AA Protein-Related Renal Amyloidosis in Drug Addicts

SIGMUND MENCHEL, MD, DANIEL COHEN, MD, ELLIOT GROSS, MD, BLAS FRANGIONE, MD, PhD, and GLORIA GALLO, MD

From the Departments of Pathology and Medicine, Irvington House Institute, and the Office of Chief Medical Examiner of the City of New York, New York University Medical Center, New York, New York

Reports of renal amyloidosis occurring among narcotic addicts have been limited, for the most part, to case reports. In a prospective survey of 150 addicts examined at autopsy in the Office of the Chief Medical Examiner of the City of New York, 7 cases of renal amyloidosis were found. Immunohistologic examination demonstrated that in all of the 7 cases, the amyloid was AA protein-related. The amyloid extracted from the kidneys of two addicts and analyzed biochemically did not differ from the AA amyloid secondary to chronic infectious and inflammatory diseases. The combined data of previous reports and the present survey demonstrate that addicts who are subcutaneous users with skin infections most frequently develop amyloidosis. Our data demonstrating renal amyloidosis in 26% of addicts with chronic suppurative skin infections suggest that such addicts are at high risk for the development of amyloidosis. (Am J Pathol 1983, 112: 195–199)

THE LIST of medical disorders associated with the chronic use of narcotics is extensive[1–4] and includes renal abnormalities such as the nephrotic syndrome and renal failure.[5–10] Although numerous studies have documented renal disease in the addict population, reports of renal amyloidosis in this group have been limited for the most part to small numbers of cases.[11–17] A previous report from this center called attention to AA protein-related amyloid in the kidneys of 4 nephrotic addicts in association with chronic subcutaneous drug abuse and extensive skin infections[17]; the characterization of the amyloid was based upon immunohistologic examination of tissue sections. The purpose of the present study was to further characterize the amyloid biochemically as well as immunohistochemically and estimate the frequency of renal amyloidosis and the possible causes in drug abusers. To do this we prospectively examined the kidneys of drug addicts subjected to autopsy examination.

Materials and Methods

The study group consisted of 150 drug addicts examined in the Office of Chief Medical Examiner of the City of New York consecutively during the period between October 1 and December 4, 1981, and during the month of February 1982. The decision as to whether the deceased was an addict was based upon routine examination of the body; signs of acute or chronic intravenous or subcutaneous injection detected at autopsy, taken as presumptive evidence of drug abuse, determined inclusion in the study. In most instances corroborative evidence included toxicologic examination of the body fluids, scene investigation, and interviews with relatives of the deceased. Clinical data regarding evidence of renal disease were not availble for analysis.

Kidney sections from each subject were fixed in 10% neutral buffered formalin for light microscopic study. Paraffin sections 2–3 μ thick were routinely stained with hematoxylin and eosin (H&E) and periodic acid–Schiff. In kidneys with glomerular abnor-

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Dr. Menchel's present address is Office of the Medical Examiner, 1050 NW 19th St., Miami, FL 33136.

Address reprint requests to Gloria Gallo, MD, Department of Pathology, New York University Medical Center, 550 First Avenue, New York, NY 10016.

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malities, silver methenamine counterstained with H&E, crystal violet, and Congo red stains were also used. Blocks of unfixed frozen kidney from all cases were stored at −70 C, and those with abnormalities were examined by routine immunofluorescence.

In kidneys with amyloid, frozen sections 4 μ thick were incubated with fluorescein-conjugated antiserum to IgG, IgA, IgM (heavy-chain-specific), kappa and lambda light chain (light-chain-specific), C3, and fibrin. Additional sections were incubated with rabbit anti-AA protein antiserum previously shown to be reactive with AA amyloid,[18] followed by fluorescein-conjugated goat anti-rabbit IgG. Appropriate negative controls included sections incubated with normal rabbit serum followed by fluorescein-conjugated goat anti-rabbit IgG and renal biopsies of glomerulopathies, including immune complex diseases and AL amyloid, which showed no fluorescent staining for AA protein-related amyloid. Sections were examined and photographed in a Leitz Dialux fluorescence microscope. For the purposes of this study, no attempt was made to determine the systemic distribution of amyloid.

Amyloid fibrils were extracted from the unfixed kidneys of two addicts by previously described methods.[19] One of the kidneys extracted (Case 7, HOL) was part of this consecutive prospective autopsy series, whereas the other kidney was from another addict (LOR) who had come to autopsy before the beginning of the study and therefore is not listed in Table 1. Briefly, 75 g of kidney was homogenized 5 times in 0.15 M saline, and the supernatants were discarded. The insoluble residue was homogenized in distilled water and subjected to ultracentrifugation for 1 hour so that the amyloid fibrils would become suspended as a mucoid mass in the upper layer. The amyloid was precipitated with saline, then dialyzed against distilled water and lyophilized. The yield from 75 g wet kidney was 1.5 g of dry amyloid fibrils. Two hundred milligrams of lyophilized material was dissolved in 6 M guanidine in Tris buffer, pH 10.2, and made 0.17 M in dithiothreitol. To this was added 2 ml of 2 M guanidine in 4 M acetic acid and the solution was centrifuged at 100,000*g*, filtered, and then fractionated on a 72 × 1-inch column of Sephadex G75 or G100 equilibrated with 5 M guanidine HCl in 1 M acetic acid. The purity of the protein fractions and the molecular weights were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).[20] The major amyloid protein subunit from each kidney was subjected to amino acid analysis, and automated amino acid sequencing was performed on a Beckman 890C sequencer (Beckman Instruments, Inc., Fullerton, Calif) using the Beckman 0.1 M Quadrol program.[21]

Results

The demographic data of the 150 addicts examined at autopsy show that the subjects were predominantly black males between the ages of 20 and 50 years, with a mean age of 35 years. The route of drug injection was intravenous in most of the addicts. In approximately one-third there were multiple circular, atrophic, and/or pigmented scars indicative of subcutaneous injection sites; these were seen alone or in combination with intravenous tracks. In one 19-year-old Hispanic male, who presumably died as a result of an overdose of orally ingested methadone, neither intravenous nor subcutaneous injection sites were evident. The mean age of the addicts with only intravenous injection sites was 35 years, whereas those with signs of subcutaneous injection usage was 42 years. Skin infections (abscesses, ulcers or both) were present in approximately one-half of those with subcutaneous injection sites but were absent in most of those with only intravenous injection tracks.

Renal amyloidosis was found in 7 of the 150 addicts (Table 1), predominantly in black males 34–57 years old, with a mean age of 42 years. Amyloidosis occurred in 6 of 44 (14%) subcutaneous users but in only 1 of 105 (1%) intravenous users and was associated with extensive skin infections in 6 of 7 (86%); of addicts with skin infections, 6 of 23 (26%) had renal amyloidosis. With one exception (Case 5), amyloid occurred in addicts with extensive chronic suppurative skin lesions involving the extremities and extending over 10–20% of the body surface. Two of the subjects (Cases 1 and 3) also had active pulmonary tuberculosis and amputations, one of which was known to be for osteomyelitis. The reason for the amputation in the other addict was not known.

Toxicologic studies revealed evidence of heroin both in the blood and bile in 5 of the 7 subjects with amyloid; cocaine was found in two of them.

In all of the 7 addicts with amyloid there were nodular mesangial deposits in the glomeruli, and heavy deposits in blood vessel walls and tubular basement membranes as seen by light microscopy. Immunofluorescence examination of the kidneys in all showed bright staining of the amyloid deposits in all locations for amyloid AA protein (Figure 1) but no staining or trace staining for Igs, C_3, or fibrin only around the surfaces of amyloid deposits in some glomeruli, presumably due to nonspecific absorption of serum proteins.

Table 1—Profiles of Drug Addicts With Renal Amyloidosis*

Case	Sex	Race	Age	Injection site	Amyloid AA protein	Clinical/ autopsy findings
1	M	B	38	SC	+	Leg ulcers, active tuberculosis, S/P amputation
2	M	B	34	SC	+	Skin abscesses, sepsis
3	M	B	57	SC & IV	+	Skin abscesses, S/P amputation for osteomyelitis, active tuberculosis, nephrotic syndrome, renal failure, amyloid in liver
4	F	B	39	SC	+	Skin abscesses, decubitus ulcer, sepsis, cirrhosis
5	M	B	34	IV	+	Penile ulcer, nephrotic syndrome
6	M	B	46	SC	+	Skin abscesses
7	M	B	47	SC & IV	+	Skin abscesses, decubitus ulcer, lymphedema, history of treated tuberculosis, sepsis, renal failure, amyloid in spleen and liver

* Seven of 150 addicts autopsied in the Office of the Chief Medical Examiner of the City of New York. SC, subcutaneous; IV, intravenous; S/P, status post.

Amyloid fibrils from the 2 cases that were extracted gave identical elution profiles after gel filtration, with predominantly one retarded peak. The SDS-PAGE of this major amyloid protein subunit extracted from the kidneys of the 2 addicts is shown in Figure 2. Their molecular weights were 8500 daltons. Their amino terminal sequences are shown in Figure 3. Fifty-one residues of protein HOL and 32 of protein LOR were obtained. The amino terminal sequences show identity with each other and with protein AA.[22] The sequences of the C terminal portions of the molecules were not determined.

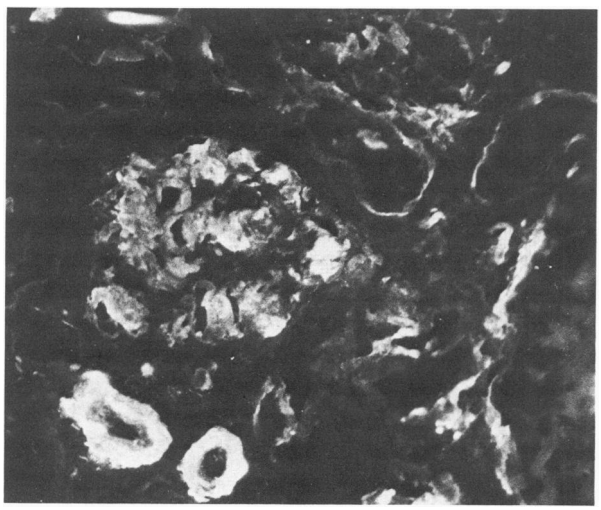

Figure 1—Section of kidney from an addict with amyloid deposits stained with rabbit anti-AA protein followed by fluorescein-conjugated goat anti-rabbit IgG. The amyloid in the glomerulus, tubular basement membranes, and vessels shows bright fluorescent staining. Stains for immunoglobulins (specific for heavy or light chains) and the third component of complement were negative. (×350) (With a photographic reduction of 14%)

Discussion

Albuminuria in addicts was known to nineteenth-century clinicians[23,24]; and since then, the nephrotic syndrome and renal failure in drug abusers have been well documented.[5-10] Of the various renal lesions observed in association with heroin abuse, focal glo-

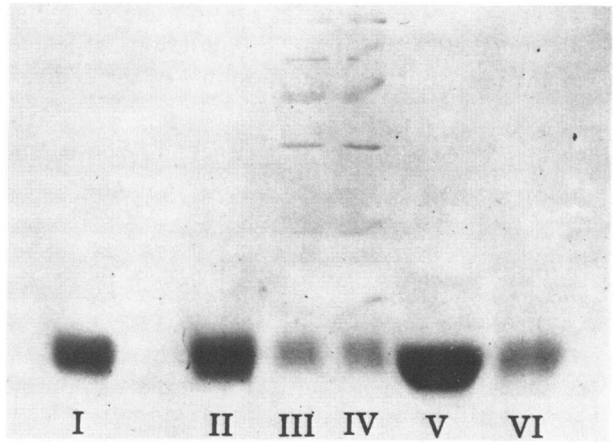

Figure 2—SDS-PAGE gel: 17% slab gel of purified amyloid protein subunits from patients LOR (Lanes I and V) and HOL (Lanes II and VI); Lanes V and VI reduced with 0.1 M dithiothreitol. Lanes I and II were unreduced. Markers (Lanes III and IV): Phosphorylase b, 94,000 mol wt; bovine serum albumin, 67,000 mol wt; ovalbumin, 43,000 mol wt; carbonic anhydrase, 30,000 mol wt; soybean trypsin inhibitor, 20,100 mol wt; lactalbumin, 14,400 mol wt; AA protein, 8500 mol wt.

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                                    10                                  20
HOL   Arg-Ser-Phe-Phe-Ser-Phe-Leu-Gly-Glu-Ala-Phe-Asp-Gly-Ala-Arg-Asp-Met-Trp-Arg-Ala
LOR   ____________________________________________________________________________

                                    30                                  40
HOL   Tyr-Ser-Asp-Met-Arg-Glu-Ala-Asn-Try-Ile-Gly-Ser-Asp-Lys-Tyr-Phe-His-Ala-Arg-Gly
LOR   ____________________________________________

                                    50
HOL   Asn-Tyr-Asp-Ala-Ala (    ) Arg-Gly-Pro-Gly-Gly
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Figure 3—The amino acid sequence of amyloid extracted from the kidneys of 2 drug addicts (patient HOL and patient LOR).—, identity; ( ), unidentified.

merular sclerosis has been the most frequent[10]; amyloidosis has been reported less frequently (Table 2). In a previous autopsy survey of renal lesions in 50 narcotic addicts who died of accidents or overdose, those with evidence of systemic disease, including amyloid, were excluded from the study.[8]

In the present prospective survey of 150 addicts subjected to examination at autopsy we found 7 unsuspected cases of renal amyloidosis, an incidence of 5% in the total addict population examined. More impressive is the observation that 14% of the subcutaneous users and 26% of those with chronic suppurative skin infections had renal amyloidosis. The majority (86%) of the cases of renal amyloidosis occurred in addicts with severe skin infections; in the remaining subject only a small ulcer was found. Three of the 7 with amyloid had other additional infections that could have been the cause of the amyloidosis. These included patient HOL, who had a history of treatment for tuberculosis but no evidence of severe or active disease at postmortem examination. Nonetheless, suppurative skin lesions appeared to be the only source of infection in 4 of the addicts.

The importance of skin infections as a cause of amyloidosis in addicts is emphasized in the work of others summarized in Table 2. Skin infections were present in 13 of 15 (87%) addicts with renal amyloidosis. In the others, skin infections were absent (Case 1, Lowenstein and Gallo[11]), or the information was not stated. If one takes together the previous and present data, 20 of 22 (90%) addicts with renal amyloid had known skin infections.

Amyloidosis has been reported in nonaddicts with secondary bacterial skin infection due to burns,[25] decubitus ulcers,[26] and dermatoses including hidradenitis and stasis ulcers in the absence of other diseases.[27] Thus it seems likely that infection, rather than the drugs per se, are responsible for the induction of amyloid in addicts. Chronic skin infection predominantly in subcutaneous users is another septic complication of chronic narcotism along with infective endocarditis, hepatitis, septic arthritis, and osteomyelitis[28-30] that likely arise as a result of nonsterile administration of the drugs.

The immunohistologic and biochemical findings demonstrated that in all of the addicts the amyloid

Table 2—Profiles of Addicts With Renal Amyloidosis Reported in the Literature

Case	Sex	Race	Age (years)	Duration of addiction (years)	Skin infections	References
1	M	B	28	Unknown	–	Lowenstein[11]
2	F	NS	50	NS	+	Jacob[12]
3	M	B	36	15	+	Novick[13]
4	M	B	40	20	+	Novick[13]
5	M	B	36	20	+	Brus[14]
6	F	NS	40	23	+	Scholes[15]
7	M	NS	56	20	NS	Scholes[15]
8	M	NS	44	27	+	Scholes[15]
9	M	NS	30	14	+	Scholes[15]
10	F	B	37	16	+	Meador[16]
11	F	B	43	19	+	Meador[16]
12	M	B	36	10	+	Rubenstein[17]
13	M	B	56	30	+	Rubenstein[17]
14	M	B	45	15	+	Rubenstein[17]
15	M	B	29	10	+	Rubenstein[17]

NS, information not stated.

was AA protein-related and, to the extent examined, did not differ from the AA amyloid secondary to chronic infections and inflammatory diseases. This lends further support to the view that chronic antigenic stimulation from cutaneous infections is the underlying cause of amyloidosis in drug addicts. AA protein and its circulating serum precursor (SAA) are found in other chronic infections and are part of a family of proteins produced in the liver in response to inflammation.[31]

Our findings, based on a study of 150 unselected addicts subjected to autopsy, suggest that addicts who are subcutaneous users with suppurative skin infections are at high risk for the development of amyloid and reemphasize earlier reports of the association.[12-17] Furthermore, addicts who are only intravenous users less frequently appear to have amyloidosis; if nephrotic, they are more likely to show glomerular sclerosis as the underlying disease.[10] The distinction is important because vigorous treatment of visible skin infections may prevent the development of amyloidosis, induce remission of the nephrotic syndrome,[11] and possibly result in the resolution of renal amyloid in patients with adequate renal function.[32,33]

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