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SPECIAL REVIEW SERIES: New ultrastructural discoveries in the pathophysiology of liver diseases

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Liver transplantation and new therapeutic approaches for familial amyloidotic polyneuropathy (FAP)

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Abstract Liver transplantation has been considered as a promising therapy to halt the progression of clinical symptoms in familial amyloidotic polyneuropathy (FAP) because most transthyretin (TTR) is produced by the liver. In addition, domino liver transplantation using an FAP patient's liver has been performed because of a shortage of donor livers. However, because the use of liver transplantation as therapy for FAP has given rise to several problems, an alternative treatment is needed. We have tried several other approaches. Recent studies suggested that certain metal ions affect amyloidogenesis. Among metal ions tested in an in vitro amyloid formation study, Cr^{3+} increased stability of both normal and mutant TTR tetramers and suppressed TTR amyloidogenesis induced by low pH. Our findings indicate that Cr^{3+} acts to suppress TTR amyloidogenesis. BSB, a Congo red derivative that binds to amyloid fibrils in FAP as well as to those in senile plaques in Alzheimer's disease, effectively suppressed TTR amyloid formation in vitro. BSB may thus be useful for preventing amyloid formation. Free radical scavenger therapy was also tried in FAP patients but yielded no conclusive results. Immunization for transgenic mice having the ATTR V30M gene using ATTR Y78P resulted in suppression of amyloid deposits. Finally, an RNA/DNA chimera and single-stranded oligonucleotides (SSOs) were tested in vitro and in vivo in an attempt to repair the amyloidogenic TTR gene in the liver and retina. On the basis of results achieved so far, SSO is a promising tool for gene therapy.

Key words FAP · Amyloidosis · TTR · Liver transplantation · Gene therapy · Antibody therapy

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Introduction

Amyloidosis is a disorder of protein metabolism in which soluble proteins are deposited in tissues as abnormal insoluble fibrils. Amyloid deposits of familial amyloidotic polyneuropathy (FAP) are also like nylon fibrils (Fig. 1). 1So far, 25 different precursor proteins have been identified in different kinds of amyloidosis (Table 1). After Andrade[1] identified patients with familial amyloidotic polyneuropathy (FAP) in Portugal in 1952, many foci of FAP cases were reported worldwide. Because patients with FAP show various serious systemic symptoms, such as cardiac and renal dysfunction, gastrointestinal disorders, glandular and autonomic dysfunction, and peripheral neuropathy, many trials have attempted to treat these symptoms.[2–6] Although liver transplantation has become a well-established treatment for halting the progression of FAP-related clinical symptoms, no truly effective therapy has been designed,[7–13] and several problems related to its use have arisen.[14,15] We cannot use liver transplantation for these patients as long-term therapy for the following reasons. (1) Medical care before and after the surgery and the surgery itself are extremely expensive. (2) Patients who have received transplants must continue lifelong use of immunosuppressants after the surgery, and these agents may have adverse side effects. (3) Carriers of the amyloidogenic transthyretin (ATTR) gene who have no clinical symptoms cannot undergo liver transplantation before the onset of the disease. (4) Clinical symptoms of FAP that were present before the surgery continue to occur after liver transplantation. (5) The worldwide shortage of liver donors means that not all FAP patients can undergo the surgery. (6) In addition, after liver transplantation, patients with FAP ATTR Val30Met or other types of FAP have been reported to develop ocular disorders, caused by vitreous amyloid deposits, and central nervous symptoms, because of leptomeningeal amyloidosis, as an effect of continuous transthyretin (TTR) production by the retina and choroid plexus, respectively. To overcome all these problems, we must develop a new noninvasive essential treatment.

The following methods can be considered potential FAP treatment strategies (Fig. 2): (1) reduction of variant TTR levels in plasma, (2) downregulation of mRNA for the TTR gene, (3) replacement of the variant TTR gene with the normal TTR gene (which can be achieved by liver transplantation or by gene therapy), (4) stabilization of the tetrameric TTR structure, (5) prevention of amyloid formation, and (6) dissolution of amyloid fibrils (see Table 1).

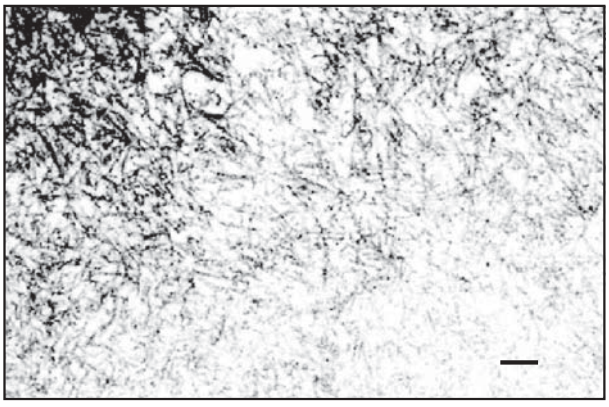

Fig. 1. Electron microscopic analysis of amyloid fibrils of familial amyloid polyneuropathy (FAP). Electron microscopy of specimens from a 38-year-old male patient with FAP amyloidogenic transthyretin (ATTR) V30M. ×135 000. *Bar* 0.07 μm

Table 1. Classification of amyloidosis

Systemic amyloidosis
Immunoglobulin amyloidosis
(1) AL amyloidosis: L chain (λ, κ)
(2) AH amyloidosis: Ig (Ig Gl (γ1))
Secondary amyloidosis: AA
Familial amyloidosis
(1) FAP I: TTR
(2) FAP II: TTR
(3) FAP III: ApoA I
(4) FAP IV: gelsolin
(5) FMF: AA
(6) Muckle–Wells syndrome: AA
(7) Familial renal amyloidosis: Lysozyme
(8) Familial renal amyloidosis: Fibrinogen
Dialysis-related amyloidosis: β_2 microglobulin
Senile amyloidosis: TTR
Localized amyloidosis
Brain amyloidosis
(1) Alzheimer's disease: β-precursor protein
(2) Amyloid angiopathy: β-precursor protein
(3) HACH: β-precursor protein
(4) HACH: Cystatine C
(5) CJD: Prion
Hormonal amyloidosis
(1) Thyroid cancer: Carcitonin
(2) DM type II: Amylin
(3) localized atrial amyloid: ANP
Skin amyloidosis: Keratin?
Localized glanular amyloidosis: L chain
Corneal Amyloidosis: Lactoferrin

AL, primary amyloid light-chain; AH, amyloid heavy-chain; AA, amyloid-A protein; FAP, familial amyloidotic polyneuropathy; TTR, trans thy retin; FMF, familial mediterranean fever; HACH, hereditary amyloidosis with cerebral hemorrage; CJD, Creutzfeldt-Jakob disease; DM, diabetes mellitus; ANP, atrial nutriuretic peptide

Here, I describe new therapeutic approaches for FAP that our group has recently investigated, as well as previously used therapies. We hope that at least one of these approaches will lead to an essential treatment of FAP.

Elimination of variant TTR in blood circulation

Plasma exchange, affinity column binding with a monoclonal antibody, and use of a special column having a specific affinity for TTR had been considered as possible methods for elimination of ATTR in blood circulation.[16–21] Sales-Luis et al. performed plasma exchange for 1.5 to 4.5 years in nine patients with FAP ATTR Val30Met, and the effect of this therapy on the disease was compared with the course of the illness in nontreated FAP patients.[16] Progression of the disease was slightly suppressed, and body weight loss and diarrhea were effectively treated.[16,17] However, Sales-Luis stopped this plasma exchange because of allergic reactions or virus infection.[17] Ikegawa and colleagues performed plasma exchange once a month with three FAP patients.[18] They reported that levels of variant TTR in the plasma decreased from 39% to 19% of the total TTR levels but returned to the values that were seen before treatment.[18] Objective and subjective evaluations indicated no improvement in laboratory and clinical data. The treatment was very expensive, and the number of treated patients was very small. In addition, no follow-up studies have been reported.

Costa and colleagues reported application of an affinity column linked with a monoclonal antibody for ATTR Val30Met to eliminate the variant TTR from the bloodstream.[19,20] However, the method was unsatisfactory because the rate of reduction was too slight to halt the progression of FAP, and rebound elevation of plasma TTR levels was observed just after treatment.[19,20] Moreover, vitamin A levels were decreased, and four patients had a TTR–TTR antibody complex in plasma because of leakage of the antibody from the column to the bloodstream.[20] Loss of skin hair throughout the whole body and allergic reactions have been reported. Tokuda et al. used the special column PA-01, which showed a specific affinity for TTR, to eliminate ATTR from the blood.[21] However, this therapy had the same defects as did the two trials just mentioned. Just after treatment, plasma TTR levels decreased by 50%, but they returned to the same values seen before treatment. From these studies, we can conclude that these methods cannot provide sufficient elimination of plasma TTR, and that regulation of TTR mRNA production does not seem to be appropriate because TTR itself is a rapid turnover protein whose plasma half-life is about 2 days.[22–24] FAP patients who want these treatments must go to a hospital every day or every hour of the day to achieve sufficient reduction of plasma TTR levels. These results also suggest that antisense or ribosomal therapies that would target variant TTR mRNA would not be advantageous treatments.

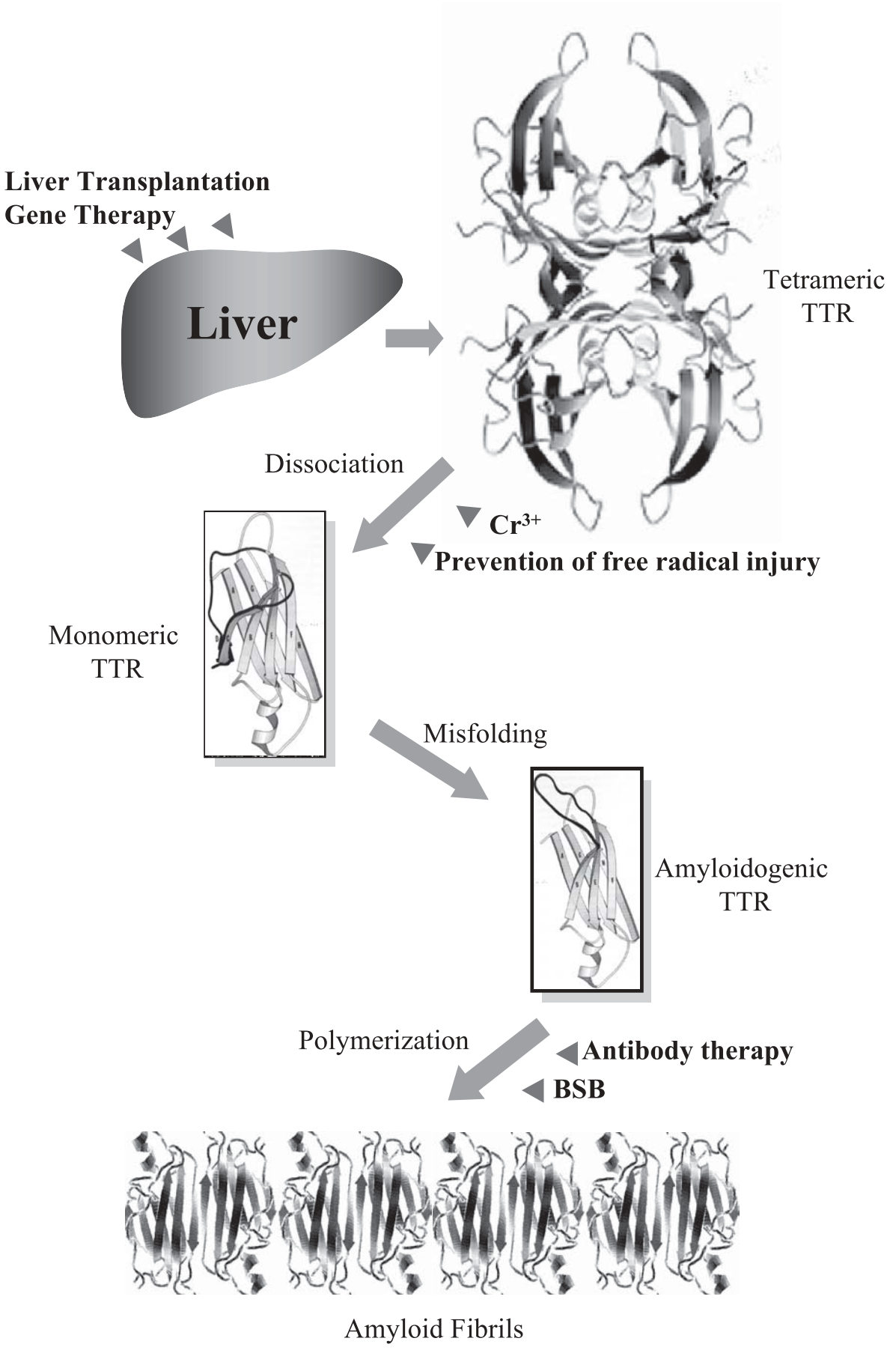

Fig. 2. Different strategies for FAP treatment. Wild-type TTR usually has a tetrameric form in blood circulation. When an amino acid substitution occurs in the TTR molecule, the tetrameric form of the molecule becomes more unstable, which leads to the amyloid formation pathway. The strategies for FAP treatment are based on this hypothesis

Downregulation of variant TTR

It is well documented that FAP patients develop systemic disorders induced by amyloid deposition in the viscera in addition to peripheral nerves. As the disease progresses, FAP patients become emaciated, and hypoproteinemia and hypoalbuminemia are often observed. To compensate for the hypoproteinemia and hypoalbuminemia, intravenous injections of fresh-frozen plasma (FFP) are often used. FFP contains a significant amount of TTR in addition to various plasma proteins. A surprising finding has been that the variant TTR levels decreased while levels of total TTR, total protein, and albumin in the plasma increased. It is possible that some genetic regulation occurs in response to the FFP injection.

Ando et al. monitored the changes in plasma TTR levels after injection of a large amount of FFP when they treated FAP patients for hypoproteinemia and hypoalbuminemia.[24] From 24 to 48 h after injection, the total plasma TTR levels were elevated, and variant TTR levels decreased, accompanied by an elevation of plasma total protein and albumin levels. To elucidate the mechanism of this phenomenon, a large amount of purified normal TTR from normal human plasma was injected intravenously into mice and into patients with FAP ATTR Val30Met. Injection of 3 mg purified TTR into C57 Black6 mice resulted in decreased expression of TTR mRNA from 6 to 24 h after injection, and then TTR mRNA expression gradually increased up to 48 h after injection. Injection of 400 mg normal human TTR into three FAP patients caused elevated total plasma TTR levels and significantly decreased variant TTR levels 24–48 h after injection. These results suggested that this method allowed downregulation of the harmful protein by means of replacement with the normal protein. This phenomenon may explain the mechanism for one of the possible methods for decreasing the amount of harmful protein in the circulation. However, because reduction of the amount of variant TTR in plasma was not sufficient, and TTR is a rapid turnover protein as described previously,[22–24] this treatment cannot be applied as an essential therapy for FAP patients.

Liver transplantation

For treatment of FAP, liver transplantation has been reported to halt the progression of clinical manifestations,[25,26] because most of serum TTR is produced by the liver and few amyloid deposits are recognized in the tissue by histopathologic examinations (Fig. 3). According to data in the Familial Amyloidotic Polyneuropathy World Transplant Registry (FAPWTR), 54 centers in 16 countries have performed orthotopic liver transplantation (OLT) for FAP. During 2003, approximately 60 OLTs were performed worldwide. During the last decade, 539 patients have undergone 579 OLTs. Survival of the patients has been excellent (overall 5-year survival of 77%) and comparable to the survival for OLT performed for other chronic liver disorders, but a longer follow-up is needed to compare the outcome after OLT with the natural course of the disease. The main cause of death was related to cardiac difficulties (39%).[27]

The use of sequential liver transplantation with resected livers from FAP patients started in Portugal; more than 50 patients have received FAP patients' livers.[28] Although no patients have started to show the clinical symptoms of FAP, careful neurological follow-up examinations should be continued.

Facilitating the stability of the tetrameric structure of TTR

It is widely believed that stabilizing tetrameric TTR, as a potential therapeutic strategy, is a prerequisite for prevention of amyloid formation, especially in FAP ATTR Val30Met.[29,30] McCutchen et al. first demonstrated this concept by using recombinant wild-type TTR and variant TTR.[31] Alves et al. reported that subjects possessing the TTR Thr119Met gene are asymptomatic carriers, and that compound heterozygotes having ATTR Val30Met and TTR Thr119Met genes show very mild FAP symptoms or have no symptoms.[32] These authors also demonstrated by semidenaturing isoelectric focusing that patients possessing TTR Thr119Met or ATTR Val30Met/TTR Thr119Met genes showed marked TTR tetrameric structural stability.[32–34] Various studies confirmed these findings, and this concept has been now widely accepted. Terazaki et al.[35] reported an interesting late-onset compound heterozygote patient with FAP ATTR Val30Met/TTR Arg104His who had very mild and slowly progressive clinical symptoms, and whose tetrameric TTR stability was greater than that of the TTR from a compound heterozygote ATTR Val30Met/TTR Thr119Met patient.[35,36]

These case reports suggest the therapeutic possibility of stabilization of the tetrameric form of TTR. Because thyroxine (T_4) is one of the most important molecules for stabilizing TTR in tetrameric form, T_4-based therapeutic drugs have been proposed. Kelly et al. also tested various nonsteroidal antiinflammatory drugs (NSAIDs) for stabilizing the tetrameric form of TTR, because the structure of NSAIDs resembles the structure of T_4 and these drugs bind to TTR via a T_4-binding site.[37] These authors reported that flufenamic acid, an NSAID, showed promise for stabilizing tetrameric TTR.[38] However, most NSAIDs bind to albumin in plasma. No human studies have yet been conducted, and the true therapeutic effect for FAP patients remains to be determined.

Recent studies suggested that certain metal ions affect amyloidogenesis in several types of amyloidosis. In FAP, metal ions may influence the stability of the tetrameric form of TTR. Mikami et al. therefore investigated whether various metal ions (e.g., Zn^{2+}, Cu^{2+}, Ca^{2+}, Fe^{3+}, Al^{3+}, Cr^{3+}) affect amyloidogenesis of wild-type TTR and ATTR. Amyloidogenesis was examined by the thioflavin T-binding assay, nonboiled sodium dodecyl sulfate–polyacrylamide gel electrophoresis, high-pressure liquid chromatography, and circular dichroism; purified human TTR protein was

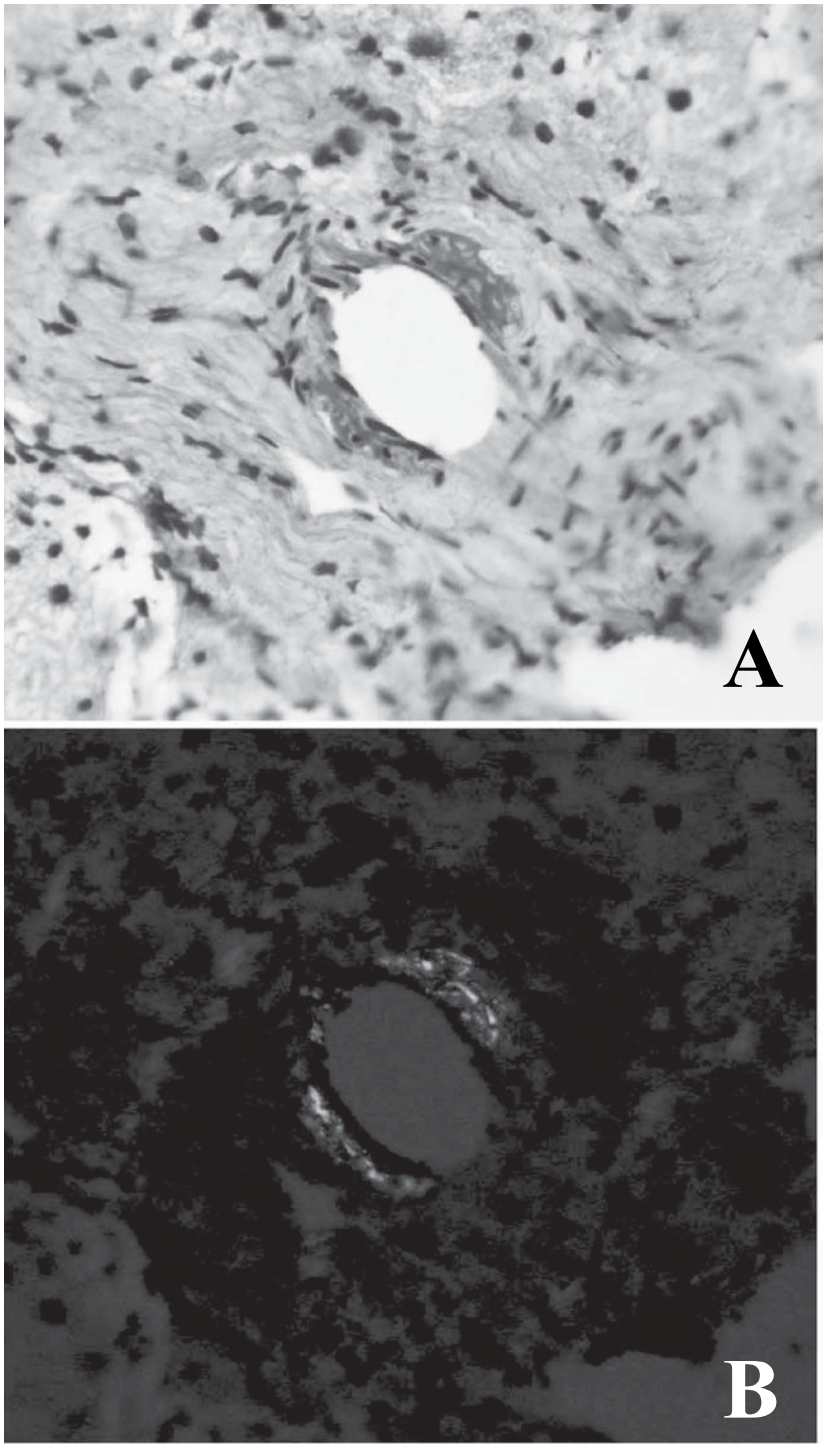

Fig. 3. Microscopic study of an FAP patient's resected liver for liver transplantation. Amyloid deposition was confirmed as only a very small amount around the vessel walls in the liver by Congo red staining (**A**) and its polarized light analysis (**B**). ×100

used. Among the metal ions, Cr^{3+} increased the tetrameric stability of both wild-type and ATTR Val30Met purified from normal subjects and homozygote FAP patients, respectively, and suppressed TTR amyloidogenesis induced by low pH in a concentration-dependent manner. In contrast, Al^{3+} decreased TTR tetrameric stability and induced TTR amyloidogenesis in a concentration-dependent manner. These findings indicate that Cr^{3+} and Al^{3+} may act as a suppressor and an inducer of TTR amyloidogenesis, respectively, although in vivo evaluation of the effects is needed.[39] Cr^{3+} is a component of health foods that is widely used throughout the world, so administration of this metal ion to FAP patients would present no problems.

Protection against amyloid deposition in tissues

A new tool for examination of amyloid deposition in tissues

In systemic amyloidoses such as FAP, AA amyloidosis, AL amyloidosis, and dialysis-related amyloidosis (DRA), biopsy samples are often obtained from the gastrointestinal tract and abdominal fat to make the diagnosis, because amyloid deposits are usually found in these tissues at the early stage of the disease.[40–42] However, in certain cases it is sometimes difficult to make the diagnosis by use of only these biopsy samples, because the pattern of amyloid deposition in the body varies in each individual. In addition, a biopsy for diagnostic purposes cannot usually be performed in patients with localized amyloidosis, such as amyloidosis in Alzheimer's disease and endocrine amyloidosis.[43] Diagnosis at the early stage of amyloidosis might be possible if a tool were available that incorporated real-time amyloid monitoring, such as radioisotope-labeled scintigraphy.[44,45]

Among several histopathological methods utilizing stains such as Congo red, fast scarlet, and thioflavine S, Congo red staining is one of the most popular for detection of amyloid deposits in tissues. Congo red is a hydrophilic chemical agent that binds to amyloid fibrils in vitro.[46] Analysis with polarized light after Congo red staining has been recommended as the most reliable method for detection of amyloid fibrils in biopsy and/or autopsy materials from patients with amyloidosis. However, judging the positivity of Congo red-positive lesions is sometimes difficult, and false-positive or false-negative results may be obtained. Moreover, Congo red staining cannot be used for in vivo studies because of its toxic effects in the human body. Chrysamine G, a Congo red derivative with a similar molecular structure used for the same purpose as Congo red, has the same limitations.[47–50] To overcome these problems, we must have a new tool for examination of amyloid deposition in tissues.

(*trans*, *trans*)-1-Bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB), which has been found to bind to amyloid plaques in postmortem samples of brains from patients with Alzheimer's disease, is also a Congo red derivative and has been the focus of recent attention.[51–55] Because this compound is lipophilic, it can traverse the blood–brain barrier, and it binds to amyloid fibrils in senile plaques when it is injected intravenously into transgenic mice with Alzheimer's disease. Moreover, this compound can detect cell inclusion bodies derived from α-synclein. However, no studies have addressed the question of whether the compound could become a useful tool for detection of amyloid deposits in systemic amyloidosis such as FAP. Various plasma proteins in the systemic circulation may disturb the binding of BSB to amyloid fibrils in tissues.

Ando et al. used BSB to detect amyloid fibrils in autopsy and biopsy samples from patients with localized amyloidosis, such as familial Creutzfeldt–Jakob disease, and systemic amyloidosis, such as FAP, AA amyloidosis, AL amyloidosis, and DRA. Histopathological methods were employed to examine the reactivity of BSB with amyloid fibrils, and the results were compared with those from studies with immunohistochemical and Congo red staining and polarized light. BSB reactivity in vivo was investigated in mice in which AA amyloidosis had been induced, because transgenic mice with a human mutant TTR gene kept in the authors' animal center at the university do not produce amyloid fibrils in their organs. The authors determined the affinity of BSB and Congo red for purified amyloid fibrils by using a highly sensitive 27-MHz quartz crystal microbalance.[56,57] BSB showed reactions in all Congo red-positive and -immunoreactive regions of the samples examined in the study, and some amyloid fibrils in the tissues could be detected more precisely with BSB than with the other stains and methods. In the mouse model of AA amyloidosis, BSB reacted with amyloid in all regions in the serial sections in which Congo red staining was positive.

BSB as a valuable therapeutic drug

To test the usefulness of BSB as a possible therapeutic agent in FAP, its inhibitory effect on the formation of TTR amyloid in vitro was examined by means of electron microscopy (Fig. 4). BSB showed a significant affinity for amyloid fibrils purified from FAP patients' samples and suppressed formation of TTR amyloid in acidic conditions in vitro. These results suggest that BSB may become a valuable therapeutic drug, although further evaluation is needed.

4′-Iodo-4′-deoxydoxorubicin (IDOX) has been reported to bind to amyloid and lead to the catabolism of amyloid in deposits. This chemical compound was first reported by Merlini et al. as an agent that would bind to amyloid fibrils found in five different types of amyloidosis.[58] A multicenter study attempted to develop a dosing schedule to confirm those results. IDOX was administered to AL amyloidosis patients at 15 mg once a week for 4 consecutive weeks, and this therapy was repeated every 3 months up to four times.[59] However, the results were not clear, and no obvious effect on the patients was seen. The authors concluded that the protocol produced insufficient activity at the dose used in the study. It is possible that IDOX does not show the same attraction for amyloid in FAP as that seen in the AL amyloidosis trial.

In an in vitro study, Sebastiao et al. observed an interaction of IDOX with ATTR Leu55Pro and reported that monoclinic ATTR Leu55Pro crystals soaked with IDOX undergo rapid dissociation.[60] Moreover, under the same conditions, the orthorhombic wild-type TTR crystals were quite stable. This result was explained by the different TTR conformations in the crystals of the two proteins: the ATTR Leu55Pro had the necessary conformation for IDOX binding, but the same structure was not present in the crystallized wild-type protein. This study presented a theoretical model of the interaction of ATTR Leu55Pro with IDOX that is consistent with the dissociation of the amyloid-like oligomer. In this model, the IDOX iodine atom was buried in a pocket located between the two beta sheets of the ATTR Leu55Pro monomer, with the long axis of the IDOX aromatic moiety nearly perpendicular to the direction of the beta sheets.[60]

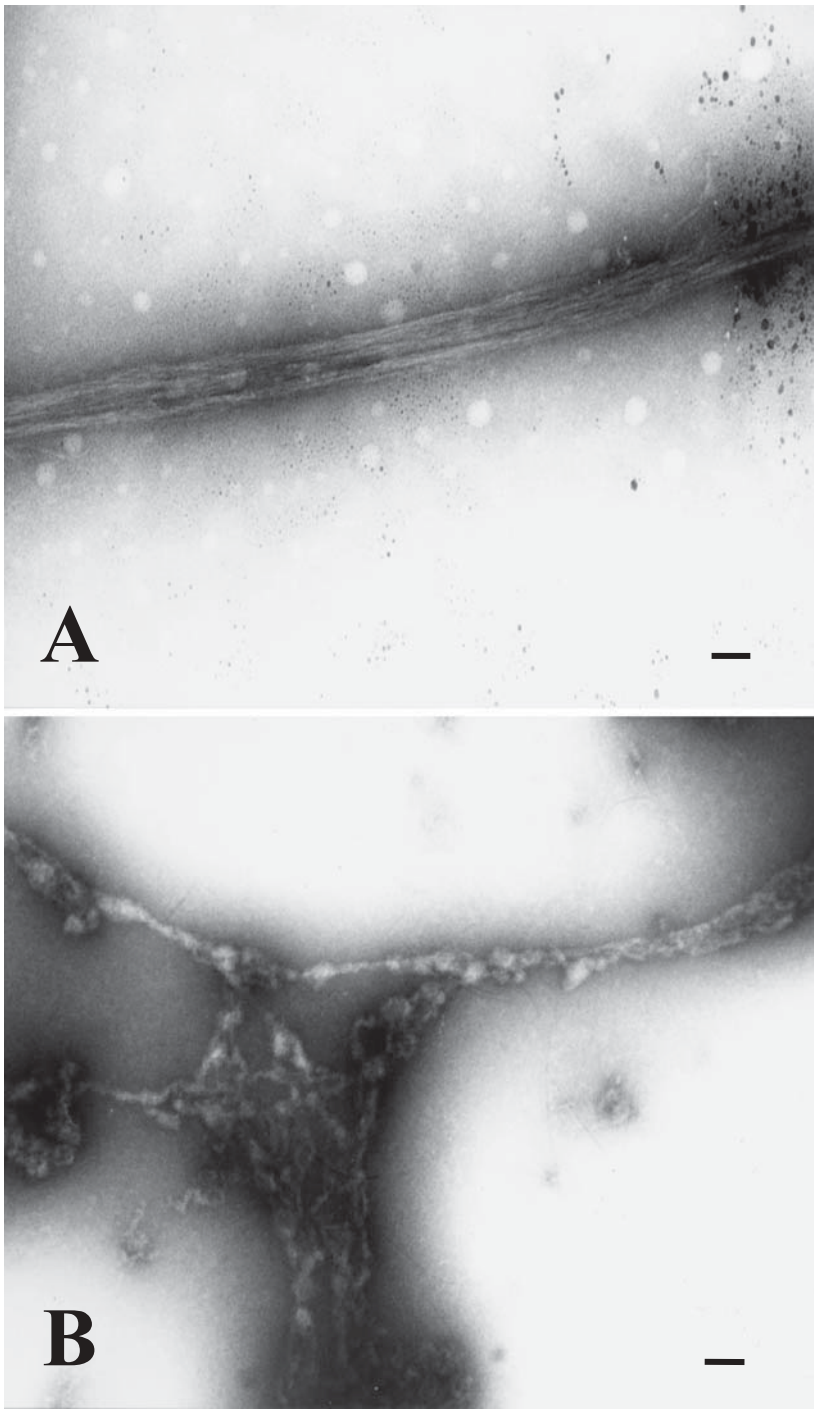

Fig. 4. Prevention of amyloid fibril formation with (*trans*, *trans*)-1-Bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB). Electron microscopic analysis of a TTR solution incubated at pH 4 for 5 days revealed that amyloid fibrils of TTR in the absence of BSB were straight and smoothly configured (**A**). In comparison, TTR amyloid fibrils in the presence of BSB were immature, had irregular beadlike shapes, and had much larger diameters (**B**). *Bars* 100 nm

This chemical compound, first developed as an anticancer drug, may be one of the most promising drugs for FAP. However, it has nephrotoxic effects, and an IDOX derivative that is less toxic to the kidneys should be sought, because one of the target organs for amyloid deposition in FAP is the kidney, and renal dysfunction often occurs during the course of the illness. In any case, we must wait for further information from in vivo studies of the effect of IDOX to derive conclusions about the usefulness of IDOX for FAP patients.

BSB, however, may show promise as a drug for preventing amyloid formation, although its cytotoxic effect was not fully examined and further study is needed. Moreover, BSB may become a valuable tool for real-time detection of amyloid deposits in systemic amyloidosis such as FAP because it binds tightly to amyloid fibrils.

Free radical scavenger therapy

Oxidative stress and amyloidosis

Oxygen toxicity has a major impact on most biomolecules, including nucleic acids, protein, lipids, carbohydrates, and other low molecular weight compounds.[61] An organism's ability to protect itself from oxidative stress is thus one of the prerequisites for aerobic life. Although intracellular levels of enzymes that protect against oxidative stress, such as superoxide dismutase (SOD), glutathione peroxidase, catalase, and other heme-containing peroxidases, are high, levels in extracellular compartments are comparatively lower.[62] Therefore, the pathogenesis of various diseases has been suggested to involve free radical injury, or free radicals acting as a propagation factor, especially in extracellular spaces, and several therapeutic trials have attempted to reduce oxidative injury.[63–66] In all types of amyloidosis, amyloid deposits are usually found in the extracellular space, although intracellular inclusion bodies, which can be detected with Congo red staining, have also been identified. These intracellular inclusion bodies were stained by Congo red, but no fibrillar substances were observed by electron microscopy.[67] Several in vitro and in vivo studies have implicated free radicals in amyloid formation in several types of amyloidosis, such as that associated with Alzheimer's disease, FAP, and β_2-microglobulin amyloidosis.[68,69] On the basis of these findings, therefore, it has been suggested that in both systemic and localized amyloidosis, free radical injury may be involved in the amyloid formation process.

Tools for detecting oxidative stress in amyloidosis

Advances in immunohistochemistry techniques and the development of new antibodies have increased the possibility of detecting injury caused by oxidative stress.[70] One of the histopathological hallmarks of Alzheimer's disease is the occurrence of senile plaques that are found in the neocortex and hippocampus. The main constituent of the core of these plaques is a 40- to 43-peptide sequence called β-amyloid (Aβ), which is capable of destabilizing calcium homeostasis and exhibits neurotoxic properties. In a number of studies, the cytotoxicity of Aβ(1–40) and its active fragment Aβ(25–35) could be suppressed by catalase and antioxidants, i.e., vitamin E and melatonin. Treatment of both cultured hippocampal neurons and synaptosomes with Aβ(25–35) produced substantially increased concentrations of the lipid peroxidation product 4-hydroxy-2-nonenal (HNE). The neurotoxic effects of both Aβ(25–35) and HNE could be prevented by treatment with glutathione ethyl ester, whereas the antioxidant propyl gallate was effective against only Aβ(25–35)-induced neurotoxicity.[71] Aβ-resistant subclones of PC12 cells, unlike the parent cells, do not accumulate peroxides after Aβ(25–35) treatment because of an overexpression of antioxidant enzymes.[72] Taken together, these data suggest that the neurotoxic effects of Aβ are mediated by hydrogen peroxide and HNE.

Proteases from polymorphonuclear leukocytes and macrophages are present in amyloid fibrils found in all types of systemic amyloidosis, and the release of free radicals from these cells may be involved in amyloid formation.[73,74] Hydroxyl radicals readily induce lipid peroxidation, so that products of this reaction should be present in amyloid deposits if free radicals are involved in the process. Several reports of accumulation of aldehydic lipid peroxidation products during oxidative stress conditions have been published.[75,76] HNE is a lipid peroxidation product that exhibits several toxic properties: cell and enzyme stimulation, and enzyme inactivation and modification. By using a purified polyclonal antibody to HNE, this toxic metabolite can be detected immunohistochemically in tissues.[70,77]

Ando et al. confirmed the detection of HNE immunoreactivity in amyloid deposits of the brain of patients with Alzheimer's disease by comparing brain specimens from Alzheimer's and non-Alzheimer's disease patients.[78] Positive HNE immunoreactivity was found in amyloid deposits in all Alzheimer's disease specimens examined. HNE immunoreactivity was noted in 89% of the vessels in perivascular areas in the Alzheimer's disease specimens, the same areas where amyloid deposits were found by Congo red staining; in the non-Alzheimer's disease specimen, in contrast, only 20% of the vessels showed HNE immunoreactivity. Congo red-negative vessels in the Alzheimer's disease specimens showed no HNE immunoreactivity. Of the senile plaques that were positive for amyloid by Congo red staining, 21% reacted with HNE antibody. Because the senile plaques change form during the course of the illness, the degree of HNE immunoreactivity may depend on the stage of the senile plaque. It should be noted that perivascular amyloid is localized outside of the blood–brain barrier, whereas senile plaques are formed within the barrier. This difference in location may affect the stability and metabolism of HNE, which is a labile molecule. HNE immunoreactivity was noted in a few perivascular areas without amyloid deposits in non-Alzheimer's disease patients, especially aged patients, which suggests that HNE adducts are generated by free radical activity in atherosclerotic vessels.[79] An increase in HNE immunoreactive neurons in APOE4 homozygote Alzheimer's disease patients has also been reported. However, the association of HNE with amyloid deposits, such as senile plaque and perivascular amyloid deposition, was not investigated. Because amyloid angiopathy has recently been reported in FAP ATTR Val30Met and other types of FAP,[80] similar findings may be obtained in such patients in the future.

Ando et al. also reported that the HNE adduct was present in amyloid deposits in tissues, and that levels of thiobarbituric acid reactive substances (TBARS) and protein carbonyl were elevated in amyloid-rich biopsy samples from FAP patients compared with levels in nonamyloidotic samples.[81,82] Nyhlin et al. reported the presence of advanced glycation end products, some of which were formed via oxidative stress.[83] These results suggest that oxidative stress is significantly involved in the amyloid formation process.

An extracellular SOD (EC-SOD) mutation and FAP

Sakashita et al. found ATTR Arg213Gly in two FAP ATTR Val30Met patients with an EC-SOD mutation, and one of them was autopsied.[84] Histochemical study revealed marked amyloid deposition, especially around the vessels. EC-SOD usually binds to endothelial cell membranes via the heparin binding domain. Sakashita et al. speculated that FAP patients with the EC-SOD mutation had increased amyloid deposition because the superoxide anion cannot be dismutated around the vessels by EC-SOD as the enzyme dissociates to the endothelial cell membranes where oxidative stress increases.[81]

Scavenger therapy in FAP

Suhr et al. attempted free radical scavenger therapy by administering 300 mg *N*-acetylcysteine, 300 mg α-tocopherol, and 500 mg vitamin C to 20 Swedish patients with FAP for 6 months.[85] The amount of amyloid deposition did not change, and prevention of amyloid deposition could not be confirmed. However, all patients had a positive feeling about the therapy, and the modified body mass index increased slightly. The doses, kinds of radical scavengers, and duration of treatment should be reviewed again.

Immunization for transgenic mice having ATTR V30M gene by ATTR Y78F

Analyses for TTR structure reveals that ATTR Y78F does not form tetrameric structure and misfolds the CD strands of TTR, which induces an antibody for amyloid fibrils formed by ATTR V30M. We immunized transgenic mice having the ATTR V30M gene by the ATTR protein. ATTR Y78F was immunized to the mice aged 6, 10, and 18 months, and they were killed by exsanguination after 4 months. Immunohistochemical analyses revealed most of amyloid fibrils disappeared in the intestine and antimacrophage antibody was immunoreacted with the lymphocytes migrating to the lesions. These results suggest that antibody therapy using misfiled TTR may be promising therapy for FAP.

Gene therapy

Because ATTR, the pathogenic protein of FAP, is synthesized predominantly by the liver, liver transplantation has been used since 1990 as therapy for FAP. By the end of 2000, more than 500 FAP patients had undergone this surgery, with about 80% of the patients surviving, and liver transplantation is now considered an important method for saving these patients' lives. However, there are many problems with this therapy, as described earlier.[8] Liver transplantation was originally suggested as a treatment that would halt the production of variant TTR in the liver: FAP does not progress when the variant TTR gene is replaced by the normal TTR gene in the liver by the surgery. This fact led to the suggestion that gene therapy that would prevent variant TTR production could become one of the most important methods for ameliorating the clinical symptoms of FAP, and thus a noninvasive therapy for replacing the abnormal TTR gene should be developed.

Ribozymes and antisense methods are possible gene therapy tools. Propsting et al. reported specific cleavage of ATTR Val30Met mRNA in a cell culture system by using hammerhead ribozymes.[86] They showed that chemically modified nuclease-stable inosine(15.1)-hammerhead ribozymes can target ATTR Val30Met mRNA with high specificity at the RNA level.[86] In an FAP gene therapy trial, they used the wild-type human normal TTR-expressing cell line HepG2 and a stable transfected cell line with the ATTR Val30Met gene. They cleaved the ATTR Val30Met and wild-type TTR mRNAs with specific nuclease-stable chemically modified inosine(15.1)-hammerhead ribozymes and analyzed the protein after immunoprecipitation and subsequent Western blotting. They were able to downregulate the TTR concentration by 54.5% (100% = 1.5 mg/l TTR) and also to specifically target ATTR Val30Met expression in the cell culture system. The therapeutic effect was improved by using cationic liposomes, resulting in a total downregulation of wild-type TTR mRNA and ATTR Val30Met mRNA of 92.1% and 62.7%, respectively. Tanaka et al. reported that another ribozyme targeting a variant TTR (E61K) degraded the variant mRNA but not the wild-type mRNA.[87] These ribozymes also reduced the amounts of TTR mRNA and protein in HepG2 cells and COS-1 cells transfected with TTR-E61K cDNA.

Although these data were interesting and the results were clear, this method cannot be applied to treatment of FAP patients, because perfect ATTR Val30Met gene suppression was not achieved, and, as described previously, TTR is a rapid turnover protein. Continued production of TTR from the liver cannot therefore be sufficiently suppressed. Additional precise in vivo studies and other TTR gene therapy methods should be considered.

Chimeric RNA/DNA oligonucleotides (chimeraplasts) have been developed to facilitate correction of single-base mutations of episomal and chromosomal targets in mammalian cells.[88,89] Chimeraplasts consist of short regions of correcting DNA bounded by long stretches of 2′-*O*-methyl RNA, hairpin loops, and GC clamps. Chimeraplasts were shown to cause a site-specific chromosomal correction or mutation in tissue culture cells and in vivo.[90–94] Because a permanent and stable gene correction by chimeraplasts was demonstrated by clonal analysis at the level of the genomic sequence, and as shown by protein and phenotypic change,[93] chimeraplast-mediated gene repair may be a powerful strategy for treatment of genetic diseases without the use of viral vectors.

The strategy of using single-stranded oligonucleotides (SSOs) for gene therapy grew from studies that attempted to characterize and improve chimeraplasty.[95,96] SSOs containing three phosphorothioate bonds at the 3′- and 5′-termini were more effective than the chimeraplasts in gene repair assays conducted with cell-free extracts and a yeast

system.[95,96] SSOs are significantly less expensive than chimeraplasts and far simpler to synthesize and purify, so they may be an invaluable resource for treating a variety of genetic diseases.

To test the feasibility of gene therapy for FAP, via chimeraplasts and SSOs, that would halt production of variant TTR in the liver and retina, Nakamura et al. first applied chimeraplasts and SSOs to HepG2 cells secreting human wild-type TTR. They then demonstrated gene conversion by SSOs in the rabbit eye expressing rabbit wild-type TTR and transgenic murine liver in which the intrinsic wild-type TTR gene was replaced by a TTR Val30Met gene. Their results are promising, although, again, perfect gene conversion could not be obtained. Further methodological development is required.

Other treatments

Kishikawa et al. reported, after an in vitro comparison of amyloid formation by the free TTR form and by a sulfated TTR,[97] that the sulfated TTR was more prone to form amyloid fibrils under acidic conditions. Altland et al. reported that the administration of thiol compounds may be beneficial for prevention of disease progression.[98] Ando et al. demonstrated no significant difference between amyloid formation by the free TTR form and amyloid formation by a cysteine-conjugated TTR in an in vitro comparison (unpublished data). The effect of posttranslational modification of TTR on amyloid formation is still controversial. Additional study is required to determine which modified form of TTR, or whether the nonmodified form of TTR, is beneficial for prevention of disease progression.

The proteinase inhibitor aprotinin has been shown to be a very important radiopharmaceutical agent for in vivo imaging of extraabdominal deposition of amyloid in amyloidosis of the immunoglobulin type. However, no information is available as to whether aprotinin binds other types of amyloid fibrils and on the nature and characteristics of the interaction between aprotinin and amyloid. Cardoso et al. reported aprotinin binding to insulin, TTR, β-amyloid peptide, and immunoglobulin synthetic amyloid fibrils by a specific dot-blot ligand-binding assay. Aprotinin did not bind amorphous precipitates or the soluble fibril precursors.[99] Thus, aprotinin may also be used as a therapeutic drug or in scintigraphy for FAP.

In the murine model of Alzheimer's disease, antibody therapy was attempted by using Aβ(1–42) to immunize transgenic mice having Alzheimer's disease.[100] After the immunization, the mice produced the antibody for the peptide, all amyloid fibrils disappeared, and the dementia resolved. However, in a phase study of Aβ(1–42) treatment of Alzheimer's disease patients in the United States,[101] acute hemorrhagic leukoencephalitis and allergic encephalitis occurred. However, there is a possibility that such treatment could be applied to FAP patients, because the immunoreactivity of amyloidogenic TTR and the mechanisms of amyloid formation are different from those in patients with Alzheimer's disease.

Conclusions

This review has described several new therapeutic approaches for FAP that hold promise for future practice. Liver transplantation, currently used to treat FAP, should be replaced by another, noninvasive method. Although we will have difficulties reaching the goal of developing this noninvasive treatment, laboratory research may be the key to an ultimately effective therapy for FAP patients.

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