Original Article

Chicken-based formula is better tolerated than extensively hydrolyzed casein formula for the management of cow milk protein allergy in infants

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The effective treatment of cow milk allergy in infants consists of elimination of cow milk protein and the introduction of formulas based on an extensively hydrolyzed protein formula or an amino acid-based formula. However, about 10% of these infants are still allergic to an extensively hydrolyzed protein formula and an amino acid-based formula is very expensive. We conducted a study to verify whether the new chicken-based formula will be better tolerated than an extensively hydrolyzed protein formula for the treatment of cow milk allergy in infants. One hundred infants, diagnosed with cow milk allergy by double-blind, placebo-controlled food challenge tests, were enrolled in a double-blind, randomized, cross-over study to compare a response to an extensively hydrolyzed protein formula and the chicken-based formula. Subjects were randomly given one of the two formulas for 2 weeks. There was a 2-week washout period of taking an amino acid-based formula before being switched to the other formula for another 2 weeks. If the subjects showed allergic symptoms during the 2 weeks of test formula, they would be announced as intolerance or allergic to that formula. Sixty seven of 80 confirmed subjects agreed to enroll their infants. Fifty-eight subjects completed the study. Twenty and 33 infants were tolerant whereas and 38 and 25 infants were intolerant to an extensively hydrolyzed protein formula and the chicken-based formula, respectively. The chicken-based formula showed significantly better tolerance than an extensively hydrolyzed protein formula in the management of cow milk allergy in infants.

Key Words: chicken-based formula, cow milk allergy, extensively hydrolyzed protein formula, infant, Thailand

INTRODUCTION

It is estimated that about 3% of all infants will suffer from cow milk allergy (CMA) within the first year of life.[1] The most common alternatives to cow milk for infants with CMA are soy milk and extensively hydrolyzed cow milk protein formula (EHF). Bishop *et al* demonstrated that 10 to 40% of children with CMA cannot tolerate soy products.[2] Bhatia *et al* on behalf of the Committee on Nutrition of the American Academy of Pediatrics, concluded that for infants with documented cow milk protein allergy, EHF should be firstly considered as a therapeutic formula, because 10 to 14% of these infants will also have soy protein allergy.[3]

Other alternatives – goat milk or a partially hydrolyzed formula – have shown less impressive results for treatment of CMA in infants.[4,5] The significant homology among milks from cows, sheep and goats results in clinical cross-reactivity.[6,7] Caffarelli *et al* determined the allergenicity of three cow's milk hydrolysates and an amino acid-derived formula in children with CMA.[8] They showed that partially hydrolyzed whey formula elicited a significantly higher number of positive skin prick test reactions than other formulas. None of the cow's milk substitutes were found to be non-allergenic.

Due to its hypoallergenicity, EHF has been widely recommended as the primary formula for the treatment of CMA in children.[3,5] However, allergic reactions to EHF have been widely reported.[9-11] Parents of infants with CMA who are allergic to EHF are then advised to feed them an amino acid-based formula (AAF) as the last option for treatment.[12,13]

Due to its high cost, most families cannot afford to buy AAF for long-term consumption for their children. We then produced a chicken-based formula (CBF) as an alternative formula for these infants. Chicken protein has rarely been reported to be a causative agent for allergic reactions, and it is readily available in all countries. Larcher *et al* reported on a group of infants with protracted diarrhea who were fed a comminuted chicken diet and showed satisfactory tolerance.[14] In a randomized controlled comparison of a comminuted chicken diet with an elemental formula based on hydrolyzed lactalbumin, Godard *et al* observed comparable recovery times for diar-

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rhea in both groups, with a clinical success rate in excess of 20%.[15] Recently in a randomized, double-blind, cross-over study, we demonstrated that CBF exhibited 8 times better results than a soy-based formula in the treatment of CMA in infants.[16]

CBF has been used in our center for several years as an alternative formula for the management of CMA in infants, and most of the parents have been very satisfied. The present study is conducted to experimentally verify whether CBF will be better tolerated than EHF for the treatment of CMA in infants.

MATERIALS AND METHODS

This is a prospective, randomized, crossover, reference-controlled study between EHF and CBF in the treatment of infants with CMA. Infants aged between 1 and 12 months old who were suspected of having CMA were diagnosed for possible CMA by a DBPCFC test. After CMA was positively diagnosed, the infant was recruited into the study. After explaining the study details and obtaining signed, informed consent from one of the parents, the infant was then given AAF for at least 14 days to clear the symptoms of CMA. After the subjects were determined to be free of symptoms of CMA, he or she received either CBF or EHF for the first 14 days. The subjects were then washed out for 14 days with AAF, after which he or she received the second formula for another 14 days.

The EHF, which was used as a control formula in this study, is the only commercially available product of this type in Thailand (Nutramigen®, Mead Johnson, USA). The CBF used is a product produced in our kitchen under aseptic conditions, and was used as reference formula. Details of CBF preparation are described in a previous publication.[16] Briefly, chicken breasts bought from the Charoen Pokphand Co (Thailand), which has exported chicken meat worldwide, were homogenized using a special technique until the meat was finely ground; then all nutrients were immediately added and the product rapidly frozen before being used in the study. A bacteriological study of the CBF was regularly performed to ensure the safety of the infants.

The number of subjects who did not accept each formula was considered for statistical significance in terms of palatability of the formulas. Nutrition contents of both EHF and CBF were listed in Table 1. The AAF used in this study was a locally available commercial formula (Neocate®, Nutricia, USA).

The subjects' complete blood count and specific IgE to cow milk protein were checked on the first day of the study. Body weights and lengths were measured at day 0 and day 15 of each formula period. The amount of test formula intake per day was recorded and reported to one of our investigators. If the assigned formula taken was less than 50 ml/kg/d after 48 hours of the study, it was interpreted that the formula was not accepted by the infant and the subjects was excluded from the study.

Subjects who accepted the tastes of both formulas were then given either one of the formulas for 14 days. Then, they were washed out by taking the amino acid-based formula for 14 days. After that the infants were crossed-over and fed the other formula for another 14 days. While taking each formula, if any one of the subjects showed signs or symptoms related to CMA, he/she was consid-

Table 1. Compositions of EHF and CBF used in the study†

Per (100 ml)		EHF	CBF
Energy	(kcal)	67	67
Protein	(g)	1.9	2
type		extensive casein hydrolysate	intact chicken protein
Fat	(g)	2.6	4
Carbohydrate	(g)	9.1	7
Sodium	(mg)	32	42
Potassium	(mg)	74	68
Chloride	(mg)	45	66
Calcium	(mg)	83	90
Phosphorus	(mg)	42	46
Magnesium	(mg)	7	7
Iron	(mg)	0.5	1.3
Zinc	(mg)	0.4	0.9
Iodine	(µg)	4.7	44
Copper	(µg)	6.3	60
Vitamin A	(µg)	50	80
Vitamin D	(µg)	0.9	1.7
Vitamin E	(mg)	4	0.8
Vitamin C	(mg)	6	12
Vitamin B1	(µg)	36	90
Vitamin B2	(µg)	63	90
Vitamin B6	(µg)	42	50
Niacin	(mg)	8.2	1.1
Folic acid	(µg)	10	14
Pantothenic acid	(µg)	3.2	0.4
Biotin	(µg)	0.1	1.8

† EHF = extensively hydrolyzed casein formula; CBF = chicken-based formula

Table 2. Characteristics of 58 infants with cow milk allergy who completed the study

Characteristics	Number (%)
Sex (male:female)	39:19*
Age (months, mean±SD)	6.5 ± 4.1
Weight (g)	7340±1567
Length (cm, mean±SD)	66.5±6.6
Presenting symptoms	
Respiratory	47/58 (81)
Dermatological	41/58 (71)
Gastrointestinal	39/58 (67)
History of allergy in parents	
Negative	20/58 (34)
Positive	38/58 (66)
Complete blood count	
Anemia (hematocrit <34%)	27/58 (47)
Eosinophilia (>700/mm^3)	12/58 (21)
Specific IgE to cow milk protein(>0.3 KUA/L)	8/58 (14)

*Significant difference (p = 0.009)

ered intolerant or allergic to that formula. In contrast, if the subjects were thriving well with a particular formula and did not have any allergic symptoms, they were considered to be tolerant to the formula. When the subjects were considered intolerant to the formula, they would be washed out of the symptoms by taking the amino acid formula for 2 weeks before being switched to the second blinded formula for another 2 weeks.

Parents were contacted daily by one of our investigators, and were asked about adverse symptoms of the subjects. After two complete crossover studies, the codes of both formulas were then opened. The investigators would then recommend an appropriate formula for the parents.

The study protocol was approved by the Ethical Committee on Research Involving Human Subjects, Faculty of Medicine, Siriraj Hospital, Mahidol University.

Two independent Student's *t*-tests were used to compare the data between each group. A chi-square test was used to compare the numbers of subjects who accepted or did not accept the tastes of the formulas, as well as clinical tolerance and intolerance to EHF and CBF. Significant difference between the two groups of each formula was set at $p<0.05$.

RESULTS

Table 2 shows demographic data of all 58 subjects who completed the study. More males than females participated in the study (p=0.009). The mean and standard deviations of the ages of the subjects in the study group were 6.5±4.1 months. Most subjects presented with more than one system of symptoms; 81%, 71% and 67% had respiratory, dermatological and gastrointestinal symptoms, respectively. Thirty-four percent of the parents had no history of allergy, while the rest had histories of allergy to varying degrees. Of the subjects, 47% were anemic and 21% had eosinophilia, while only 14% of the study group tested positive for specific IgE to cow milk protein.

As shown in Figure 1, 100 subjects suspected of CMA

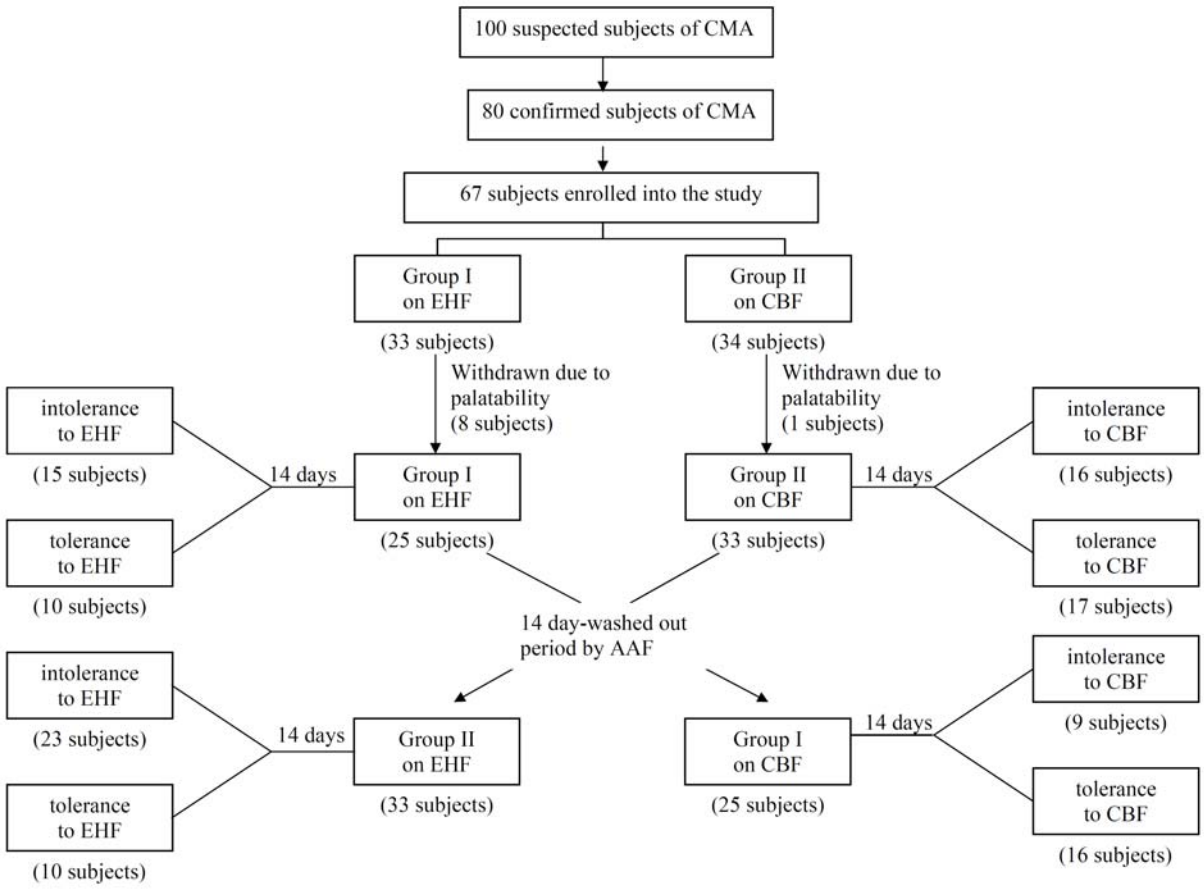

Figure 1. Schematic procedure and responses of infants to extensively hydrolyzed casein formula (EHF) and chicken-based formula (CBF)
AAF = amino acid-based formula

had DBPCFC tests performed to diagnose CMA; 80 subjects were confirmed as being CMA positive. However, only 67 subjects agreed to enroll the study. Of these, 8 and 1 subjects did not accept the tastes of EHF and CBF, respectively. Thus, the subjects accepted the taste of CBF significantly more than that of EHF (p=0.04). Ultimately, 58 subjects completed the crossover study.

Figure 1 also shows details of responses of the subjects to the test formulas. Twenty-five subjects were allocated into Group I to first receive EHF for 14 days. Fifteen subjects in this group were intolerant to EHF. Of the 33 subjects in Group II who were given CBF initially, 16 showed intolerance to CBF, while the other 17 subjects in Group II showed tolerance to CBF. When Group I (25 subjects) was crossed-over to take CBF, 9 subjects showed intolerance to CBF. When Group II members (35 subjects) were crossed-over to receive EHF, 23 subjects showed intolerance to EHF while the other 10 subjects were tolerant to EHF. In summary, 38 and 20 subjects showed intolerance and tolerance to EHF respectively; while 25 and 33 subjects showed intolerance and tolerance to CBF, respectively. CBF was thus significantly more tolerated than EHF in this study (p = 0.02).

DISCUSSION

An extensively hydrolyzed cow milk protein formula (EHF) has been shown to be effective in reducing the incidence of CMA, and is also recommended for use in the management of CMA in infants.[17] However, there are two serious problems that frequently occur when using EHF. Firstly, highly allergic infants react to even the very low amount of residual allergens in EHF.[9,10] Secondly, EHF has a bitter taste which is probably rejected by older infants when it is introduced. An amino acid-based formula (AAF) then can solve most of the first problem, but sometimes it cannot solve the second problem because it also has a bitter taste.[18] Another problem occurs when using AAF, is that it is very expensive. The majority of parents in developing countries cannot afford long-term use of AAF for their infants.

Chicken-based formula developed by Jirapinyo is designed to be used as an alternative formula for management of CMA.[16] The composition of this CBF complied with the guidelines of CODEX and the European Regulation for Infant Formula.[19] Chicken-based formula is better tasting than EHF, based on this study, and can be used by infants who are allergic to EHF. Moreover, the price is much cheaper than AAF.

In this study, CBF was proven to be more effective than EHF when fed to infants with CMA. Many subjects did not accept the taste of EHF and also showed greater intolerance to EHF than CBF. Since our center is known by many parents as the center for management of CMA in infants in Thailand, most of the subjects we encounter are more severe in terms of clinical appearance and more highly allergic to other food proteins. Surprisingly, we have very low rate of positive tests of specific IgE to cow milk protein which is contrast to the severity of symptoms in our subjects. However, this figure is almost the same as in our previous study.[16] It may be explained that most of our subjects may have delayed-type hypersensitivity to cow milk protein. It is essential to perform double-blind and crossover food challenge tests to determine which of the two formulas performs better. Also, the parents can then inform the investigators without bias as to which of the test formulas they would prefer their infants to take after the study.

Furthermore, it is imperative to wash out the subjects for 14 days with AAF before taking any test formulas. The subjects should exhibit no signs or symptoms of CMA before taking the test formulas. Thereafter it will be easier to detect any allergic reactions from the test formula. Also it is important to have the subjects take the test formulas for 14 days, since some allergic reactions may develop many days later. However in some extreme subjects, they may exhibit allergic symptoms after 2 weeks. In that case, we have to re-evaluate them after the study.

In conclusion, CBF has been shown to be more effective than EHF when used as a substitute formula in the management of CMA in infants.

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AUTHOR DISCLOSURES

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REFERENCES

1. Venter C, Pereira B, Voigt K, Grundy J, Clayton CB, Higgins B, Arshad SH, Dean T. Prevalence and cumulative incidence of food hypersensitivity in the first 3 years of life. Allergy. 2008;63:354-9.
2. Bishop JM, Hill DJ, Hosking CS. Natural history of cow milk allergy: clinical outcome. J Pediatr. 1990;116:862-7.
3. Bhatia J, Greer F, Committee on Nutrition (American Academy of Pediatrics). Use of soy protein-based formulas in infant feeding. Pediatrics. 2008;121:1062-8.
4. Tavares B, Pereira C, Rodrigues F, Loureiro G, Chieira C. Goat's milk allergy. Allergol Immunopathol. 2007;35:113-6.
5. Giampietro PG, Kjellman NI, Oldaeus G, Wouters-Wesseling W, Businco L. Hypoallergenicity of an extensively hydrolyzed whey formula. Pediatr Allergy Immunol. 2001;12:83-6.
6. Ah-Leung S, Bernard H, Bidat E, Paty E, Rancé F, Scheinmann P, Wal JM. Allergy to goat and sheep milk without allergy to cow's milk. Allergy. 2006;61:1358-65.
7. Järvinen KM, Chatchatee P. Mammalian milk allergy: clinical suspicion, cross-reactivities and diagnosis. Curr Opin Allergy Clin Immunol. 2009;9:251-8.
8. Caffarelli C, Plebani A, Poiesi C, Petroccione T, Spattini A, Cavagni G. Determination of allergenicity to three cow's milk hydrolysates and an amino acid-derived formula in children with cow's milk allergy. Clin Exp Allergy. 2002;32: 74-9.
9. Wahn U, Wahl R, Rugo E. Comparison of the residual allergenic activity of six different hydrolyzed protein formulas. J Pediatr. 1992;121:80S-4S.
10. Rosendal A, Barkholt V. Detection of potentially allergenic material in 12 hydrolyzed milk formulas. J Dairy Sci. 2000; 83:2200-10.
11. Kaczmarski M, Wasilewska J, Lasota M. Hypersensitivity to hydrolyzed cow's milk protein formula in infants and young children with atopic eczema/dermatitis syndrome

with cow's milk protein allergy. Rocz Akad Med Bialymst. 2005;50:274-8.
12. Sicherer SH, Noone SA, Koerner CB, Christie L, Burks AW, Sampson HA. Hypoallergenicity and efficacy of an amino acid-based formula in children with cow's milk and multiple food hypersensitivities. J Pediatr. 2001;138:688-93.
13. de Boissieu D, Dupont C. Allergy to extensively hydrolyzed cow's milk proteins in infants: safety and duration of amino acid-based formula. J Pediatr. 2002;141:271-3.
14. Larcher VF, Shepherd R, Francis DE, Harries JT. Protracted diarrhoea in infancy. Analysis of 82 cases with particular reference to diagnosis and management. Arch Dis Child. 1977;52:597-605.
15. Godard C, Bustos M, Muñoz M, Nusslé D. Value of a chicken-based formula for refeeding of children with protracted diarrhea and malnutrition in a developing country. J Pediatr Gastroenterol Nutr. 1989;9:473-80.
16. Jirapinyo P, Densupsoontorn N, Wongarn R, Thamonsiri N. Comparisons of a chicken-based formula with soy-based formula in infants with cow milk allergy. Asia Pac J Clin Nutr. 2007;16:711-5.
17. Niggemann B, von Berg A, Bollrath C, Berdel D, Schauer U, Rieger C, Haschke-Becher E, Wahn U. Safety and efficacy of a new extensively hydrolyzed formula for infants with cow's milk protein allergy. Pediatr Allergy Immunol. 2008; 19:348-54.
18. Pedrosa M, Pascual CY, Larco JI, Esteban MM. Palatability of hydrolysates and other substitution formulas for cow's milk-allergic children: a comparative study of taste, smell, and texture evaluated by healthy volunteers. J Investig Allergol Clin Immunol. 2006;16:351-6.
19. 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.

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牛奶蛋白過敏兒對於雞肉配方的耐受性優於高度水解酪蛋白配方

對於牛奶過敏兒治療之組成配方，主要是將牛奶蛋白去除，改以高度水解蛋白配方或是胺基酸配方取代之。然而，使用高度水解蛋白配方之過敏兒仍約有10%未得到改善，而胺基酸配方非常昂貴。因此，本研究欲釐清，對牛奶過敏兒來說，是否使用新的雞肉配方相對於高度水解蛋白配方有較好的耐受性。共招募 100 位牛奶過敏兒，先經過双盲、安慰劑對照的食物攝取測試，以確定診斷。利用双盲、隨機的交叉實驗設計比較攝食高度水解蛋白配方與雞肉配方之過敏兒反應。首先受試者被隨機分派至其中一種配方組別中，持續 2 個星期，接著有 2 星期的洗滌期，在此期間內所有受試者皆攝取胺基酸配方。洗滌期過後，再交換使用另一組不同的配方，一樣維持 2 星期。若受試者在接受測試配方的期間，出現過敏症狀，則會被認為對於此配方有耐受不良或過敏的情形。在 80 位確診的受試者中，有 67 位的父母同意配合實驗進行，而最後共有 58 位完成試驗。結果發現，共 38 位過敏兒對於高度水解蛋白配方產生耐受不良，而有 25 位對於雞肉配方耐受不佳。因此本篇結論為，對牛奶過敏兒而言，雞肉配方相較於高度水解蛋白配方，其耐受性明顯較佳。

關鍵字：雞肉配方、牛奶蛋白過敏、高度水解蛋白配方、嬰兒、泰國