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Original article

Prebiotics and synbiotics: two promising approaches for the treatment of atopic dermatitis in children above 2 years

Background: Appropriate use of prebiotics and optimal combinations of probiotics and prebiotics (synbiotics) could allow significantly better results to be obtained in the treatment of atopic dermatitis (AD).
Objective: To evaluate the efficiency of synbiotics when compared with prebiotics alone (control group) in the treatment of moderate and severe AD in children aged 2 years and over.
Methods: Double-blind prospective randomized study performed on children aged at least 2 years presenting AD with a minimum SCORing Atopic Dermatitis (SCORAD) score of 15. A dose of 1.2×10^9 colony-forming units *Lactobacillus rhamnosus* Lcr35 plus prebiotic preparation or an identically appearing prebiotic preparation alone was given three times a day for 3 months. Patients' diet and usual treatment for AD remained unchanged during the study period. Efficiency was evaluated using the SCORAD score. Use of topical drugs was noted.
Results: A total of 48 patients were originally enrolled; nine did not complete the study. In synbiotic group, the mean values of the total SCORAD score was 39.1 before treatment *vs* 20.7 after 3 months of treatment ($P < 0.0001$). In the prebiotic group, the mean of the total SCORAD score was 39.3 before the treatment *vs* 24.0 after 3 months ($P < 0.0001$). After 3 months of treatment, no statistical differences between the two treatment groups with regard to the total SCORAD score were noted ($P = 0.535$). Neither were there any statistical differences in the total use of ointment between patients receiving prebiotics or synbiotics ($P = 0.966$) over the study period. Tolerance was excellent in both groups.
Conclusions: Both synbiotics and prebiotics used alone seem able to significantly improve the manifestations of AD in children aged 2 years and over.

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Probiotics are defined as viable micro-organisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance (1). On the contrary, prebiotics are indigestible substances that beneficially affect the host by selectively stimulating the growth and/or the activity of a limited number of bacterial strains already established in the gut flora. The low viability and survival rates of probiotics remain a problem for their therapeutic use. Appropriate prebiotics and optimal combinations of probiotics and prebiotics (synbiotics) could allow significantly better efficiency to be obtained. Such a combination presents obvious theoretical advantages for the treatment of atopic manifestations, especially atopic dermatitis (AD). To date, no data are available on the use of prebiotics or synbiotics in treating AD.

AD is a chronic disorder affecting 10–25% of children in western countries (2, 3). The management of AD in childhood is challenging. Daily applications of emollients are necessary. The administration of topical corticosteroids or immunosuppressive ointments allows controlling flares, especially in children with mild and moderate eczema. However, relapses are common, chronicity can alter the quality of life of both the patient and his family, and prolonged use of the treatments can induce side-effects.

In AD, a dysregulation of cellular immunity leads to an imbalance of the T-helper lymphocyte (Th) ratio (Th1/Th2) in favour of Th2. The steady increase in the incidence of AD in developed countries might be linked to hygiene improvements whereby children might be less exposed to infectious agents during the first months or years of life and might therefore develop a predominant Th2 response. The steady increase in the incidence of AD in developed countries might be linked to hygiene improvements whereby children might be less exposed

to infectious agents during the first months or years of life and might therefore develop a predominant Th2 response (4–7). To date, there is no treatment able to correct this. Probiotics are live, commensal micro-organisms of the digestive tract, which may have antiallergic properties while promoting Th1 response (8, 9). Not all probiotics have the same immunological properties, however. In a recent study performed on mouse dendritic cells, *Lactobacillus rhamnosus* appeared significantly to induce the production of cytokines promoting Th1 response [i.e. interleukin (IL)-12, IL-6, tumour necrosis factor (TNF)-α] without increasing the production of IL-10 (which promotes the Th2 response) (10). This strain would therefore be of interest in treating AD. Accordingly, it has been shown that *L. rhamnosus* GG was effective in the primary prevention of AD (11, 12) and, to a lesser extent, in the treatment of young infants (13–15). These studies were conduced in very young children and the efficacy of the treatment might be due to the initial colonization of intestinal microflora. However, immunomodulatory effects of probiotics are also observed in children and adults, and thus could be efficient in these age groups (16). To date, only one study has evaluated the use of probiotics for 6 weeks only in children aged 12 months and over (17). Objective assessment suggests no improvement of probiotic over placebo, but results suggested that probiotics could be somewhat beneficial in the treatment of AD.

The purpose of this study was to evaluate the efficiency of synbiotics (probiotic *L. rhamnosus* Lcr35 plus prebiotics) when compared with prebiotics alone as a control, in the treatment of moderate and severe AD in children above 2 years.

Methods

Patients and study design

The study was performed over the period from February 2003 to June 2004. Patients aged between 2 and 12 years presenting with AD at the Department of Dermatology (Archet 2 Hospital, Nice, France) were consecutively enrolled after written consent had been obtained from the parents. The diagnosis of AD was made according to standardized criteria (18). Only patients with a total SCORAD score above 14 were included. The patients were chronically followed by our department and all were included at distance of a flare of their AD. The patients were randomized in a double-blind design and received either prebiotics alone or synbiotics. Randomization was performed before the onset of the study according to severity of AD (total SCORAD score $<$ or ≥ 40) and season (winter, spring, summer, fall). Noninclusion criteria were: congenital or acquired immunosuppression and administration of systemic corticosteroids or other immunosuppressive drugs in the previous 3 months. The bacterial preparation contained *L. rhamnosus* Lcr35 and was manufactured by Lyocentre© laboratory (Aurillac, France). Each dose (weight: 1.5 g) was stored in airtight alu-bags. The synbiotic doses contained 1.2×10^9 colony-forming units of *L. rhamnosus* Lcr35 + prebiotic-specific preparation and metabolites secreted by the bacterium. The prebiotic preparation was derived from the fermentation broth for *L. rhamnosus* Lcr35, and contained skimmed milk powder (0.344 g – bovine protein 33%, lactose 52%), potato starch (0.759 g) and lactose (0.397 g). Doses of the synbiotic or prebiotic preparations were given three times a day for 3 months. Patients were asked to dissolve the preparation in water or any cold liquid preferred by the patient. None of the patients changed diet during the study period.

During the study period, the patient's usual treatments for AD remained unchanged; all of the patients applied emollients and almost all used topical corticosteroids or topical tacrolimus for treating the flares. A quantitative estimate of the use of topical corticosteroids or topical tacrolimus was made at each visit by weighing the medication tubes.

The study protocol was approved by the local committee for person protection (Comité Consultatif de Protection des Personnes se prêtant à des Recherches Biomédicales de Nice, France).

Evaluation

The patients, their parents and the dermatologist were blinded to the treatment the patient was receiving. Clinical evaluations were performed before starting the treatment and at one (M1), two (M2) and three (M3) months. Each patient was examined by the same dermatologist at each visit. The standardized SCORAD scoring system (SCORing Atopic Dermatitis) developed by the European Task Force for Atopic Dermatitis, was used to evaluate patients (19). The SCORAD score combines the clinical evaluation of intensity and extent of the eczema with a subjective itch and insomnia score indicated by the patient, parents, or both on a visual analogue scale. The objective SCORAD ranges from 0 to 83. When pruritus and insomnia are added, the total SCORAD score can extend to a maximum of 103. At the initial visit, patients were classified into moderate (SCORAD score: 16–39), and severe (SCORAD score: ≥ 40) groups. At each visit, objective and total SCORAD scores were calculated. The Investigator's Global Assessment (IGA; a 6-category scale including the following categories: 0, clear; 1, almost clear; 2, mild; 3, moderate; 4, severe; 5, very severe) was also evaluated at each visit. The number of flares and use of topical steroids or tacrolimus during the previous month were noted along with possible side-effects. At the final visit, the patients' and/or parents' opinions on the efficacy of the treatment (AD worse, unchanged, improved) and tolerance were recorded. Finally, all the parents were called 6–12 months after the end of the study to determine if they observed a relapse of the disease after the interruption of the treatment.

Statistical analysis

The sample size of 22 per group was calculated to provide 80% power with a two-sided test for detecting a difference of 15 (assuming a standard deviation of 16) for the total mean SCORAD score between the two groups, with a type I error of 5%, when taking into account a proportion of patients lost to follow up of 20%.

Potential differences because of gender, other atopic diseases and use of topical treatments between the two groups were analysed using a chi-square test (or Fisher's exact test as appropriate). Baseline age, number of flares per months, use of topical treatments, total SCORAD score and objective SCORAD score for the two groups were compared using the *t*-test (or Kruskal–Wallis test for nonparametric data).

At the end of the study period, the changes in objective and total SCORAD scores from baseline within each treatment group were analysed using a paired *t*-test; objective and total SCORAD scores between the two groups at M3 were compared using a *t*-test for

independent samples. This analysis was also conducted in the subgroup of patients presenting with other atopic manifestations. Only the patients who completed study (primary outcome and total SCORAD score available at M3) were included in these analyses. However, the analyses were repeated on the whole study population after replacing the missing SCORAD scores at M3 with the mean of the SCORAD scores in each treatment group based on available data at M3. The numbers of patients in each treatment groups achieving a decrease in the SCORAD scores ≥50% and ≥90% from baseline to M3 were also analysed using a Fisher's exact test. The IGA scores were similarly analysed by using tests of Wilcoxon and Mann–Whitney. Finally, the total numbers of flares and the quantities of topical treatments used during the study period were compared both between the groups and for the month before the study and for the last month of the study within each group (Kruskal–Wallis test and Friedman test for paired samples). Statistical analyses were performed using the commercially available SPSS for Windows program (version 11.0; SPSS Inc., Chicago, IL, USA).

Results

Study population

Among the 48 patients included, nine (synbiotic group, $n = 7$; prebiotic group, $n = 2$; $P = 0.13$) did not complete the study. The reasons for exclusion were nonattendance to scheduled visits – (synbiotic group, $n = 5$; prebiotic group, $n = 2$) – and withdrawal of former informed consent (synbiotic group, $n = 2$). Thirty-nine patients (mean age 5.82 years, mean total SCORAD score = 39.7) completed the study. The characteristics of the study population are summarized in Table 1. No statistical differences were noted between the two groups based on age, presence of other atopic manifestations, number of monthly flares, use of topical steroids or topical tacrolimus and objective and total SCORAD scores. The mean baseline objective SCORAD score was 29.1 (synbiotic group = 28.7 *vs* prebiotic group = 29.4), and the mean baseline total SCORAD score was 39.2 (synbiotic group = 39.1 *vs* prebiotic group = 39.3).

Clinical effects

The evolution of the objective and total SCORAD scores in both groups is set out in Tables 2 and 3, respectively. In the synbiotics group, the total SCORAD score mean was 39.1 before treatment ($n = 24$) *vs* 20.7 after 3 months of treatment ($n = 17$; $P < 0.0001$, based on the 17 complete observations). In the prebiotics group, the mean total

Table 1. Characteristics of the study population (total and in each treatment group)

	Total	Synbiotics	Prebiotics	*P*-value
Number of patients	48 (100.0)	24 (100.0)	24 (100.0)	
Age [years; mean (range)]	5.85 (2–12)	5.38 (2–11)	6.33 (2–12)	NS
Other atopic diseases	25 (52)	12 (50)	13 (54)	NS
Asthma	17 (68)	9 (75)	8 (62)	NS
Allergic rhinitis	19 (76)	8 (67)	11 (85)	NS
Allergic conjunctivitis	11 (44)	5 (42)	6 (46)	NS
Flares [months; mean (range)]	7.5 (0–30)	8.83 (0–30)	6.13 (1–30)	NS
Treatment				
Topical steroids	34 (71)	16 (67)	18 (75)	
Class 2	29 (60)	15 (63)	14 (58)	
Class 3	5 (11)	1 (4)	4 (17)	
Tacrolimus 0.03%	11 (23)	6 (25)	5 (21)	
Emollients alone	3 (6)	2 (8)	1 (4)	
Monthly usage [g; mean (range)]	1.29 (0–5)	1.25 (0–4)	1.32 (0–5)	NS
SCORAD objective baseline [median (IQR)]	29.0 (19.9–37.4)	28.7 (16.1–36.6)	29.0 (21.6–37.7)	NS
SCORAD total baseline [median (IQR)]	39.7 (27.6–46.2)	39.7 (26.5–46.1)	39.2 (30.6–48.1)	NS

Values are *n* (%) except where stated otherwise.
SCORAD, SCORing Atopic Dermatitis; IQR, interquartile range.

Table 2. Evolution of objective SCORAD score

Objective SCORAD score	Baseline	1 month	2 months	3 months
Synbiotic group	$n = 24$	$n = 20$	$n = 17$	$n = 17$
Mean (95% CI)	29 (22.9–34.5)	26 (20.2–32.1)	19 (13.0–25.4)	16 (8.9–23.8)
Median (IQR)	29 (16.1–36.6)	28 (15.7–37.2)	22 (7.5–25.7)	8 (7.2–32.2)
Prebiotic group	$n = 24$	$n = 23$	$n = 22$	$n = 22$
Mean (95% CI)	29 (25.3–33.6)	25 (18.5–31.1)	21 (16.0–25.2)	20 (14.2–25.7)
Median (IQR)	29 (21.6–37.7)	19 (14.5–38.0)	19 (13.5–27.4)	17 (10.1–30.2)

SCORAD, SCORing Atopic Dermatitis; IQR, interquartile range; CI, confidence interval.

Table 3. Evolution of total SCORAD score

Total SCORAD score	Baseline	1 month	2 months	3 months
Synbiotic group	$n = 24$	$n = 20$	$n = 17$	$n = 17$
Mean (95% CI)	39 (31.9–46.4)	35 (28.5–42.5)	26 (18.1–34.0)	21 (11.7–29.8)
Median (IQR)	40 (26.5–46.1)	37 (23.2–48.6)	30 (11.5–35.5)	14 (8.0–39.5)
Prebiotic group	$n = 24$	$n = 23$	$n = 22$	$n = 22$
Mean (95% CI)	39 (34.7–43.9)	32 (24.6–39.2)	26 (20.8–31.6)	24 (17.3–30.7)
Median (IQR)	39 (30.6–48.1)	26 (19.0–46.5)	26 (18.4–32.5)	21 (13.4–36.4)

SCORAD, SCORing Atopic Dermatitis; IQR, interquartile range; CI, confidence interval.

SCORAD score was 39.3 before treatment ($n = 24$) *vs* 24.0 after 3 months ($n = 22$; $P < 0.0001$). No statistical differences between the two groups of treatment could be noted with the objective SCORAD score and total SCORAD score after 3 months of treatment ($P = 0.418$ and $P = 0.535$, respectively). These results were consistent with those obtained in the analyses performed after replacement of missing values for total and objective SCORAD at M3 (data not shown). When the number of patients who reached at least 50% and 90% improvement were compared, the two groups again showed no statistical difference ($P = 0.408$ and $P = 0.184$ respectively). Moreover, no differences between the two groups were observed when comparing the total numbers of flares during the entire study or the mean numbers of flares in the month before the study and M3.

As treatment response was more pronounced in allergic patients compared with nonallergic patients in the first study performed in children aged over 1 year (17), we analysed our results according to the presence, or not, of other atopic manifestations. Twenty-five patients presented with other atopic manifestations (13 in prebiotic group and 12 in synbiotic group). At baseline, the mean total SCORAD score was 41.7 ± 11.4 in the prebiotics group *vs* 49.6 ± 16.8 in the synbiotics group ($P = 0.181$). Both groups showed a significant decrease in total SCORAD score after 3 months of treatment but no statistical difference was observed between the two groups.

The evolution of IGA scores was similar to SCORAD scores. In the synbiotics group, the mean IGA score was 3.33 (IC: 2.97–3.70) before treatment ($n = 24$) *vs* 2.41 (IC: 1.75–3.07) after 3 months of treatment ($n = 17$; $P = 0.002$, based on the 17 complete observations). In the prebiotics group, the mean total IGA score was 3.42 (IC: 3.20–3.63) before treatment ($n = 24$) *vs* 2.73 (IC: 2.31–3.14) after 3 months ($n = 22$; $P = 0.006$). No statistical differences between the two groups of treatment at M3 could be noted ($P = 0.457$). Finally, global assessment of the treatment by the patients showed no differences between the two groups.

Use of topical drugs

Topical treatments used by the patients were classified in three groups: topical steroids ($n = 34$), calcineurin inhibitor ointments ($n = 11$) and emollients only ($n = 3$). Tacrolimus 0.03% ointment was the only product used in the calcineurin inhibitor group. When the total quantities of ointment used over the study period were compared between patients receiving prebiotics or synbiotics, no statistical difference was observed ($P = 0.966$). Similar results were obtained when comparing use during the last month of the study ($P = 0.919$). In both groups, the use of steroid or tacrolimus ointments during the month before the study was higher than use during M3 (prebiotics, $P = 0.005$; synbiotics, $P = 0.07$).

Tolerance of the treatment

The treatment was well-tolerated in both groups. No serious side-effects were observed; only three episodes of mild abdominal pain were noted – two in the synbiotics group and one in the prebiotics group.

Follow up

All the children who had an improvement of their AD during the treatment but two, had a relapse of the disease or at least a deterioration in their skin status 2–4 months after the end of the treatment. This course was similar between in both groups of treatment.

Discussion

Both groups of patients (prebiotics and synbiotics group) had a significant improvement of their AD at the end of the treatment with a high rate of satisfaction, even though no changes in their usual topical treatments were made. However, the synbiotics appear not superior to the prebiotics that served as our control group.

Each probiotic bacterial strain has specific properties and ability to proliferate and survive in the intestinal tract. The *L. rhamnosus* Lcr35 strain used in the present study has many interesting properties. It presents 96% homology with *L. rhamnosus* GG by comparison of intergenic 16S–23S sequences. Moreover, *L. rhamnosus* Lcr35 strain has the ability to hydrolyse lactose, *L. rhamnosus* GG cannot. Finally, previous studies have demonstrated the ability of *L. rhamnosus* Lcr35 to achieve

lasting colonization of the intestinal mucosa after oral consumption (20). Our decision to evaluate the efficiency of synbiotic treatments in AD was based on the fact that appropriate prebiotic adjuncts can improve the viability and survival of probiotic strains following ingestion (21). Thus, we hypothesized that adding prebiotics to probiotics could give better results in treating AD than probiotics used alone. Current evidence shows that lactose and potato starch, as used in this study, can be considered as true prebiotics (22, 23). However, the use of a specific prebiotic preparation (as described above) relies on its ability to enhance the proliferation and survival of the *L. rhamnosus* Lcr35 strain *in vitro* (unpublished data obtained by Lyocentre® laboratories). Interestingly, as the prebiotic preparation is used for the fermentation broth of *L. rhamnosus* Lcr35, synbiotic treatments also contain the metabolites secreted by *L. rhamnosus* Lcr35. These metabolites can be defined as eubiotics. This combination is used in France for a number of years in Bacilor® (Awillac, France). Its efficiency has been demonstrated in diarrhoea and the product has been available for this indication in many countries for some time. The choice of a length of the study of 3 months was decided to allow a sufficient period of time for immunomodulation and to prevent spontaneous variation of disease intensity interfering with results due to treatment.

Our results show that synbiotic treatment [prebiotics + metabolites + *L. rhamnosus* Lcr35 strain (1.2×10^9 CFU three times a day)] is not superior to prebiotic preparation alone when treating AD in children aged 2 years or over. This was apparent both when using the total SCORAD score and the subjective patients' assessment as criteria of efficiency. It is possible that although the severity and extent of lesions remained the same, synbiotic treatment could have decrease the numbers of flares or the use of topical drugs. However, no statistical difference in these two parameters could be shown between the two treatment groups. Although synbiotic treatment induced a greater decrease of total SCORAD score mean (20.5 *vs* 15.6) the difference was not statistically significant. Up to now, no study has evaluated the efficiency of synbiotics and prebiotics in AD or in other atopic diseases. However, it has recently been demonstrated that prebiotics, like probiotics, can significantly decrease the generation and accumulation of potentially toxic fermentation products by comparison with placebo in healthy human volunteers (24). Thus, prebiotics by themselves could have a dramatic impact on gut flora and could also induce immunological changes. Although further investigations are necessary, preliminary data suggest that the consumption of prebiotics can modulate immune parameters including Th1/Th2 balance (25, 26). Thus, specific prebiotics could act on the gut flora to induce an immunomodulation that could be beneficial to the decrease of AD manifestations.

Although these experimental data possibly explain the potential role of prebiotics in treating AD, the importance of the clinical improvement observed in this study with both synbiotics and prebiotics alone, should consider other factors than these treatments that could have improved the clinical status of our patients. A seasonal effect can be ruled out because patients' enrolments were spread over 1 year and randomization was stratified depending on season. However, the frequency of visits and inclusion in a clinical study with the concomitant hope of an improvement in disease status may have provided a psychological support to the patients and their families. This, combined with the placebo effect, could induce significant improvement in AD. The absence of data comparing treatment with placebo prevents us from making definitive conclusions about the true efficacy of these treatments; however, although real, the efficacy of placebo in treating AD is limited, and to the best of our knowledge, never allows to induce more than a 5-point improvement in total SCORAD score (13, 17, 27–29). In the present study, treatments with prebiotics and synbiotics each induced more than 15-points of decrease in total SCORAD score (15.6 and 20.5 points, respectively). Moreover, this improvement cannot be linked with increased use of topical steroid or tacrolimus. On the contrary, the use of topical treatments had significantly decreased at the end of the study. Finally, the relapse of the disease 2–4 months after discontinuation of treatment observed in almost all patients of the two groups who were improved during the study also suggests that this improvement was not due to chance or spontaneous course of AD. All of these data support the efficacy of both prebiotic and synbiotic treatments in AD.

Our results corroborate with clinical improvement observed in other studies which have evaluated the use of probiotics in treating AD. Although a dramatic effect was noted in the first study evaluating probiotics in children under 2 years of age (13), the improvement observed in the second study, which studied children aged more than 1 year, was less significant (17). Interestingly, the first study was performed in highly selected patients (age < 2 years with proven allergy to cow's milk) that could explain the difference of efficiency. The improvement in total SCORAD score observed in this study with both prebiotics and synbiotics also appears to be higher than that observed in the study of Rosenfeldt et al. (17). This difference could be due to the length of treatment [6 weeks in Rosenfeldt et al. (17) study and 3 months in ours]. We think that using prebiotics and metabolites of probiotics as adjuncts might also play a role in this difference. However, few data are available concerning the respective role of probiotics, prebiotics and synbiotics in modulating the immune system. A recent study suggests that probiotics and prebiotics could act via different mechanisms (30). Two randomized placebo-controlled trials, both performed with probiotics for treating children with AD aged below 18 months, have been recently reported. In the first one, *Lactobacillus* GG, a mixture of four probiotic strains, or placebo were given

in AD patients with suspected cow's milk allergy for 4 weeks (14). No differences were shown between treatment groups immediately or 4 weeks after the treatment, but in a subgroup [immunoglobulin E (IgE)-sensitized infants], the *Lactobacillus* group showed a statistical significant greater reduction in SCORAD than did the placebo group. In the second study, *L. fermentum* and placebo were given for 8 weeks(15). The reduction in SCORAD index over time was significant in the probiotic group but not the placebo group. Interestingly, in both studies a marked decrease of SCORAD score was noted in placebo group (−20 and −10 points, depending on study). When looking at the placebos used in these two studies, one could find an explanation to this very unusual effect of placebo in AD. Cellulose and maltose dextran were respectively used in the studies of Weston et al. (15) and Viljanen et al. (14). Substances chosen as placebo both could be considered as prebiotics. Thus, results obtained with 'placebo' in these two studies are very similar to those observed in the prebiotic group in the present trial emphisizing the importance of the choice of a true placebo (and not a compound with prebiotic characteristics) when conducting therapeutic studies of AD with prebiotics, probiotics or synbiotics.

In conclusion, synbiotics (*L. rhamnosus* LCR35 plus prebiotics), as well as prebiotics alone, seem to be able to significantly improve the manifestations of AD in children aged 2 years and over. Although further investigations should be done with a higher number of patients to get more reliable results and to compare these new therapeutic approaches with both probiotics alone and with placebo, the importance of the improvement observed in this study underlines the potential interest in prebiotics and synbiotics in the management of AD.

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