

Ass's milk in children with atopic dermatitis and cow's milk allergy: Crossover comparison with goat's milk

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Cow milk allergy is a common disease of infancy, often associated with atopic dermatitis (AD). Avoidance of cow milk (CM) implies the use of alternative dietary supports such as mammalian milks. In this study, we assessed the tolerability and clinical effect of ass's milk (AM), when compared with the largely used goat's milk (GM) in a single-blind, controlled, randomized crossover. Twenty-eight children with AD and ascertained allergy to CM were enrolled. The children were randomized to AM or GM for 6 months, then switched to the other milk for further 3 months. The SCORAD index (SI) and a visual analog scale (VAS) were evaluated blindly. After termination of the study, food challenges with GM and AM were performed. An SDS-PAGE analysis of different milks was performed. Two children from the GM group dropped out after randomization and 26 completed the study. Ass milk invariably led to a significant improvement of SI and VAS of symptoms ($p < 0.03$ vs. baseline and inter-group), whereas GM had no measurable clinical effect. At the end of the study 23 of 26 children had a positive food challenge with GM and one of 26 with AM. Ass's milk had a protein profile closer to human milk than GM. Ass milk is better tolerated and more effective than GM in reducing symptoms of AD. It may represent a better substitute of CM than the currently used GM.

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Cow's milk allergy (CMA) is a common disease in infancy and childhood and its prevalence approximates 2.5% during the first 3 yr of life (1). One of the most frequent clinical presentations of CMA is atopic dermatitis (AD), followed by urticaria/angioedema, gastrointestinal symptoms and, less frequently, wheezing and asthma. The only effective treatment for CMA is the total avoidance of CM, which has to be replaced with appropriate substitutes. Currently, the most used substitutes are either soy formulas or casein and whey extensively hydrolyzed formulas that have a good nutritional value. On the other hand, hydrolyzed formulas products have an unpleasant taste and are quite expensive, whereas soy

formulas have the potential to evoke allergic reactions (2–5). For these reasons, other mammalian milks can be used to replace CM, including goat's milk (GM), ass's milk (AM), or mare's milk. In particular, GM is available in several countries and it is recommended by some physicians for infants and young children with CMA (6, 7). In spite of this, there are few clinical studies concerning the allergenicity and tolerability of GM or mare's milk, and their results are controversial (8–10). Ass's milk is a potential candidate to replace CM as its composition is similar to human milk, it has an acceptable taste and is less expensive than hydrolyzed formulas. Nevertheless, there is so far no controlled trial

about the tolerability of AM milk in children with CMA and AD. For these reasons we carried out a crossover randomized-controlled trial in order to objectively compare the tolerability of AM and GM. This latter milk was chosen as control since it is still widely used as a substitute of CM in clinical practice. The protein composition of the two milks was also assessed.

Material and methods

General plan

The study was designed as investigator-blind, randomized and crossover, using GM as control (Fig. 1). The study was approved by the local ethical committee and all the parents gave their informed consent. After obtaining the consent and assessing the baseline clinical situation, children were randomized to receive for 6 months either GM or AM in an open fashion. Subsequently, they were switched to the other treatment for further 3 months. This timing was chosen in order to avoid the summer period, when AD usually improves spontaneously. During the trial, the SCORAD index (SI) and a visual analog scale (VAS) of symptoms were recorded. A double-blind placebo-controlled food challenge (DBPCFC) with different mammalian milks was carried out at the end of the study.

Patients and diagnosis

Children were enrolled between April and July 2004, with the co-operation of the GP pediatricians of Sicily and Calabria (southern Italy), and seen at the Allergy Unit of the pediatric clinic of the Messina University. Enrolment criteria were:

- age between 6 months and 3 yr, both sexes,
- clinical history of CMA,
- positive prick by prick test to cow milk (CM) extract,
- positive DBPCFC with CM,
- active AD with a SI > 20, and
- children who had been previously fed with AM or GM were excluded.

At the enrolment, all children underwent skin tests, CAP-RAST assay, and DBPCFC with fresh CM and soy formula (Humana Sinelac, Milan, Italy) as placebo. Skin tests were also performed with GM and AM as well as CAP-RAST for GM.

Skin tests were carried out on the volar forearm surface with the prick by prick using undiluted CM and soy formula. The sterile lancet was immersed in the milk before pricking and the results were read after 15 min. A wheal of 3 mm or greater was considered positive. Negative (saline solution) and positive (histamine 10 mg/ml) controls were also used (Stallergenes, Milan, Italy). *In vitro* allergy testing by fluorescent enzyme-linked immunoassay (AutoCAP, Pharmacia, Upssala, Sweden) was also carried out for CM. Levels of specific IgE > 0.35 kU/l were considered positive. The DBPCFC were performed at the clinic, with full facilities for resuscitation available. Fresh CM, or soy formula were administered at increasing doses of 0.1, 0.3, 1.0, 3.0, 10.0, 30.0 and 100 ml. The time interval between each dose was 20 min (11). The challenge procedure was stopped when clinical symptoms appeared or when the highest dose was reached. After completing the DBPCFC, children were kept under observation for at least 6 h and then discharged. During the observation period a physician was always ready on call. The food challenges were scored as positive by a pediatric allergist if a single symptom or a combination of the following objective clinical reactions was observed: urticaria, angioedema, wheezing, vomiting, diarrhea, abdominal pain, exacerbation of AD, or anaphylaxis.

Interventions. After confirming the diagnosis of CMA, the children were randomized to receive either GM (Sapori Montani s.r.l., Milazzo, Italy) or AM (Asilat s.r.l., Giarre, Italy) for 6 months, then the patients were switched to the other treatment for further 3 months. Randomization was made according to a computer-generated list. The coordinator, who was blinded to the treatment, was in charge of patients' supervision and adjustment of rescue medications according to symptoms. He was also responsible for

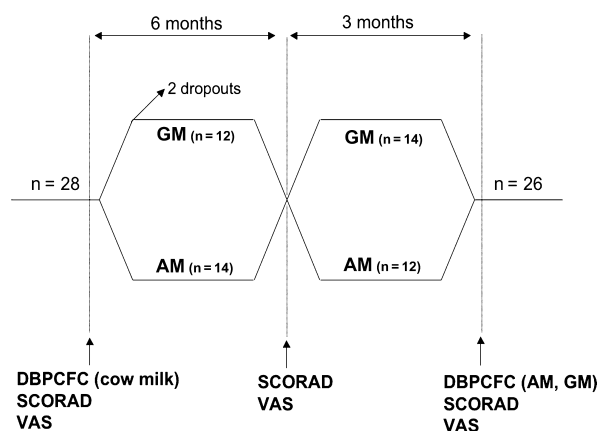


Fig. 1. Study design. DBPCFC, double-blind placebo-controlled food challenge; AM, ass's milk; GM, goat's milk.

reporting any reaction certainly or possibly related to the treatment with mammalian milks. Allowed medications were short courses (3 days) of hydroxyzine, topical steroids, or clarithromycin, when needed.

Evaluated parameters. Primary outcome measure was the SI, assessed at baseline and the end of each treatment course. The SI evaluates disease's extension (head and neck, each arm, front and back of the legs, the four trunk quadrants, and genitalia), clinical aspect (erythema, edema, papulation, oozing, crusts, excoriation, lichenification, xerosis), and subjective symptoms (pruritus and sleep loss; 12). AD was considered mild with a $SI < 25$, moderate for $25 \leq SI < 50$ and severe if $SI \geq 50$. At each visit parents were asked to report the overall severity of skin symptoms during the last 4 wk on a VAS from 0 (no symptoms at all) to 10 (very severe symptoms).

Protein identification in the milks. SDS-PAGE assays were carried out by a 12% polyacrylamide gel at 80 V through the stacking gel and at 100 V through the separating gel in reducing condition. CM, GM, AM, and human milk (at 10 and 60 days of breastfeeding) were reduced by 5% mercaptoethanol at 100°C for 5 min. Then, 10 μ l per well were loaded on the gel and run together with purified markers: caseins, β -lactoglobulin, α -lactalbumin and 14–66 kDa molecular weight markers (Sigma, St Louis, MO, USA). The gel was stained with Gel Code Blue Stain Reagent (Pierce, Rockford, IL, USA).

Statistical analysis. The analysis of non-parametric data was performed by means of the Mann–Whitney *U*-test for intergroup comparisons. Parametric data for intragroup comparisons for SI and VAS were analyzed by means of the Wilcoxon matched pairs signed rank test. The *p*-values < 0.05 were considered significant. Tsai and Patel's method for analyzing the period crossover design was used to compare the SI obtained during GM and AM treatments (13). All statistical evaluations were performed by using a commercial software package (Minitab release 8; Minitab Inc., State College, PA, USA).

Results

Twenty-eight children (15 male, age range: 0.6–3.8 yr, median age 2.5 yr), with a median disease's duration of 1.8 ± 1.2 yr fulfilled the inclusion criteria and were enrolled. All children suffered from AD (mean SCORAD 45, range: 21–66) and nine of them had also asthma and/or

rhinitis. All children displayed a positive SPT response to CM (mean wheal diameter = 8 mm, erythema = 14 mm) and positive DBPCFC and 25 of them had also a positive CAP-RAST to CM. None of the patients had positive SPT to AM. Eight children had positive skin prick test to GM (five with positive CAP-RAST, specific IgE 3.75 ± 1.57 kU/l).

At baseline all patients had a positive DBPCFC with CM, whereas none of them reacted to soy formula. During the CM challenge, the observed symptoms were urticaria and/or angioedema or exacerbation of eczema in 26, wheezing and/or rhinitis in two, vomiting and abdominal pain in three. Only one patient had diarrhea. All the positive responses to the challenges occurred within 4 h (range: 3 min to 4 h). The median dose of the mammalian milks that gave a positive response to the challenge test was 30 ml (range: 1–100). The demographic data and diagnostic assessments are summarized in Table 1. Two patients dropped out immediately after being randomized to the GM group. In one case withdrawal was due to family problems, and in the other to systemic symptoms as shortness of breath, sneezing, and severe generalized urticaria.

The SI after each separate phase of the crossover, and the cumulative at the end of the study are illustrated in Fig. 2. There was invariably a significant decrease in SI after using AM, whereas with GM, the change vs. baseline was never significant. In detail, the mean SI of the AM group decreased from 48 at baseline to 8 ($p = 0.001$) after 6 months of dietary intervention, whereas the SI of the GM changed from 43 to 39 ($p = \text{ns}$). The intergroup difference was $p = 0.002$. After the crossover, children on AM (previously on GM) reduced their SI to 14 ($p < 0.003$), whereas children on GM (previously on AM) increased their SI from 8 to 32 ($p < 0.003$). The intergroup difference was therefore significant ($p < 0.03$). The baseline

Table 1. Baseline characteristics of the children

	Total	AM	GM
Number	28	14	14
Age (mean)	2.5	2.7	2.5
Age (range)	0.6–3.8	0.8–3.8	0.6–3.7
Sex M/F	15/13	8/6	6/8
Mean SCORAD (range)	45 (21–66)	44 (23–65)	47 (21–66)
Concomitant asthma	7	4	3
Concomitant asthma + rhinitis	2	1	1
Positive CAP-RAST to cow milk	25	13	12
Positive skin test to AM	0	0	0
Positive skin test to GM	8	3	5
Positive CAP-RAST to GM	5	2	3
Mean specific IgE to CM (kU/l)	6.25 ± 3.50	6.14 ± 2.80	6.25 ± 3.57

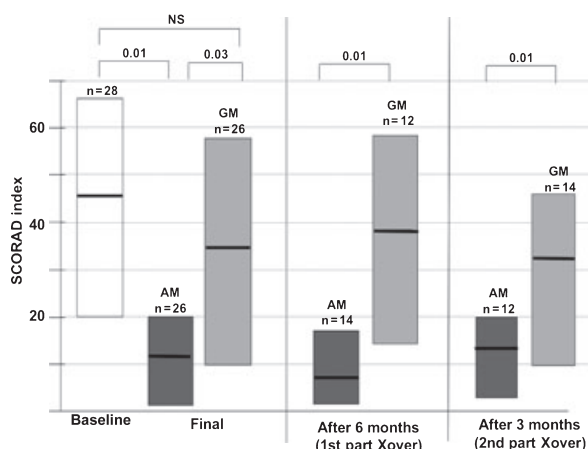


Fig. 2. SCORAD index at baseline, after completion of the crossover and after each single part of the crossover. p-Values are reported at the top of the graphs. The boxes represent the minimum-maximum interval and the thick bar is the median value.

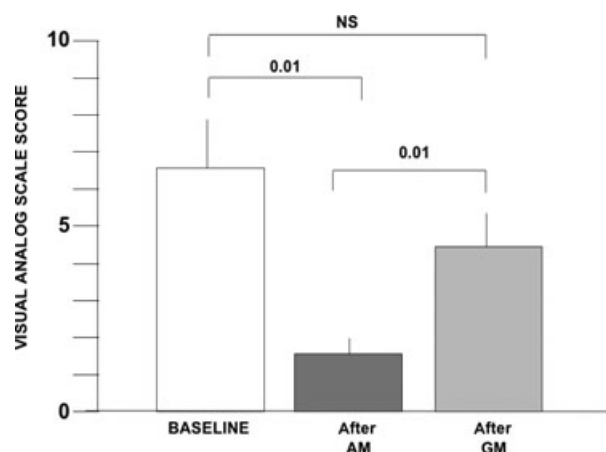


Fig. 3. Visual analog scale scores (median and s.e.m.) at baseline and at the end of the study. p-Values are reported at the top of the graphs.

VAS was 6.0 ± 1.5 , and it decreased to 1.5 ± 0.5 after AM ($p = 0.02$) and to 5.5 ± 1 after GM ($p = \text{ns}$), with a significant intergroup difference ($p = 0.03$) as illustrated in Fig. 3. At the end of the study the DBPCFCs proved positive for GM in 23 of 26 patients and for AM in one of 26 patients. All the positive patients had mild symptoms, such as exacerbation of eczema and/or urticaria. None had respiratory symptoms or systemic reactions with GM and AM.

Concerning the protein profiles, obtained by SDS-PAGE, AM contained lower amounts of casein proteins, but higher amounts of β -lactoglobulin than human milk, with only weak differences in whey components. On the contrary, CM and GM had a greater amount of caseins and less β -lactoglobulin and α -lactalbumin.

Discussion

The problem of dietary treatment of children with CMA is critical. Currently, there are several CM substitutes available, including soy formulas, rice formulas (14), extensively hydrolyzed formulas, GM and equidae' milks. The choice of an alternative milk should take into account its palatability and cost together with the clinical profile of the allergic child (severity of symptoms, age, degree of sensitivity to CM). In addition, another crucial and not negligible aspect is the nutritional adequacy of CM substitutes (15). Among mammalian milks, GM is generally considered to be a safe alternative for children suffering from CMA, it is therefore marketed in many countries and quite largely used (6, 7). Nonetheless, there are concerns about the tolerability and safety profile of GM, and even an anaphylactic reaction to GM in children with CMA has been reported (16). Clein (17) noticed that individuals with CMA may also be allergic to GM and Belloni-Businco et al. (8) documented a cross-reactivity between CM and GM in placebo-controlled food challenges. The clinical experience is confirmed by biochemical studies, showing that the casein proteins in CM and GM are quite similar (18). According to these observations it was suggested that individuals with CM allergy should avoid GM (8).

Ass belongs to the Equidae family and its milk has been shown to have a protein composition similar to human milk (19), at variance with GM, which seems to be more similar to CM. AM is particularly rich in whey proteins, whereas casein components occur in lower quantity than in CM. Finally, AM has a pleasant taste due to the high lactose content (20–22). AM has been previously used in CMA with favorable results. Iacono et al. (9) showed that AM was optimally tolerated in nine infants with severe symptoms (vomiting, diarrhea, failure to thrive, shock) caused by CMA. Another Italian group performed a controlled study on the tolerability of Mare's milk in CMA children (10) and found that 96% of the children with CMA could tolerate MM. This was confirmed by the cross-inhibition immunoblotting.

The present controlled crossover trial aimed at answering the question if in clinical practice the best choice among mammalian milks, in children with IgE-mediated CMA, is GM or AM. In other words we choose GM as control in order to evaluate a milk that is used in clinical practice. The results of our study indicate that AM is tolerated by 88% of the children with CMA and produces a significant improvement in AD. On the contrary, in all the children receiving GM, symptoms remained unchanged or even wor-

sened. In particular, all children previously on AM had a relapse of AD after switching to GM. The sample size is not quite large, but this is the consequence of the very strict inclusion criteria that were adopted to have a clean experimental model, without confounding factors. In fact, all the subjects enrolled were highly homogeneous as far as their sensitization and clinical aspects were concerned. Although eight of the children had a positive skin test to GM, they had never been fed with that milk, therefore we interpreted the sensitization as a cross-reactivity and included them in the trial. In fact, true GM allergy has different characteristics from CMA and CM does not provoke reactions in children allergic to GM (23). Concerning the age, it is well known that CMA often improves or disappears within the first 3 yr of life (24), nevertheless in our children of 3 yr or more, CMA was still active as testified by the DBPCFC. Moreover, by terminating the crossover study before the summer, the bias of the spontaneous improvement of AD in the summertime could be avoided. The study is, indeed, not double-blind, but completely blinding the milks to patients was not feasible for practical reasons and the different taste of the two milks. Of note, at the end of the study, the majority of the children had a positive DBPCFC also for GM, although none of them had been previously fed with GM-containing foods. We speculate that this may be due to the protein profile of GM that is quite similar to that of CM, as confirmed by the SDS-PAGE analysis.

This is not the first report documenting that Equidae' milk could be an appropriate alternative to CM, but is the first demonstration that AM is better tolerated than GM, which is in turn still largely used. In conclusion, our results suggest that GM should not be used in children with CMA (25) and AD, whereas AM, if available, may be an effective and safe alternative substitute.

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