Original article

Better management of cow's milk allergy using a very low dose food challenge test: A retrospective study

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CM cow's milk
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OIT oral immunotherapy
VL very low dose

A B S T R A C T

Background: Low dose reactive cow's milk (CM) allergic children are at high risk of persistent CM allergy and a positive oral food challenge (OFC). The present study aimed to evaluate if the results of a very low dose (VL) OFC with these children contributes to better management of CM allergy.

Methods: We retrospectively reviewed subjects with CM allergy who underwent a VL OFC with 3 mL heated CM and had a previous allergic reaction to <25 mL heated CM in the 2 years before the OFC. Subjects who passed the OFC were defined as VL tolerant, and subjects who failed were defined as VL reactive. VL tolerant subjects increased the dose to 25 mL heated CM either during an OFC in our hospital or gradually at home.

Results: Of the 83 subjects (median age, 4.3 years; range, 1.0–12.9 years) who were included, 41 (49.4%) were VL tolerant, and 42 (51.6%) were VL reactive. Thirty-nine VL reactive subjects had skin and/or respiratory symptoms during the OFC. Most reactions could be treated with an antihistamine and/or a nebulized β2 agonist. The VL tolerant subjects consumed 3 mL heated CM or 10 g butter. Within the year following the OFC, 18 VL tolerant subjects (45.0%), but none of the VL reactive subjects, were able to consume 25 mL heated CM (p < 0.001).

Conclusions: A VL OFC allows the management of some low dose reactive CM allergic children to change from complete avoidance to partial intake of CM.

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Introduction

IgE-mediated cow's milk (CM) allergy is a common food allergy in infancy.1–5 Many children tend to outgrow a CM allergy in early childhood;5,6: 50% by age 5 and 75% by the early teenage years based on a review of natural history.4 However, some children continue to suffer from CM allergy, and an oral food challenge (OFC) is needed to assess the achievement of tolerance.5 Low dose reactive CM allergic children are at a high risk of persistent CM allergy6 and a positive OFC.7 Because high dose intakes for these children cause severe reactions,8 the OFC must be conducted carefully.

Baked milk9,10 and milk oral immunotherapy (OIT)11–12 are possible approaches for CM allergy. Because many CM allergic children tolerate baked milk,9–11 it can improve the dietary variety in these children, who then generally have a good prognosis with their unheated CM allergy.12 However, the challenge food for baked milk contains 0.5–1.3 g CM protein (equivalent to 15–40 mL CM),9–11 and children who react to baked milk avoid CM completely.12

Milk OIT for CM allergic children reportedly contributes to desensitization or threshold elevation13–15 but it might be impractical or inconvenient in real life because of the need for daily ingestion and risk of possible adverse reactions.13 In addition, it is difficult to achieve desensitization with milk OIT for low dose reactive CM allergic children, and there is a high rate of adverse reactions.14,15

Therefore, to identify strategies for better management of CM allergy, we performed a very low dose (VL) OFC (3 mL heated CM) and CM dose progression in CM allergic children who had experienced a previous reaction to <25 mL heated CM, based on our daily practice.

Methods

Study design

We retrospectively reviewed subjects with low dose CM reactions who underwent a VL OFC, which involves 3 mL heated CM...
actions were defined as VL tolerant, and subjects who failed the VL OFC were defined as VL reactive.

The results of the VL OFC are presented as the OFC positive rate and symptoms and treatments administered during the OFC. The results of CM dose progression based on our daily practice during the year after the OFC are compared using the time to reach 25 mL heated CM between the VL tolerant and VL reactive subjects.

Informed consent for the OFC and publication of the data was obtained from the children’s guardians. This study was approved by the Sagamihara National Hospital Ethics Committee and was conducted in accordance with the Declaration of Helsinki. The research plan was posted at Sagamihara National Hospital. However, because this study was retrospective, registration in an internationally certified registry was not required.

Subject selection

Eligible subjects were children who underwent VL OFC between July 2012 and December 2013, had a previous allergic reaction to CM containing CM, which was prepared by mixing 3 mL CM, 3 g pumpkin, 2 g sorghum bicolor, 1 g sugar, 0.02 g baking soda, and 1 mL water. The mixture was heated to 90 °C for 1.5 min in a 1000-W microwave. For the OFC with 25 mL heated CM, we increased the ingredients by approximately 8 times the amount for the VL OFC.

Oral food challenge protocol

The challenge food used in the VL OFC was pumpkin cake containing CM, which was prepared by mixing 3 mL CM, 3 g pumpkin, 2 g sorghum bicolor, 1 g sugar, 0.02 g baking soda, and 1 mL water. The mixture was heated to 90 °C for 1.5 min in a 1000-W microwave. For the OFC with 25 mL heated CM, we increased the ingredients by approximately 8 times the amount for the VL OFC.

Statistical analysis

Differences in characteristics at the time of the VL OFC were compared between the VL tolerant and VL reactive subjects using Mann–Whitney tests for continuous variables (expressed as median and range) and chi-square or Fischer’s exact tests for categorical variables (expressed as number and percentage).

Cow’s milk dose progression and follow-up

Subjects who passed the VL OFC were advised to consume a food containing 3 mL heated CM or 10 g butter (equivalent to 2.9 mL CM) at home at least once a week. One to three months after the OFC was passed, the CM dose was increased to 25 mL heated CM either during an OFC in our hospital or gradually at home. With the latter method, the heated CM dose was increased by 1 mL every few consumptions. If adverse reactions appeared, the previous dose was repeated. When the previous dose was passed, the scheduled increase was attempted. Subjects who failed the VL OFC underwent a second OFC at least 6 months from the first OFC.

We prescribed antihistamines for all subjects, adrenaline auto-injectors for the subjects with a history of anaphylaxis, and other medications depending on complications. All subjects received instructions on when and how to administer emergency medications and visit the emergency department.

Results

Baseline subject characteristics

Of the 131 subjects who underwent the VL OFC between July 2012 and December 2013, 48 subjects were excluded for a previous allergic reaction to CM more than 2 years prior, resulting in 83 subjects (median age, 4.3 years; range, 1.0–12.9 years) in the analyses (Fig. 1). The median CM-specific IgE level was 19.5 kUA/L (range, 0.66–284 kUA/L) (Table 1). Baseline subject characteristics were not significantly different between the VL tolerant (n = 41, 49.4%) and VL reactive (n = 42, 51.6%) subjects (Table 1).

The subjects’ previous allergic reactions were caused by accidental ingestion (61.4%) or an OFC with CM (38.6%). The median threshold dose at the previous OFC with CM was 12.5 mL (range, 3.0–25.0 mL) (Table 2). The threshold dose in the previous OFC with CM was higher in the VL tolerant subjects than in the VL reactive subjects, and rate of skin symptoms was lower in the VL tolerant subjects than in the VL reactive subjects (Table 2).

Results of the very low dose oral food challenge

Respiratory symptoms were the most common symptom, occurring in 83.3% (n = 35) of the VL reactive subjects, followed by skin symptoms, occurring in 81.0% (n = 34) of the VL reactive subjects. The majority of reactions were treated with antihistamines and/or nebulized β2 agonists. Among the 35 subjects with

(equivalent to 100 mg CM protein). Subjects who passed the VL OFC were defined as VL tolerant, and subjects who failed the VL OFC were defined as VL reactive.

The results of the VL OFC are presented as the OFC positive rate and symptoms and treatments administered during the OFC. The results of CM dose progression based on our daily practice during the year after the OFC are compared using the time to reach 25 mL heated CM between the VL tolerant and VL reactive subjects.

Informed consent for the OFC and publication of the data was obtained from the children’s guardians. This study was approved by the Sagamihara National Hospital Ethics Committee and was conducted in accordance with the Declaration of Helsinki. The research plan was posted at Sagamihara National Hospital. However, because this study was retrospective, registration in an internationally certified registry was not required.

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Statistical analysis

Differences in characteristics at the time of the VL OFC were compared between the VL tolerant and VL reactive subjects using Mann–Whitney tests for continuous variables (expressed as median and range) and chi-square or Fischer’s exact tests for categorical variables (expressed as number and percentage).

Cow’s milk dose progression and follow-up

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We prescribed antihistamines for all subjects, adrenaline auto-injectors for the subjects with a history of anaphylaxis, and other medications depending on complications. All subjects received instructions on when and how to administer emergency medications and visit the emergency department.

Results

Baseline subject characteristics

Of the 131 subjects who underwent the VL OFC between July 2012 and December 2013, 48 subjects were excluded for a previous allergic reaction to CM more than 2 years prior, resulting in 83 subjects (median age, 4.3 years; range, 1.0–12.9 years) in the analyses (Fig. 1). The median CM-specific IgE level was 19.5 kUA/L (range, 0.66–284 kUA/L) (Table 1). Baseline subject characteristics were not significantly different between the VL tolerant (n = 41, 49.4%) and VL reactive (n = 42, 51.6%) subjects (Table 1).

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Results of the very low dose oral food challenge

Respiratory symptoms were the most common symptom, occurring in 83.3% (n = 35) of the VL reactive subjects, followed by skin symptoms, occurring in 81.0% (n = 34) of the VL reactive subjects. The majority of reactions were treated with antihistamines and/or nebulized β2 agonists. Among the 35 subjects with
respiratory symptoms, 4 (11.4%) received only antihistamines, 27 (77.2%) received 1 dose of a nebulized $\beta_2$ agonist, 2 (5.7%) received 2 doses of nebulized $\beta_2$ agonist, and 2 (5.7%) received 1 dose of adrenaline. One subject received 1 dose of adrenaline for general-ized urticaria with agitation (Table 3).

Cow’s milk dose progression based on our daily practice within the year after the very low dose oral food challenge

Of the VL tolerant subjects, 14 underwent an OFC with 25 mL heated CM, and 9 of these subjects passed the OFC. The remaining 27 VL tolerant subjects gradually increased the CM dose at home, and 9 of these subjects reached 25 mL heated CM. Therefore, a total of 18 (45.0%) VL tolerant subjects reached 25 mL heated CM.

Of the VL reactive subjects, 24 underwent a second VL OFC, and only 3 subjects passed the second OFC. None (0.0%) of the VL reactive subjects reached 25 mL heated CM ($p < 0.001$) (Fig. 2).

Adverse reaction to cow’s milk consumed at home

A 7-year-old boy had 2 reactionary episodes to 3 mL heated CM. In the first episode, moderate abdominal pain and persistent cough occurred after CM intake that was followed by walking. The reaction was treated with oral steroids and 1 dose of a nebulized $\beta_2$ agonist. In the second episode, mild abdominal pain occurred after CM intake. The subject did not consume more than 4 mL heated CM during the year after the VL OFC.

The heated CM dose could be increased in 40 subjects. Three subjects (7.5%) each had 1 reactionary episode to 4–25 mL heated CM. One subject had generalized urticaria, one subject had mild abdominal pain, and the remaining subject had an intermittent cough (Table 4). None of the adverse reactions required adrenaline or a visit to the emergency department. There was no worsening of eczema or asthma.

Discussion

To the best of our knowledge, this is the first study to indicate that VL OFC could be used to help manage food allergies in children with a low dose reactive CM allergy. Based on the OFC results, half of the children in the present study could begin consuming very low doses of CM products on a daily basis. Furthermore, approximately half of these VL-tolerant children could consume 25 mL heated CM within the year following the VL OFC.

Regarding the definition of low dose reactive CM used in the present study, we believe that it is appropriate because previous studies have used 25–30 mL CM for low dose OFCs; we defined low dose reactive CM allergy based on previous allergic reactions to <25 mL heated CM.

Cianferoni et al. reported that non-skin symptoms at the previous reaction are predictors of OFC. However, the frequency of skin symptoms at the previous reaction in the present study was lower in the VL tolerant subjects than in the VL reactive subjects. In the VL reactive subjects, 29 (72.5%) with skin symptom at the previous reaction were predictive of OFC.

We conducted the OFC with 3 mL heated CM, which is a very low dose (VL) OFC. In many previous studies of food allergies, the OFC dose was 25–30 mL. However, there were no advantages of using a high OFC dose compared with a VL OFC. In the present study, we retrospectively compared the results of the OFC with 3 mL heated CM (VL OFC) and the OFC with 25 mL heated CM (high OFC) (Table 4). The number of subjects who passed the OFC with the VL OFC was significantly higher than those who passed the OFC with the high OFC ($p < 0.001$) (Fig. 2). The rate of non-rejectors who passed the OFC with the VL OFC (61.5%) was higher than the rate of non-rejectors who passed the OFC with the high OFC (33.3%), suggesting that the VL OFC was more effective than the high OFC.

None of the adverse reactions required adrenaline or a visit to the emergency department. There was no worsening of eczema or asthma.

Table 1

Baseline characteristics of subjects with cow’s milk allergy who underwent a very low dose oral food challenge.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All subjects (n = 83)</th>
<th>VL tolerant (n = 41)</th>
<th>VL reactive (n = 42)</th>
<th>p value$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>4.3 (1.0–12.9)</td>
<td>4.3 (1.0–12.9)</td>
<td>4.0 (1.2–8.2)</td>
<td>0.774</td>
</tr>
<tr>
<td>Male sex</td>
<td>52 (62.7)</td>
<td>24 (58.5)</td>
<td>28 (66.7)</td>
<td>0.444</td>
</tr>
<tr>
<td>History of anaphylaxis to CM</td>
<td>49 (59.0)</td>
<td>21 (51.2)</td>
<td>28 (66.7)</td>
<td>0.152</td>
</tr>
<tr>
<td>Other food allergy, current</td>
<td>58 (69.9)</td>
<td>27 (65.9)</td>
<td>31 (73.8)</td>
<td>0.430</td>
</tr>
<tr>
<td>Eczema, current</td>
<td>55 (71.1)</td>
<td>28 (68.3)</td>
<td>27 (64.3)</td>
<td>0.579</td>
</tr>
<tr>
<td>Asthma, current</td>
<td>24 (28.9)</td>
<td>11 (26.8)</td>
<td>13 (31.0)</td>
<td>0.679</td>
</tr>
<tr>
<td>Allergic rhino-conjunctivitis, current</td>
<td>20 (24.1)</td>
<td>8 (19.5)</td>
<td>12 (28.6)</td>
<td>0.335</td>
</tr>
<tr>
<td>Total IgE (kUA/L)</td>
<td>429 (28.5–7290)</td>
<td>521 (35.8–6090)</td>
<td>380 (28.5–7290)</td>
<td>0.906</td>
</tr>
<tr>
<td>CM-specific IgE (kUA/L)</td>
<td>19.5 (0.66–284)</td>
<td>19.7 (0.66–228)</td>
<td>19.2 (4.6–284)</td>
<td>0.112</td>
</tr>
</tbody>
</table>

$^*$Comparisons between the VL tolerant and VL reactive subjects were conducted using Mann–Whitney tests for continuous variables or chi-square or Fischer’s exact tests for categorical variables.

Values are reported as median (range) or n (%). VL, very low dose; CM, cow’s milk.
Values are reported as median (range) or n (%).

Subjects who underwent an OFC with baked milk in previous tests for categorical variables. Statistically significant p values (<0.05) are in bold. Values are reported as median (range) or n (%).

### Table 2

Characteristics of previous allergic reaction to cow’s milk.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All subjects (n = 83)</th>
<th>VL tolerant (n = 41)</th>
<th>VL reactive (n = 42)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reason for previous allergic reaction to CM</td>
<td>Accidental ingestion</td>
<td>32 (38.6)</td>
<td>16 (39.0)</td>
<td>16 (38.1)</td>
</tr>
<tr>
<td></td>
<td>OFC to CM</td>
<td>12.5 (3.0–25.0)</td>
<td>22.5 (9.4–25.0)</td>
<td>9.7 (3.0–25.0)</td>
</tr>
<tr>
<td></td>
<td>previous OFC</td>
<td>(n = 32)</td>
<td>(n = 16)</td>
<td>(n = 16)</td>
</tr>
<tr>
<td></td>
<td>Symptom at previous allergic reaction to CM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skin</td>
<td>73 (88.0)</td>
<td>33 (80.5)</td>
<td>40 (95.2)</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal (%)</td>
<td>29 (34.9)</td>
<td>15 (36.6)</td>
<td>14 (33.3)</td>
</tr>
<tr>
<td></td>
<td>Respiratory (%)</td>
<td>41 (49.4)</td>
<td>17 (41.5)</td>
<td>14 (33.3)</td>
</tr>
<tr>
<td></td>
<td>Cardiovascular (%)</td>
<td>5 (6.0)</td>
<td>3 (7.3)</td>
<td>2 (4.8)</td>
</tr>
<tr>
<td></td>
<td>Anaphylaxis (%)</td>
<td>49 (59.0)</td>
<td>21 (51.2)</td>
<td>28 (66.7)</td>
</tr>
</tbody>
</table>

Comparisons between the VL tolerant and VL reactive subjects were conducted using Mann–Whitney tests for continuous variables or chi-square or Fischer’s exact tests for categorical variables. Statistically significant p values (<0.05) are in bold. Values are reported as median (range) or n (%).

### Table 3

Symptoms and administered treatments during the very low dose oral food challenge with cow’s milk.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>VL reactive (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin (%)</td>
<td>34 (81.0)</td>
</tr>
<tr>
<td>Gastrointestinal (%)</td>
<td>13 (31.0)</td>
</tr>
<tr>
<td>Respiratory (%)</td>
<td>35 (83.3)</td>
</tr>
<tr>
<td>Cardiovascular (%)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Anaphylaxis (%)</td>
<td>30 (71.4)</td>
</tr>
<tr>
<td>Treatments</td>
<td></td>
</tr>
<tr>
<td>Antihistamine (%)</td>
<td>41 (97.6)</td>
</tr>
<tr>
<td>β2 agonist, nebulized (%)</td>
<td>31 (73.8)</td>
</tr>
<tr>
<td>Steroids (%)</td>
<td>5 (11.9)</td>
</tr>
<tr>
<td>Adrenaline (%)</td>
<td>3 (7.1)</td>
</tr>
</tbody>
</table>

The very low dose (VL) reactive subjects failed the VL oral food challenge, which was conducted with 3 mL heated cow’s milk.

### Table 4

Adverse reaction to heated cow’s milk at home after passing the very low dose oral food challenge.

<table>
<thead>
<tr>
<th>VL tolerant</th>
<th>Dose escalation (4–25 mL) (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%) of subjects</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td>No. of adverse reactions</td>
<td>2</td>
</tr>
<tr>
<td>Skin</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>2</td>
</tr>
<tr>
<td>Respiratory</td>
<td>1</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>0</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td>1</td>
</tr>
</tbody>
</table>

The very low dose (VL) tolerant subjects passed the VL oral food challenge, which was conducted with 3 mL heated cow’s milk.

One subject who had a reaction to 3 mL heated cow’s milk at home did not consume >4 mL heated cow’s milk within the year following the very low dose oral food challenge.

### Table 5

Absolute reaction to heated cow’s milk at home after passing the very low dose oral food challenge.

### Fig. 2

Kaplan–Meier curves showing the time to achieve consumption of 25 mL heated cow’s milk during the year after very low dose oral food challenge for cow’s milk allergy. VL, very low dose; CM, cow’s milk.

In conclusion, VL OFC helps to identify those low dose reactive CM allergic children who can shift from complete avoidance to partial intake of CM, providing better management of these children. Future randomized controlled trials are needed to determine if partial intake of CM improves CM allergy.

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Conflict of interest
The authors have no conflict of interest to declare.

Authors’ contributions
YO performed research, analysed data, and wrote the paper. NY supervised YO’s work. SS and ME contributed to the data analysis and the preparation and revision of the manuscript.

References