Original Article

Reconfirmation of improved tolerance to a new amino acid-based formula by infants with cow's milk protein allergy

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Background and Objectives: Reasons for intolerance to commercial amino acid-based formulas (cAAF) in infants diagnosed with cow's milk protein allergy (CMA) remain unknown. We assume that minute amounts of proteins, presenting in the glucose polymers derived from corn starch (cGPs), can elicit the intolerance to the cAAFs observed in some infants with CMA. By replacing cGPs with glucose polymers derived from rice starch (rGPs), a new amino acid-based (nAAF) formula has been shown to be better tolerated than an existing cAAF. This study was carried out to corroborate the superiority of nAAF over a different commercially available cAAF. **Methods and Study Design:** Infants with CMA aged less than 4 months underwent a double-blind, placebo-controlled food challenge. They consumed each of the 2 test formulas for 14 days before switching to the other one. Following the 28-day challenge period, infants consumed the tolerated formula for 4 weeks as an at-home open challenge. **Results:** Out of 36 infants who completed the study, 18 were intolerant to the cAAF, seven of whom (38.8%) were also intolerant to the nAAF. Eleven of the 18 infants who were intolerant to the cAAF tolerated the nAAF ($p<0.01$). **Conclusions:** This study reconfirms that substitution of rGPS for cGPs in the amino acid-based formula improves tolerance of young infants with CMA.

Key Words: amino acid-based formula, corn glucose polymer, cow's milk protein allergy, immunotolerance, rice glucose polymer

INTRODUCTION

The prevalence of cow's milk protein allergy (CMA) has been increasing steadily. The diagnosis of CMA relies on subsidence of infants' symptoms after being fed with either an extensively hydrolyzed formula (eHF) or an amino acid-based formula.[1] In theory, the number of infants intolerant to a commercial amino acid-based (cAAF) formulas should be very low or nil. However, we have encountered a considerable number of infants who were intolerant to a cAAF during the past decade. One possible factor is that we may be biased as we work at the only center for the management of infants with CMA in the country. The difficult cases, therefore, come to our center for definite diagnosis and appropriate management of CMA.

As the double-blind, placebo-controlled food challenge (DBPCFC) is the gold standard for the diagnosis of CMA,[2] tolerance to the cAAF is an indicator of its hypoallergenicity. If an infant is intolerant to the cAAF, either the diagnosis of CMA cannot be made, or it is the wrong diagnosis. In this situation, we administer total parenteral nutrition (TPN) for at least 2 weeks as an additional tool to prove that the infant is intolerant to the cAAF.

As all cAAFs currently available contain glucose polymers derived from corn starch (cGPs), even minute amounts of proteins in the cGPs can elicit allergic reactions in sensitive infants with CMA. Therefore, we produced a nAAF in which the source of glucose polymer is rice starch, and eliminated the protein fraction before using such ingredient in this formula

We have demonstrated that this nAAF is tolerated by a significantly larger number of infants with CMA than a cAAF (Neocate®).[3] As another cAAF (Puramino Nutramigen®) marketed worldwide also contains glucose polymers from corn strach, we conducted the second double-blind, prospective, randomized, crossover, reference-controlled study in infants with CMA to compare the efficacy of a cAAF (Puramino Nutramigen®) to that of nAAF.

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Manuscript received 23 June 2016. Initial review completed 13 August 2016. Revision accepted 13 August 2016.
doi: 10.6133/apjcn.022017.14

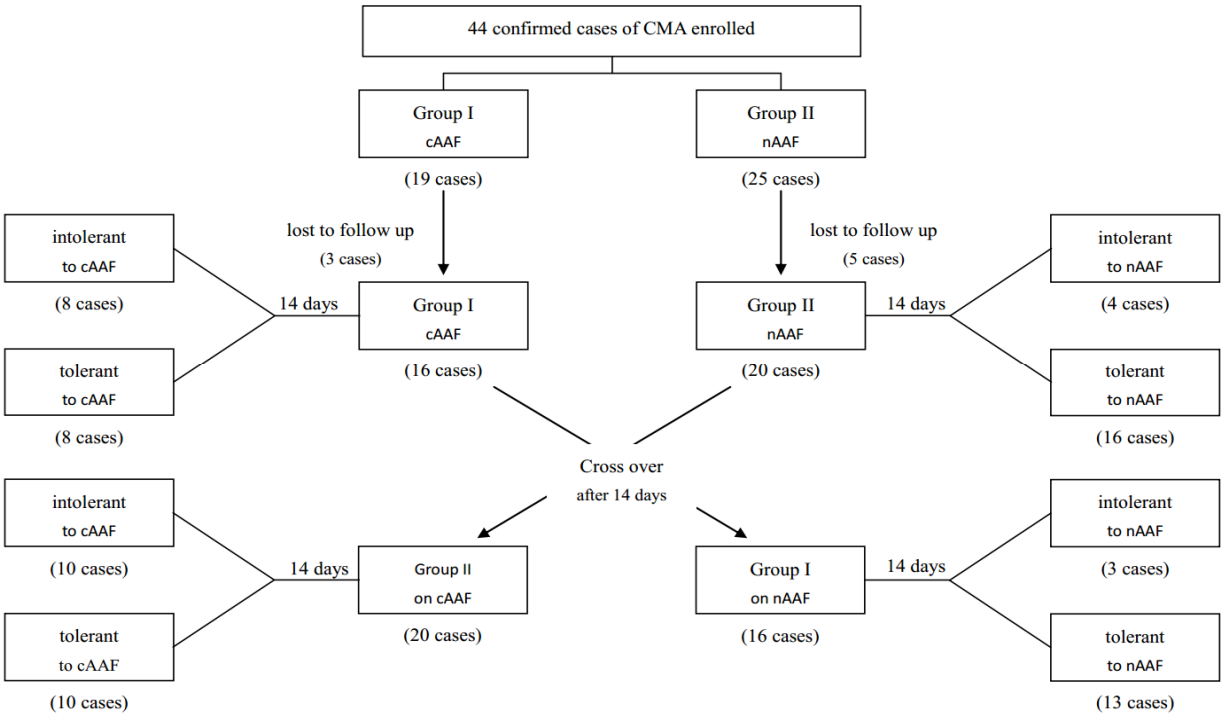

Figure 1. Scheme of the study and responses of infants to the commercial amino acid-based formula (cAAF) and the new amino acid formula (nAAF).

The aim of this study was to compare tolerance of the nAAF versus a different cAAF in the management of difficult cases of CMA in young infants.

MATERIALS AND METHODS

Subjects

The study is a double-blind, prospective, randomized, crossover, reference-controlled study. The detailed study was explained to the parents, who signed an informed consent. The protocol was approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University. Infants aged between 0 and 4 months suspected of having CMA had the diagnosis confirmed by a double-blind, placebo-control food challenge (DBPCFC) test performed in our hospital. Those who developed symptoms compatible with CMA while fed with infant formulas were placed on an elimination diet and, then were fed a commercially available amino acid-based formula (Neocate®). Infants whose symptoms persisted while being fed with this formula were given total parenteral nutrition (TPN). Once infants became symptom-free, they were randomized to receive either the nAAF or the cAAF. EHFs were not prescribed to these infants because most of them had histories of allergic reactions to eHF before being referred to our center.

Study design

Infants were recruited immediately after diagnosis of CMA was made. Infants were blindly and randomly assigned to receive either the cAAF or the nAAF for 14 days (Figure 1). Assignments were concealed in envelopes prepared before the study. If symptoms attributable to CMA occurred during that period, the formula was discontinued and TPN was given until the infant became symptom-free. Subsequently, the other test formula was given for a 14-day period. If the infant tolerated the formula assigned during the first 14-day period, the other formula was given for an additional 14-day period. If the infant did not tolerate the first formula but did tolerate the second one, that infant underwent a second challenge with the first formula to confirm the diagnosis of intolerance. However, if the infant showed any signs or symptoms of intolerance upon introduction of the second formula, this was discontinued. The process was then restarted by giving the first formula for 14 days. If there was no evidence of intolerance, the second formula was given for an additional 14 days. If the infant showed signs and/or symptoms of CMA similar to those of first challenge, the diagnosis of allergy to the second formula was confirmed. After completion of the second period of formula testing, parents decided to continue with either of the two amino acid formulas and, then, their infants ingested that formula for one month as an at-home opened challenge. Three experienced clinicians simultaneously assessed the patients for reactions of intolerance to the formulas during the DBPCFC and after the opened challenge.

Study formula

The nutrient composition of both formulas is shown in Table 1. Both formulas are suitable for young infants, according to the recommendations of an ESPGHAN coordinated international expert group and CODEX.[4,5] The only difference between the cAAF (Puramino Nutramigen®, Mead Johnson Nutrition, Evansville, IN, USA) and the nAAF is the source of glucose polymers, which are from corn and rice starch, respectively. We also eliminated most of the rice proteins contained in our rice starch before being hydrolyzed to GPs. The new rice-derived glucose polymers (rGPs) were analyzed by a high-

Table 1. Nutrient composition of cAAF and nAAF used in the study

Per 1,000 mL	cAAF	nAAF
Energy, kcal	670	680
Protein equivalent, g	19	15
Type	Amino acids	Amino acids
Fat, g	36	40
Carbohydrate		
Glucose polymers, g	70	70
Source	Corn starch	Rice starch
Sodium, mg	320	198
Potassium, mg	740	680
Chloride, mg	580	440
Calcium, mg	630	688
Phosphorus, mg	350	460
Magnesium, mg	74	70
Iron, mg	12	13
Zinc, mg	6.7	7
Iodine, mcg	100	400
Copper, mcg	510	600
Vitamin A, mcg	610	550
Vitamin D, mcg	8.5	20
Vitamin E, mg	12	17
Vitamin C, mg	81	78
Vitamin B-1, mcg	540	220
Vitamin B-2, mcg	610	850
Vitamin B-6, mcg	410	1688
Niacin, mg	6.8	8.8
Folic acid, mcg	108	158
Pantothenic acid, mg	3.4	4
Biotin, mg	0.02	0.02

cAAF: commercial amino acid-based formula; nAAF: new amino acid-based formula.

Table 2. Demographic data of infants with CMA who completed the study[†]

Characteristics	Value
Sex, male/female, n	22/14
Age, mean±SD, months	2.62±1.68
Symptoms	
Dermatological	32 (89)
Respiratory	32 (89)
Gastrointestinal	33 (92)
History of allergy in parents	
None	9 (25)
Father	6 (17)
Mother	10 (28)
Both	11 (30)
Complete blood count	
Hematocrit <34%	23 (64)
Eosinophil count (cells/mm3)	
<450	23 (64)
450-700	6 (17)
>700	7 (19)
Specific IgE to cow's milk protein	
Positive (≥0.35 kUA/L)	6 (17)
Negative (<0.35 kUA/L)	30 (83)

CMA: cow's milk protein allergy; IgE: immunoglobulin E; kUA/L: kilo allergen specific units per litre.
[†]Values are presented as number (%) unless otherwise indicated.

performance liquid chromatography, which showed to have most glucose polymers of not more than 10 molecules of glucose.

The proportions of amino acids in both formulas are similar to those in breast milk. However, total amino acid content is 19 g/L in the cAAF and 15 g/L in the nAAF. The fat in the cAAF is derived from sunflower, coconut and soya oils while those of the nAAF are from sunflower, palm and soya oils.

Statistical analysis

McNemar's test of equality of paired proportions with a 0.050 two-sided significance level was used in analysis. A sample size of 45 pairs would have an 80% power to detect a difference in proportions of 0.250 when the proportion of discordant pairs is expected to be 0.400. However after 36 pairs of cross-over study, we performed an interim analysis and stopped the study because there was a significant difference between the two formulas.

RESULTS

Forty-four infants with confirmed CMA were enrolled in the study between January 2014 and June 2015. Nineteen infants were assigned to begin the trial with cAAF (Group 1) and 25 with the nAAF (Group 2) (Figure 1). Three patients in group 1 and 5 in group 2 were lost to follow up. Sixteen patients in group 1 and 20 in group 2, respectively, completed the study.

The demographic data of the 36 patients are shown in Table 2. There were more male (22 infants) compared to female (14 infants). The dermatological, respiratory and gastrointestinal symptoms accounted for 89%, 89% and 92%, respectively. Thirty percent of this study group had a history of allergy in both parents, while 45% had in one of the parents. Thirty-six percent of the group had peripheral blood eosinophilia (absolute eosinophil count greater than 450 cells/mm^3) and 64% had anemia (hematocrit less than 34%). Specific IgE of cow's milk protein was positive in 17% of the study group.

After completion of the double-blind, cross-over study, 18 patients were found to be intolerant to cAAF while only 7 to the nAAF (p<0.01) (Table 3). All patients who were intolerant to the nAAF were also intolerant to the cAAF.

Symptoms and laboratory data of patients who were intolerant to the formulas are shown in Table 3. Patients who were intolerant to both formulas had almost identical symptoms. There were 8 patients and 6 patients who had eosinophilia and positive tests for specific IgE to cow's milk protein, respectively.

DISCUSSION

The results of this study are similar to those of our previous one in which our product was compared to a different commercially available AAF (Neocate®)[3]. In this study the comparison was against Puramino Nutramigen®. One of the components commonly found in both cAAF are glucose polymers derived from corn starch. It is most likely that trace amounts of proteins might be the cause of the allergic symptoms in infants who are intolerant to the cAAF.[6]

Sopo et al[7] reported a 7-month-old infant with food protein-induced enterocolitis symptoms allergic to corn. Periodically, corn has been reported to be the cause of allergy in infants.[8,9] Only one of the proteins in the kernels, called the lipid transfer protein, has been firmly de-

Table 3. Symptoms and laboratory results of subjects who were intolerant to nAAF and cAAF

Number	Age (months)	Symptoms of intolerance*		Peripheral blood eosinophilia ≥450 cells/mm^3	Specific IgE to cow's milk protein ≥0.35 kUA/L
		nAAF	cAAF		
1	1	-	Vomiting, constipation	Negative	Negative
2	2	MPR, AS	Constipation, MPR	Negative	Negative
3	4	-	MPR, constipation	Positive	Positive
4	4	-	MPR, constipation, AS	Negative	Negative
5	4	MPR, AS	MPR, AS	Negative	Negative
6	2	-	MPR, rhinorrhea, colic	Negative	Negative
7	1	MPR,MBS, AS	AS	Positive	Negative
8	3	MPR, AS, constipation	MPR, constipation	Positive	Positive
9	4	-	MBS, MPR, AS	Negative	Negative
10	3	-	MPR	Negative	Negative
11	2	AS, bloating	Constipation	Positive	Positive
12	2	-	MPR, constipation	Positive	Negative
13	4	-	MPR, constipation	Positive	Positive
14	4	AS, MPR	MPR	Negative	Negative
15	2	-	AS, MPR	Positive	Positive
16	1	Constipation, vomiting	Constipation, vomiting	Negative	Negative
17	3	-	MPR	Positive	Positive
18	3	-	Constipation	Negative	Negative

cAAF: commercial amino acid-based formula; nAAF: new amino acid-based formula; AS: airway secretion; MBS: mucous-bloody stool; MPR: maculopapular rashes.
*Number of infants who were intolerant to nAAF was significantly less than those who are intolerant to cAAF ($p<0.01$).

termined to be the allergen involved in corn allergy.[10] This protein was first identified as an important allergen in fruits, but it is also present in nuts, various vegetables and in cereals.[11,12] Nevertheless, other proteins that may contaminate food ingredients in the cAAF should be carefully searched.

Although the process of producing glucose polymers and getting rid of rice protein from rice starch for the nAAF used in this study is very meticulous, there may be trace amounts of rice proteins remaining. These trace amounts of rice proteins may be the cause of allergic reactions in very sensitized infants. However, as in the previous study[3] by using this nAAF, we were able to reduce the cases of intolerance to the cAAFs to less than 30%.

The reasons why some infants were still intolerant to the nAAFs cannot be explained by our study. There is the possibility that we enrolled infants with complex CMA. Nevertheless, the nAAF is well tolerated by up to 70% of the cases who were intolerant to the tested cAAF.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Suthipol Udompunthurak for statistical assistance and Dr Carlos Lifschitz for manuscript review.

AUTHOR DISCLOSURES

The authors declare that they have no conflict of interest. This study was funded by the Siriraj Research Development Fund and a Chalermphrakiat Grant from the Faculty of Medicine Siriraj Hospital, Mahidol University.

REFERENCES

1. Rosenthal E, Schlesinger Y, Birnbaum Y, Goldstein R, Benderly A, Freier S. Intolerance to casein hydrolysate formula. Clinical aspects. Acta Paediatr Scand. 1991;80: 958-60.
2. Kleinman RE. Cow milk allergy in infancy and hypoallergenic formulas. J Pediatr. 1992;121:S116-21.
3. Jirapinyo P, Densupsoontorn N, Kangwanpornsiri C, Wongarn R, Tirapongporn H, Chotipanang K, Phuangphan P. Improved tolerance to a new amino acid-based formula by infants with cow's milk protein allergy. Nutr Clin Pract. 2016;31:824-8.
4. Koletzko B, Baker S, Cleghorn G, Neto UF, Gopalan S, Hernell O et al. Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. J Pediatr Gastroenterol Nutr. 2005;41:584-99.
5. Codex, Alimentarius. CODEX Standard 72 on infant formula 1981. Revision. 2007. [cited 2016/04/28]; Available from: http://www.codexalimentarius.net/download/standards/288/cxs-072e.pdf.
6. Frisner H, Rosendal A, Barkholt V. Identification of immunogenic maize proteins in a casein hydrolysate formula. Pediatr Allergy Immunol. 2000;11:106-10.
7. Sopo SM, Filoni S, Giorgio V, Monaco S, Onesimo R. Food protein-induced enterocolitis syndrome (FPIES) to corn: a case report. J Investig Allergol Clin Immunol. 2012;22:391-2.
8. Sanchez-Borges M, Fernandez-Caldas E. Hidden allergens and oral mite anaphylaxis: the pancake syndrome revisited. Curr Opin Allergy Clin Immunol. 2015;15:337-43. doi: 10.1097/ACI.0000000000000175.
9. Maniu CM, Faupel U, Siebenhaar G, Hunzelmann N. Maize: a new occupational allergen in the pharmaceutical industry. Allergy. 2010;65:930-1. doi: 10.1111/j.1398-9995.2009.02264.x.
10. Pastorello EA, Farioli L, Pravettoni V, Ispano M, Scibola E, Trambaioli C et al. The maize major allergen, which is responsible for food-induced allergic reactions, is a lipid transfer protein. J Allergy Clin Immunol. 2000;106:744-51. doi: 10.1067/mai.2000.108712.
11. Zuidmeer L, van Ree R. Lipid transfer protein allergy: primary food allergy or pollen/food syndrome in some cases. Curr Opin Allergy Clin Immunol. 2007;7:269-73. doi: 10.1097/ACI.0b013e32814a5401.
12. Kuppannan K, Albers DR, Schafer BW, Dielman D, Young SA. Quantification and characterization of maize lipid transfer protein, a food allergen, by liquid chromatography with ultraviolet and mass spectrometric detection. Anal Chem. 2011;83:516-24. doi: 10.1021/ac102201m.