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Effects of Dimethyl Sulfoxide and Colchicine on the Resorption of Experimental Amyloid

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Summary. The induction of amyloid in C_3H mice by either casein solution or complete Freund's adjuvant emulsion with *Mycobacterium butyricum* was confirmed by partial splenectomy. The animals were autopsied after treatment with dimethyl sulfoxide (550 mg/kg, 50 times), colchicine (0.02 mg/kg, 15–37 times), or saline solution as a control. Detailed histological comparisons of biopsy and autopsy spleens provided evidence that dimethyl sulfoxide was significantly effective in the resorption of amyloid, while in the animals treated with colchicine amyloid deposition was increased. The effect of dimethyl sulfoxide was discussed with reference to the modification of amyloid fibrils.

Key words: Amyloid – Resorption – Dimethyl sulfoxide – Colchicine.

Introduction

Although a number of studies have dealt with the resorption of experimental amyloid (Richter, 1954; Williams, 1967; Polliac et al., 1970; DeLellis et al., 1970), only a few reports have been related to effective treatment. Colchicine, which has been used for the treatment of Familial Mediterranean fever (Zemer et al., 1974; Dinarello et al., 1974) was found to reduce the urinary protein level in amyloid nephropathy (Zemer et al., 1976). Later it was shown that colchicine could affect amyloidogenesis (Shirahama and Cohen, 1974; Kedar et al., 1976). Dimethyl sulfoxide (DMSO), which is generally used as a solvent because of its unique dissociative action, has been recently reported to inhibit amyloid formation in mice (Isobe and Osserman, 1976; Kedar et al., 1977).

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Clinically, the effectiveness of DMSO has been observed in the treatment of patients with primary or secondary amyloidosis (Osserman et al., 1976). However, little is known about the mechanism of action of these two compounds.

The present study was undertaken to obtain further evidence of the effects of DMSO and colchicine in the resorption of experimental amyloid, and to find a clue for the elucidation of the mechanism of these agents.

Material and Methods

Inbred C_3H mice, six weeks old (weight approximately 25 g), were used. Amyloid was induced by the following methods; daily subcutaneous injections of 0.5 ml of 5% casein in 0.3 M $NaHCO_3$ or a single intraperitoneal injection of 0.2 ml of the emulsion composed of complete Freund's adjuvant and Mycobacterium butyricum (Difco Laboratories, Detroit Michigan). The emulsion was prepared according to the method of Ram et al. (1968). In order to confirm amyloid deposition in the spleen, partial splenectomy was performed in the mice received the casein solution 60 times or the emulsion two weeks previously. The mice having amyloid were divided into three groups, and were given the following treatments; (1) subcutaneous injection of DMSO (550 mg/kg body weight) 50 times in total, (2) oral administration of colchicine (0.02 mg/kg body weight) 15–37 times in total and (3) subcutaneous injection of saline solution 32–50 times in total. All of the groups of animals were fed laboratory diet and tap water.

All of the animals were examined by autopsy. The resected spleens were fixed in 5% formalin and embedded in paraffin. The paraffin sections of biopsy spleen and corresponding autopsy spleen were mounted on the same slide and were stained with haematoxylin-eosin and Congo red. The occurrence of amyloid was confirmed by examining Congo red-stained sections under a polarizing microscope. Three or four photomicrographs ($\times 120$) were taken of each specimen in order to make the composite photograph. The amyloid areas in these photographs were measured by the system for quantitative evaluation of images (Mop/Digiplan, Kontron). The intensity of staining by Congo red was measured using the scanning microdensitometer (M85, Vickers) at 500 nm. This measurement was performed at 10–15 points on each section and also done on 3–8 serial sections from the same block to confirm reproducibility. The measuring range of each point was equal to the scanning spot size and Congo red-stained amyloid alone was included in the scanning spot for the measurement.

Results

Amyloid occurred predominantly in the spleen, when compared with the liver and the kidney under these experimental conditions. In the spleen the extent of amyloid deposition, which was particularly observed in the perifollicular zone, was graded from I to V according to the modified method of Christensen's classification (Christensen and Hjort, 1959) (Figs. 1–4). The extent of amyloid in the autopsy spleen was compared with that in the biopsy spleen of the same animal as shown in Table 1. In DMSO-treated animals the amount of amyloid was significantly decreased in contrast with that in control animals (according to the Mann-Whiteny U test, $P<0.05$), while in colchicine-treated animals it was increased ($P<0.05$). The ratio of the area of amyloid deposition to the entire field examined was calculated in each animal by the method as described above. The grade I, II, III, and IV corresponded to the area ratio of $3.8\pm2.6\%$ (mean$\pm$S.D.), $16.6\pm8.1\%$, $45.2\pm15.3\%$, and $82.4\pm15.4\%$, respectively. As shown in Table 2, amyloid areas in the autopsy spleens were

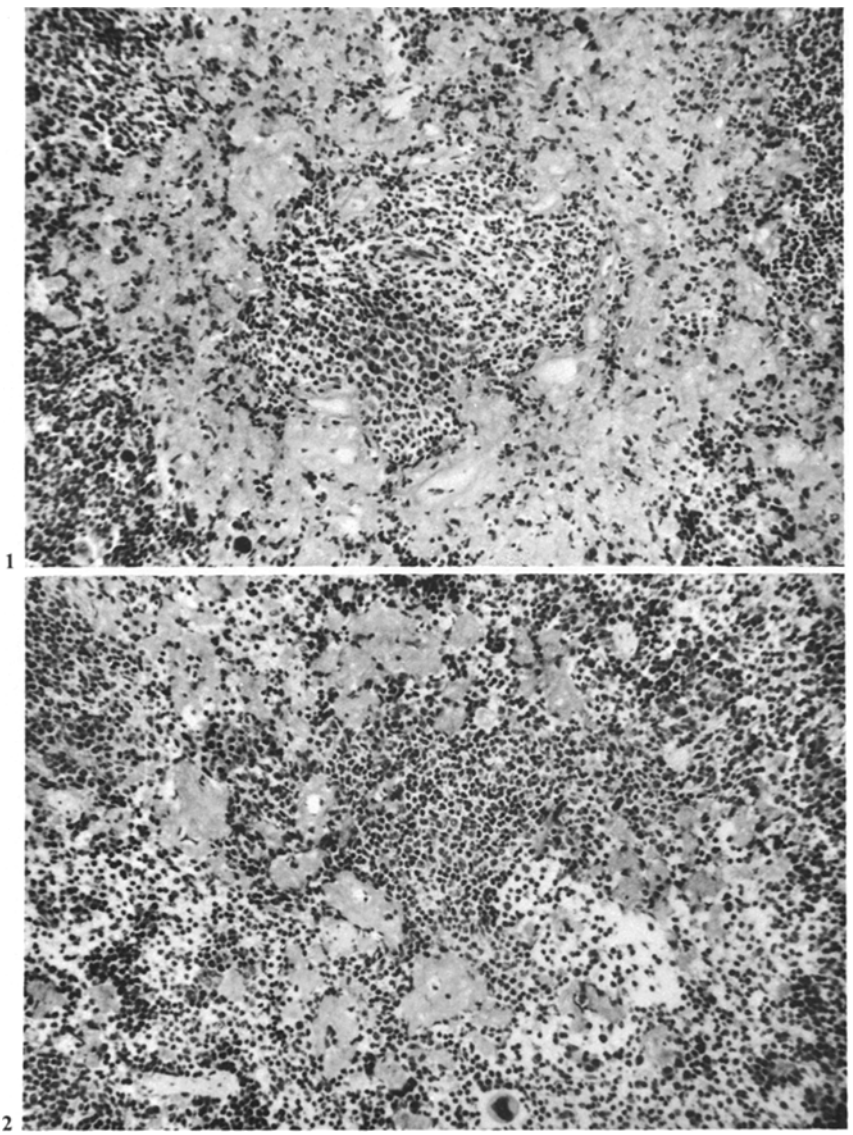

Fig. 1. Biopsy spleen two weeks after a injection of complete Freund's adjuvant emulsion with Mycobacterium butyricum. The grade of amyloid deposition is classified as Grade II-III. Broad band of amyloid in the perifollicular zone. (×300, Congo red)

Fig. 2. Autopsy spleen from the same animal as shown in Fig. 1. Small masses of amyloid around the follicle after DMSO treatment. The grade of amyloid deposition is classified as Grade I–II. (×300, Congo red)

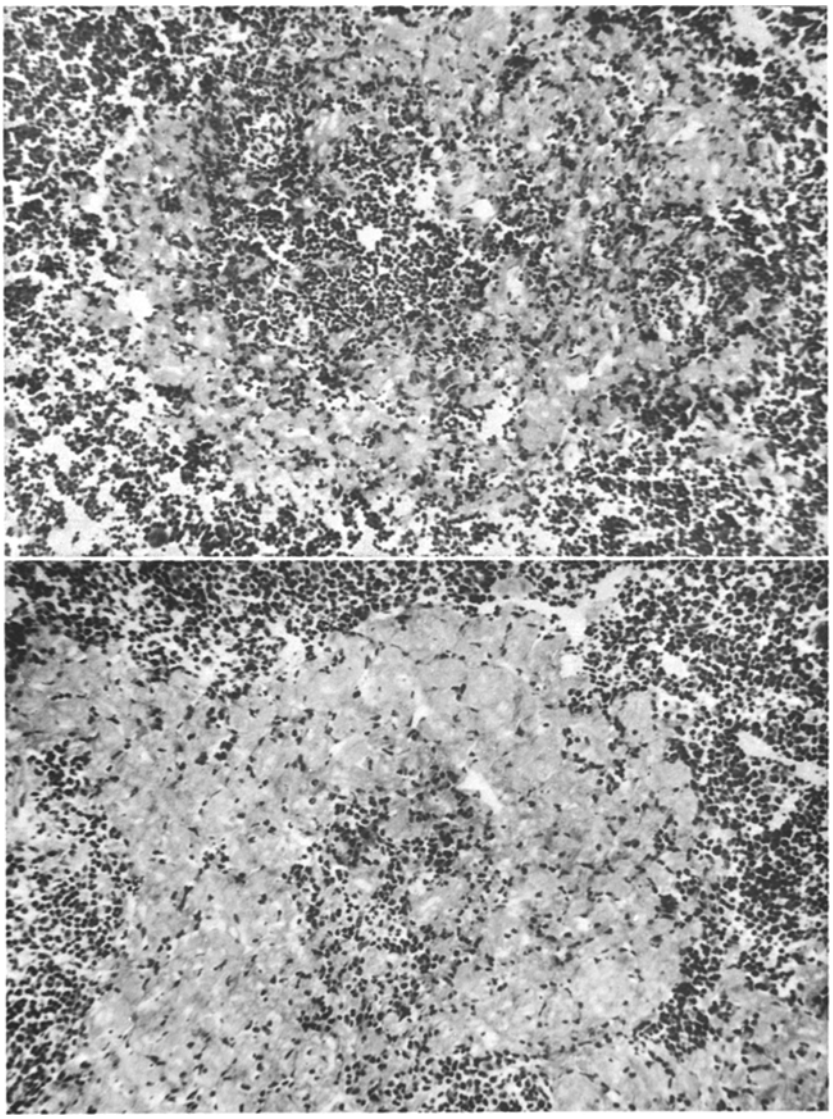

Fig. 3. Biopsy spleen after injections of casein suspension 60 times. The grade of amyloid deposition is classified as Grade II. Amyloid band in the perifollicular zone. (×300, Congo red)

Fig. 4. Autopsy spleen from the colchicine-treated animal, the biopsy spleen of which is shown in Fig. 3. Broad band of amyloid around the atrophic follicle, this corresponds to Grade III. (×300, Congo red)

Table 1. Changes of the extent of amyloid deposition

Group No.	Treatment	Number of animals		
		Decrease	Constant	Increase
1	DMSO[a]	8	2	3
2	Colchicine[b]	1	3	13
3	Saline	8	4	13

[a] Significantly decreased compared with control ($P<0.05$)
[b] Significantly increased compared with control ($P<0.05$)

Table 2. Area ratios (mean value) of amyloid deposition in the specimens

Group No.	Treatment	Biopsy	Autopsy	Difference
1	DMSO	17.3% ± 8.9	9.9% ± 7.4	− 7.4% ± 10.8[a]
2	Colchicine	26.9% ± 12.6	52.4% ± 21.1	+25.5% ± 22.6[b]
3	Saline	15.9% ± 12.2	23.4% ± 15.9	+ 7.5% ± 17.3[c]

± standard error

[a] Significantly decreased ($P<0.05$)
[b] Significantly increased ($P<0.0025$)
[c] Significantly increased ($P<0.05$)

Table 3. Relation of the grade and the area ratio of amyloid deposition in each specimen of DMSO treated animal

Animal number	Biopsy		Autopsy		Difference area ratio
	Grade	Area ratio	Grade	Area ratio	
1	I–II	19.4%	II–III	21.5%	+ 2.1%
2	II–III	36.6%	II	19.9%	−16.7%
3	I	7.3%	I	3.9%	− 3.4%
4	II	19.0%	I	1.7%	−17.3%
5	II	10.3%	I–II	8.7%	− 1.6%
6	II	28.2%	I	1.2%	−27.0%
7	I–II	16.8%	I	0.6%	−16.2%
8	II–III	20.1%	I–II	5.3%	−14.8%
9	II–III	20.0%	II	12.6%	− 7.4%
10	II	17.4%	II–III	19.9%	+ 2.5%
11	II	16.7%	I	6.4%	−10.3%
12	II	10.3%	II	13.2%	+ 2.9%
13	I	2.2%	II–III	13.2%	+11.0%
Mean		17.3%		9.9%	− 7.4%

Table 4. Density (mean value) of Congo red in the specimens

Group No.	Treatment	Biopsy	Autopsy	Difference
1	DMSO	68.1 ± 9.8	58.8 ± 8.4	− 9.3 ± 10.5[a]
2	Colchicine	73.2 ± 9.2	84.8 ± 10.3	+ 11.6 ± 10.7[b]
3	Saline	65.7 ± 10.0	69.9 ± 13.3	+ 4.2 ± 11.9[c]

(The results are shown in arbitrary 'machine' unit.)
± standard error

[a] Significantly decreased ($P < 0.05$)
[b] Significantly increased ($P < 0.0025$)
[c] Significantly increased ($P < 0.05$)

significantly smaller than those in the biopsy spleens in DMSO-treated animals (according to Student's T test, $P < 0.05$). On the other hand, control ($P < 0.05$) and colchicine-treated animals ($P < 0.0025$) gave converse results. The interrelation of the grade and the area ratio of amyloid deposition in each specimen of DMSO-treated animals is shown in Table 3. The density of Congo red in biopsy and autopsy specimens of each group is shown in Table 4. DMSO-treated animals gave a significantly lower value in autopsy specimens compared with that in biopsy specimens (according to Student's T test, $P < 0.05$), while both control ($P < 0.05$) and colchicine-treated animals ($P < 0.0025$) gave a significantly higher value in autopsy specimens.

Discussion

The present experiments demonstrate that DMSO is effective in the resorption of splenic amyloid. Although the mechanism of action of DMSO is not completely elucidated, it can be suggested from the results of several studies that DMSO modifies the extracellular amyloid fibrils. Isobe and Osserman (1976) reported that DMSO partly dissolved amyloid fibrils in vitro. Kedar et al. (1977) and Ravid et al. (1977) found that amyloid fibril-like materials were excreted after DMSO administration in the urine of the animals and the patients with amyloidosis. As shown in Table 4, reduced intensity of staining by Congo red was observed in the spleens of DMSO-treated animals. This suggests that the administration of DMSO results in the partial modification of the extracellular amyloid in the spleen.

It is known that the amyloid fibril proteins have different chemical properties depending on the different types of amyloidosis (Glenner and Page, 1976). A cross-β pleated sheet conformation is the only demonstrable common feature (Eanes and Glenner, 1968; Bonar et al., 1969), which has been also observed in the tissues of experimental amyloidosis (Glenner et al., 1971). Congo red, which stains amyloid specifically, most likely binds to the cross-β pleated sheet protein of amyloid fibrils (Cooper, 1974). Change in amyloid fibrils by DMSO

could be explained by a partial destruction of its cross-β pleated sheet conformation followed by solubilization or degradation of amyloid fibrils.

An important role for macrophages in amyloid resorption has been described by several investigators (Shirahama and Cohen, 1971; Ishihara and Uchino, 1975). However, intact amyloid fibrils may be removed with difficulty even by macrophages, especially when the amyloid is abundant. The present study suggests that the amyloid fibrils can be modified by DMSO so as to be easily phagocytized by macrophages. In addition, it is known that DMSO can simulate the activity of lysosomal enzymes by promoting membrane permeability (Mish and Mish, 1967). Therefore, it seens possible that DMSO may promote the degradation of amyloid both intracellularly and extracellularly.

In contrast with the effect of DMSO, colchicine was not effective in removing amyloid in this study. This finding was consistent with the studies of Shirahama and Cohen (1974) and Kedar et al. (1976). The increase of amyloid in colchicine-treated animals could be explained by the inhibition of its resorption.

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