Hypothesis

Apolipoprotein A-I induced amyloidosis

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Abstract Amyloidosis is characterized by extracellular deposits of protein fibrils with a high content of β-sheets in secondary structure. The protein forms together with proteoglycans amyloid fibrils causing organ damage and serious morbidity. Intact apolipoprotein A-I (apoA-I) is an important protein in lipid metabolism regulating the synthesis and catabolism of high density lipoproteins (HDL). Usually, apoA-I is not associated with amyloidosis. However, four naturally occuring mutant forms of apoA-I are known so far resulting in amyloidosis. The most important feature of all variants is the very similar formation of N-terminal fragments which were found in the amyloid deposits (residues 1–83 to 1–94). The new insights in the understanding of the association of apoA-I with HDL, its metabolism, and its hypothesized structural findings may explain a common mechanism for the genesis of apoA-I induced amyloidosis. Here we summarized the specific features of all known amyloidogenic variants of apoA-I and speculate about its metabolic pathway, which may have general implications for the metabolism of apoA-I.

Key words: Apolipoprotein A-I; Mutation; Amyloidosis; Lipoprotein; In vivo metabolism; Proteolysis

1. Introduction

Amyloidosis is characterized by extracellular accumulation of protein fibrils forming β-pleated sheets. The form of amyloidosis depends on the protein involved as the basic subunit of the amyloid fibril [1–3]. Systemic forms of hereditary amyloidosis may be associated with variant forms of normally soluble proteins such as transthyretin, fibrinogen, gelsoline, lysozyme and apolipoprotein A-I [1,2,4–8]. Non-hereditary forms of amyloidosis can also occur, like the deposits of variable fragments of the immunoglobulin light chains or degradation products of the acute phase protein serum amyloid A [9,10].

The association of amyloid fibrils with plasma and extracellular matrix proteins and proteoglycans leads to amyloid deposits which invade the extracellular spaces of organs destroying the tissue and its function, which usually leads to death over time.

2. Apolipoprotein A-I

Apolipoprotein A-I (apoA-I) is the major protein component of high density lipoproteins (HDL) [11–15]. ApoA-I has several functions: (i) structural properties of HDL; (ii) cofactor for the enzyme lecithin:cholesterol acyltransferase (LCAT), which is essential for the reverse cholesterol transport; and (iii) interacting with membranes, which is required for cholesterol efflux, hepatic uptake and interaction with other lipoprotein particles [16–20]. The protein is synthesized both in the intestine and in the liver as a 267 amino acid prepropeptide. The prepropeptide is intracellularly cleaved to the 249 amino acid propeptide and secreted in the plasma. The cleavage from the propeptide to the native apoA-I with 243 amino acids occurs within the plasma [21]. The mature protein contains no carbohydrates; the only reported post-translational modifications represent acylation and phosphorylation [22,23]. The comparison of the kinetics of human isolated apoA-I and recombinant apoA-I derived from *E. coli* revealed identical radioactive decay curves in normolipemic rabbits [24]. Therefore, the human acylated and phosphorylated apoA-I and the non-posttranslational modified *E. coli* derived apoA-I behave kinetically identical. Thus, these post-translational modifications of apoA-I do not seem to affect the in vivo metabolism of apoA-I, still they may play a role in intracellular trafficking. Mature plasma apoA-I is associated with phospholipids forming a lipid poor HDL particle, the so-called pre-β-1 HDL. This lipid poor HDL takes up phospholipids and cholesterol forming the larger discoidal HDL (pre-β-2 HDL). Finally, the esterification of cholesterol within the HDL particle by the enzyme lecithin:cholesterol acyltransferase leads to the conversion of the discoidal HDL to the spheric HDL (α-HDL) [25–27].

3. Amyloidogenic mutations of apolipoprotein A-I

Four apoA-I variants have been reported so far which cause hereditary systemic amyloidosis in patients. The important features of these amyloidogenic variants are summarized in Table 1. The first case was reported by van Allen et al. describing a kindred in which a single base mutation of guanine to cytosine to the first base of codon 26 on exon 3 of the apoA-I gene was identified as the underlying cause [28]. This point mutation leads to a change in the corresponding amino acid sequence. The amino acid glycine at position 26 was replaced by an arginine (apoA-I_{Iowa}). The protein content of the amyloid fibrils contained an N-terminal fragment (residues 1–83) of mutant apoA-I_{Iowa}. The affected patients suffered from neuropathy and nephropathy [28–32]. Interestingly, the affected patients had in addition low plasma levels of HDL and apoA-I [33].

The second amyloidotic apoA-I variant, described by Soutar et al., resulted from a single base mutation in exon 4 of the apoA-I gene [34]. The base thymine was displaced by guanine resulting in displacement of the amino acid leucine with arginine at amino acid position 60 ($Leu^{60} \rightarrow Arg$). The amyloid deposits were made of N-terminal fragments (residues 1–88,

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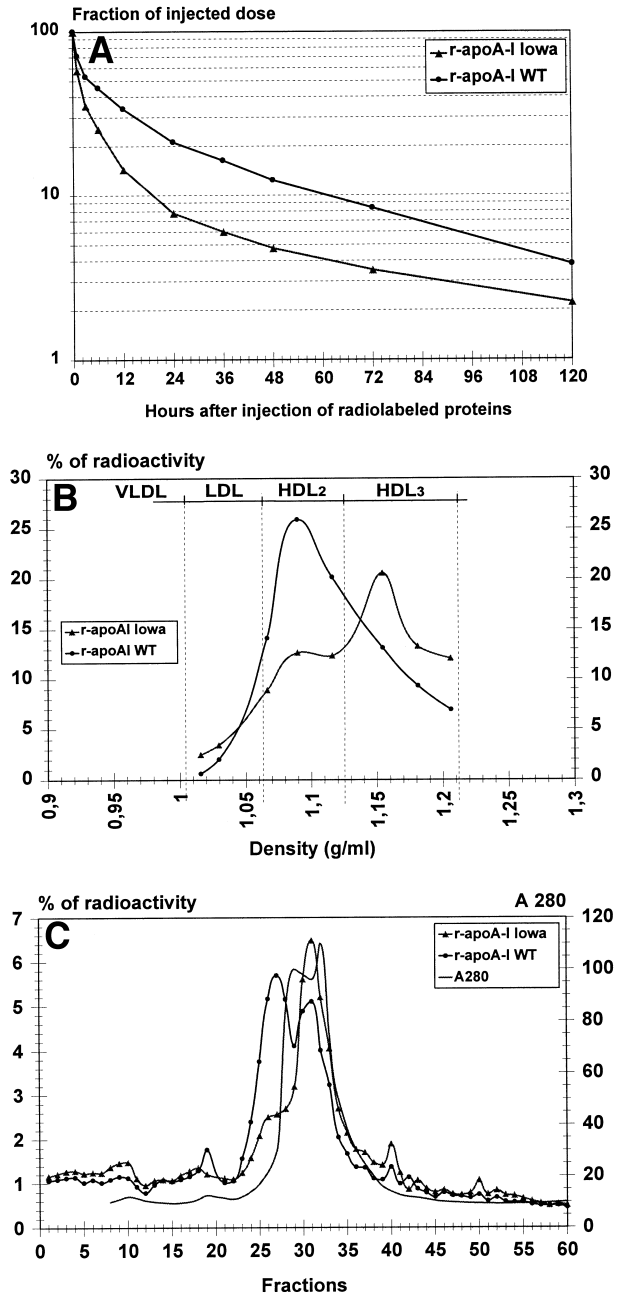

Fig. 1. A: The in vivo kinetics of human recombinant apoA-$I_{wild-type}$ (●) and human recombinant apoA-I_{Iowa} (▲) after radioiodination and injection into NZW rabbits are illustrated. All data represent the mean of triplicates. The in vivo kinetics showed a decreased catabolism of apoA-I_{Iowa} compared to apoA-$I_{wild-type}$. B: The analysis of density gradient ultracentrifugation of a serum sample obtained 1 h after simultaneous injection of radiolabeled human recombinant apoA-$I_{wild-type}$ (●) and human recombinant apoA-I_{Iowa} (▲) into NZW rabbits are shown. Two peaks of radioactivity appeared in the density range of HDL (HDL_2: $d = 1.063–1.125$ g/ml; HDL_3: $d = 1.125–1.21$ g/ml), which indicated that both apoA-I forms were associated with HDL. The majority of apoA-$I_{wild-type}$ was associated with the HDL_2 fraction, whereas the majority of apoA-I_{Iowa} was associated with the HDL_3 fraction. C: An FPLC analysis of a serum sample obtained 1 h after injection of radiolabeled human recombinant apoA-$I_{wild-type}$ (●) and human recombinant apoA-I_{Iowa} (▲) into NZW rabbits is illustrated. Two peaks of radioactivity reflect the different association of the studied apoA-I forms with HDL. The majority of apoA-$I_{wild-type}$ appeared in the first peak at fraction 23–29 and in the second peak at fraction 30–34. The majority of apoA-I_{Iowa} appeared in the second peak at fraction 30–34, indicating an association with smaller HDL particles. ←

1–92, 1–93, 1–94) of the mutant apoA-I. The intact mutant apoA-I was also found in the amyloid fibrils coprecipitated with serum amyloid A in trace amounts. However, no wild-type apoA-I was seen in the studied tissues. Neuropathy was not reported in these patients. The HDL and apoA-I serum concentrations were not reported in these patients.

Another variant apoA-I causing hereditary amyloidosis was reported by Booth et al. [35]. The mutation is a result of a substitution of thymine to cytosine in exon 4 of the apoA-I gene. The corresponding protein contains an arginine instead of tryptophan (Trp^{50}-Arg). The amyloid deposits were composed of N-terminal fragments of the variant apoA-I corresponding to residues 1–86, 1–92, 1–94. Neuropathy was not detectable. The liver was the primary site of amyloidosis in the studied patients. The HDL and apoA-I serum concentrations were not reported in the patients.

The last reported amyloidogenic apoA-I mutation (Booth et al.) [36] was caused by a mutation in exon 4 in which 35 nucleotides had been deleted and 5 nucleotides inserted. The mutation in the apoA-I gene resulted in an amino acid sequence in which the amino acids 60–71 were substituted by the two amino acids valine and threonine. The detected apoA-I fragments in the amyloid fibrils contained the N-terminal residues 1–83 and 1–92 according to the residue numbers of the wild-type sequence. The patients' plasma levels of apoA-I and HDL were significantly lower than in unaffected individuals. The affected patients had primarily hepatic involvement.

4. In vivo characterization of amyloidogenic apoA-I

The metabolic pathway of amyloidogenic apoA-I causing tissue deposition has not been elucidated yet. In this respect the work of Rader et al. is of great interest. This group isolated apoA-I from plasma of patients with the heterozygous state of apoA-I_{Iowa} induced familial amyloidosis. They radiolabelled the isolated apoA-I containing both the Gly^{26}-Arg variant and wild type, and studied its in vivo metabolism in

Table 1
Amyloidogenic apo A-I variants

Mutation	Exon	Fragments	Clinical symptoms	Ref.
Gly^{26}-Arg	3	1–83	Neuropathy, nephropathy, peptic ulcer, low HDL, low apoA-I	[28–33]
Trp^{50}-Arg	4	1–86, 1–92, 1–93	Non-neuropathic amyloidosis, renal failure	[35]
Leu^{60}-Arg	4	1–88, 1–92, 1–93, 1–94	Non-neuropathic amyloidosis, renal failure	[34]
Δ(60–71)InsValThr	4	1–83, 1–92	Non-neuropathic amyloidosis, liver failure, low HDL, low apoA-I	[36]

The currently known amyloidogenic variants are summarized. The different mutations, their genetic locations, the length of fragments within the amyloidotic fibrils, and their clinical symptoms are depicted.

both normal subjects and patients, subsequently. Their results revealed both an increased catabolism and altered association with the HDL fraction of apoA-I isolated from affected patients [33]. To gain further insight into the metabolism of apoA-I_{Iowa}, we established recombinant expression of this protein and its wildtype in transfected CHO cells [37]. After purification by reversed phase chromatography we radiolabelled the recombinant form of apoA-I_{Iowa}, and injected it simultaneously with wild type in New Zealand White (NZW) rabbits using ^{131}I and ^{125}I, respectively [38,39]. The radioactive decay curves confirmed the in vivo findings of Rader et al. ApoA-I_{Iowa} was catabolized at a much higher rate than wild type as shown in Fig. 1A. Serum samples were also analyzed by density gradient ultracentrifugation and FPLC analysis [24]. The analysis of serum samples taken after injection of radiolabelled proteins revealed an altered association of apoA-I_{Iowa} with HDL in contrast to apoA-$I_{wild-type}$. The density gradient ultracentrifugation analysis showed an increased association of apoA-I_{Iowa} with the more dense HDL_3 particles, whereas apoA-$I_{wild-type}$ was primarily associated with the HDL_2 fraction (Fig. 1B). The FPLC analysis showed two apoA-I containing peaks. The first one contained the larger HDL particles, which were composed of apoA-$I_{wild-type}$ and trace amounts of apoA-I_{Iowa} whereas the second peak contained the majority of apoA-I_{Iowa} (Fig. 1C).

5. Discussion

The mechanisms how specific mutations of apoA-I lead to amyloid deposition have not been revealed yet. Interestingly, there are some similar features of all four known amyloidogenic apoA-I variants. The genetic trait is autosomal dominant. All three point mutations are based on a substitution of a neutral amino acid by the cationic amino acid arginine. This change in the net charge of the protein results in a characteristic shift in its isoelectric point [30,34–36]. The deletion/insertion mutation also resulted in an additional positive charge of the protein with the corresponding shift in isoelectric focussing. All four amyloidogenic mutations are located in the N-terminal end of apoA-I (Fig. 2). All four mutations resulted in amyloid deposits containing N-terminal fragments of the variant protein. The identified N-terminal fragments are of similar length (residues 1–83 to 1–94) (Fig. 2). Since the HDL levels were not reported for all affected subjects, it is not known whether decreased HDL is a characteristic finding in all patients.

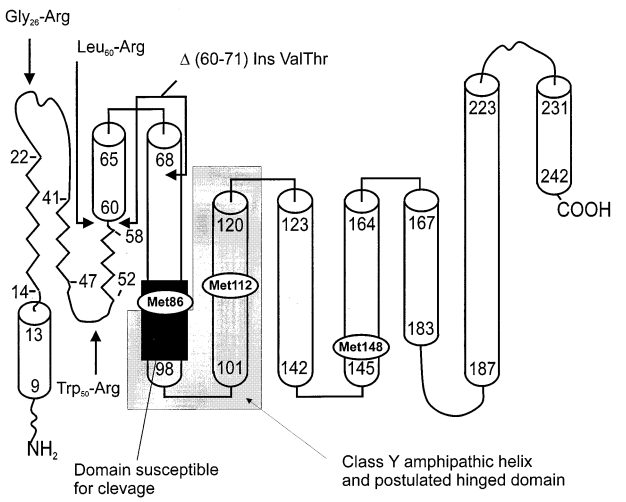

Fig. 2. The predicted secondary structure of apoA-I is presented. The location of the amyloidogenic mutations of apoA-I are indicated by arrows. The domain which is susceptible for cleavage is marked with a dark rectangle. The three methionine residues of apoA-I are also indicated. The domain containing one of the class Y amphipathic helices and the postulated hinged domain is marked with light grey.

There are also reports about other mutants of the apoA-I gene resulting into an additional positive charge, which in contrast do not cause amyloidosis. The variants apoA-$I_{Giessen}$ (Pro^{143}-Arg), apoA-I_{Fin} (Leu^{159}-Arg), and apoA-I_{Oslo} (Leu^{160}-Arg) have also a substitution of a neutral amino acid with the positive charged arginine. The affected members have a decreased HDL concentration presumably due to rapid catabolism. However, they do not have any signs of amyloidosis [40–42]. This observation clearly shows that the location of the mutation determines whether the apoA-I is amyloidogenic. The arginine substitution within the N-terminal end of apoA-I, but not within the middle or carboxy-terminal end, results in amyloid deposits.

In this respect the observation of Matsunaga et al. is very interesting. They reported about a mutation of apoA-I which is defined by a nonsense mutation in codon 84 of the apoA-I gene, predicting the synthesis of a truncated 1–83 N-terminal apoA-I. The clinical picture of the patient includes apoA-I and HDL deficiency, atherosclerosis, but no amyloidosis so far. Since the apoA-I of the patient is not detectable in plasma or in the HDL fraction by electrophoretic methods or radial immunodiffusion techniques, this truncated apoA-I may not be secreted at all. This may explain why the patient is not affected by amyloidosis [43]. The crucial question if this mutant of apoA-I is secreted has to be addressed in the future. The detection of secreted apoA-I_{1-83} within the serum, but not within the tissue as amyloid, would underline the importance of the additional positive charge of the reported amyloidogenic mutations. In this respect there is some evidence that even a secreted apoA-I_{1-83} may not cause amyloidosis. Kunitake et al. detected fragments of apoA-I in vivo, including one N-terminal fragment of a length of about 10 kDa, which is very similar to the fragments seen in apoA-I induced amyloidosis. However, this fragment was detected in healthy subjects with no clinical signs of amyloidosis. In addition, we also observed the generation of an apoA-I fragment of the exact size in vitro. We expressed apoA-I in *E. coli*, CHO cells, and in Sf9 cells [15,24,44]. ApoA-I was purified either from cell lysates (*E. coli* and Sf9 cells) or from cell medium. The isolation of apoA-I revealed in all applied techniques the presence of an N-terminal 10-kDa fragment, although tissue deposition in radiolabeled in vivo studies was not detectable [24]. Thus the appearance of the 10-kDa apoA-I fragment may reflect the physiologic pathway of catabolized apoA-I.

There is some evidence that the reported amyloidogenic mutations of apoA-I alter its structural conformation. The residue methionine within polypeptides is in general sensitive to oxidation during purification procedures. The amino acid sequence of apoA-I contains three methionine residues at position 86, 112, and 148 (Fig. 2). Von Eckardstein et al. showed that the methionine residues at position 112 and 148 are usually modified to methionine sulfoxide during purification procedures using chromatographic methods, but not methionine

at position 86. This methionine residue seems to be highly protected against oxidation, presumably as a result of the conformational structure of the protein [45]. In contrast, mass spectroscopic analysis of apoA-I fragments isolated from amyloid fibrils showed an oxidized methionine residue at amino acid position 86. These data confirm the hypothesized alteration in the conformational structure of the amyloidogenic apoA-I.

We revealed an increased catabolism of apoA-I_{Iowa} and altered association of apoA-I_{Iowa} with the HDL fraction compared to apoA-$I_{wild-type}$ confirming the data of Rader et al.. The apoA-I_{Iowa} seems to be associated with smaller and more dense HDL particles than apoA-$I_{wild-type}$ as could be shown in the density gradient ultracentrifugation and FPLC analysis. The phenomenon of decreased association of apoA-I with HDL and increased metabolism of apoA-I has been shown before [15,24]. There is accumulating evidence that once apoA-I dissociates from HDL it is rapidly catabolized [46]. We therefore believe that the amyloidogenic apoA-I variants with the additional positive charge in their N-terminal end result into a change of their secondary and/or tertiary structure. This altered conformation decreases the ability of apoA-I_{Iowa} to associate with the HDL particle, which in turn causes rapid catabolism. Since apoA-I is very sensitive to proteolysis it may be cleaved intra- and/or extravascularly. The amyloid deposits caused by human apoA-I were all composed of variant apoA-I, but not of apoA-$I_{wild-type}$. Thus, the additional positive charge within the β-pleated domain of apoA-I determines the fate of amyloid deposits. There is one report in which in trace amounts the intact mutant apoA-I (Leu^{60}-Arg) was found besides the mentioned fragments in the fibrils. Therefore, the location of the cleavage (blood vs. tissue) of amyloidogenic apoA-I remains to be elucidated.

Interestingly, the amyloidogenic mutations of apoA-I occur distantly from the site of proteolysis. The mutations occur between residue 26 and 60 (71) resulting all in fragments of almost identical size (apoA-I_{1-83} to apoA-I_{1-94}). Remarkably, apoA-I is not predicted to be an amyloidogenic protein by its secondary structure. The majority of amyloid proteins have extensive β-sheet configuration, whereas in apoA-I the α-helix is the dominating structural component. The content of α-helix in the lipid free apoA-I was about 40–50%, which increases up to 70–80% once it associates with phospholipids [47]. Only the very N-terminal end of apoA-I is composed of β-sheet configuration. The predicted structural features of apoA-I are greatly reviewed in [47]. The recently published crystal structure of a truncated apoA-I (Δ(1–43)A-I) confirmed the predicted structural data [48].

The domain of apoA-I which seems to be sensitive for cleavage contains some interesting structural features. One of the general characteristic features of apoA-I are repeats of 11 amino acids forming amphipathic helices. Segrest et al. first described the amphipathic helix as a structure/function motif involved in lipid interaction [49]. An amphipathic helix is defined as an α-helix with polar amino acids on one site and non-polar amino acids on the opposite site orientated along the axis of the helix. Anatharamahiah et al. grouped the amphipathic helices upon their physico-chemical and structural properties into several classes. The most prevalent and characteristic amphipathic helix for apoA-I is the class A amphipathic helix [50]. Class Y amphipathic helices were proposed at amino acid positions 88–121 and 209–241. A modified Class G amphipathic helix is only present in the very N-terminal end of apoA-I [47]. Interestingly, a change from class A-helix to class Y-helix occurs exactly in the domain where proteolysis was observed (residue 88, Fig. 2). A change in the class of amphipathic helices is correlated with a change in the hydrophobic moment. The distribution of positively, negatively or neutral amino acids on the helix alters the helix class from A to Y. This modified distribution of amino acids on the helix is known to change the ability to associate with lipoprotein surfaces [51]. Another aspect is interesting for this domain. ApoA-I has a postulated hinged domain, that describes a mobile region of one or two amphipathic helices which can either be associated with the lipoprotein surface or can be released from the lipoprotein surface [52,53]. The hinged domain is a proposed LCAT-activating domain, and a domain probably involved in the cholesterol efflux [47]. The hinged domain has been hypothesized to play an important role in both the conversion from nascent HDL particles to spheric HDL particles and the regulation of size and composition of the HDL particle [50,53]. It is of interest that the hinged domain is discussed to be a part of the class Y amphipathic helix region of the apoA-I structure (amino acids 88–121) (Fig. 2) [50,53,54]. One can speculate that the altered structural conformation of amyloidogenic apoA-I may result in a fixed position which makes the protein susceptible for cleavage and may be even susceptible for tissue deposition.

6. Conclusions

The similar features of the four so far known amyloidogenic apoA-I variants suggest a similar mechanism for cleavage and deposition of the observed apoA-I fragments. The additional positive charge within the N-terminal domain of apoA-I leads presumably to an alteration in the conformation of the variant proteins. This conformational change may cause an altered association with the HDL particle, which in turn results in increased catabolism, as shown in our experiments. It has been reported that the methionine residue at position 86 of apoA-I is usually protected against oxidation with the exception of the studied amyloidogenic apoA-I variant confirming the altered conformation. This new conformation may explain an increased susceptibility for apoA-I proteolysis, which is known for non-lipid bound apoA-I. The postulated hinged domain may be positioned at an open angle as a target for proteases. However, the proteolysis may also occur once the amyloidogenic apoA-I is extravascular. Finally, whether apoA-I is regularly catabolized or deposited within the tissue forming amyloid is determined within its N-terminal domain.

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