

A randomized, single-blind and crossover study of an amino acid-based milk formula in treating young children with atopic dermatitis

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Cow's milk and soy protein allergies are commonly associated with atopic dermatitis (AD) in young children. Amino acid (AA)-based elemental milk formula may improve AD control in these patients. This study investigates the efficacy of AA-based formula in treating young AD patients irrespective of their food allergy status. AD patients younger than 3 yr old were eligible. Sensitization to food allergens was ascertained by skin prick tests and allergen-specific immunoglobulin E (IgE) assay. Patients were then randomly allocated to take either active treatment or pre-existing formulae (placebo) for 6 wk. They were allowed a 6-wk washout period before crossed over to the other intervention for another 6 wk. Fifteen AD patients, with median (interquartile range, IQR) age of 1.4 (0.6–2.6) yr, were recruited. Their median (IQR) SCORAD score was 23.9 (10.5–29.7). Seven of them were sensitized to cow's milk or soybean. Among 11 patients who completed the study, the median changes for all scores and urinary eosinophil protein X (EPX) concentration were not statistically significant. There was also no evidence of carry-over effects for SCORAD and its various components and global health score, except for urinary EPX concentration ($p = 0.05$). Our results do not support the use of AA-based elemental milk formula in treating young children with AD irrespective of their food allergy status.

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Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease commonly associated with food and inhalant allergies (1). Ninety percentage of AD patients presents before 5 yr of age. About one-third of children with moderate-to-severe AD have immunoglobulin E (IgE)-mediated clinical reactivity to food proteins (2). Cow's milk and soy protein are among the most common food allergens in infants and young children. The two conditions may also coexist, and 14% of 93 children aged <3.5 yr with cow's milk allergy also developed allergic reaction to soy (3). Extensively hydrolyzed or amino acid (AA)-derived formulae are efficacious and safe in treating infants with cow's milk allergy with or without AD (4, 5). Apart from the avoidance of

incriminated foods, these formulae may exert immunomodulatory effects either directly on AD itself (6, 7) or by altering the intestinal microflora (8). The use of extensively hydrolyzed whey formula in AD infants was associated with a reduction of faecal tumour necrosis factor- α levels, indicating an improvement in intestinal inflammation. The addition of *Lactobacillus* GG further lowered serum and faecal markers of allergic inflammation (7). Cow's milk avoidance in sensitized patients also reduced proinflammatory cytokine production from their peripheral blood mononuclear cells (9). The objective of this study is to investigate the therapeutic efficacy of an AA-based elemental milk formula in treating AD in infants and young children.

Materials and methods

Study population

This was a randomized, single-blind, placebo-controlled and crossover clinical trial to study the usefulness of a hypoallergenic, milk-free, lactose- and sucrose-free, complete elemental formula with free AA (Neocate, SHS International, Liverpool, UK) (4, 5, 10) in treating Chinese children with AD. This study recruited AD patients, younger than 3 yr of age, from paediatric allergy and dermatology clinics of our hospital. AD is diagnosed according to Hanifin criteria (11), whereas the severity of AD was assessed by SCORAD (12). The responsible dietitian (LTFC) conducted detailed nutritional assessment of our subjects at baseline, and all participants had to take ≥ 500 ml cow's milk- or soy-based formulae in order to be eligible. Parents of these patients gave informed written consent, and Clinical Research Ethics Committee of our University approved this study.

Evaluation for allergen sensitization

Skin prick tests (SPT) with six food allergens (cow's milk, soy, whole egg, peanut, wheat and mixed fish) and *Dermatophagoides pteronyssinus* (Hollister-Stier, Spokane, WA, USA) were performed at baseline. Any wheal ≥ 3 mm larger than that of negative control was considered positive. Besides, serum concentrations of cow's milk- and soybean-specific IgE were measured by fluorescent enzyme immunoassay (AutoCAP analyzer; Pharmacia, Uppsala, Sweden). Specific IgE ≥ 0.35 kAU/l was considered positive. Total IgE in serum was measured by microparticle immunoassay (IMx analyzer, Abbott Laboratories, Abbott Park, IL, USA).

Study design and protocol

Following a 4-wk run-in, subjects were randomly allocated to either active treatment (Neocate) or placebo (patients' pre-existing formulae) for 6 wk. Subjects were allowed a 6-wk washout period (pre-existing formulae) after this phase before they crossed over to the other intervention for another 6 wk. The study dietitian not involved in AD assessment distributed Neocate to patients' parents during their respective active treatment phase. They were also advised not to give any dairy or soy-based product to patients throughout the study. All drugs including topical corticosteroid (mometasone furoate for all patients as needed) and oral sedating antihistamines remained unchanged. None of the patients

required oral steroid or antibiotic during this study. Before each visit, these caregivers were required to fill out a 3-day food record. The dietitian then calculated fluid and caloric intake on these days by computerized nutrient analysis.

Clinical and laboratory outcomes

The paediatric dermatologist (KCM) unaware of patients' interventions (i.e. single blinding) evaluated AD severity using SCORAD before and after each phase. Patients' caregivers also rated the global health score in the form of a visual analogue scale from '1' (worst) to '9' (best). Spot urine samples were collected between 08:00 and 11:00 for eosinophil protein X (EPX) measurement (EPX RIA; Pharmacia), which was expressed as a quotient to creatinine (Cr). The sensitivity of this EPX assay was 3 μ g/l. The laboratory staff in this study was unaware of the clinical status of our subjects.

Statistical analysis

Results were expressed in median and IQR. Tsai and Patel's method for analysing a two-period crossover design with baseline measurements was used to compare the clinical scores obtained during active and placebo treatments (13). This non-parametric method provides a robust analysis of a 2×2 crossover design with baseline measurements of responses prior to both periods. Using baseline measurements as covariates, the locally weighted robust regression (LOWESS) (14) was applied to fit a regression line of the treatment response on the baseline measurement for each period separately. Hodges-Lehmann procedures were then used to estimate the treatment $\times$ period interaction and treatment difference for each clinical score. Wilcoxon rank sum test was used to compare the first and second baselines to test the carry-over effect of the two sequences. S-PLUS 2000 for Windows was used to conduct the analyses. The level of significance was set at 5% in all comparisons, and all statistical testing was two-sided.

Results

Patient characteristics

Fifteen patients (median age 1.4 yr, IQR: 0.6–2.6 yr; eight male, seven female) were recruited. Their median (IQR) SCORAD was 23.9 (10.5–29.7). The median (IQR) baseline urinary EPX concentration was 72 (35–130) μ g/mmol Cr, and this marker did not differ between patients with

mild and moderate AD (median: 96 µg/mmol Cr vs. 65 µg/mmol Cr; $p = 0.814$).

The median (IQR) serum log-transformed total IgE concentration was 1.73 (1.27–2.43) kIU/l. The allergenic skin prick test results are listed in Table 1. Five patients had cow's milk-specific IgE and five patients had soybean-specific IgE in serum. Overall, seven (47%; three with both and four with either SPT or serum IgE) patients were sensitized to cow's milk or soybean.

Efficacy of Neocate on AD severity

Eleven (73%) of the 15 recruited patients completed the 5-month study. Four dropout cases were excluded from the analysis. The reasons for dropout included: two for 'drank less than daily milk requirement', one for 'refused to drink Neocate' and one for 'discontinued because of too mild AD'. In the remaining subjects, daily intake of fluid ($p = 0.563$ for active treatment and $p = 0.449$ for placebo) or energy ($p = 0.477$ for active treatment and $p = 0.814$ for placebo) did not differ before and after interventions.

The results on treatment $\times$ period interaction and treatment difference of study outcomes are shown in Table 2. The median changes for SCORAD and its area, intensity, pruritus and sleep loss components, as well as global health score and urinary EPX levels were not statistically significant during the active or placebo phase. Significant treatment $\times$ period interaction effect was found for SCORAD and its intensity and pruritus components. On the contrary, carry-over effect was not observed ($p = 0.689$, 0.388, 0.456, 0.689, 0.388 and 0.147; Wilcoxon rank sum test) for SCORAD and its area, intensity, pruritus and sleep loss components and global health score respectively, except for urinary EPX concentration ($p = 0.05$). The results remained insignificant when analyses were repeated for the seven AD patients with coexisting sensitization to cow's milk or soybean (data not shown).

Table 1. Skin prick test results to purified allergen extracts

Allergens	Number of positive (%)
Cow's milk	1 (7)
Soybean	1 (7)
Whole egg	11 (73)
Wheat	1 (7)
Mixed fishes	1 (7)
Mixed peanuts	4 (27)
<i>Dermatophagoides pteronyssinus</i>	6 (40)

Table 2. Results of treatment $\times$ period interaction and treatment difference using Hodges-Lehmann's procedures

Outcomes	Treatment $\times$ period interaction		Treatment difference	
	Estimates*	p-Value†	Estimates*	p-Value†
Total SCORAD score	7.23	0.012	3.97	0.274
SCORAD components				
Area	1.09	0.955	2.62	0.689
Intensity	0.32	0.036	0	0.829
Pruritus	1.78	0.036	2.07	0.173
Sleep loss	1.17	0.328	−0.19	0.864
Global health score	0.01	0.958	0	0.792
Urinary EPX	13.5	0.607	−17.6	0.328‡

*Negative value indicates decrease in median score.

†By Wilcoxon rank sum test.

‡Analysed only for results between active treatment and placebo in the first intervention phase because of significant carry-over effect.

Discussion

A recent study suggested that a 6-month treatment with either AA-based or extensively hydrolysed formula resulted in more than 50% improvement in SCORAD in AD patients with coexisting cow's milk allergy (5). Nonetheless, the authors did not study any mechanistic link between these formulae and the immunopathology of AD. In the present randomized, crossover study, we could not detect any improvement in clinical or laboratory marker of AD severity when these young AD patients were treated with a 6-wk course of Neocate. About half of our patients were sensitized to cow's milk or soybean. We did not intentionally include any criteria on coexisting food allergy in order to investigate whether the possible benefits observed in the recent study (5) is due to avoidance of incriminated foods. DBPCFC is time-consuming, tedious and potentially anaphylactogenic. Thus, the present study did not try to diagnose food allergy in our subjects using this approach. Instead, we provide efficacy data on the elemental formula in treating AD irrespective of the food allergy status of the patients. However, this study could not eliminate the possibility that this formula might cause any subclinical improvement in AD pathology in our recruited subjects.

The elemental AA-based formula did not improve urinary EPX levels in our patients. Pucci et al. reported that urinary concentrations of EPX were elevated in 54 AD children younger than 4 yr of age, and this marker also showed significant correlation with disease activity (15). These findings prompted us to include urinary EPX as one of the outcomes. Nonetheless, urinary EPX did not differ between our patients

with mild and moderate AD, or show any correlation with their total SCORAD or its components. This difference is probably due to smaller sample size in this study. Thus, we cannot confidently conclude whether AA-based formula had any immunomodulatory effect on eosinophil-related activity in AD patients. As AD is a heterogeneous disease, other inflammatory markers (e.g. chemokines, cytokines, leukotrienes) may be included in any future study to better delineate the efficacy of AA-based formula in treating AD (16, 17).

This study has several limitations. The first, and probably the most important, limitation is the small number of recruited subjects. However, we tried to minimize this by using the crossover study design. As shown in Table 2, the treatment difference associated with AA-based formula was only 4 when compared with placebo whereas the median SCORAD in our patients was 24. Besides, none of the outcomes reached even any borderline significance. It is thus unlikely that our active milk formula brings about any clinically significant improvement in AD severity. The crossover study design is another drawback. It is possible that confounders such as seasonal changes in temperature, humidity or environmental exposure to allergens and pollution may influence AD severity in subjects who received active formula treatment at different time-points. Indeed, our group has shown significant treatment $\times$ period interactions for SCORAD and its intensity and pruritus components. This finding may explain in part the difference in treatment efficacy of Neocate when compared with the previous report (5).

In conclusion, a 6-wk treatment with an AA-based milk formula did not improve clinical severity or urinary EPX concentration in infants and young children with AD irrespective of their coexisting food allergy status.

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