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Original contribution

Relationship between amyloid deposition and intracellular structural changes in familial amyloidotic polyneuropathy☆,☆☆

Yohei Misumi MD, PhD[a], Mitsuharu Ueda MD, PhD[b], Konen Obayashi MD, PhD[b], Hirofumi Jono PhD[b], Yu Su MD[b], Taro Yamashita MD, PhD[a], Toshinori Ohshima MD[a], Yukio Ando MD, PhD[b,]*, Makoto Uchino MD, PhD[a]

[a]*Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto 860-0811, Japan*
[b]*Department of Diagnostic Medicine, Graduate School of Medical Sciences, Kumamoto University, Kumamoto 860-0811, Japan*

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Summary Transthyretin-related familial amyloidotic polyneuropathy is a systemic amyloidosis caused by mutations in the transthyretin gene. Extracellular deposition of amyloid is the common pathologic hallmark of amyloidoses including Alzheimer disease, AL amyloidosis, AA amyloidosis, and familial amyloidotic polyneuropathy. However, the exact relationship between amyloid deposition and cell death has not yet been clarified. To elucidate this relationship, we studied the effect of transthyretin amyloid fibrils and prefibrillar aggregates on cells by using autopsy tissues obtained from 8 patients with familial amyloidotic polyneuropathy, as well as cultured cell lines. Ultrastructural studies of amyloid-laden cardiomyocytes showed that intracellular structural changes correlated with the degree of amyloid deposition and may reflect metabolic disturbances caused by physical limitations imposed by the amyloid deposits. Amyloid-laden vascular endothelial cells, mesangial cells, smooth muscle cells, Schwann cells, and cardiomyocytes, however, had well-preserved cell nuclei and showed no apoptotic changes, even when cells were completely surrounded by prefibrillar transthyretin aggregates and amyloid fibrils. Synthesized prefibrillar transthyretin aggregates, transthyretin fibrils, and amyloid fibrils obtained from patients with familial amyloidotic polyneuropathy evidenced no cytotoxicity in cell culture experiments. Our data thus indicate that neither transthyretin amyloid fibrils nor prefibrillar transthyretin aggregates directly induced apoptosis. However, cellular metabolic disturbances caused by cells' being physically confined by amyloid deposits may induce cell degeneration.
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1. Introduction

Familial amyloidotic polyneuropathy (FAP) is a disease, inherited in an autosomal dominant fashion, that is characterized by systemic extracellular deposition of transthyretin (TTR) amyloid fibrils, particularly in peripheral nerves, autonomic nervous system tissues, heart, kidneys, eyes, and

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* Corresponding author.
E-mail address: andoy709@kumamoto-u.ac.jp (Y. Ando).

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gastrointestinal tract [1]. TTR normally exists as a soluble plasma protein that transports thyroxine and retinol-binding protein [2]. In vitro studies of TTR amyloidogenesis led to the hypothesis that soluble TTR self-assembles into amyloid fibrils as a result of point mutations or deletions in the TTR gene that thereby lead to amyloidogenic TTR (ATTR) [3].

Many other proteins, such as Aβ, β_2-microglobulin, and prion protein, also form amyloid fibrils. These fibrils induced by different precursor proteins have common structural and biochemical features, however: nonbranching fibrils with diameters of 6 to 16 nm, apple-green birefringence when stained with Congo red and visualized under polarized light [4], and the presence of universal amyloid-associated proteins, such as apolipoprotein E, serum amyloid P component, and proteoglycans [5].

The accumulation of amyloid fibrils in the extracellular space is the common pathologic feature in all types of amyloidosis. However, the relationship between amyloid deposition and the mechanism of cell degeneration and cell death remains largely unclear [6-8]. In Alzheimer disease, which is the most comprehensively studied amyloidosis, apoptosis has often been proposed as a possible mechanism of neuronal death [9-12]. Recent studies have shown that fibril-free oligomeric aggregates, rather than mature amyloid fibrils, cause cytotoxicity. A number of hypotheses of toxicity related to Aβ oligomers and fibrils have been proposed: oxidative stress [13], mitochondrial dysfunction [14], impaired synaptic transmission [15], disruption of membrane integrity [16], and impaired axonal transport [17]. Host responses such as an inflammatory process mediated by activated microglial cells and astrocytes reportedly played an important role in the pathway leading to neuronal cell death [18,19]. However, these hypotheses were mainly studied under in vitro conditions, all hypotheses had opposing views, and controversy has surrounded the mechanism and cause of cell death in amyloidosis [6-8]. Moreover, direct evidence that neuronal apoptosis is induced by Aβ fibrils or oligomers in vivo has not been provided [20,21]. Similarly, findings showing specific colocalization of apoptotic nuclei with Aβ plaques are lacking [22]. Detecting a direct interaction between neuronal death and Aβ deposition in vivo is difficult, because neurons have very complicated structures with an axon and dendrites, and physiologic neuronal apoptosis also frequently occurs as a normal process of aging.

In FAP, however, amyloid deposition was observed around comparatively simple-shaped cells, including fat cells, myocytes, and Schwann cells; and the number of cells clearly decreased as amyloid accumulated [23]. Therefore, the direct impact of amyloid deposits on cells can be analyzed in FAP.

To elucidate the relationship between amyloid deposition and cell death mechanisms, we analyzed intracellular structural changes in amyloid-laden cells by using autopsy tissues, and we determined the toxicity of TTR aggregates and fibrils by means of cultured cell lines.

2. Materials and methods

2.1. Tissue specimens

Autopsy tissue specimens of the kidney, sciatic nerve, heart, liver, and intestine from 8 patients with FAP ATTR Val30Met were examined. Table 1 summarizes the characteristics of these patients.

Approval for this study was obtained from the ethical committee of Kumamoto University.

2.2. Congo red staining

We performed Congo red staining of specimens of the kidney, sciatic nerve, heart, liver, and intestine obtained from 8 patients with FAP. Paraffin-embedded sections were stained with Congo red according to the method of Puchtler et al [24] and were viewed by bright field or polarized light microscopy.

2.3. Terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling assay

Terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) staining of specimens was performed by using the In Situ Apoptosis Detection Kit (Takara Bio Inc, Shiga, Japan) according to the manufacturer's instructions. Specimens included kidney, sciatic nerve, and heart from 5 patients with FAP (numbers 1-5 in Table 1). The percentage of apoptotic cells in each specimen was determined by means of an apoptotic index (number of TUNEL-positive nuclei per field/total number of nuclei per field). For each myocardial specimen, tissue sections were examined at ×100 magnification and at least 200 cells were counted in 10 high-power fields.

2.4. Electron microscopy

Samples of kidney (patients 3, 4, and 5 in Table 1), sciatic nerve (patients 4, 6, and 7), and heart (patients 2, 3, and 4)

Table 1 Characteristics of autopsied patients with FAP

Patient no.	Sex	Age at onset of FAP (y)	Duration of disease (y)	Cause of death
1	F	35	11	CRF, CHF
2	M	60	7	CHF
3	F	27	11	CVD, CRF
4	M	40	6	ND
5	M	27	11	CRF
6	F	32	15	CRF, CHF
7	F	51	9	SD
8	M	30	12	CHF

Abbreviations: CHF, chronic heart failure; CRF, chronic renal failure; CVD, cerebrovascular disease; ND, not determined; SD, sudden death.

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