The Turkish Journal of Pediatrics 28:243 - 248, 1986

THE EFFECTS OF ASCORBIC ACID AND KETOTIFEN IN THE PREVENTION OF MURINE AMYLOIDOSIS*

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Key words: ascorbic acid, ketotifen, murine amyloidosis

Systemic amyloidosis is an incurable disease characterized by the widespread extracellular deposition of the protein fibrils which disrupt the function of the organs and tissues. Although the pathogenesis of amyloidosis is not clear, it has been suggested that the amyloid degrading factor is defective in these patients[1-3]. It has been reported that *in vitro* addition of ascorbic acid, citric acid and EDTA to amyloidotic serum restores the amyloid degrading activity[4] (Figure 1), and on the basis of *in vivo* experimental studies it has been claimed that ascorbic acid would be useful in the treatment of amyloidosis[5-7]. Another report, however, has failed to confirm this suggestion[8]. So the aim of this study has been to evaluate the contradictive data in the literature concerning the effect of ascorbic acid on the prevention of murine amyloidosis. Since ketotifen appears to

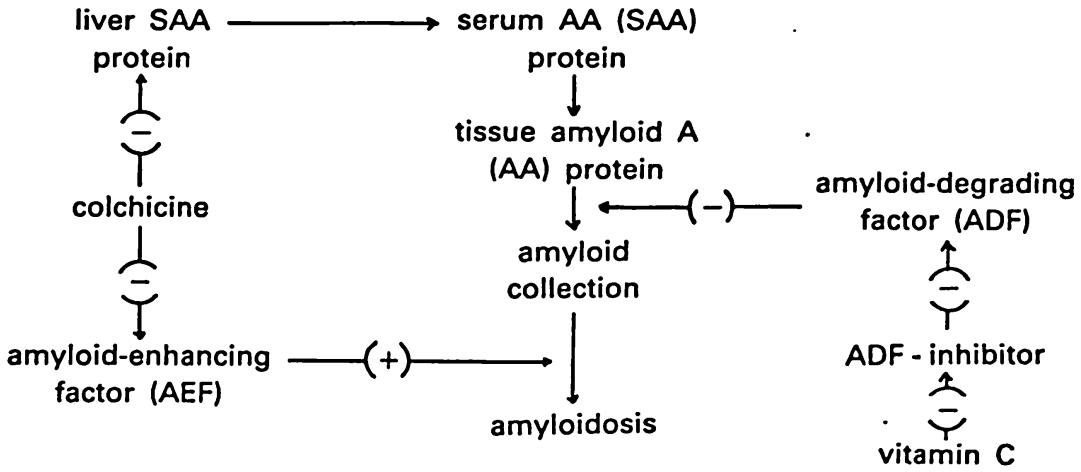

Figure 1

Pathogenesis of amyloidosis and the mechanisms of drugs used in the treatment of amyloidosis.

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block the release of some mediators from the polymorphonuclear leukocytes[9,10], the present study was also designed to determine whether the addition of ketotifen to the diet of experimental animals would result in any appreciable degree of protection against the murine amyloid deposition.

Material and Methods

Male albino mice, 6-8 weeks old and weighing approximately 30 g each, which had been obtained from the Laboratory Animals' Breeding Unit of the Ministry of Health and Social Welfare, were used in this study. The mice were housed five per cage and were given water and food without restriction. They were fed on the Turkish Mouse Maintenance Diet, in pellet form, which contains 12-15 mg ascorbic acid per kg.

Amyloidosis was induced in 45 mice with 14 consecutive daily i.m. injections of 0.5 ml 13% vitamin-free casein (NBC, Cleveland, Ohio) in 0.05 M NaOH[11]. The mice were divided, at random, into three equal groups. The first was the control group. The second received ascorbic acid as 3.5% sodium ascorbate at a dose of 5 g/kg body weight and the third received ketotifen (Zaditen®) as 0.2% at a dose of 0.3 mg/kg body weight daily through the study period. In each case this was administered in the drinking water.

The plasma levels of the ascorbic acid and the ketotifen could not be determined. All the animals were killed on the fifteenth day and their spleen examined for the presence of amyloid[12-15]. Amyloid was confirmed by alkaline Congo Red staining, which gives the characteristic apple-green birefringence when viewed under polarized light[16]. The results were classified into three categories: no amyloid, sporadic microdeposits of amyloid around the blood vessels only and diffuse deposition of amyloid. The X^2 test was used for statistical analysis.

TABLE I

Splenic Amyloidosis in Normal Control Mice and in Mice Treated with Vitamin C or Ketotifen

Group	Splenic amyloidosis		
	Diffuse	Microdeposits	None
Control (n : 15)	7	5	3
Vitamin C (n : 15)	–	6	9
Ketotifen (n : 15)	5	7	3

Results

The results are summarized in Table I. There was a significant decrease ($p<0.05$) in the degree of amyloid deposition in the vitamin C (second) group in comparison with the control (first) group and the ketotifen (third) group. There was no difference between the control and the ketotifen groups with respect to the amount of amyloid deposited in the spleen. Seven of the control animals and five of the ketotifen group had abundant splenic amyloidosis on the fifteenth day (Figure 2). In the vitamin C group, the spleens of nine animals were free of amyloid, microdeposits were seen in six.

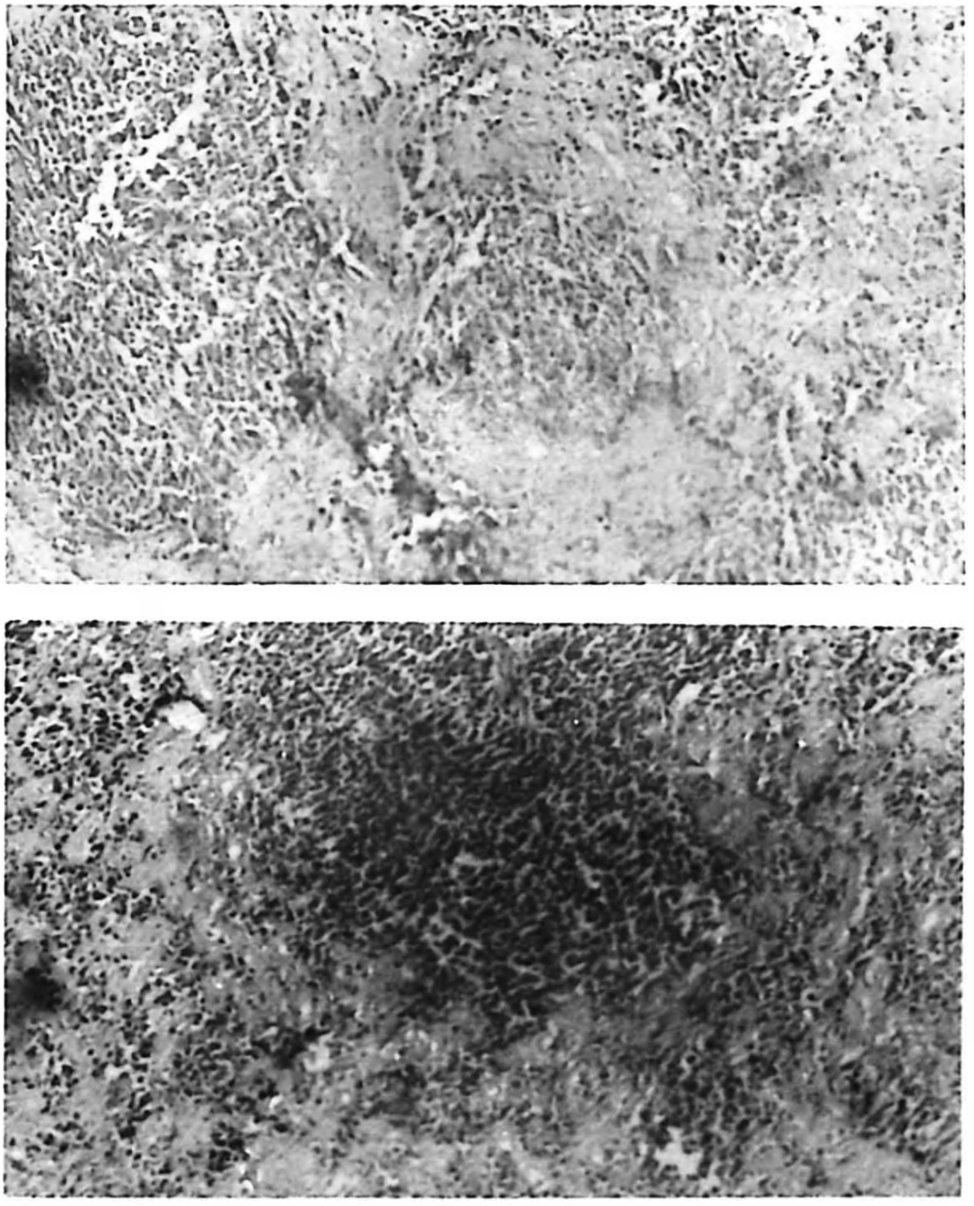

Figure 2 (a, b)

Splenic amyloidosis induced by daily injections of casein (HE).

Discussion

The amyloid substance in the secondary type of amyloidosis in certain familial forms (such as familial Mediterranean fever), and in animals (whether naturally occurring or experimentally induced), consists primarily of a protein known as amyloid A (AA) protein. The AA protein is related to a serum component called serum A (SAA) protein and it seems likely that SAA is a circulating precursor of AA. Amyloid A is thought to arise from proteolysis of SAA, an acute phase reactant synthesized in the liver in response to macrophage production of an SAA inducer known to be closely related to interleukin I. The concentration of SAA increases markedly in many acute and chronic diseases, including all types of amyloidosis. Though SAA levels tend to return to normal as the illness resolves, it seems that during many chronic diseases the prolonged liberation of SAA may exceed the body's capacity to degrade it, resulting in deposition of AA fibrils[1-3].

Both normal human serum and the serum of amyloidotic patients contain an amyloid-degrading factor. The serum of amyloidotic patients also contains an inhibitor of the amyloid degrading factor[1-3]. Accumulation of amyloid may therefore become possible when the degrading factor is suppressed by the inhibitor. Ascorbic acid was found to neutralize the inhibitor factor, thus partially restoring the initial amyloid-degrading activity[4]. Colchicine probably acts on polymorphonuclear leukocytes to prevent the release of a filtrable factor termed the amyloid-enhancing factor (AEF) which is necessary for amyloid synthesis, and/or acts on liver cells to reduce protein synthesis, including SAA[17,18].

In the present study, we observed that ascorbic acid blocks experimental amyloidosis induced by injections of casein; this finding has also been determined in several other studies[5,7]. We also tested the value of ketotifen on the inhibition of accelerated amyloid deposition. Ketotifen is an orally-active cycloheptathiophene antihistamine, with prolonged duration of action[19]. In a number of animal models, antihistamines, including ketotifen, have been shown to block *in vitro* release of mediators from mast cells and polymorphonuclear leukocytes by antigenic and non-antigenic stimuli[9,10]. Although there is no report indicating that ketotifen blocks the release of the amyloid-enhancing factor, we have investigated the effectiveness of it in the inhibition of accelerated amyloid deposition induced by the injection of casein and we have determined that ketotifen has no such effect.

These experiments confirm previous observations concerning the effectiveness of ascorbic acid in the treatment of amyloidosis obtained from human models *in vitro* and experimentally in animal models[5,7,8,17]. Although there is a homology of human AA and murine amyloid, it is possible that the human response to high doses of ascorbic acid may be different from that of the mouse. Even so the use of ascorbic acid in amyloidotic patients to prevent the progression of their illness, might well be effective.

Summary

Amyloidosis was induced in 45 mice by 14 consecutive daily injections of casein solution. The mice were divided at random into three equal groups. The first group was the control. The second group received ascorbic acid and the third group received ketotifen in the drinking water throughout the study. On the fifteenth day there was no difference between the control and the ketotifen groups with respect to splenic amyloidosis. This observation has not previously been reported in the literature, but the protective value of vitamin C in splenic amyloidosis, shown in this study confirms the findings of previous studies.

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