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Effect of albumin on transthyretin and amyloidogenic transthyretin Val30Met disposition and tissue deposition in familial amyloidotic polyneuropathy☆

Kazuaki Taguchi [a,e], Hirofumi Jono [g], Tomoe Kugimiya-Taguchi [a], Saori Nagao [a], Yu Su [c], Keishi Yamasaki [e,f], Mineyuki Mizuguchi [h], Toru Maruyama [a,b], Yukio Ando [d], Masaki Otagiri [a,e,f,*]

[a] Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan
[b] Center for Clinical Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan
[c] Department of Diagnostic Medicine, Kumamoto University, Kumamoto, Japan
[d] Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan
[e] Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto, Japan
[f] DDS Research Institute, Sojo University, Kumamoto, Japan
[g] Department of Pharmacy, Kumamoto University Hospital, Kumamoto, Japan
[h] Faculty of Pharmaceutical Sciences, University of Toyama, Toyama, Japan

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ABSTRACT

Aims: Transthyretin (TTR)-related familial amyloidotic polyneuropathy (FAP) is characterized by the systemic accumulation of amyloid fibrils caused by amyloidogenic. Our previous studies demonstrated that albumin played a role in the inhibition of TTR amyloid-formation. The aim of this study was to evaluate the effect of albumin on TTR disposition and tissue deposition *in vivo*.

Main methods: For pharmacokinetic studies, recombinant wild-type TTR (rTTR) and recombinant amyloidogenic TTR Val30Met (rATTR V30M) were labeled with iodine and administered to Sprague–Dawley rats and analbuminemia rats (NAR: Nagase Analbuminemia Rats). The deposition of ATTR V30M was also analyzed by immunohistochemistry in the transgenic (Tg) rats possessing a human *ATTR V30M gene* (ATTR V30M Tg rats) and NAR possessing a human *ATTR V30M gene* (ATTR V30M Tg NAR).

Key findings: The presence of albumin had no effect on the tissue distribution of either rTTR or rATTR V30M. However, more ATTR V30M was deposited in the hearts, stomachs and small intestines of ATTR V30M Tg NAR rats, compared to ATTR V30M Tg rats.

Significance: Although the disposition of TTR and ATTR V30M was unaffected by the presence of albumin, the deposition of ATTR V30M in some organs was apparently increased in the absence of albumin compared to the presence of albumin. These results show that albumin would contribute to suppressing the tissue deposition of TTR in pathogenesis of FAP, but does not affect the disposition of TTR.

Introduction

Albumin is the most abundant protein in the blood, and an important carrier of endogenous and exogenous ligands in the circulation. It contributes to the maintenance of osmotic pressure and plasma pH and to the Donnan-effect in capillaries (Otagiri et al., 2013; Otagiri and Chuang, 2009). In addition, the cysteine residue at position 34 (Cys34) in albumin provides antioxidant activity (Stewart et al., 2005), which, in turn, influences the plasma thiol-dependent antioxidant status of albumin, as well as the extent of oxidative damage to proteins (Quinlan et al., 1998, 2004). These multiple functions of albumin have a key role in the maintenance of physiological homeostasis. In fact, it was reported that hypoalbuminemia is strongly associated with the progression of and mortality in a number of disorders (Okamura et al., 2013; Staples et al., 2010; Zisman et al., 2009). We also reported that serum albumin levels and the reduced form at Cys34 of albumin were decreased in familial amyloidotic polyneuropathy (FAP) patients as the disease progressed (Kugimiya et al., 2011).

Transthyretin (TTR)-related FAP is an autosomal dominant form of a fatal form of heredity amyloidosis characterized by the systemic accumulation of amyloid fibrils in organs (Ando et al., 1993, 2005). To date, more than 100 different points of mutation in the TTR gene have

Abbreviations: TTR, transthyretin; FAP, familial amyloidotic polyneuropathy; ATTR, amyloidogenic transthyretin; NAR, Nagase Analbuminemia Rats; Tg, transgenic; ^{125}I, iodine; % of ID, % of injection of dose; $t_{1/2\ \alpha}$, distribution-phase half-life; $t_{1/2\ \beta}$, elimination-phase half-life; CL, clearance; AUC, area under the concentration-time curve; V_1, distribution volume of central compartment; V_2, distribution volume of peripheral compartment; V_{dss}, distribution volume.

☆ Albumin plays key roles in transthyretin disposition.

* Corresponding author at: Faculty of Pharmaceutical Sciences, Sojo University, 4-22-1 Ikeda, Kumamoto 862–0082, Japan. Tel.: +81 96 326 3887; fax: +81 96 326 5048.
E-mail address: otagirim@ph.sojo-u.ac.jp (M. Otagiri).

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been reported, and most of these mutations are amyloidogenic (Ando et al., 2005; Benson and Kincaid, 2007; Connors et al., 2003). Of the different types of amyloidogenic transthyretin (ATTR), ATTR Val30Met (ATTR V30M) is the most common (Benson and Uemichi, 1996). Although many attempts have been made to identify various types of ATTR-related FAP (Connors et al., 2003), the mechanisms responsible for the self-assembly of naturally occurring proteins into amyloid deposits remain a mystery even given the progress made in biochemical and diagnosis methods. Our previous *in vitro* studies showed that *S*-nitrosylated TTRs and ATTR V30M induced more amyloid fibrils than did unmodified TTRs and wild-type TTR, respectively, which indicates that oxidative stress is a facilitator of amyloid formation (Saito et al., 2005). Furthermore, we also revealed that albumin, which maintains antioxidant activity, may possibly play an inhibitory role in the TTR amyloid-formation process in organs (Kugimiya et al., 2011). However, the precise roles of albumin in the deposition of TTR in organs remain unknown.

The objective of this study was to evaluate the role of albumin in TTR disposition and tissue deposition *in vivo*. In initial experiments, we labeled recombinant TTR (rTTR) or recombinant ATTR V30M (rATTR V30M) with iodine (^{125}I) to produce ^{125}I-labeled TTR (^{125}I-rTTR) or ATTR V30M (^{125}I-rATTR V30M) and subsequently examined changes in the pharmacokinetic properties of rTTR or rATTR V30M using Sprague–Dawley (SD) rats and analbuminemia rats (NAR: Nagase Analbuminemia Rats). Next, to evaluate in detail the effect of albumin on TTR deposition, we compared the extent of TTR deposition between the transgenic (Tg) rats possessing the human *ATTR V30M gene* (ATTR V30M Tg rats) and NAR possessing the human *ATTR V30M gene* (ATTR V30M Tg NAR).

Materials and methods

Expression and purification of the rTTR and rATTR V30M

The rTTR and rATTR V30M were expressed and purified as reported in a previous study (Matsubara et al., 2003). In this preparation, the TTR or ATTR V30M vector (pQE30) was mixed with the *E. coli* strain M15. A preculture was grown overnight at 37 °C in Luria broth medium with ampicillin and kanamycin and was inoculated to 100 mL of the fresh Luria broth medium. Protein expression was induced with 1 mM isopropyl-β-*D*-thiogalactopyranoside when the absorbance at 600 nm reached 0.6. After growth for 7 h at 37 °C with shaking, the cells were immediately chilled on ice and harvested by centrifugation at 5000 *g* for 20 min at 4 °C. The pelleted cells were resuspended and lysed by sonication at 4 °C. After centrifugation at 5000 *g* for 40 min at 4 °C, the supernatant was filtered and the proteins were isolated by passage through DEAE sepharose gel. Finally, the proteins were purified and concentrated by high performance liquid chromatography.

rTTR and rATTR V30M labeling with ^{125}I

The rTTR and rATTR V30M were labeled with ^{125}I as previously reported, with minor modifications (Matsushita et al., 2006). ^{125}I-rTTR and ^{125}I-rATTR V30M were prepared by incubation of rTTR and rATTR V30M with Na^{125}I (PerkinElmer Inc., Piscataway, NJ, USA) in an Iodo-Gen (1, 3, 4, 6-tetrachloro-3a, 6a-diphenylglycoluril) tube for 30 min at room temperature. Thereafter, ^{125}I-rTTR and ^{125}I-rATTR V30M were isolated from free ^{125}I by passage through a PD-10 column (GE Healthcare Bio-Sciences AB; Patent Department Björkgatan 30, 75184 Uppsala, SE).

The pharmacokinetic experiments

All animal experiments were performed according to the guidelines, principles, and procedures for the care and use of laboratory animals of Kumamoto University. All rats were given water containing 5 mM sodium iodide for the duration of the experiment to avoid specific accumulation in the glandula thyreoidea. Male SD rats (Kyudou Co., Kumamoto, Japan) or NAR (Kyudou Co., Kumamoto, Japan) were anesthetized with ether and injected with the ^{125}I-rTTR or ^{125}I-rATTR V30M *via* the tail vein at a dose of 0.1 mg/kg. Blood samples were collected at multiple time points after the ^{125}I-rTTR or ^{125}I-rATTR V30M (10 min, 15 min, 30 min, 1.5 h, 3 h and 4 h) and the plasma was separated by centrifugation (3000 g, 5 min). Degraded rTTR or rATTR V30M and free ^{125}I were removed from the plasma by centrifugation in 1% bovine serum albumin and 40% trichloroacetic acid. After obtaining the last blood sampling, the organs were excised (kidneys, liver, spleen, lungs, heart, stomach, and small intestine), rinsed with saline, and weighed. The radioactivity of the samples was determined by means of a γ-counter (ARC-5000, Aloka, Tokyo, Japan).

Preparation of ATTR V30M Tg rats and ATTR V30M Tg NAR

NAR were isolated from SD rats of CLEA Japan (Japan CLEA Co., Kanagawa, Japan) (Nagase et al., 1979). ATTR V30M Tg rats were generated as previously described (Ueda et al., 2007). The ATTR V30M Tg NAR were developed by mating NAR and V30M Tg rats and were genotyped by a PCR analysis of ear DNA. The animals were maintained in a specific pathogen-free environment at the Center for Animal Resources and Development, Kumamoto University.

Immunohistochemical staining

9-month-old ATTR V30M Tg rats and ATTR V30M Tg NAR were sacrificed by acute bleeding from the abdominal aorta and selected organs (kidneys, liver, spleen, lungs, heart, stomach and small intestine) removed. The organs were then resected *en bloc* for immunohistochemical staining. The organs were fixed in 4% paraformaldehyde overnight and embedded in paraffin.

Paraffin-embedded 4 μm-thick sections were prepared and deparaffinated in xylene and rehydrated in a graded series of alcohols. Deparaffinized sections were pretreated by heating for 20 min in an autoclave apparatus. The slides were then treated with periodic acid for 10 min at room temperature, after which they were incubated in 5% normal serum for 1 h at room temperature in a moist chamber. The primary antibodies were rabbit polyclonal anti-TTR (Dako, Glostrup, Denmark) and were used at a 1:50 dilution. The secondary antibody was a horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin antibody (Dako, Glostrup, Denmark) diluted 1:100 in buffer. Reactivity was visualized with the DAB Liquid System (Dako, Glostrup, Denmark). Sections were counterstained with hematoxylin.

Histopathological scoring system of TTR deposition in organs

The sections were examined by light microscopy. For each organ, 5 different selected areas per specimen used for semiquantitative immunochemistry were analyzed by two independent investigators blind manner. The deposition of TTR in organs was assessed according to the degree of deposition using a 0 to 2 grading system. A score of 0 indicates the absence of deposition, a score of 1 indicates mild deposition (<50% of the total muscle layer in visual field), a score of 2 corresponds to severe deposition (>50% of the total muscle layer in visual field). Scores for each organ were then averaged per group (ATTR V30M Tg rats and ATTR V30M Tg NAR).

Data analysis

A two-compartment model was used for the pharmacokinetic analysis after the administration of ^{125}I-rTTR or ^{125}I-rATTR V30M. Each parameter was calculated by fitting using MULTI, a normal least-squares program (Yamaoka et al., 1981). Data are reported as the mean ± SD