

BLOCKAGE OF AMYLOID INDUCTION BY COLCHICINE IN AN ANIMAL MODEL*

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Amyloidosis, characterized by the deposition of extracellular material exhibiting green birefringence under the polarized microscope after Congo red staining and thin, rigid, nonbranching fibrillar profiles by electron microscopy, can occur de novo (primary), in relation to chronic inflammatory or infectious diseases (secondary) or tumors (i.e. myeloma), and is now known to be far more widespread than previously believed (1, 2). The nature of amyloid has become increasingly clear, and recent amino acid sequence analyses have shown primary and myeloma amyloid to consist of a protein homologous to the variable portion of immunoglobulin light chain and secondary amyloid to be a unique nonimmunoglobulin protein (A-protein) (3, 4). Nevertheless, the pathogenesis of amyloid remains unsolved and effective methods for prevention and treatment are not yet established (1-4). Attempts to prevent the occurrence of amyloidosis in experimental animal models using a variety of treatments including anti-inflammatory, immunosuppressive, and anticarcinogenic treatments have not been consistently effective, or indeed have accelerated amyloid induction under certain circumstances (2, 5, 6).

We report here our experimental data in a "casein-induced" mouse amyloid model which demonstrate that amyloid induction can be successfully blocked by the administration of colchicine, not only when colchicine is given for the entire course of the amyloid induction regimen but, more significantly, when it is given only in the late pre-amyloid and the amyloid phase of the regimen or to the recipients after "transfer of amyloid (2)."

Materials and Methods

Animals. Animals used in the present study were 6-8-wk old female CBA/J mice (Jackson Laboratories, Bar Harbor, Maine) and were maintained on Purina Chow pellets (Ralston Purina Co., St. Louis, Mo.) and water ad libitum.

Standard Amyloid Induction. Mice were given daily subcutaneous injections of 0.5 ml of 10% casein solution (7) and sacrificed on the day after the last injection.

Colchicine Administration. Colchicine (as "colchicine injection," Eli Lilly & Co., Indianapolis, Ind.) was diluted with physiological saline to make a final concentration 0.05 mg/ml, and injected intraperitoneally into mice.

Amyloid Induction by "Transfer of Amyloid." Amyloidosis has been shown to be "transferred" in several ways (2). The necessary duration for amyloid induction can be significantly reduced in the recipients by the injection of tissue (especially spleen) homogenate or extracts from the animals in the late pre-amyloid or the amyloid phase of the amyloid induction regimen. With this "transfer of

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amyloid" experiment, various side effects of the amyloid induction regimen in the early phases, which may not be directly related to amyloid production, can probably be eliminated from the recipients. In the present study, donor mice received nine daily casein injections and were sacrificed on the day after the last injection. Spleens were removed and homogenized thoroughly in cold physiological saline with a Potter-Elvehjem homogenizer (Potter Instrument Co., Inc., Melville, N. Y.). By examination of frozen sections after Congo red staining, none of the spleens demonstrated amyloid deposition, so that the mice were judged to be in the late pre-amyloid phase of the amyloid induction regimen. The spleen homogenate was frozen and thawed, and then injected intraperitoneally to the recipients at a rate of one donor spleen to one recipient. The recipients received six daily casein injections commencing on the same day of the "transfer," and were sacrificed on the day after the last casein injection.

Evaluation of Results. Spleens, livers, and kidneys were examined for amyloid deposition by light and electron microscopy (7).

Results

As our accumulated data indicate (7, 8) and as confirmed by the present control study (Exp. 1, 2, and 11 in Table I), our standard amyloid induction schedule

TABLE I
Effects of Colchicine on Amyloid Induction in Mouse Model

Exp. no.	No. of casein inj.*	Colchicine‡		No. of mice			Incidence of amyloid	
		Daily dose	No. of inj.	Splenic amyloid	Hepatic amyloid	Total mice used	Spleen	Liver
		<i>mg</i>					<i>%</i>	
Standard amyloid induction								
1	0	0	0	0	0	12	0	0
2	14	0	0	10	0	12	83	0
3	14	0.005	14	2	0	10	20	0
4	14	0.010	14	0	0	8	0	0
5	14	0.015	14	0	0	9	0	0
6	14	0.005	6	3	0	10	30	0
7	14	0.010	6	1	0	9	11	0
8	14	0.015	6	0	0	10	0	0
9	14	0.020	6	0	0	10	0	0
10	14	0.025	6	0	0	7	0	0
11	21	0	0	9	8	9	100	89
12	21	0.015	13	0	0	12	0	0
13	21	0.020	13	0	0	7	0	0
14	21	0.015	7	8	1	9	89	11
"Transfer of amyloid" induction§								
15	6	0	0	10	7	10	100	70
16	6	0.015	6	0	0	10	0	0
17	6	0.020	6	0	0	8	0	0

* Daily subcutaneous injection of 0.5 ml of 10% casein solution.

‡ Colchicine was intraperitoneally injected concurrently with casein for the entire course or the last days of the casein injections.

§ Spleen homogenate from donor mice which had received nine casein injections was intraperitoneally injected into recipient mice, i.e. one donor spleen to one recipient.

onto CBA/J mice usually induces amyloid in the spleen after 12-18 casein injections, in the liver after 16-22 injections and in the kidney after 20-26 injections. For example, none develops amyloid after eight casein injections, 70-90% of the animals have splenic amyloid after 14 injections (Fig. 1), and after 21 injections amyloid is found in all spleens and 80-90% of the livers.

Preliminary study showed that daily injections of more than 0.025 mg of colchicine were lethal—all animals died within 7 days. With 0.020 mg per day, the mice survived for 5-15 days. Most of the mice tolerated daily injections of less than 0.015 mg colchicine (up to 21 injections).

When the casein and the colchicine injections were given concurrently, the

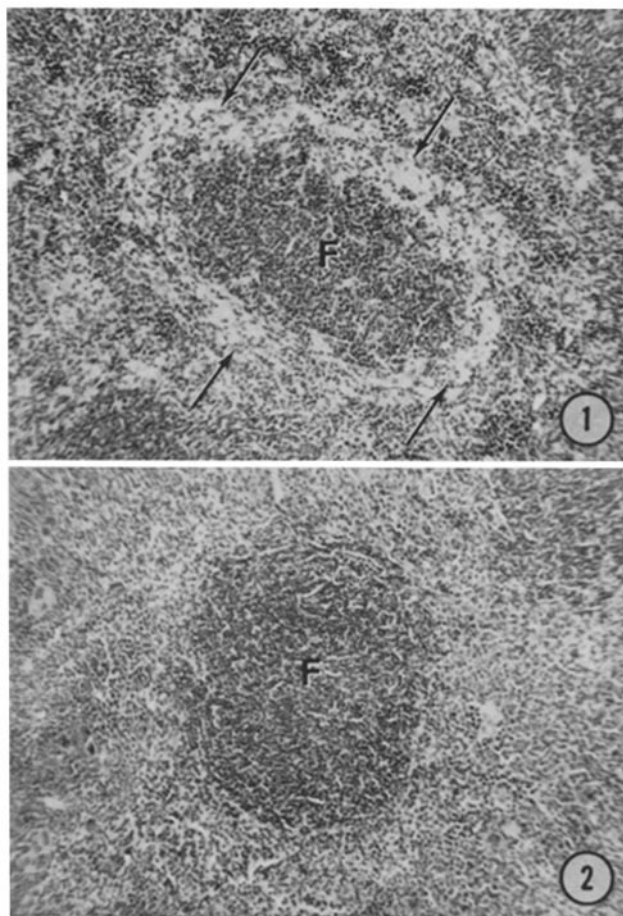


FIG. 1. Light micrograph of a mouse spleen after 14 daily casein injections without colchicine. Amyloid, which stains well with Congo red and demonstrates green birefringence under the polarized light, deposits in the marginal zone (arrows). F, lymph follicle. Congo red and hematoxylin stain. $\times 80$.

FIG. 2. Mouse spleen after 14 daily casein injections and 6 daily injections of 0.015 mg colchicine starting on the 9th day of the casein injection schedule; comparable area to that shown in Fig. 1. No amyloid is demonstrable. F, lymph follicle. Congo red and hematoxylin stain. $\times 80$.

incidence of amyloidosis was significantly decreased or completely blocked depending upon the dosage of colchicine. For example, 14 daily casein injections accompanied by 14 daily injections of 0.005 mg colchicine induced amyloidosis in only 2 out of 10 animals. None of the mice receiving more than 0.010 mg colchicine per day along with casein developed amyloid (Exp. 3-5 in Table I). Moreover, the colchicine injections at the late pre-amyloid or the amyloid phase of the amyloid induction regimen, which is considered to relate directly to amyloid formation (2, 6), were found to be equally effective (Table I). For example, six daily injections of 0.005-0.010 mg colchicine started on the 9th day of the 14 casein injection schedule (late pre-amyloid phase) reduced significantly the incidence of amyloidosis (Exp. 6 and 7 in Table I), and with more than 0.015 mg colchicine, the amyloid induction was prevented completely (Exp. 8-10 in Table I) (Fig. 2). Daily injections of high doses of colchicine starting with the late pre-amyloid phase (on the 9th day) prevented amyloid induction even when the casein injections were extended to 21 days (Exp. 12 and 13 in Table I). When seven colchicine injections were given during the last days of 21 casein injections, i.e. commencing on the 15th day of the casein injections (amyloid phase), deposition of amyloid was found in 89% of spleens and in 11% of livers (Exp. 14 in Table I), suggesting that additional amyloid deposition was inhibited by the colchicine administration after the 15th day of the casein injection schedule, though amyloid which had already deposited may not have been significantly absorbed during the colchicine treatment.

With the "transfer of amyloid" followed by six daily casein injections, amyloidosis was induced in all the recipients (Exp. 15 in Table I). However, when daily doses of 0.015-0.020 mg of colchicine were administered concurrently with the casein, none of the recipients developed amyloidosis (Exp. 16 and 17 in Table I).

Discussion

Despite considerable efforts made by a number of investigators, effective methods for prevention and treatment of amyloidosis have not yet been established either clinically or experimentally (1, 2). A few treatments apparently effective in inhibiting amyloid induction in experimental animal models depended on their application in the early pre-amyloid phase of the amyloid induction regimen (6). Various treatments applied to the animals in the late pre-amyloid or the amyloid phase of standard amyloid induction regimen or to the recipients after the "transfer of amyloid" were always found either to be inconsistently effective or to accelerate amyloid induction (2, 5, 6).

The present results demonstrate that colchicine inhibits amyloid induction in the experimental mouse model, not only when it is given for the entire course of the amyloid induction regimen but also when it is administered only at the late pre-amyloid phase, at the amyloid phase or after the "transfer of amyloid." Moreover, the data suggest that colchicine is probably effective in blocking amyloidogenesis at its final stage(s). Colchicine may, however, not affect significantly amyloid already deposited in the tissue.

Although the pathogenesis of amyloid is not yet clear, there is a large body of evidence indicating that the reticuloendothelial cells play an important role in its formation (2, 6, 8). Colchicine, widely used clinically, is known to disrupt microtubules (including neurotubules) in vivo and in vitro or inhibit their assemblage through its specific binding to the protein subunit, "tubulin". It also has a strong effect on endocytosis including "reverse endocytosis" (9, 10). No available data indicate that colchicine has a significant effect on the biosynthesis of proteins and other substances. One may speculate that colchicine inhibits the endocytic activity of the reticuloendothelial cells and eventually affects amyloid fibril formation, or, as a remote possibility, it binds with amyloid fibril subunits and prohibits their polymerization.

Finally, the present observation is of importance since (a) it may lead to the establishment of effective methods for the prevention and treatment of amyloidosis, (b) this method for blocking amyloid production in experimental animals will be helpful for further exploration of the mechanism of amyloidogenesis, and (c) the system will contribute toward further clarification of the biological effects of colchicine.

Summary

Colchicine was found to have a strong inhibitory effect on amyloid induction in an animal model. When CBA/J mice were treated with colchicine concurrently with the amyloid induction regimen, the incidence of amyloidosis was, depending upon the dosage of colchicine, significantly decreased (0.005–0.010 mg colchicine per day) or completely blocked (more than 0.015 mg colchicine per day). The colchicine treatment was effective not only when colchicine was given for the entire course of the amyloid induction regimen but also when it was given only in the late pre-amyloid or the amyloid phase of the regimen or to the recipients after the transfer of amyloid. The data suggest the colchicine is effective in blocking amyloidogenesis at its final stage(s), while it may not affect significantly amyloid already deposited in the tissue.

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Note added in proof. While this paper was in press, the following studies were brought to our attention at the International Symposium on Amyloidosis held on August 26–28, 1974 at Helsinki, Finland. E. Sohar and J. Gafni presented data on the familial Mediterranean fever with colchicine and its possible preventive effect on amyloidosis (*N. Engl. J. Med.* In press) and the prophylactic effect of colchicine on experimental murine amyloidosis was also independently observed (Kedar (Keizman), I., M. Rabid, E. Sohar, and J. Gafni. 1974. *Israel J. Med. Sci.* 10:787).

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