

Comparison between two maintenance feeding regimens after successful cow's milk oral desensitization

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Abstract

Background: Cow's milk allergy is common in infancy, and total avoidance of this food is the only effective approach. In alternative, oral immunotherapy has been proposed to achieve tolerance. Once desensitization is achieved, daily intake of milk is recommended to maintain it, but this may be impractical for children/parents. We assessed whether a twice weekly maintenance regimen is effective.

Methods: Children who were successfully desensitized with oral immunotherapy were randomized to two maintenance regimens for 1 year: group A had to eat 150–200 ml milk daily, group B had to eat 150–200 ml milk twice weekly. Both regimens were associated to a totally free diet. Maintenance of tolerance and adverse events were recorded during 1 year. Specific IgE, IgG4 and prick-by-prick test to milk were carried out before immunotherapy (T0), before maintenance (T1), and after 1 year (T2).

Results: Recorded episodes included asthma, oral itching, urticaria, rhinitis, abdominal pain variously combined, usually associated with concomitant illness or exercise. The episodes were 8 in group A and 9 in group B, with no difference. None of the children discontinued the feeding maintenance. Specific IgG4 increased at T1 and remained high at T2. Specific IgE and skin reactivity significantly decreased at T2. There was no difference in those parameters between the groups.

Conclusion: After achieving desensitization to cow milk with oral immunotherapy, a maintenance regimen with milk given twice weekly is as effective as the daily maintenance.

Immune-mediated adverse reactions to cow's milk (CM) proteins can range from immediate, potentially life-threatening reactions to chronic disorders. Cow's milk allergy (CMA) is the most common food allergy in infants and young children, affecting 2–3% of general population (1–4). Many studies have suggested that the prognosis of CMA is overall favorable, because a vast majority of children outgrow their allergy by the age of 3 years (5–7). However, recent reports have indicated that a longer time may be needed to outgrow CMA and that, to some extent, tolerance develops in adolescence rather than in early school age as previously believed (8–10). Avoidance of CM and derivatives is still considered the first-choice approach in the management of CMA, where hydrolyzed formulas can be used in alternative. Also, other mammalian milks can be used to replace CM, but this does not exclude the possibility of cross-reactions. In addition to the nutritional concerns and the costs, this approach does not exclude the risk of accidental ingestion of milk, for instance by the involuntary consumption of foods

containing milk or milk derivatives. Another practical problem is the duration of milk avoidance, for which recommendations are not uniform (11–13). Usually, children should be reevaluated yearly to assess whether their food allergy has been outgrown.

Based on these considerations, therapeutic approaches different from the simple avoidance are being explored. The more attracting strategy is to induce a tolerance, with the so-called oral immunotherapy (OIT), consisting in the administration of progressively increasing doses of CM, until the food can be assumed safely at a normal amount. In principle, the goal of OIT (as happens with specific immunotherapy with inhalant or hymenoptera allergens) is to achieve a long-lasting tolerance, meaning that the food can be safely consumed despite periods of withdrawal. Indeed, it has been repeatedly reported that a regular consumption of the food allergen(s) is needed to maintain a tolerant status. Else, when ingestion is discontinued, the protective effect of OIT may be lost or significantly decreased. The effectiveness of OIT has been

demonstrated, but it is not still clarified which is the best maintenance dietary regimen after tolerance is achieved. This is of practical relevance, because the mandatory daily consumption of CM could be impractical or inconvenient for children and their families (14, 15).

In this randomized controlled study, we evaluated the tolerance in real life, defined as the ability, after OIT, to consume milk no more than two times per week with a totally free diet. This would be more convenient and suitable for children and adolescents, in comparison with the mandatory daily consumption of milk.

Methods

Overall design

This study was randomized, controlled, with two parallel groups. Patients who had undergone a successful OIT for the treatment of their CMA (full tolerance achieved) were randomly allocated to two different dietary regimens for 12 months. The primary outcome of the study was the assessment of the maintenance of tolerance to CM in the two groups. Secondary outcomes included: (i) the occurrence of adverse events in the two groups, and (ii) the changes in selected immunological markers. The study, not placebo controlled, was simply notified to the Ethical Committee of the University of Messina.

Patients and diagnosis

Children of both sexes, aged 4–13 years, with an ascertained IgE-mediated CMA and eligible for OIT were enrolled at the allergy unit of the department of pediatrics of Messina University between January 2005 and December 2009. The diagnosis of CMA was based on clinical history, presence of CM-specific IgE (>0.35 kUA/l), positive skin prick test to CM (>3 mm wheal), and positive response to double-blind controlled food challenge (DBPCFC). The DBPCFC was considered positive if one or more signs or symptoms (urticaria, angioedema, wheezing, rhinitis, vomiting, diarrhea, exacerbation of atopic dermatitis, hypotension) occurred with a cumulative dose of <2 g of CM proteins. After the diagnosis of CMA was well established, the children underwent OIT, with a weekly up-dosing protocol, as described elsewhere (16, 17). All children included in this study underwent DBPCFC before and after the desensitization protocol and before starting the two different maintenance regimens. OIT was considered successful if the children reached the target dose of 200 ml of milk (corresponding to about 7 g of CM proteins), that is the daily target dose use in most studies, and appropriate in real life.

Dietary regimens

After terminating the desensitization to CM, patients were randomly allocated to two groups. Group A was prescribed a CM regimen involving the ingestion of 150–200 ml of milk once a day, plus a totally free diet. Group B received a maintenance involving the ingestion of 150–200 ml of milk two

times a week plus free diet. Free diet included foods containing CM proteins such as cheese, pizza, desserts, cakes, biscuits, ice creams, yoghurt. Sports and exercise were permitted. If an illness occurred (e.g., common cold, fever, or suspect relapse of CMA) during the maintenance, appropriate treatments were given, and milk feeding was temporarily stopped up to 3 days.

Immunological markers

Blood samples were collected at the time of the diagnosis of CMA (T0), after completing the desensitization (T1) and after 1 year of maintenance feeding (T2). CM-specific IgE and IgG4 were assayed using the ImmunoCAP System (Phadia, Uppsala, Sweden). The sum of alpha-lactalbumin-, beta-lactoglobulin- and casein-specific antibody levels were used as a surrogate for CM-specific IgG4, to avoid the interference with IgG antibodies specific for bovine albumin, which are found in most sera. The lower limit for assay detection was 0.35 kU/l for specific IgE and 0.3 µg/ml for specific IgG4.

A prick-by-prick test with undiluted fresh milk was carried out on the volar surface of the forearm, and a wheal diameter of 3 mm or greater was considered positive.

Statistical analysis

The Shapiro–Wilk test was applied, to assess the normal distribution of parameters. Differences among baseline, T1, and T2 were evaluated with the student's *t*-test. Laboratory parameters were evaluated by linear regression models, with generalized estimating equations to account for repeated measures over time. The intergroup differences at T0, T1, and T2 were evaluated by the Mann–Whitney *U* test. A *p* value <0.05 was considered significant.

Results

Population

Of 45 subjects receiving OIT, 38 (21 male, age range: 4–13 years) successfully completed the desensitization, and tolerated the ingestion of 200 ml of whole milk. In seven children, the desensitization failed because of side severe adverse events (1 asthma/rhinitis, 3 asthma, urticaria, and hypotension, 2 urticaria/relapse of atopic dermatitis, 1 anaphylaxis). Of the 38 eligible children, four were excluded because of a new diagnosis of allergy to hen's egg and/or peanuts, and two declined to continue in the study after desensitization. Thirty-two subjects were therefore randomized, 16 in group A and 16 in group B (Table 1). Three subjects and their families withdrew during maintenance because of personal problems, not related to the study procedures. The subjects' disposition is shown in Fig. 1. Twenty-nine children completed the 12-month study, 15 in group A and 14 in group B.

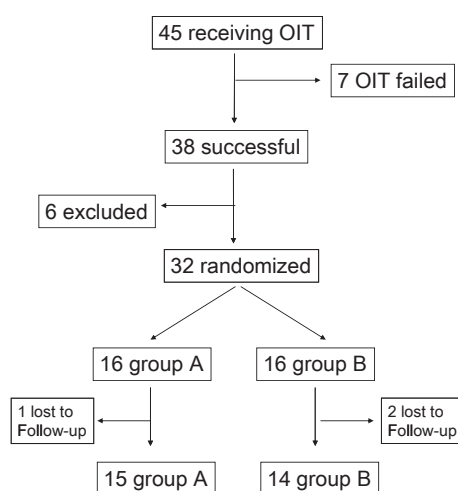
Clinical outcome

In group A, two patients (no. 2 and 5) had mild adverse events (itching in mouth, itching in mouth and throat). Patient 9 had

Table 1 Characteristics of the randomized patients

Parameter	Group A	Group B
N	16	16
Male/female	9/7	10/6
Median age	7	8
Age range	4–12	4–13
Clinical presentation		
Urticaria angioedema	4	3
Atopic dermatitis	3	3
Asthma/rhinitis	1	0
Anaphylaxis	2	1
Multiple symptoms*	6	9
CM-specific IgE, kU/L, median (range)	41.8 (8.3–84.3)	39.8 (6.8–98.3)
CM-specific IgG4, µg/mL, median (range)	3.3 (1.2–5.6)	3.4 (1.3–6.1)

*Two or more of the symptoms listed above.

**Figure 1** Patients' disposition.

two episodes of abdominal pain after a viral illness. Patient 12 had one episode of urticaria during exercise (sport activity). Patient 6 reported an episode of wheezing during a football match. Patient 13 reported multiple symptoms (urticaria, wheezing, diarrhea) during an episode of pneumonia. Patient 5, who had previously had mild local symptoms displayed pharyngeal pruritus, sneezing, and generalized urticaria during exercise (Table 2). None of the participants discontinued permanently the maintenance diet.

In group B, one patient had itching and lip swelling. Patient 7 reported three episodes of gastroenteritis with abdominal pain during viral illness, and urticaria/angioedema during a rugby match. Patient 10 had 2 episodes with multiple symptoms (wheezing and hives) during a football match. Patient 4 had diarrhea and abdominal pain during urinary tract infection. Patient 9 had two episodes of asthma during menses (Table 2). Also, in this group, none permanently discontinued the dietary regimen. No statistical difference was observed

between two groups concerning the rate of appearance of adverse events ($p = 0.08$).

Immunological parameters

In group A, CM-specific IgE slightly decreased at T1 in comparison with T0 ($p = 0.05$) and then significantly decreased again at T2 ($p < 0.0001$). CM-specific IgG4 increased at T1 ($p < 0.0001$) and T2 ($p < 0.0001$) vs. baseline. Skin prick test reactivity vs. T0 was not statistically different at T1 ($p = 0.7$), but it reached a statistical significance at T2 ($p = 0.005$). The difference between T1 and T2 was significant for CM-specific IgE ($p = 0.001$) and for SPT reactivity ($p = 0.005$), but not for CM-specific IgG4 ($p = 0.08$; Figs 2 and 3).

In group B, CM-specific IgE significantly decreased at T2 ($p = 0.0003$) but not at T1 ($p = 0.08$) vs. baseline. CM-specific IgG4 were significantly increased at T1 ($p = 0.0002$) and at T2 ($p < 0.0001$) vs. baseline, with no difference between T1 and T2. Skin test reactivity to CM significantly decreased only at T2 ($p = 0.005$) vs. baseline. The difference between T1 and T2 was significant for CM-specific IgE ($p = 0.02$) and for skin test ($p = 0.02$), but not for IgG4 ($p = 0.09$; Figs 2 and 3). There was no significant intergroup difference (regimen A vs. regimen B) for all the considered parameters at the three time-points.

Discussion

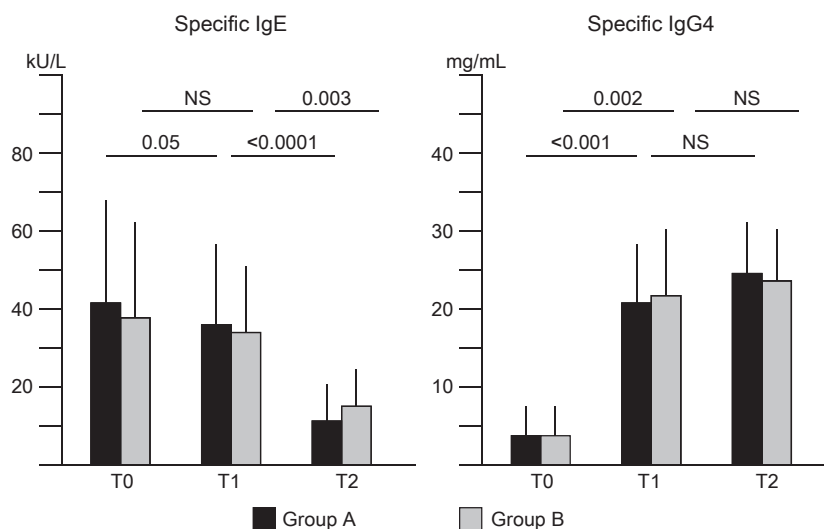
Oral desensitization is a promising approach for the treatment of CMA, because its effectiveness was confirmed in several trials. Nonetheless, there are still some gray areas and open questions. First, how frequently should CM be consumed, once an effective tolerance has been achieved? Second, a long-lasting daily CM administration is really needed? Third, are different maintenance regimens equally safe?

So far, there are only sparse data about the fact that desensitization for food allergy can lead to a permanent tolerant status, rather than to a temporary tolerance, and few data are available on the dosage interval required to maintain tolerance (14, 18, 19). It is generally accepted that even after an apparently successful immunotherapy, patients might be at risk for allergic reaction(s) if they do not consume the allergen regularly. In general, a daily consumption of a standard dose of CM is suggested to maintain tolerance, but this may be impractical or inconvenient in real life, because children are often compelled to the daily ingestion of the culprit food (20, 21). In addition, indefinite daily consumption of milk may represent a burden for children and their families and may induce an aversion to CM. Thus, more flexible maintenance regimens would represent an advance from the patient's viewpoint.

In the present study, we explored the possibility to simplify the dietary regimen in children successfully desensitized to milk. According to the data obtained in a selected population of patients, it seems that an extended maintenance dose of cow's milk (i.e., twice weekly) is as effective and safe as the daily administration is. The extended maintenance dose does not increase the risk of adverse events, still yet maintaining the tolerance previously induced. In addition, the immunological

Table 2 The events recorded during 12 months of follow-up in the two dietary regimens

No.	Group A		Group B	
	Sex, age	Event	Sex, age	Event
1	M, 4.5	None	M, 8.4	None
2	F, 9.2	Oral itching	M, 4.4	None
3	F, 7.4	None	F, 9.1	Oral itching, lips' swelling
4	M, 5.9	None	M, 9.0	Diarrhea during urinary tract infection
5	F, 9.5	Oral/throat itching	F, 6.5	None
6	M, 10.1	Urticaria/rhinitis on exercise	F, 8.6	None
7	M, 5.10	Asthma during exercise	M, 11.3	Three episodes of gastroenteritis during viral illness. One urticaria on exercise
8	M, 12.2	None	M, 5.7	None
9	F, 11.6	2 episodes of abdominal pain during viral illness	F, 13.2	2 asthma on menses
10	M, 4.2	None	M, 10.7	2 episodes of urticaria with wheezing on exercise
11	M, 5.3	None	F, 7.3	None
12	F, 6.0	Urticaria during exercise	F, 7.11	None
13	M, 6.8	Urticaria, wheezing, diarrhea on pneumonia episode	M, 8.10	None
14	F, 8.1	None	M, 4.8	None
15	F, 6.9	None		
Total events		8		9

Figure 2 Cow's milk -specific IgE (left panel) and IgG4 (right panel) at baseline, at the end of OIT and after 1 year of maintenance. Means and SD are shown, with the p values upon the bars.

parameters (e.g., specific Ig response) show no significant difference between the two regimens. Thus, the ingestion of CM either once a day, or two times per week are similar in the

induction of tolerance. Indeed, in westernized countries and in real life, a simplified maintenance regimen, after the achievement of desensitization, is easier for patients and their families.

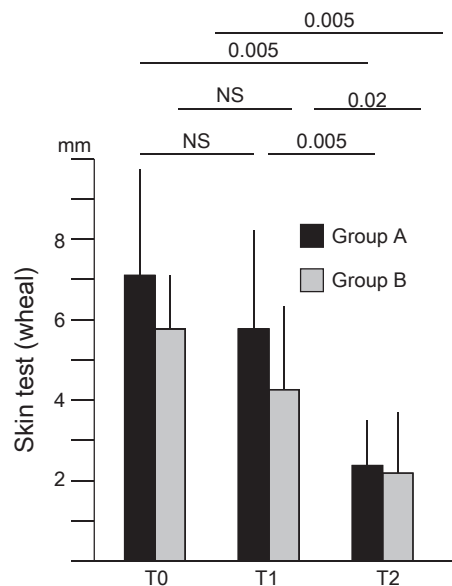


Figure 3 Skin prick test wheal diameter at baseline, at the end of OIT and after 1 year of maintenance. Means and SD are shown, with the p values upon the bars.

The safety of both regimens was satisfactory, although some patients had a temporary relapse of their allergy, especially during viral illness or exercise. One girl had asthma during menses. These patients prudentially stopped their usual diet up to 3 days, then continued with CM and dairy products.

There are several methodological limitations in this study. The most important one is the absence of a placebo arm, but we could not know how many subjects would have spontaneously improved, although a previous placebo controlled study of CM immunotherapy, carried out with a similar population found no spontaneous improvement (22). Also, due to the small sample size, more rarely occurring effects could have been missing. In addition, the real mechanisms of action of OIT remain unclear. It was observed that CM-specific IgE remained high during desensitization, but after 12 months of

consumption of CM significantly decreased, with both regimens. Similarly, the wheal size remained unchanged during OIT, but it decreased after 12 months with both regimens. This could be assumed as an indirect evidence of the suppression of mast cells activity (23). On the other hand, IgG4 levels against CM protein exhibited a significant increase overtime in both groups. An increase in specific IgG4 antibody levels, with or without a concomitant decrease in IgE, has been previously associated with the clinical improvement in OIT-treated patients (23, 24). After immunotherapy, the blocking activity present in serum is usually associated with allergen-specific IgG4 antibody. This effect, that is maintained also when CM is not consumed daily, may be responsible for long-term immune tolerance. In a strict sense, allergen immunotherapy implies the administration of gradually increasing doses of the allergen, thus reducing symptoms due to natural allergen exposure. In addition, it induces measurable immunological changes (e.g., IgE, IgG4, Th1/Th2 balance, T regulatory cells) (25). In the case of CMA, the immunological changes have investigated less in depth than for aeroallergen immunotherapy, the induction protocols are largely variable, and long-term data are still scarce.

In summary, this study showed in real life that the achieved tolerance to CM can be maintained without a mandatory daily consumption. Certainly, prolonged periods of CM avoidance after successful OIT would be necessary to confirm the achievement of a true tolerance. In this field, promoting and evaluating post-desensitization strategies will be fruitful for the success of this treatment of IgE-mediated food allergy.

Authorship

GBP, LC, GS, GC TA, MFM, and MW did the clinical work, collected and analyzed the data, and contributed to draft the MS. GBP and GP designed the study, analyzed the data, and prepared the final version of the article.

Conflict of interest and funding

The authors declare no conflict of interest and funding.

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