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COMBINED TREATMENT WITH TERBUTALINE AND AMINOPHYLLINE INHIBITS EXPERIMENTAL AMYLOIDOSIS IN MICE

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Objective. **To investigate the effects of drugs known to elevate adenosine 3′:5′-cyclic monophosphate (cAMP) on experimental amyloidosis.**

Methods. **A β_2-agonist, terbutaline, and a phosphodiesterase inhibitor, aminophylline, were administered in combination in a mouse model of amyloidosis induced by inflammatory stimulation with silver nitrate. Amyloidosis was quantitated by radioimmunoassay for splenic amyloid A (AA) protein.**

Results. **At the doses selected, aminophylline/terbutaline inhibited splenic amyloid deposition more potently than did colchicine, a known inhibitor of amyloidosis.**

Conclusion. **Drugs known to elevate cAMP inhibit experimental mouse AA amyloidosis.**

Amyloidosis is an infiltrative disorder caused by the tissue deposition of one of a diverse group of peptides derived from serum proteins, including immunoglobulin light chains, acute-phase proteins, transthyretin, and β_2-microglobulin, and deposited in a β-pleated sheet configuration to form insoluble linear fibrils (1). Amyloidosis may occur as a complication of chronic inflammatory diseases such as rheumatoid arthritis (RA), juvenile RA, ankylosing spondylitis, and chronic infection, with deposition occurring in reticuloendothelial organs such as spleen and liver (1). This form of amyloidosis is due to the deposition of amyloid A (AA) protein, which is proteolytically cleaved by macrophage surface serine proteases (2) from an acute-phase serum precursor, SAA, an apoprotein of serum high-density lipoprotein (3).

Immunopharmacologic agents which modulate macrophage function may potentially block amyloid deposition. We previously observed that colchicine (4) and parenterally administered prostaglandin E_1 (PGE_1) (5) inhibit amyloidosis in mice. Both colchicine (6) and PGE_1 (7) increase intracellular adenosine 3′:5′-cyclic monophosphate (cAMP) levels, while colchicine also induces PGE_1 release from macrophages (8). Agents that increase cAMP levels decrease inflammatory cell activity (9). The purpose of this pilot study was to determine if other agents known to increase cAMP levels, specifically a β_2-agonist and a phosphodiesterase inhibitor, inhibit experimental amyloidosis.

MATERIALS AND METHODS

Animals. Female CBA/J mice (age 15–16 weeks, body weight 20–25 gm) were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were fed standard laboratory chow and water ad libitum.

Reagents. All solutions were prepared with endotoxin-free water (Sigma, St. Louis, MO). Silver nitrate was obtained from Fisher Scientific (Fairlawn, NJ), colchicine (1 mg/2 ml) injectable from Eli Lilly (Indianapolis, IN), aminophylline (250 mg/10 ml) injectable from Elkins-Sinn (Cherry Hill, NJ), and terbutaline sulfate (1 mg/ml) injectable from Geigy Pharmaceuticals (Ardsley, NY).

Experimental protocol. Inflammation was induced by injection of 0.2% (0.5 ml) $AgNO_3$ subcutaneously (SC) daily for 10 consecutive days, starting at day 0. All drugs were administered as loading doses 16 hours prior to the first injection of $AgNO_3$. Daily injections of test substances were given 1 hour prior to and 10 hours after injection of $AgNO_3$.

Mice (n = 8 per group) were injected SC with test substances as follows: group A 0.5 ml of water twice daily (control); group B 10 μg (0.5 ml) of colchicine 1 hour prior to $AgNO_3$ and 0.5 ml of water 10 hours after $AgNO_3$; group C

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125 μg of aminophylline and 100 ng of terbutaline (A/T) 0.5 ml twice daily. The dose of colchicine selected was the highest previously demonstrated to effectively inhibit murine amyloidosis without drug toxicity (4). The doses of A/T were pharmacologically relevant human doses adjusted for mouse body weight.

All mice were bled serially (50–100 μl) from the retroorbital venous plexus on day 0 (prior to $AgNO_3$) and days 1, 4, 7, and 10 after induction of inflammation. Sera were stored at −20°C prior to assay for SAA. All mice tolerated the procedures well and were killed after 10 days. Spleens were frozen at −20°C in phosphate buffered saline (PBS), pH 7.2, for quantitation of AA protein.

Determination of SAA and splenic AA. SAA levels (μg/ml) were measured by solid-phase radioimmunoassay (RIA), as the ability of heat-denatured sera (60°C, 1 hour) to inhibit the binding of ^{125}I-labeled AA to rabbit anti-mouse AA antibodies immobilized on polyvinyl chloride microtiter plates (10). Splenic AA concentrations were determined as previously described (4,5). Spleens were homogenized (100 mg spleen/ml PBS) in a Tekmar Tissumizer (Tekmar, Cincinnati, OH), and 25-μl aliquots were denatured in 1 ml of 10% formic acid at 37°C for 12 hours. The samples were lyophilized to remove formic acid, and reconstituted in 2% casein-barbital buffer. AA was determined by RIA and expressed as ng of AA/100 mg spleen.

Congo red–stained splenic squash specimens were examined in a blinded manner under polarized light microscopy for apple-green birefringence. Histopathologic severity was scored according to a semiquantitative assessment of the degree of amyloid deposition: 0 = no detectable amyloid; 1 = mild amyloid deposition in perivascular or interstitial regions or both; 2 = moderate amyloid deposition; and 3 = severe amyloid deposition.

Statistical analysis. SAA levels were expressed as the mean ± SEM and compared by analysis of variance using Scheffe's multiple comparison procedure (11). Splenic AA levels were expressed as the median (range) and compared by nonparametric methods, using the Kruskal-Wallis statistic for overall comparison and Mann-Whitney rank sum test with Bonferroni's correction for pairwise comparisons (11). Splenic AA levels were correlated with the histopathologic severity score for amyloid deposition using Spearman's rank sum correlation (11).

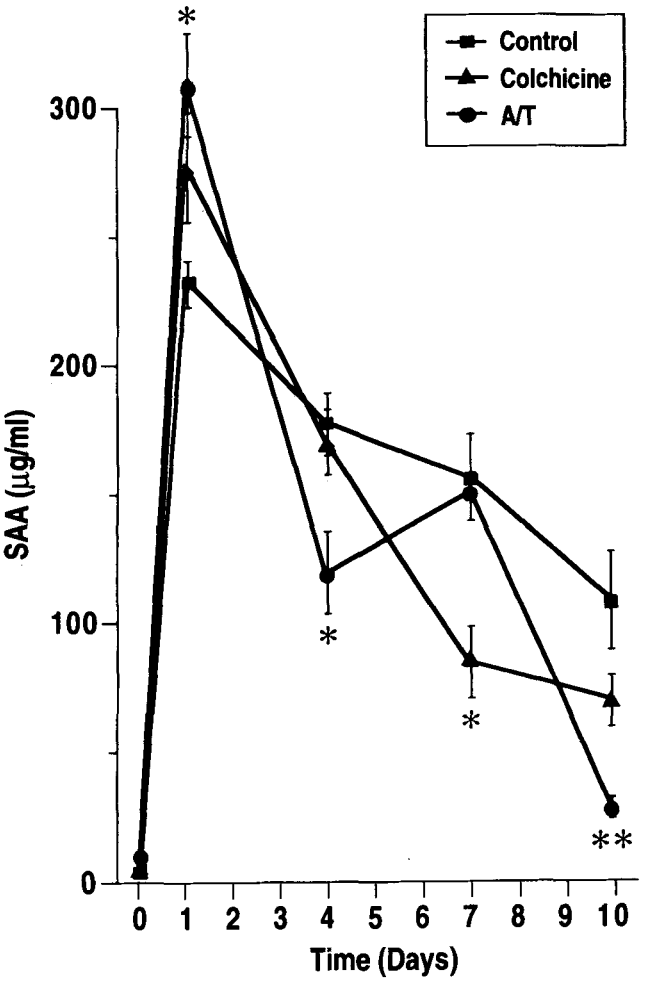

Figure 1. Effect of treatment with aminophylline plus terbutaline (A/T) compared with colchicine or water (control) on serum amyloid A (SAA) protein kinetics (μg/ml) during induction of amyloidosis with $AgNO_3$. * = $P < 0.05$ and ** = $P < 0.01$ versus control. Values are the mean ± SEM. See Materials and Methods for details.

RESULTS

Acute-phase SAA response. Injection of $AgNO_3$ on day 0 resulted in an acute-phase SAA response by 24 hours, which was not blocked by colchicine or A/T (Figure 1). Between days 1 and 10, there was a decline in SAA levels in all groups. Statistically significant differences were observed between both the A/T and colchicine groups and the control group at several points. In particular, at day 10 (when splenic amyloid was measured), A/T lowered SAA levels to 28 ± 4 μg/ml compared with 108 ± 19 in the control group ($P < 0.01$) and 69 ± 10 in the colchicine-treated group (P not significant). However, the impression is that the overall acute-phase response was similar in the 3 groups.

Splenic amyloid. The lower limit of detectability of AA by RIA was 10 ng of AA/100 mg of spleen. AA levels < 10 ng were defined as equal to 10 ng for the purpose of analysis. Although SAA and AA are both detected by polyvalent rabbit anti-mouse AA antibodies, we previously made use of the different denaturing properties of these two molecules to heat and formic acid to demonstrate that splenic sequestration of SAA is minimal and does not significantly influence splenic AA levels determined by RIA (4). This was confirmed

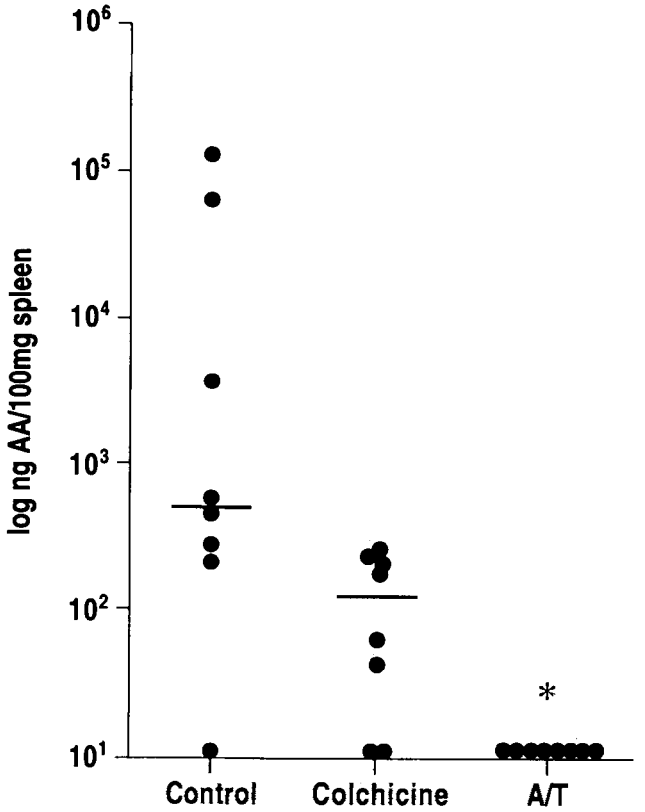

Figure 2. Effect of treatment with aminophylline plus terbutaline (A/T), colchicine, or water (control) on splenic amyloid A (AA) protein concentrations (log ng AA/100 mg spleen) induced by 10 daily injections of $AgNO_3$. * = $P < 0.05$ versus control. Horizontal bars are the medians. See Materials and Methods for details.

in the present study, in which individual mice had serum SAA levels as high as 96 μg/ml at the same time as splenic AA concentrations <10 ng/100 mg.

Control mice had a median AA concentration of 389 ng/100 mg of spleen (range 10–114,540) at day 10 (Figure 2). Colchicine lowered splenic AA to 114 ng/100 mg (range 10–210), although this did not achieve statistical significance. A/T lowered splenic AA to <10 ng/100 mg in all spleens ($P < 0.05$).

The histopathologic severity scores (mean ± SEM) for amyloid deposition were as follows: group A 2.38 ± 0.32; group B 0.38 ± 0.26; and group C 0.25 ± 0.16. There was a positive correlation between splenic AA determined by RIA and the histopathologic severity score ($r_s = 0.62$, $P < 0.002$).

DISCUSSION

This pilot study suggests that drugs known to elevate cAMP, such as aminophylline (a phosphodiesterase inhibitor) and terbutaline (a β_2-agonist), may reduce murine amyloid deposition secondary to inflammation. This is consistent with previous observations of the immunomodulatory and antiinflammatory effects of cAMP as an intracellular second messenger (9).

We used an immunoassay to quantitate precisely splenic amyloid protein. It appeared that aminophylline and terbutaline in combination were more potent than colchicine, in the doses used. These drugs were used in combination, rather than as single agents, in order to optimize the increase in cAMP levels in vivo. Since we did not quantitate intracellular cAMP in inflammatory cells and correlate these with the degree of inhibition of amyloidosis, this relationship remains speculative. This could be addressed in future studies by assaying cAMP in macrophages.

The mechanism by which cAMP might inhibit amyloidosis is uncertain. One possibility is suppression of SAA production by hepatocytes. We previously observed that dibutyryl cAMP, isoproterenol (a nonselective β_1- and β_2-agonist), and isobutylmethylxanthine block macrophage release of interleukin-1, an SAA-inducing cytokine (12). Although we observed lower SAA levels at day 10 in the A/T-treated mice than in the controls, this is an unlikely explanation for inhibition of amyloidosis, because the exuberant acute-phase SAA response in the A/T group earlier on would have provided adequate amounts of SAA for conversion to AA. A more likely explanation is that increased intracellular cAMP, which modulates the activity of inflammatory cells such as macrophages, may have interfered with the proteolytic conversion of SAA to AA and the deposition of amyloid fibrils by these cells.

This study addresses the AA model of amyloidosis. There are no comparable animal models of light chain (AL) amyloidosis in which to assess novel treatments. The latter disease in humans is associated with multiple organ failure and increased mortality (1). The processing of light chains to amyloid fibrils may also involve macrophages (13). It would be of interest to determine the effects of cAMP-elevating drugs on this in vitro model of AL amyloid formation. Finally, brain lesions in Alzheimer's disease contain vascular amyloid deposits composed of β-amyloid peptide (βA4 or Aβ), derived from amyloid precursor protein, and an acute-phase protein, α_1-antichymotrypsin, both of which are inducible by interleukin-6 (14). Moreover, a clinical response has recently been demonstrated in patients with Alzheimer's disease treated with antiinflammatory drugs (15). Hence, antiinflammatory

cAMP-elevating drugs may represent another investigational therapeutic option in this disorder, a possibility that could be addressed if transgenic animal models of Alzheimer's disease currently under development become available in the future.

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