Doxycycline and Ursodeoxycholic acid in the treatment of AA amyloidosis

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Abstract
Amyloidoses are disorders of protein folding and metabolism in which insoluble fibrils are deposited in body organs, causing organ dysfunction. Till date, more than 36 different proteins have been identified as amyloidogenic; and at least 17 of them can cause systemic disease. Out of these, AA amyloidosis is probably the most common type of amyloidosis worldwide, as most reported cases from developing countries are associated with underlying infections. Renal involvement is common (nearly 95 %) in patients with AA amyloidosis, patients present with proteinuria and/or renal failure. The optimal treatment strategy of AA amyloidosis includes control of the underlying inflammatory disease and thereby complete suppression of SAA production. Several drugs have shown anti-amyloid properties in recent times. Recently doxycycline (a tetracycline antibiotic) and ursodeoxycholic acid (UDCA) have been shown to have an anti-amyloid effect through different mechanisms.

Keywords: AA amyloidosis, Proteinuria, Doxycycline, Ursodeoxycholic acid.

Introduction
Amyloidoses are disorders of protein folding and metabolism in which insoluble fibrils are deposited in body organs, causing organ dysfunction. The fibrils are composed of proteins that often have been mutated, partially fragmented, or otherwise produced in excess, which predisposes them to adopt an abnormal conformation. This contributes to resistance to catabolism resulting in progressive tissue amyloid accumulation. To date, more than 36 different proteins have been identified as amyloidogenic; and at least 17 of them can cause systemic disease,[1] in which the amyloidogenic protein is produced in one site and is deposited at distant site(s). The most common subtypes of systemic amyloidosis are AL amyloidosis, amyloid A (AA) amyloidosis, familial amyloidosis, and ß2-microglobulin-related amyloidosis. Out of these, AA amyloidosis is probably the most common type of amyloidosis worldwide, as most reported cases from developing countries are associated with underlying infections. AA amyloidosis is characterized by the tissue deposition of amyloid A protein (SAA) fibrils, a hepatic acute phase reactant.[2] Various genetic factors also regulate susceptibility to the deposition of SAA with substantial ethnic differences. The optimal treatment strategy of AA amyloidosis includes control of the underlying inflammatory disease and thereby complete suppression of SAA production. We describe two patients with AA amyloidosis who had a significant reduction in proteinuria on treatment with doxycycline (a tetracycline antibiotic) and ursodeoxycholic acid (UDCA)

Case History
First clinical case
A 38-year-old male caucasian patient presented to the hospital with swelling of his legs for 3 months which was gradually progressive. Further, he had history of cough with expectoration on and off for the last three years. He was a chronic smoker for the last 15 years and not an alcoholic. He had no history of pulmonary tuberculosis and had no medical attention in the past.

On admission, patient was stable with bilateral pedal edema. His renal and liver function tests were normal except for low serum albumin of 2.4 g/dl. C-reactive protein was mildly elevated. Echocardiogram was normal. Urine routine revealed significant proteinuria without active deposits. Further 24-hour urine protein excretion was 4.1 grams per day. He was evaluated for nephrotic syndrome. His complement levels were normal with negative anti-nuclear antibodies. Kidney biopsy revealed histopathology compatible with amyloidosis and subtyping with immunohistochemistry was AA type (Fig 1). Chest imaging done for chronic respiratory symptoms revealed bronchiectasis of the upper lobe of the right lung suggesting a probable cause of AA amyloidosis in this patient. Pulmonary tuberculosis being the most common cause of bronchiectasis in India, acid-fast bacilli staining on induced sputum done was negative. This patient was not affordable for anti-inflammatory antibody therapy typically used to treat AA amyloidosis. Doxycycline and Ursodeoxycholic acid (UDCA) were recently shown to have beneficial effects on amyloidoses. Consequently, the same (Doxycycline 100 mg b.d and UDCA 300 mg t.d.s p.o) were started in this patient. Proteinuria decreased over the following 4-6 months and he had a 24-hour urine proteinuria of 740 mg with a stable renal function.

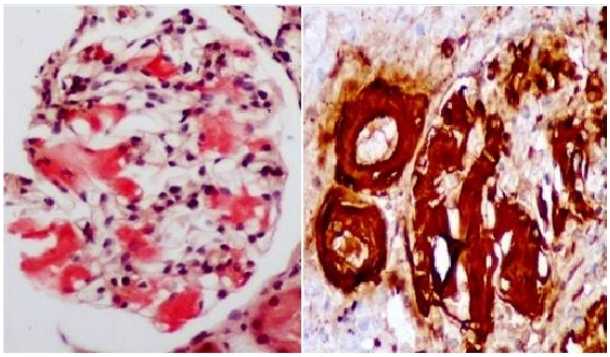

Fig. 1: (a) Congo red staining showing homogenous amyloid deposits in the mesangium; (b) Immunohistochemistry showing positive staining for AA amyloid in the mesangium and vessel wall.

Second clinical case

A 30-year-old male caucasian patient presented with swelling of his legs for 1 month. He was a non-smoker, non-alcoholic and not a drug abuser. He had no history of tuberculosis. He had no history of recurrent fever, joint pain, weight loss, or gastrointestinal symptoms.

On admission, examination was normal except for pedal edema. His creatinine was mildly elevated (1.5 mg/dl). Serum albumin was 2.1 g/dl. Urine routine revealed significant proteinuria and 24-hour proteinuria was 5.2 g. Complement levels were normal. A kidney biopsy was done revealing a picture consistent with amyloidosis and was identified to be AA subtype with immunohistochemistry (Fig 2). Further investigations were done to identify the cause. Hepatitis B and C serology, anti-nuclear antibody and C-reactive protein yielded negative results. Whole-body imaging revealed no abnormality. The cause for AA amyloidosis could not be found. This patient was also started on doxycycline and ursodeoxycholic acid similar to the first clinical case. This patient achieved clinical improvement at 4 months and his proteinuria declined to 1.5 g/day at 6 months of follow-up with a creatinine of 1.6 mg/dl.

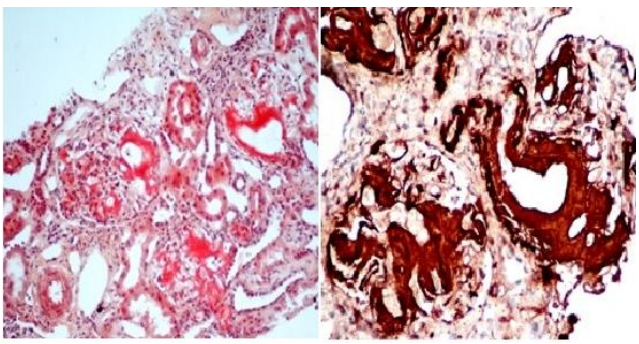

Fig. 2: (a) Congo red staining showing amyloid deposits in the mesangium and vessel wall; (b) Immunohistochemistry showing positive staining for AA amyloid in the mesangium and vessel wall.

Discussion

The precursor amyloidogenic protein is serum amyloid A (SAA), an acute-phase reactant protein, and synthesis can be upregulated by 1000-fold in response to inflammatory cytokines. SAA is produced by hepatocytes in response to pro-inflammatory cytokines, such as TNF, IL-1 and IL-6. Various diseases that trigger chronic inflammation have been implicated as a cause for AA amyloidosis, like chronic infections such as tuberculosis, osteomyelitis and bronchiectasis, rheumatological/autoimmune conditions, hereditary conditions such as familial Mediterranean fever (FMF), cryopyrin-associated periodic syndrome (CAPS) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), benign tumors like Castleman's disease and various hematological and solid cancers.

Renal involvement is common (nearly 95 %), patients present with proteinuria and/or renal failure.[3] Liver is the second most commonly involved organ (approximately 20% of the patients), whilst cardiac involvement is rare (~2% of patients). Gastrointestinal and neurological involvement are also rare in AA amyloidosis.

Diagnosis is confirmed by histological demonstration of amyloid deposits. Staining with hematoxylin-eosin reveals homogeneous and eosinophilic amyloid deposits. Amyloid material is identified based on its metachromatic properties with Congo red. It is still considered the gold standard owing to its higher sensitivity and specificity for differentiating amyloid from other protein deposits.[4] The classic apple-green birefringence under polarized light can only be seen under ideal circumstances is characteristic. Adding phenol to the classic Congo red dye and using fluorescence microscopy may help improve the sensitivity of this technique for the detection of amyloid deposits.

After the initial diagnosis of amyloidosis, the subtype must be identified, and systemic organ involvement evaluated. The amyloid deposits in all forms of amyloidosis look alike. Therefore, amyloid typing is done on the tissue sample containing amyloid. The most reliable method is mass spectrometry (MS)and its sensitivity and specificity are close to 100%, making it the gold standard for typing.[5] However, is an expensive typing method and is not widely available. The use of antigen-antibody-based typing methods, such as immunohistochemistry and immunofluorescence, although less sensitive and specific than MS, is acceptable alternative typing method. Immunohistochemistry was used for subtyping amyloid in both of our patients.

There are 2 main approaches to treating AA amyloidosis – stopping further production of the precursor protein and reducing the burden of already deposited amyloid.

The best way to achieve a normal basal serum value of SAA is by eradication of the underlying chronic inflammatory disease. This means the elimination of the infection by antibiotic treatment and sometimes combined with surgery in patients with infectious diseases such as tuberculosis, leprosy, recurrent pulmonary infections, and osteomyelitis. In conditions where eradication is not possible like chronic inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, and Crohn's disease, can be treated by effective anti-inflammatory drugs such as methotrexate and biologicals, especially those directed against tumor necrosis factor (TNF),

interleukin-1 (anakinra) and interleukin-6(tocilizumab). If these drugs can be used, effective suppression of SAA to low serum concentrations is often possible.

Dimethyl sulfoxide is a molecule derived from intracellular low-density lipoprotein, it disrupts hydrogen bonding. It has been tested in patients with renal amyloidosis and can lower acute-phase reactant levels. Eprodisate is a negatively charged low molecular-weight molecule that is very similar to heparan sulfate. By binding competitively to GAG union sites, it inhibits the polymerization of amyloid fibrils and prevents the stabilization of amyloid deposits. Heparins and statins also have beneficial effects on the outcome of AA amyloidosis. The former can slow progression by breaking the stabilizing bonds between GAG and SAA in the deposits, in a manner analogous to that of eprodisate; the latter seem to exert their effect through inhibition of the isoprenoid pathway by specifically blocking farnesyl transferase. This pathway is shared by some autoinflammatory diseases.

Doxycycline has shown to have beneficial properties in amyloidosis for many years. After mature fibrils have been deposited, physical disruption of amyloid fibrils by intercalating agents such as doxycycline can be beneficial. Doxycycline inhibits matrix metalloproteinases[6,7] and causes fibril disruption, inhibition of amyloid fibril deposition preventing the progression of the disease. A modified form of doxycycline covalently linked to poly-L-glutamic acid has been shown to further improve bio-distribution and fibril degradation.[8] Though it has been shown to be useful in AL, ATTR, and dialysis-related amyloidosis, some proposed mechanisms may be beneficial in AA amyloidosis. Dose administered in various studies was 100 mg b.d. of p.o. doxycycline. The same was used in our patients.

Tauroursodeoxycholic acid (TUDCA), a biliary acid can reduce amyloid fibril aggregation.[9] Ursodeoxycholic acid is a bile acid with an efficacy similar to that of tauroursodeoxycholic acid and has been used as an alternative in some studies.[10] We used UDCA at a dose of 300 mg three times per day.

Though photosensitivity, rash, and gastrointestinal symptoms can occur in patients taking doxycycline on long term, both patients tolerated the drug well. Similarly, UDCA was also well tolerated except for an event of diarrhea which resolved on transient discontinuation of the drug in the second patient. At 12 months, both of them were taking these drugs with 60-70% reduced proteinuria and a stable renal function

AA amyloidosis should be treated by decreasing serum amyloid A levels to near normal values (<3 mg/l). If this can be reached and maintained below 10 mg/l, the 10-year survival rises to 90%, whereas in the group with median SAA levels above 10 mg/l the 10-year survival falls down below 40%.

Conclusion

Doxycycline and ursodeoxycholic acid can be beneficial in patients with AA amyloidosis. Large-scale randomized control studies are required to confirm the inhibitory effect on disease progression. Though treatment of the underlying disease is of proven cardinal significance, doxycycline and/or ursodeoxycholic acid may be considered as an add-on therapy for patients in AA amyloidosis.

Statement of Ethics

Institutional Ethics Committee (Madurai Medical College) review board in our hospital requires approval only for original research and case reports with trial/experimental interventions (procedure/drugs) administered to patients. This patient has not been treated with trial/experimental therapy and has been administered only the approved form of treatment for the condition. All treatment and examinations followed the guidance of the Declaration of Helsinki.

Informed consent

Informed consent for treatment was obtained from the patient. And written informed consent was obtained from the patient for publication of this case report.

Conflict of Interest

The authors have no conflicts of interest to declare.

Source of Funding

None.

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