

Univariate analyses using the 3 measures of emotional well-being obtained from TAPE patients at baseline showed that the Juniper Mini Asthma Quality of Life emotional dimension scores were well correlated with the CES-D depression scores ($r = -0.44$; $P < .0001$; $N = 480$), and that SF-36 mental score correlated even better with CES-D depression scores ($r = -0.63$; $P < .0001$; $N = 480$). The latter results were similar to those reported by Hann et al.⁸ Therefore, we are reasonably confident in our ability to use the Juniper AQLQ emotional dimension scores and the SF-36 mental component score as indicators of emotional well-being and depression in the aforementioned 3 trials.

Table 1 shows that there was no evidence of any significant deterioration of emotional well-being as measured by the Juniper AQLQ in any of the 3 trials, either in the adults or the children who received montelukast, nor were there differences among treatment groups. This was true for the adults who were followed for 2 to 24 weeks, and for the children who were followed for 4 to 16 weeks. On the contrary, we observed a positive effect of montelukast compared with the placebo group on the emotional dimension of the Mini AQLQ in the TAPE² trial after 2 weeks of treatment that did not persist. Similarly, when SF-36 mental component score was used as an index of well-being in the TAPE² trial, we observed a greater increase with montelukast after 2 weeks, and no significant difference after 4 weeks. We saw no evidence of a different effect when we limited our analysis to patients younger than age 26 years. In the 3 trials, we have no adverse event reports of psychiatric disturbances, suicide, or depressive episodes in any of the patients receiving montelukast.

These findings lead us to conclude that despite the recent publicity regarding the adverse effects of montelukast on suicide or emotional well-being, we did not find evidence to suggest that this is a general concern. We acknowledge that despite the strength of having randomized comparison groups, there are certain limitations: (1) our quality of life instruments were not directly designed to evaluate depression even though we found good correlation between our measures of well being and recognized measures of depression; (2) patients participating in clinical trials may be excluded for significant psychiatric illness; (3) our studies up to 24 weeks cannot exclude long-term effects; and (4) younger children may be more vulnerable,⁹ and our trials included a small number of children, all over the age 5 years.

In conclusion, we did not find evidence of a negative effect of montelukast on emotional well being in any of the 3 ACRC trials. Although our review of ACRC trial data is reassuring, we obviously cannot exclude the possibility of idiosyncratic reactions to montelukast.

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Administration of oral vitamin D induces cathelicidin production in atopic individuals

To the Editor:

Atopic dermatitis (AD) is a chronic skin condition that affects 10% to 20% of children and 1% to 3% of adults.¹ Individuals with atopic dermatitis are at an increased risk for cutaneous infections with *Staphylococcus aureus*, herpes simplex, and the small pox or vaccinia virus.² Recently, it has been shown that defects in the innate immune system, such as the capacity to increase the production of broad spectrum antimicrobial peptides like cathelicidin, may account for this increase in infections.^{3,4}

Important insight into the mechanism responsible for the production of antimicrobial peptides came with the discovery of the vitamin D response element in the cathelicidin promoter and the finding that the conversion of 25-hydroxyvitamin D to the active 1,25 dihydroxyvitamin D by CYP27B1 can occur in keratinocytes and monocytes and is under the control of Toll-like receptor 2.^{4,6} Thus, with infection or wounding, activation of Toll-like receptor 2 results in expression of CYP27B1, causing conversion of 25-hydroxyvitamin D to the active 1,25 dihydroxyvitamin D, and subsequent induction of cathelicidin.^{5,6} Our study sought to examine whether supplementation with oral cholecalciferol could provide the CYP27B1 enzyme enough substrate to overcome this relative deficiency in induction of cathelicidin in atopic patients.

This was a single-center, controlled study of 14 normal controls and 14 atopic subjects with moderate to severe atopic dermatitis with an average Rajka-Langeland score of 6 (range, 4-9). Two-millimeter punch biopsies of uninvolved skin were collected from all subjects, and subjects with AD also received 2-mm punch biopsies of lesional skin. Baseline calcium and 25-hydroxyvitamin D levels were obtained. Supplementation with oral vitamin D3 (cholecalciferol) at 4000 IU per day was given for 21 days. At 21 days, subjects with AD received a 2-mm punch of involved skin, and all subjects had repeat biopsies of uninvolved skin, repeat serum calcium, and vitamin D levels. The study was approved by the Human Research Protection Program at the University of California, San Diego. All subjects gave written informed consent.

Analysis of cathelicidin expression in skin was performed by qRT-PCR and normalized to GAPDH. Total RNA was isolated

from the 2-mm skin biopsy samples from the subjects by homogenization. Briefly, the biopsy samples were placed into 2.0-mL polypropylene tubes (Biospec Products, Bartlesville, Okla) with 1.0 mL Trizol (Invitrogen Corp, Carlsbad, Calif). Zirconia/Silica beads (Biospec Products) were added to the tubes, and the skin samples were homogenized by using a Mini-beadbeater-8 (Biospec Products). Homogenized Trizol solution was transferred to a ribonuclease-free tube for RNA extraction according to the manufacturer's instructions, and RNA was reversed-transcribed by using iScript (Bio-Rad, Munchen, Germany). Real-time, quantitative PCR assay for cathelicidin was performed with the ABI 7000 Sequence Detection system (PE Applied Biosystems, Foster City, Calif). Expression of cathelicidin was evaluated by using a FAM-CAGAGGATTGTGACTTCA-MGB probe with primers 5'-CTTCACCAGCCCGTCCTTC-3' and 5'-CCAGGACGACA CAGCAGTCA-3'. For GAPDH expression, a VIC-CATCCATG ACAACTTTGGTA-MGB probe with primers 5'-CTTAGCACC CCTGGCCAAG-3' and 5'-TGGTCATGAGTCCTTCCACG-3' was used. Fold induction was calculated by using the 2-delta CT method, where delta CT is CT cathelicidin – CT GAPDH and CT is the cycle at which arbitrary detection threshold is crossed. The 1-way ANOVA test from the GraphPad Prism statistical package (La Jolla, Calif) was used to determine significant differences. We constrained error rates to the standard α level of 0.05 by the Newman-Keuls method.

After supplementation with 4000 IU/d oral vitamin D for 21 days, AD lesional skin showed a statistically significant increase in cathelicidin expression from a median of 3.53 relative copy units (RCU) before supplementation to a median of 23.91 RCU postsupplementation ($P < .01$; Fig 1). Normal skin showed a more modest increase from a median of 1.0 RCU to 1.78 RCU ($P > .05$), and AD nonlesional skin, mildly increased from a median value of 1.50 RCU to 1.75 RCU postsupplementation ($P > .05$). These results suggest that supplementation with oral vitamin D dramatically induces cathelicidin production in AD lesional skin and may also induce production in normal skin. Immunofluorescence staining of duplicate skin biopsies from lesional skin confirmed results from RT-PCR and demonstrated an increase in cathelicidin protein after oral vitamin D supplementation.

Serum calcium and vitamin D levels were measured in atopic and normal subjects at baseline and after 3 weeks of supplementation with 4000 IU/d of oral vitamin D₃. 4000 IU of vitamin D₃ has been well documented to effectively increase 25-hydroxyvitamin D levels⁷ while maintaining normal serum calcium levels, suggesting that the current recommendation for vitamin D supplementation may be too conservative. Indeed, serum calcium in our normal control subjects did not rise but actually decreased from a median of 9.8 mg/dL to a median of 9.4 mg/dL postsupplementation ($P > .05$). During this time, their serum 25-hydroxyvitamin D levels rose from a median of 24.5 mg/mL to 37 mg/mL ($P > .05$). Similarly, atopic subjects' serum calcium levels also decreased from a median of 9.6 mg/dL to a median of 9.4 mg/dL postsupplementation ($P > .05$), and their serum 25-hydroxyvitamin D levels rose from a median of 22.5 mg/mL to 35.5 mg/mL ($P > .05$). No subject complained of any adverse event during supplementation.

Analysis of previous data has shown that with wounding or disruption of the epidermal barrier, cathelicidin is induced.⁶ Our data show that there is a small (but not statistically significant) increase in cathelicidin in AD lesional skin before treatment with vitamin D (Fig 1) indicative of some ability of the atopic

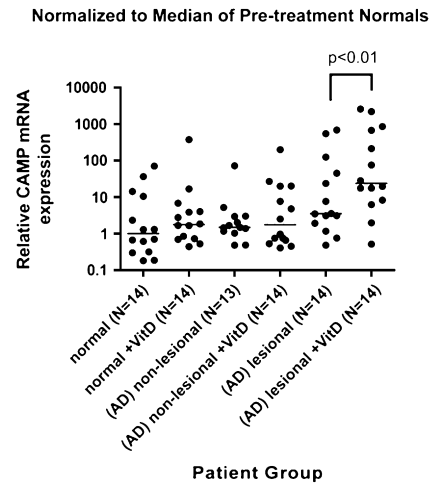


FIG 1. Increase in cathelicidin expression in lesional atopic skin. Cathelicidin mRNA measured by qRT-PCR in lesional and nonlesional skin of atopic subjects and nonlesional skin of normal subjects. Atopic involved skin showed significantly higher hCAP18 mRNA expression with vitamin D supplementation compared with normal skin and AD nonlesional skin ($P < .01$ by 1-way ANOVA). Data are shown as relative expression as normalized by the median of pretreatment controls (bars, median of each group).

subject to induce cathelicidin with disruption of the epidermal barrier. This small increase is aided immensely with oral vitamin D₃, allowing the AD lesional skin value to rise significantly. Because this increase is primarily seen in lesional skin, it is hypothesized that only with the supplementation with oral vitamin D can AD lesional skin be allowed to increase cathelicidin to its normal level seen postinjury. The smaller induction of normal skin and nonlesional skin suggests that these are already in their normal uninduced state (nonwounded and noninfected).

Vitamin D deficiency has been linked to increased rates of multiple cancers, autoimmune diseases, infectious diseases, cardiovascular diseases, and hypertension.⁸ We believe our study has shown that supplementation of oral vitamin D can result in correction of defects in cathelicidins in the innate immune system of atopic subjects. Larger studies examining the incidence and risks of infections in atopic subjects while on vitamin D supplementation, and supplementation for a longer duration, will be necessary in the future to see if this increase in cathelicidin is adequate in the prevention of infections in these patients.

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Usefulness of induced sputum in investigating occupational asthma with normal responsiveness to methacholine: A case report

To the Editor:

Current international guidelines suggest stopping investigations of occupational asthma (OA) in the case of normal nonspecific bronchial hyperresponsiveness (NSBH) in a subject currently exposed to the suspected agent(s) in the workplace.¹ Noninvasive markers of inflammation have been increasingly used in the diagnosis and management of obstructive airway diseases over the past 15 years. Induced sputum (IS) analysis is a noninvasive method for studying airway inflammation. The monitoring of sputum eosinophil counts has been shown to be useful in the investigation of OA.^{2,3}

We report the case of a 33-year-old man, an exsmoker, who was referred to our allergy unit for evaluation of work-related respiratory symptoms. He had been working as a stock handler in a food industry for 12 years. Six months before our investigations, he started a job as a technician in an electric industry. Dyspnea, productive cough, chest tightness, and wheezing appeared a few days after starting this work, with significant improvement during weekends and holidays. Respiratory symptoms appeared a few hours after starting work, persisting at home during the evening. The worksheets showed exposure to cyanoacrylate through the inhalation route at the workplace, without exposure to any other known sensitizer.

The patient underwent the common diagnostic pathway of OA.¹ Skin prick test responses to common aeroallergens were positive for house dust mites, several pollens, latex, and cat's epithelium (although he did not own a cat). These findings were confirmed based on specific IgE count. The result of a reversibility test with albuterol was negative. Peak expiratory flow (PEF) monitoring and methacholine (Mch) challenge during periods at work and off work were in the normal range.

According to current diagnostic algorithms for the diagnosis of OA,¹ investigations should have been stopped at this step (normal Mch assessment at work), but because of the suggestive occupational history, the patient was submitted to IS challenge.³ IS was carried out 2 days after the last exposure at the workplace and showed bronchial eosinophilia (27.7%; normal value, <2%).² Therefore we performed a specific inhalation challenge (SIC) with cyanoacrylate. The SIC was carried out 3 months later, when the patient had not been exposed at the workplace for 2 months. On day 1, the patient was exposed to isobutyl acetate (control day). Twenty-four hours later, an IS test was carried out, showing 11.7% eosinophils. On day 3, SIC with cyanoacrylate was carried out by using the occupational method.^{4,5}

The patient was exposed to cyanoacrylate by painting a paper surface with the cyanoacrylate glue (occupational-like method). A total of 8 mg of cyanoacrylate was used, which, in an inhalation chamber of 7.46 m³, corresponded to the time-weighted average limit (1 mg/m³). The subject was exposed in the inhalation chamber for 30 minutes.

Both on the control day and during specific exposure, spirometric and PEF results were measured before and 5, 15, 30, and 60 minutes after the end of exposure, then hourly for 7 hours and after 24 hours (Fig 1). A late response with a maximum 16% FEV₁ decrease at 300 minutes and a 25% PEF decrease 10 hours after the end of exposure was registered. At the same time points, the patient reported respiratory symptoms (dyspnea, productive cough, chest tightness, and wheezing). The day after SIC, the results of Mch challenge were negative (FEV₁ PD₂₀ >2376 µg before and after SIC), whereas IS showed an increase in total cellular count (2640 cells/mg after SIC vs 1320 cells/mg before SIC) and eosinophilia (54.5%), as shown in Fig 2. The serum eosinophil cationic protein level was 26.4 ng/mL before and 30.7 ng/mL after the specific challenge.

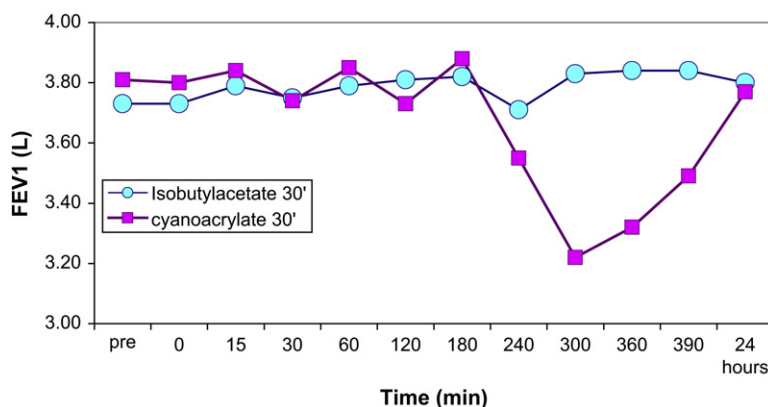


FIG 1. Time course of FEV₁ (in liters) during SICs.