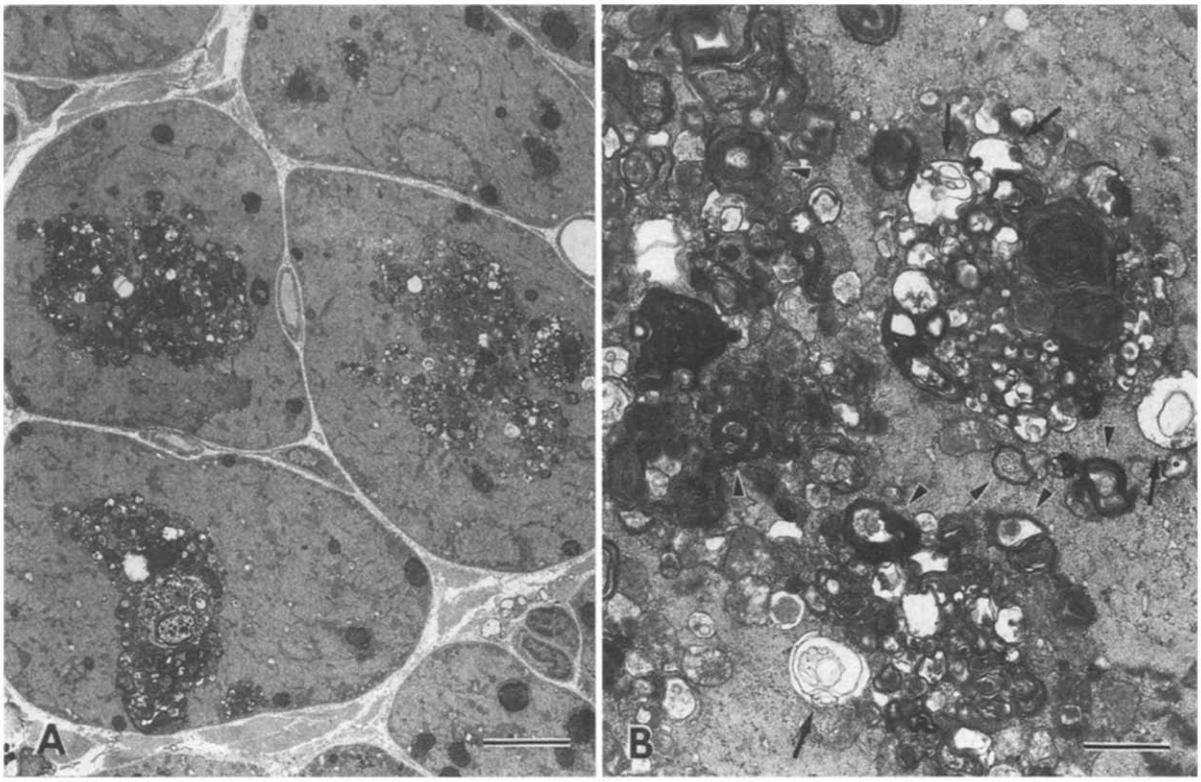
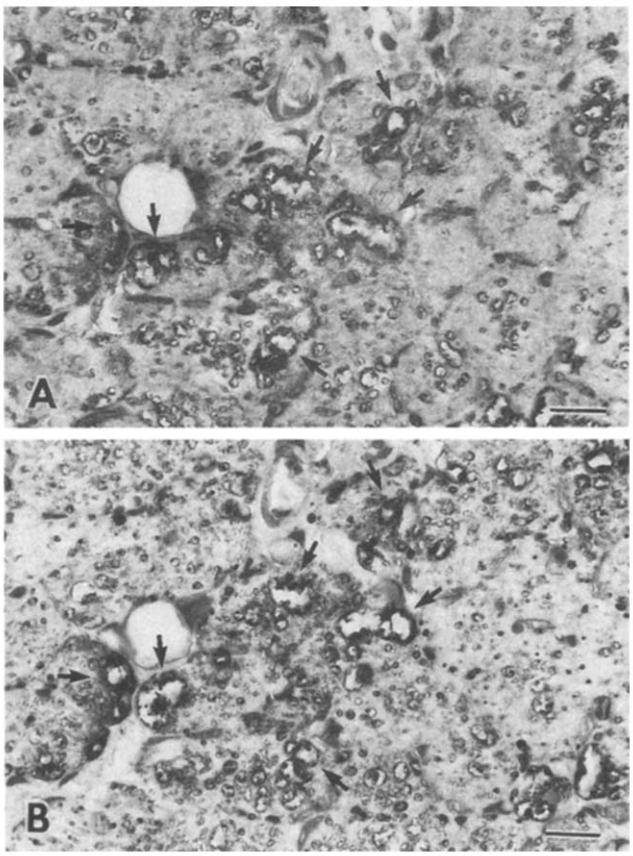


Fig. 2. Electron micrographs of denervated soleus muscle fibers on day 14. Note conglomerates composed of heterogeneous cytoplasmic degeneration products. Arrowheads indicate dense bodies, membranous profiles, myeloid bodies (B) and arrows indicate vacuoles (B). Bar, 5 μm in A, and 1 μm in B.



with RV myopathy, lysosomal proteolytic enzymes were found in RV and the activities of these enzymes in these cells were elevated [12]. In a study using human IBM and myopathy with RVs, APP and cathepsin D were found in RV [21]. It is conceivable that the lysosomal/endosomal pathway participates in RV formation, either as a primary or secondary event.

Our data suggest that a lysosomal/endosomal pathway is involved in Aβ production under these experimental conditions. This chloroquine-induced myopathy provides not only insight into the basic mechanisms leading to RV formation but also is useful for understanding APP processing to Aβ accumulation in regard to the pathogenesis of AD.

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