

Efficacy of Oral Immunotherapy Protocol for Specific Oral Tolerance Induction in Children with Cow's Milk Allergy

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ABSTRACT: **Background:** In the last two decades milk oral immunotherapy has gained interest as an effective treatment option for milk-allergic patients.

Objectives: To report on the efficacy of a milk oral immunotherapy.

Methods: Children with immunoglobulin E-mediated cow's milk allergy were included in the protocol. The treatment consisted of an induction phase in which milk doses were increased weekly in the hospital, while the tolerated dose was continued daily at home. The goal was to achieve a minimum milk intake of 200 ml a day. During the maintenance phase, patients ingested at least 200 ml of milk in a single dose every day.

Results: The protocol was applied to 105 milk-allergic children diagnosed by specific IgE to milk and controlled oral food challenge. The mean duration of the induction phase was 19 weeks. Of the 105 subjects, 86 (81.9%) successfully complied with the protocol and 19 (19.1%) failed. Causes of failure were moderate/severe reactions in 12 patients (12.44%) and personal reasons in 7 (6.66%). A total of 182 adverse reactions occurred during the induction phase, most of them mild. Baseline specific IgE to milk and casein was significantly lower ($P < 0.05$) in the successfully treated group compared to the group in which the treatment failed.

Conclusions: Milk oral immunotherapy is a safe and effective treatment for milk-allergic children, although adverse reactions may occur. Baseline milk and casein-specific IgE may be useful to predict a good response to milk oral immunotherapy.

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KEY WORDS: food allergy, milk, children, oral immunotherapy, immunoglobulin E-mediated allergy

Milk allergy is considered a transient disorder and usually develops in the first 3 years of life [5]. However, some reports described more than 19% of children over 5 years old with ongoing milk allergy and 11% who had not recovered by age 8 years [6]. Patients with persistent cow's milk allergy have more severe reactions and a different immunological pattern than those with transient allergy [7].

Traditionally, the only treatment for milk-allergic children was a strict avoidance diet and the rescue treatment of acute reactions after accidental ingestions [8]. The potential fatality risk of a severe reaction after an unknowing ingestion of contaminated food, together with the frequent difficulty to understand food allergen advisory labelling [9] necessitate seeking a more effective treatment than merely a milk-free diet [10].

Milk oral immunotherapy is a promising treatment that has advanced considerably in the last few years and its efficacy is supported by growing evidence. The treatment regimen consists of step-dose milk administration. Depending on the goal of the treatment, the final tolerated dose can be a small protective dose in case of an accidental exposure, or a larger dose for following a diet with no restrictions and a minimum milk daily amount intake ranging from 150 to 250 ml. In both cases, the aim is to reach milk tolerance, either by tolerance induction where permanent tolerance is achieved, or by desensitization where a continued and regular intake of the allergen is mandatory to maintain the protective effect [8].

We describe the efficacy of a milk oral immunotherapy protocol designed to achieve tolerance at a minimum dose of 200 ml of milk per day. This protocol has been applied in our outpatient clinic for the last 5 years in 105 pediatric patients.

PATIENTS AND METHODS

In this prospective open-label study, consecutive patients who attended the Allergy Department of Niño Jesús Paediatric Hospital between 2006 and 2011 were enrolled according to the following inclusion criteria:

- Children aged 2 to 18 years old
- A history suggestive of immediate milk allergy within the first 2 hours of ingesting milk

During the last two decades food allergy has become an important public health disorder, affecting both children and adults [1]. The severity and frequency of food allergy are growing problems and a cause of impaired quality of life [2].

Cow's milk allergy is the most frequent food allergy in infants, affecting 2%–3% of children under 1 year of age [3,4].

- IgE-mediated allergy to cow's milk diagnosed by at least one of the following:
 - ▷ Positive skin-prick test to whole cow's milk or at least one cow's milk protein: casein, alpha-lactalbumin, beta-lactoglobulin (Leti, Barcelona, Spain), with a wheal diameter 3 mm larger than the saline control
 - ▷ Detection of specific IgE > 0.35 kU/L to whole milk or any of its proteins, measured by fluorescence enzyme immunoassay (Phadia, CAP System, Uppsala, Sweden)
- Positive milk oral challenge: anaphylactic children or those who experienced an immediate and unequivocal reaction after milk ingestion within the previous 3 months, who did not overcome the challenge and were considered allergic to milk.

The exclusion criteria were malignant or autoimmune disease or associated diseases contraindicating the use of epinephrine, such as severe hypertension, and a poor compliance to protocol. The study was approved by the hospital's ethics committee and informed consent was obtained from all parents/guardians and children over 12 years old prior to their participation.

MILK ORAL CHALLENGE

Both challenges and immunotherapy were performed with commercially available milk with a protein concentration of 0.03 g/ml. Parents were advised to use the same brand throughout the protocol, and fatted, skimmed or semi-skimmed milk to avoid reactions due to different protein concentrations. Calcium-enriched milk was to be avoided since it contains a higher quantity of protein that may lead to overdosing and the resultant adverse reactions.

The milk oral challenge was performed in children who met the inclusion criteria with increasing amounts of milk as follows: 0.2, 0.5, 1, 2.5, 5, 10, 25, 60 and 145 ml on the same day. It was considered negative if children tolerated a total ingestion of 250 ml of milk. The challenge was considered positive if an objective symptom was observed. Patients whose challenge was negative were not included in the protocol and they were considered not allergic.

MILK ORAL IMMUNOTHERAPY PROTOCOL

The milk oral immunotherapy was performed in the Allergy Department under the supervision of the medical and nursing staff. Cardiopulmonary resuscitation measures and a pediatric intensive care unit were available for the treatment of possible adverse reactions. The milk oral immunotherapy consisted of two phases: the induction phase and the maintenance phase.

- **Induction Phase:** Initiated 1 week after a positive oral milk challenge, this phase involved increasing the milk doses weekly at the clinic and continuing at home with a daily

IgE = immunoglobulin E

Table 1. Induction phase of the two methods used (dilutions were performed in water)

Induction Phase*			Induction Phase Slow escalation dose**				
Day	ml	Protein (g)	Day	Drops diluted 1/25	Drops	ml	Protein (g)
1	0.05	0.0015	1	1	–	–	0.00006
1	0.1	0.003	7	2	–	–	0.00013
1	0.3	0.009	14	4	–	–	0.00026
1	0.6	0.018	21	8	–	–	0.0005
2	0.6	0.018	28	16	–	–	0.001
2	1	0.03	35	32	–	–	0.002
2	2.5	0.08	42	64	–	–	0.004
7	4	0.13	49	–	5	–	0.008
14	6	0.19	56	–	10	–	0.016
21	8	0.26	63	–	20	–	0.03
28	10	0.32	70	–	–	2	0.06
35	12	0.38	77	–	–	4	0.13
42	15	0.475	84	–	–	6	0.19
49	20	0.64	91	–	–	8	0.26
56	25	0.795	98	–	–	10	0.32
63	30	0.95	105	–	–	12	0.38
70	40	1.28	112	–	–	15	0.475
77	50	1.59	119	–	–	20	0.64
84	75	2.385	126	–	–	25	0.795
91	100	3.18	133	–	–	30	0.95
98	125	3.975	140	–	–	50	1.59
105	150	4.77	147	–	–	75	2.385
112	200	6.36	154	–	–	100	3.18
–	–	–	161	–	–	140	4.45
–	–	–	168	–	–	200	6.36

* Modified from Martorell et al. [13]

**Modified from Meglio et al. [14]

intake of the tolerated dose. Dairy products were not permitted during the induction phase. The dose-up methodology is shown in Table 1 [12,13]. The protocol was designed to achieve a total amount of 200 ml in 16 weeks. However, the induction phase was longer in patients who experienced adverse reactions. When a dose in the induction phase was not tolerated, the patient was treated and referred home for 1 more week with the previous tolerated dose, and a second attempt was made later on. Patients were treated with antihistamines (cetirizine) once a day throughout the entire induction phase. Patients were instructed to take the dose at a quiet time of the day; exercise was prohibited for 2 hours before and after the intake to avoid a predisposing

factor that may precipitate a reaction. A symptoms diary was given to the patient for noting any adverse reaction during the week. Written instructions were given and the parents were trained to treat any reaction that may occur during the immunotherapy. Parents were also advised to contact their allergist if any reaction occurred. A so-called slow dose-up protocol was performed [14] in patients at “high risk”: namely, patients over 10 years old with a previous anaphylactic reaction and/or sIgE >100 kU/L, and those who failed the afore-mentioned protocol.

- **Maintenance Phase:** Once a 200 ml intake of milk was achieved in the hospital, treatment with cetirizine was discontinued and a minimum home daily intake of at least 200 ml of milk was advised. Larger amounts of milk and dairy products were also allowed. Cheese was forbidden based on the observation of adverse reactions following goat and sheep milk ingestion [15] and the report of cross-sensitization between milk proteins from other species [16].

Following the guidelines of Clark and Ewan [17], adverse reactions were registered and classified according to severity as mild (oral allergy syndrome, erythema, rhinitis, conjunctivitis, local urticarial, vomiting), moderate (generalized urticaria and angioedema, mild bronchospasm), and severe (moderate/severe bronchospasm, shortness of breath, anaphylactic shock).

Milk oral immunotherapy was interrupted and classified as a treatment failure when two or more severe reactions occurred or repeated moderate reactions appeared. It was also stopped if a decrease in quality of life was detected, if parents/patient decided to stop, or if compliance was poor according to the clinician.

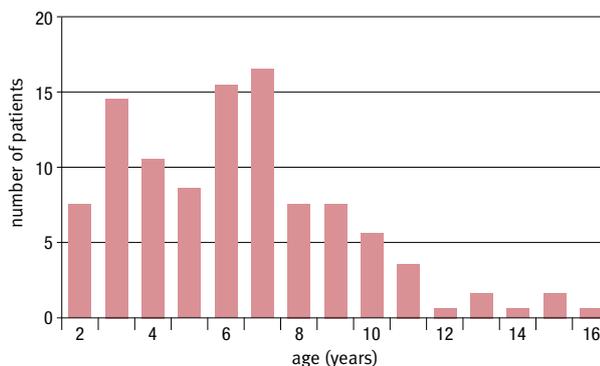
VARIABLES MEASURED AND STATISTICAL ANALYSIS

Skin-prick tests were performed following European guidelines [11]. Blood samples were collected to measure total IgE and sIgE (UNICAP, Phadia, Sweden) to cow’s milk and proteins. Immunological tests performed before the induction phase were considered as baseline values. Baseline skin-prick tests and sIgE to whole milk, casein, α-lactalbumin and β-lactoglobulin were compared between patients who successfully completed the treatment and those who failed, using the *t*-test. Failures for personal or family reasons were not included in the analysis. Differences were considered significant when *P* < 0.05.

RESULTS

The study population comprised 105 children who met the inclusion criteria and were aged 2–15 years old (mean SD 6.37 ± 3.09). Distribution by age (in years) is shown in Figure 1. Sixty-three of the 105 were male (60%). Doses eliciting symptoms in the inclusion oral milk challenges varied from 0.2 to

Figure 1. Distribution by age (years) of the children treated with milk oral immunotherapy



145 ml (mean SD 10 ± 45.7 ml) and their frequency, according to severity of reaction, was 66.66% mild, 32% moderate, and 1.33% severe. Fourteen patients did not perform the challenge and they were included in the protocol based on unequivocal symptoms or anaphylaxis after milk intake. Mean results of baseline skin-prick test (mm) were: whole milk 5, ALA 6, BLG 6, casein 5. Baseline sIgE (kU/L) to milk ranged from 0.35 to 216 (mean SD 22.5 ± 25.7), ALA from 0.35 to 220 (mean SD 9.6, ±SD 17.5), BLG from 0.35 to 100 (mean SD 6.27 ± 13.6) and casein from 0.35 to 267 (mean SD 19.9 ± 25.33).

Of the 105 patients, 86 (81.9%) successfully completed the treatment and 19 (19.1%) failed. Twelve patients withdrew from the treatment due to moderate/severe reactions and 7 for personal reasons. A 12 year old child achieved partial tolerance (60 ml) for 4 months. Due to poor compliance and oral allergy syndrome, the dose of milk was reduced to 40 ml for a further 4 months. However, he refused to follow the treatment and was classified as a failure due to family/patient decision.

Adverse reactions during the induction phase led to extending the time, thus the total mean duration of the induction phase was 19 weeks (± SD 8.7). Thirty-one patients (29.5%) did not suffer any reaction at all. During the induction phase 182 adverse reactions occurred in 74 patients (70.5%); 98.3% were mild or moderate. Severe symptoms were observed in 3 children (1.7% reactions, 2.85% patients) who suffered severe bronchospasm immediately after milk intake. All of these reactions occurred in the hospital and were reverted with adrenaline.

Baseline skin-prick test comparison between the successful and failed groups showed no differences. Specific IgE to milk, ALA, BLG and casein is shown in Table 2, and no differences were found in ALA and BLG-sIgE between the two groups. However, a significant lower baseline sIgE to milk and casein was found in the successful group (*P* < 0.05).

Two patients were classified as high risk. After two failures with the regular protocol due to repeated moderate/severe reac-

sIgE = specific IgE

ALA = alpha-lactalbumin
BLG = beta-lactoglobulin

Table 2. Comparison of specific IgE to milk, ALA, BLG and casein between patients who successfully completed the treatment and those who failed

	Milk sIgE	ALA-sIgE	BLG-sIgE	Casein-sIgE
Success	19.24 (n=73)	9.75 (n=73)	6.30 (n=73)	16.07 (n=73)
Failures	42.91 (n=9)	9.44 (n=9)	5.29 (n=9)	43.32 (n=9)
Statistical difference	$P=0.007^*$	$P=0.962$	$P=0.837$	$P=0.001^*$

sIgE = specific immunoglobulin E, ALA = alpha-lactalbumin, BLG = beta-lactoglobulin

tions they were treated with a slow dose-up method [14]. One of them was an anaphylactic 10 year old boy whose baseline milk IgE was 13 kU/L and casein sIgE 10 kU/L. The use of the slow dose-up protocol in this patient took 39 weeks and no adverse reactions were recorded. During the maintenance phase he did not experience any adverse reaction and today he ingests 200 ml of milk daily without symptoms. The second patient was a non-asthmatic 14 year old boy who experienced anaphylactic reactions after dietetic transgressions. Regular milk oral immunotherapy treatment failed twice with an interval of 18 months. Milk and casein sIgE was > 100. The induction phase of the slow dose-up method lasted 39 weeks. He experienced moderate reactions including mild bronchospasm, generalized urticaria and severe bronchospasm that were treated with two doses of adrenaline. Cetirizine and inhaled fluticasone were administered throughout the remaining induction phase. After 2 months in maintenance phase, he tolerated a daily dose of 200 ml of milk.

DISCUSSION

Elimination diet represents the current treatment for milk-allergic children; this treatment is difficult to comply with, especially in the younger population, and any unknowing contact with the culprit food may unleash a severe reaction. Among other therapeutic approaches, milk oral immunotherapy is an interesting optional treatment and is applied widely. Although an increasing number of allergists and researchers have contributed to our current knowledge on this topic during the last few years [12-14,18], there remain several unmet needs that require further investigation. First, there is a discrepant terminology to name this treatment, currently known as oral immunotherapy, tolerance induction, and desensitization. Second, there are no recommendations for the long-term clinical and immunological follow-up. Third, many different protocols have been published, from 3 days (rush milk oral immunotherapy) [19,20] to a 6 month regimen (slow dose-up oral immunotherapy) [14]. Fourth, there is a lack of studies both comparing different oral immunotherapy regimens and defining different patients' pheno-

types in order to perform tailored oral immunotherapy to minimize risks and maximize adherence.

For the last 5 years we have been applying this treatment to milk-allergic children with a mean age of 6 years old and a sIgE to milk and casein of 22.5 and 19.9 kU/L, respectively. That means the treatment was performed in older children with high levels of sIgE to milk and casein, which implies a risk of adverse reactions. In our experience, this is an effective treatment (81.9% success rate). Skripak et al. [12] report a success rate of 95%; we think this difference may be due to the smaller number of patients treated, pointing to a possible selection bias. The average age was also younger than in our study, which suggests a higher probability of children in their study having transient rather than persistent allergy [12]. We found that baseline milk and casein sIgE were significantly lower in patients who successfully completed treatment, which is consistent with previous publications related to the natural outcome of milk allergy. Rottem and co-authors [21] observed that milk sIgE during the first year of life can serve as a predictor of the persistence of milk allergy and they reported a positive predictive value of 82.6% to casein sIgE > 3 IU/ml at age 3 years. Furthermore, most of the patients with persistent milk allergy showed IgE binding epitopes on caseins as compared to none of the patients with transient milk allergy [22]. Although we did not establish a cutoff point, we conclude that milk and casein-sIgE are useful tools to determine whether a patient will be successfully treated with milk oral immunotherapy. No differences were observed in baseline ALA and BLG sIgE.

Both patients and parents reported a subjective improvement in their quality of life after the treatment. However, it is noteworthy that 6.66% of the patients (36.89% of the failures) failed due to family or personal reasons. This reflects the difficulties that parents and children have in adhering to the protocol.

Adverse reactions were observed in 69.5% of patients, most of which were mild, and the majority of patients did not receive rescue medication or were treated with oral antihistamines. Administration of antihistamines throughout the induction phase could be argued for, but in our experience this treatment did not mask any symptom apart from oral allergy syndrome and facilitated patient compliance. We found major differences in the literature regarding adverse reactions, ranging from 45.4% [12] to 80% [13]. We think this may be due to a misclassification of severity of adverse reactions or differences in grading systems.

The introduction of milk oral immunotherapy to patients with severe cow's milk allergy is controversial. Although it implies exposing the patient to the risk of a severe reaction with every increase in dosage, the possibility of an anaphylactic reaction following the accidental ingestion of a small amount of allergen at any time and not in a controlled manner as occurs in oral immunotherapy mandates seeking new therapeutic approaches. The combination of omalizumab and milk oral

immunotherapy has shown encouraging results [18]. Longo et al. [23] observed that 36% of a selected group of “very severe milk allergic children” who underwent 10 days of a dose-up protocol could tolerate larger volumes of milk. Meglio and team [14] described a slow dose-up protocol of 6 months duration to achieve a total amount of 200 ml, reaching a rate of 71.4% successful treatments. Kaneko et al. [24] recently reported their experience of up-dosing every 2 weeks, resulting in an 80% success rate. In our series, 2 of the 105 patients suffered repeated moderate to severe reactions that required adrenaline treatment several times during the induction phase of our 16 week protocol. One of these two patients was successfully treated with a slow dose-up regimen. Our limited experience with long-term protocols does not allow us to infer whether it should be applied instead of the shorter regimen.

Since we began to implement milk oral immunotherapy we have observed a growing demand from patients and allergic patient organizations to include it in daily clinical practice. However, and taking into account that this treatment is not without risk, we believe that milk oral immunotherapy should be implemented as a routine treatment only in experienced allergy departments that dedicate a portion of their time exclusively to food oral immunotherapy and where resuscitation measures are available. Although milk oral immunotherapy is a time-consuming treatment and necessitates a considerable effort by both clinicians and patients, the risk-benefit balance is positive. The disadvantages of milk oral immunotherapy are the following: risk of an adverse reaction, parents’ fear, low compliance, poor accessibility to allergists, and distance to the hospital. In contrast, the benefits observed were dietary improvement [25] and the general subjective perception of a better quality of life.

Milk oral immunotherapy is an effective treatment option for milk-allergic children. Further studies are needed to assess biomarkers of good response to milk oral immunotherapy and to optimize the best method in terms of efficacy and safety. Finally, an international position paper is essential before milk oral immunotherapy can be implemented in routine practice.

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