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The Effect of Dimethyl Sulfoxide (DMSO) upon Spontaneous Amyloidosis in Mice[1] (41254)

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Abstract. The effect of dimethylsulfoxide (DMSO) on removing amyloid deposition was tested on low leukocyte count (LLC) mice which spontaneously develop amyloidosis after 1 year of age, characterized by amyloid deposition in the spleen, liver, and kidney. Ten LLC mice 13 months old or older were given subcutaneous injections of 100 mg DMSO three times per week for 2 to 5 weeks, and 10 untreated mice were used as control. Eight of ten control LLC mice had amyloid in the liver, spleen, and kidneys; 2 mice had no amyloid. These same organs were devoid of amyloid in 8 of 10 DMSO-treated mice, and the remaining 2 mice had minimal amyloid deposition. Thus, the incidence and degree of amyloidosis in the DMSO-treated LLC mice were significantly reduced.

The effect of dimethylsulfoxide (DMSO) upon amyloidosis in low leukocyte count (LLC) mice was tested. At 3 to 6 months of age, LLC animals develop reticular cell hyperplasia, and after about 1 year of age develop amyloidosis, with amyloid deposits occurring primarily in the kidneys, spleen, and liver. The animals eventually die of renal failure (1, 2).

DMSO has been used in several investigations to treat amyloidosis in animals (3, 4) and human patients (5–7) afflicted with diverse forms of this disease. Previous animal studies with DMSO required prior or simultaneous induction of amyloidosis, using agents such as casein. In contrast, amyloidosis in the LLC mouse line develops without artificial induction. As an animal model, this characteristic makes LLC mice uniquely appropriate for studying the effect of drugs upon amyloidosis.

The intent of this study was to investigate two major parameters: the effectiveness of DMSO in removing amyloid deposits and the usefulness of urine testing for the presence of amyloid as an evidence of drug response.

Materials and Methods. *Mice.* LLC mice were derived by selective breeding for low leukocyte counts from a population produced by intercrossing six inbred strains—C57BL/6J, C57BR/cdJ, A/J, BALB/cJ, LG/Ckc, and SM/Ckc. After 22 generations of selection, the line is being maintained by sib mating. The leukocyte count averages about 5000/mm^3 of peripheral blood (8). Mice of the LLC strain are not as prolific as those of ordinary inbred strains and their longevity is shorter. Twenty mice 13 months old or older were used in this experiment. Large numbers of experimental mice are difficult to acquire as aged populations of the LLC mice are difficult to maintain. The animals were housed one to four per cage and fed Old Guilford Jax Lab Diet 234.

Treatment. Ten mice were injected subcutaneously with 100 mg of DMSO, three times per week, for 2 to 5 weeks. Ten untreated mice were used as control.

Microscopic examination. Two experimental and two control animals were sacrificed after the second, third, and fourth week of treatment with the remainder being sacrificed after 5 weeks of treatment. Standard histologic preparations of kidney, liver, and spleen were made and sections were stained with either crystal violet or Congo red in order to determine the presence or absence of amyloid.

Urine test. Urine was collected once a week immediately following the administration of DMSO or H_2O, and precipitates were prepared according to the technique of Ravid *et al.* (5). The urine and precipi-

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tated urine samples were examined for the presence of amyloid using (i) Congo red staining viewed under polarized light, (ii) Ouchterlony gel diffusion plates with rabbit anti-mouse (LLC) amyloid antiserum diluted 1:100 and, (iii) indirect fluorescent antibody staining with the primary rabbit anti-mouse amyloid serum used at a 1:100 dilution and the secondary fluorescein-labeled goat anti-rabbit IgG used at a 1:40 dilution.

Results. Histological sections from control animals showed splenic amyloidosis in one of three patterns, representing stages in the temporal sequence of amyloid accumulation: (i) isolated foci at the periphery of the spleen follicles, (ii) encirclement of the follicles, and (iii) infiltration of all splenic tissue. These degrees of amyloid deposition are designated as 1+, 2+, 3+. Liver deposition exhibited a similar pattern: foci adjacent to central veins (1+), encirclement of central veins (2+), and diffuse infiltration of the liver parenchyma (3+) (1). Deposits in the kidney were present in glomeruli, renal papillae, and the interstitium of the cortex and medulla. The scale used in Table I for renal deposition of amyloid refers to the relative amount deposited, not to any specific site of deposition. An arbitrary scale was used: 1+ means minimal, 2+ moderate, and 3+ maximal deposition. In all tissues, 0 stands for the absence of amyloid deposits.

Eight of the ten control mice exhibited varying degrees of amyloid deposition in liver, spleen, and kidney (see Table I). No amyloid deposits were detected in the remaining two control animals. In 8 of 10 DMSO-treated mice, the spleen, liver, and kidney were free of amyloid. In the remaining 2 mice, minimal amounts of amyloid deposits were present in all three organs of 1 mouse and in the spleen and liver of the other.

No amyloid-like material could be detected in the urine or urine precipitate of either control or treated animals when examined by Congo red staining under polarized light, Ouchterlony gel difussion plates, or fluorescent antibody staining.

Discussion. Mice of the LLC strain treated with DMSO showed a significant reduction in the frequency of amyloidosis and the degree of amyloid deposition. The organs devoid of amyloid in the treated mice appear normal in size and histological morphology. It would appear, therefore, that normal cellular development can recur after the removal of the amyloid deposit.

An attempt to determine if degraded amyloid was being excreted in the urine met with negative results. Methods employed to identify this excreted material depend upon detecting the β-pleated sheet conformation of the amyloid fibrils (Congo red staining) and the antigenic determinant of the amyloid (techniques using anti-amyloid an-

TABLE I. DEPOSITION OF AMYLOID IN SPLEEN, LIVER, AND KIDNEY OF DMSO-TREATED AND CONTROL MICE

Weeks of DMSO treatment	DMSO treated				Control			
	Mouse number	Spleen	Liver	Kidney	Mouse number	Spleen	Liver	Kidney
2	1	0	0	0	11	1+	0	0
	2	0	0	0	12	3+	3+	3+
3	3	0	0	0	13	0	0	0
	4	0	0	0	14	1+	1+	0
4	5	0	0	0	15	2+	3+	3+
	6	0	0	0	16	1+	2+	1+
5	7	0	0	0	17	0	0	0
	8	0	0	0	18	1+	2+	1+
	9	1+	1+	0	19	3+	3+	3+
	10	1+	1+	1+	20	2+	2+	1+

Note. See text for scale.

tiserum). DMSO has been shown to dissociate amyloid protein subunits (3). It is possible that the application of DMSO in the LLC mice caused an abolition of the β-pleated sheet conformation and a change in the antigenic properties of the reactive substance. Thus, the drug may have caused alteration of amyloid fibrils to a form as excreted in urine, but not detectable by the methods here used.

An *in vitro* test has shown that DMSO dissociates amyloid fibrils consisting of immunoglobulins into subunits, but results of an *in vivo* test of DMSO in induced cases in mice are inconsistent (3). Immunochemical studies of amyloid proteins identified to date delineate two major types, fragmented immunoglobulin light chains and amyloid A or AA protein, each of which occurs in different forms of amyloidosis. In the induced cases in animals, only AA has been observed. Partial amino acid sequences of the LLC amyloid protein show homology to the mouse κ-light chain of immunoglobulin. On the other hand, there are inconsistencies between different determinations, and only minimal staining utilizing the immunoperoxidase procedure with IgG and IgM as the primary antibody has been found according to Gorevic (P. C. Gorevic, personal communication). Immunochemical analyses have established, however, that LLC amyloid protein is not AA. We believe that a variant form or forms of κ-light chains may compose LLC amyloid. If this is the case, the present results seem to show the effect of DMSO on this type of amyloidosis. In any case, this study shows some promise of this drug for treatment of amyloidosis.

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