

Effect of disodiumcromoglycate on intestinal permeability changes and clinical response during cow's milk challenge

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Oral disodiumcromoglycate (DSCG) has been used for many years in the prevention of food allergic reactions. The reported effectiveness varies widely and little is known about the mode of action. The aim of this study was to evaluate the effect of DSCG pretreatment (2×100 mg) on intestinal permeability (IP), as measured with a sugar absorption test (SAT), in relation to the clinical response during food challenge in 30 children suspected of cow's milk allergy. In the SAT the urinary Lactulose/Mannitol (L/M) ratio was measured after oral administration of these compounds. DSCG pretreatment did not alter the number of clinically positive challenges. Children with clinically positive challenges showed a significant increase in the L/M ratio with placebo pretreatment as compared to children with clinically negative challenges ($p = 0.0008$). This difference was reduced to nonsignificant levels after DSCG pretreatment ($p = 0.07$). We conclude that DSCG in this dosage probably reduces the local intestinal response but does not prevent the extra-intestinal reactions.

**R. M. Van Elburg¹, H. S. A. Heymans¹,
J. G. R. De Monchy²**

¹Beatrix Children's Hospital, ²Department of Allergology, University Hospital of Groningen, The Netherlands

Ruurd M. Van Elburg, Beatrix Children's Hospital, University of Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands

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When the diagnosis of food allergy is established, avoidance is the treatment of choice (1). However, sometimes symptoms persist despite optimal dietary treatment or because dietary treatment is impossible or impractical (1,2). In such situations pharmacologic agents can be used for the local treatment of the skin and airways but this may be insufficient to control symptoms in the face of ongoing allergen exposure. Systemic agents provide a theoretical opportunity to intervene earlier in food allergic reactions by local action at the intestinal level as well as systemic effects on target organs. A variety of drugs, such as disodiumcromoglycate, ketotifen and other antihistamines, steroids, aspirin and other non-steroids are advocated in the treatment of food allergy (1,3). Of these, disodiumcromoglycate (DSCG), which is considered a safe drug with very few side-effects, has been studied most extensively (1–8). Most of these studies examined clinical responses to food challenges, and results have been conflicting: in some studies DSCG prevented clinical reactions during food challenge while in other studies it had no effect (1). Differences in study design, patient characteristics

and dosage of DSCG may account in part for the observed differences in outcome. However, further analysis of this work is hampered by a lack of understanding of the site and exact mode of action of DSCG, which have not yet been elucidated.

Jalonen recently presented data which indicate that intestinal permeability (IP) is altered after 3 days of cow's milk challenge in children with cow's milk allergy (CMA) irrespective of the clinical symptoms (9). Several tests have been developed to measure IP using different probes: polyethylene glycols (PEG), ⁵¹Cr-EDTA and sugars (10–12). We use the differential sugar absorption test (SAT) with two sugars of different molecular size as molecular probes. Results are expressed as a ratio between the absorption of both sugars. Compared to other methods this test is less sensitive for errors in urine collection, differences in gastric retention, intestinal dilution, rapid transit time and impaired renal excretion (12, 13). The IP for large molecules (>0.5 nm, e.g. lactulose, cellobiose) is increased by small intestinal damage whereas the IP for small molecules (<0.4 nm, e.g. mannitol, L-rhamnose) is unchanged or decreased. Therefore slight

Table 1. Patient characteristics. TOT IgE = IgE level, IgE Milk = IgE to cow's milk (RAST), Eo's = total peripheral eosinophil count

Patient (n°)	Age (months)	TOT IgE (IU/ml)	IgE MILK (PRU/ml)	Eo's (10 ⁶ /L)
01	28.0	3	< 0.35	191
02	57.0	3110	>17.5	ND
03	98.5	28	< 0.35	121
04	31.0	828	1.9	3685
05	20.0	355	1.1	44
06	45.0	84	< 0.35	990
07	51.0	149	< 0.35	704
08	15.0	741	1.1	110
09	17.0	50	2.2	ND
10	14.0	2	< 0.35	902
11	86.0	833	1.3	462
12	15.0	35	< 0.35	660
13	52.0	14	< 0.35	220
14	5.0	1	< 0.35	143
15	27.5	35	< 0.35	397
16	51.0	6	< 0.35	286
17	12.0	8	< 0.35	3179
18	30.0	2972	0.9	143
19	6.5	4	< 0.35	209
20	46.0	23300	>17.5	1947
21	8.0	7	< 0.35	44
22	4.5	4	< 0.35	741
23	13.0	8	< 0.35	88
24	24.0	259	3.3	209
25	74.0	297	2.6	1122
26	120.0	380	1.0	396
27	64.5	5782	13.5	506
28	30.0	100	8.6	297
29	35.5	19	< 0.35	242
30	42.0	2831	11.2	1386

changes in both result in a more significant change in the ratio, making it a more sensitive test for IP (12–16).

In addition to being a marker of clinically positive reactions in children with food allergy undergoing food challenge, changes in IP could be involved in the mechanism of food allergic reactions. An increased IP for macromolecules might reflect a reduced intestinal barrier function which may lead to increased uptake of protein antigens with subsequent sensitisation for these antigens. An ongoing local allergic inflammatory reaction may enhance IP for antigens for which sensitisation has already taken place and could cause aggravation of the symptoms. Hence, measuring IP with the SAT in relation to clinical responses with and without DSCG pretreatment may provide insight into the events following food challenge in responsive individuals.

The aim of this study was thus to evaluate the effect of pretreatment with oral DSCG (Nalcrom®)

versus placebo on IP, as measured with the SAT, during food challenges in relation to the clinical response.

Patients and methods

Thirty children, 9 girls and 21 boys, visiting the outpatient department of Paediatrics and suspected of CMA entered the study. Median age was 30.0 months, range 4.5–120 months. The study was approved by the hospital medical ethical committee. Written informed consent for the study was given by the parents. A child was eligible for entry to the study if the clinical history (including the family history) and physical examination were suggestive of CMA. The most frequently recorded symptoms were skin- (eczema, rash, occasionally hives), gastrointestinal- (vomiting, diarrhoea, colic) and/or respiratory symptoms (rhinitis, wheezing, asthma) while taking a diet containing cow's milk. Often this was accompanied by general signs or symptoms including failure to thrive and irritability. One child had only gastrointestinal symptoms which did not improve on a lactose free diet. A clinical improvement by elimination of cow's milk from the diet, documented by a physician, was required to enter the study and all children remained on this diet during the study. Those with a history of suspected anaphylaxis to cow's milk or adverse reactions to DSCG were excluded.

Patient characteristics are given in Table 1. Except for patients 27 and 30, who were on maintenance of Ketotifen, no child used oral antihistamines, steroids or cromoglycate during the study. Blood samples for total IgE (TOT IgE) and IgE RAST against cow's milk (IgE MILK) and eosinophils (Eo's) were taken from all children when on the cow's milk-free diet.

Fifteen healthy children with no signs or symptoms of gastrointestinal disease, not known to have suffered from (food) allergies and asymptomatic while taking a diet containing cow's milk served as controls. Mean age and sex ratio of the controls was not significantly different from the patients.

Sugar absorption test (SAT)

The SAT was performed using a solution containing 2 g of mannitol, 5 g of lactulose and 40 g of sucrose with demineralized water made up to a volume of 100 ml, with an osmolarity of 1650 mosm/L. After an overnight fast (of at least 4 h for children under 2 years of age and 8 h for older children) each child received 2 ml/kg of the solution, up to a maximum of 100 ml. Urine was collected over a 5-h period, to which 0.5 ml chlorohexidine digluconate 20% was added as a deconta-

Table 2. List of symptoms scored at cow's milk challenge. Major symptoms count for 2 points each, minor symptoms for 1 point. A challenge is considered clinically positive if ≥ 2 points are scored

Clinical symptoms	
Major symptoms	Minor symptoms
Eczema	Itching
Hives	Nausea
Rash	Abdominal pain, colic
Angioedema	Feverish feeling
Conjunctivitis	Swollen feeling in throat
Dyspnoea, wheezing	Arthralgia
Coughing	
Rhinitis	
Vomiting	
Diarrhoea	
Anaphylaxis	

minant. All children continued to fast for 2 h after drinking the solution. The urine volume was measured and aliquots were stored frozen at -20°C . In order to calculate the lactulose/mannitol ratio (L/M) samples were analyzed for lactulose and mannitol by gas chromatography as previously described (17). For quality control the variation between 35 consecutive series was measured at three levels and was found to be (mean $\pm$ SD): level 1: 0.00823 ± 0.00108 , level 2: 0.0612 ± 0.0069 and level 3: 0.1201 ± 0.0144 .

The L/M ratio measured on a separate day before challenge while on a cow's milk free diet (L/M_{baseline}) and the L/M ratio during challenge ($L/M_{\text{challenge}}$) were used to calculate the change in L/M ratio ($\Delta L/M$) with the formula:

$$\Delta L/M = \frac{(L/M_{\text{challenge}} - L/M_{\text{baseline}})}{L/M_{\text{baseline}}} \times 100\%$$

Food challenges

Food challenges were performed with whole cow's milk in children older than 2 years of age. A formula, Nutrilon Premium® (Nutricia BV Holland), was used in younger children, as they had reacted to this type of feeding in the past. The cow's milk challenge was a slightly modified version of the method described by Falth-Magnusson et al. (8): On the challenge day the child was given the test solution. After 5 min the child received 2 ml of cow's milk/formula with 3 ml of water. If no severe reaction occurred, then the child received 10, 20 and finally 50 ml of cow's milk/formula at intervals of 20 min. With the exception of the challenge liquids, the child continued to fast for 2 h after drinking the test solution.

Drug administration

DSCG (Nalcrom®) was provided by Fisons BV Holland. Our hospital pharmacy provided the placebo (sucrose). Each patient received 2 times 100 mg of DSCG (or placebo): 60 and 15 min prior to the challenge. The powder was dissolved in 20–30 ml of water. No parent or patient believed they could distinguish between the two solutions.

Study design

IP was tested before challenge and during 2 cow's milk challenges, following pretreatment with either DSCG or with placebo. To prevent a treatment order effect half of the patients received DSCG followed by placebo, while the order was reversed in the other half of the patients. In order to exclude effects of the previous challenge the interval between the challenges was at least two weeks.

The clinical signs and symptoms were recorded by the same observer at the start of the baseline day and 0, 1, 2 and 5 h after the start of the challenge. Special attention was given to skin-, gastrointestinal- and respiratory symptoms. The challenge was considered positive if clinical reaction(s) occurred within 5 h with at least one major or two minor symptoms according to Table 2. The assessment of the response to challenge was made immediately after the 5 h observation period. Children with clinically positive challenges are further referred to as "reactors", while those with clinically negative challenges are referred to as "non-reactors".

Data analysis

Because of the skewed distributions of most variables, nonparametric techniques were used for data analysis: Mann Whitney U test for independent variables. A p-value of <0.05 was considered statistically significant.

Results

All children tolerated the 2 challenges without serious symptoms except one who developed dyspnoea, wheezing, vomiting and angioedema requiring intravenous antihistamines and inhalation with beta-2-agonists after the first challenge (following pretreatment with DSCG). Because of this reaction he was not challenged a second time.

One child developed a short bout of diarrhoea on the baseline day and both challenge days 24 h after drinking the solution. In this patient diarrhoea was not recorded as a symptom induced by the challenge.

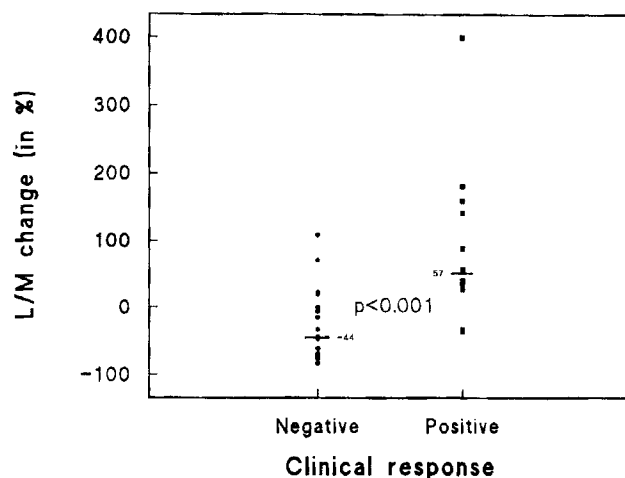


Fig. 1a. Δ L/M after placebo pretreatment in clinically negative versus clinically positive cow's milk challenges. Significance determined by Mann Whitney U test.

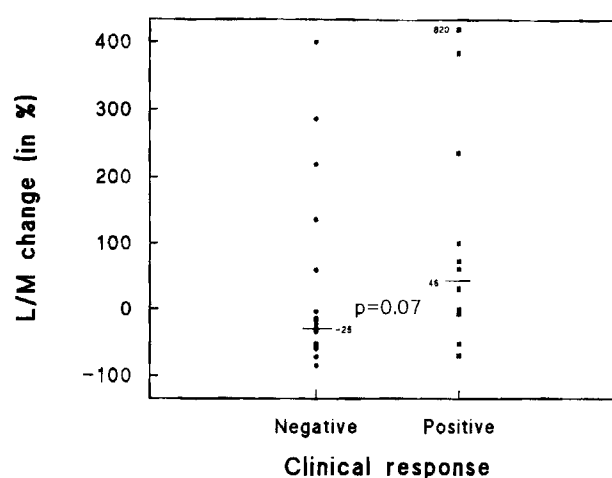


Fig. 1b. Δ L/M after DSCG pretreatment in clinically negative versus clinically positive cow's milk challenges. Significance determined by Mann Whitney U test.

Table 3. L/M_{baseline} , L/M change (Δ L/M), and clinical response to cow's milk challenge (Chal) after placebo- and DSCG pretreatment (+/- = clinically positive/negative challenge respectively)

Patient (n°)	L/M_{baseline}	After placebo		After DSCG	
		Δ L/M (%)	Chal (+/-)	Δ L/M (%)	Chal (+/-)
01	0.104	- 71	-	- 4	-
02	0.010	+160	+	+ 30	+
03	0.011	+109	-	+136	-
04	0.035	+ 57	+	0	+
05	0.017	+182	+	+ 59	-
06	0.059	+ 71	-	- 32	-
07	0.023	+ 35	+	+ 61	+
08	0.162	- 73	-	- 59	-
09	0.031	- 6	-	+100	+
10	0.055	+ 40	+	- 7	+
11	0.120	- 76	-	- 85	-
12	0.056	+ 89	+	- 14	-
13	0.071	- 61	-	- 34	-
14	0.076	- 47	-	- 55	-
15	0.039	- 44	-	+236	+
16	0.044	0	-	+286	-
17	0.026	+ 27	+	+219	-
18	0.031	+ 23	-	+384	+
19	0.228	- 75	-	- 72	-
20	0.003	+400	+	+400	-
21	0.095	- 15	-	+ 73	+
22	0.053	+142	+	- 17	-
23	0.025	+ 20	-	- 28	-
24	0.117	- 83	-	- 70	+
25	0.036	- 33	-	- 22	-
26	0.031	- 45	-	- 52	-
27	0.042	- 69	-	- 60	-
28	0.031	- 35	+	- 52	+
29	0.045	- 33	+	+820	+
30	0.016	ND	ND	+ 31	+

Challenges after placebo pretreatment

Of 29 challenges after placebo pretreatment 11 (38%) were clinically positive with all 11 developing skin symptoms. This was accompanied by gastrointestinal symptoms in 5, by respiratory symptoms in 2 and in 1 case by both.

Clinically positive challenges were related to a significant increase in L/M ratio ($p = 0.0008$) (Fig. 1a, Table 3). Neither the presence of IgE antibodies to cow's milk (>0.35 PRU/ml; $p = 0.96$), the levels of total IgE ($p = 0.64$) nor the age of the child ($p = 0.46$) were related to clinically positive challenges. The baseline level of eosinophils was, however, significantly higher in reactors, median $722.5 \times 10^6/L$ (44-3685) than in non-reactors, median $209.0 \times 10^6/L$ (44-1122) ($p = 0.017$). Furthermore,

L/M baseline controls vs patients

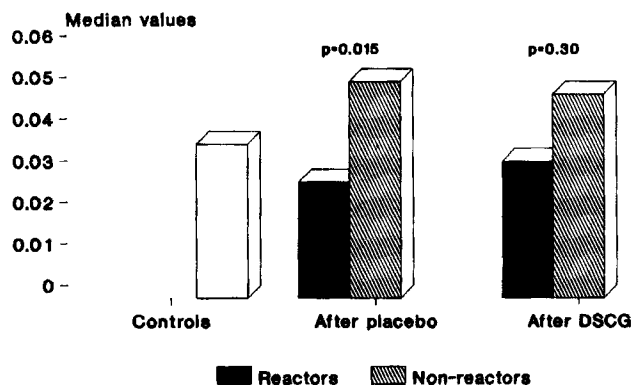


Fig. 2. Baseline values of L/M ratio of controls, patients with subsequent clinically negative cow's milk challenges (non-reactors), and patients with subsequent clinically positive cow's milk challenges (reactors) after placebo- and after DSCG pretreatment. Significance determined by Mann Whitney U test.

the L/M ratio before challenge on a cow's milk free diet, (L/M_{baseline}) in reactors although not different from controls, was significantly lower than in the non-reactors ($p = 0.015$) (Fig. 2).

Challenges after DSCG pretreatment

Of 30 challenges after DSCG pretreatment, 12 (40%) were clinically positive. Eleven children developed skin symptoms, in 4 cases accompanied by gastrointestinal symptoms, in 4 by respiratory symptoms and in 1 case by both. One child had only gastrointestinal symptoms after challenge.

Clinically positive challenges after DSCG pretreatment were not significantly related to an increase in L/M ratio ($p = 0.07$) (Fig. 1b, Table 3).

As with challenges after placebo pretreatment, the presence of IgE antibodies to cow's milk ($p = 0.23$), the levels of total IgE ($p = 0.36$) and the age of the child were not related to clinically positive challenges. Furthermore, the levels of eosinophils were not related to the outcome of the challenge after DSCG pretreatment ($p = 0.65$).

The L/M ratio before challenge on a cow's milk free diet, L/M_{baseline} , was not significantly different in reactors compared to non-reactors ($p = 0.30$) (Fig. 2).

Controls

No adverse reactions were recorded while performing the SAT in controls. The median L/M ratio in 15 controls was 0.037 (range 0.000–0.101) (Fig. 2).

Discussion

In this study we evaluated the effect of pretreatment with DSCG during open cow's milk challenges with a standardized procedure in children suspected of cow's milk allergy. Although in general double-blind, placebo-controlled food challenges are recommended (18), we chose to perform a natural oral ingestion in order not to miss local, perioral reactions. Open challenges can induce a certain degree of bias however in pediatric practice this bias is considered to be small since placebo-related reactions are rare in (young) children (9, 18). To minimize the effect of other variables we studied the effect of DSCG on allergic reactions after standardized challenges with fixed doses of cow's milk or a cow's milk formula given with fixed intervals. All children were observed by the same observer (RMvE) during the challenges.

In order to study the mechanism of DSCG we determined IP changes during food challenges by means of the SAT. Testing IP with sugars in a moderately hyperosmolar solution will better dis-

criminate between normal and abnormal small bowel function than tests employing iso-osmolar solutions (12, 13, 19, 20). Theoretically, the use of a hyperosmolar solution can induce osmotic diarrhoea in children. In this study, however, only 1 of 30 children had a short bout of diarrhoea 24 h after drinking the test solution on all 3 occasions. In our opinion a moderately hyperosmolar solution can be used safely in children if the dosage of the solution is adjusted for the weight of the child: 2 ml/kg with a maximum of 100 ml.

Although the SAT is promising in children with food allergy, clinical studies using this method have been limited and results have been conflicting (9, 21–24). We found that after placebo pretreatment reactors had a significant increase in the IP, as measured with the SAT, in comparison to non-reactors. The change in the L/M ratio was mainly due to the change in the IP for lactulose. Mannitol excretion was not significantly influenced by the challenge. The IP change from baseline levels in non-reactors might be related to the substantial extra fluid (cow's milk or formula) given during the challenge. The ingestion of extra fluid during the challenge could decrease the osmolarity of the ingested test solution. In an iso-osmolar solution the uptake of lactulose is lower than in a hyperosmolar solution (19). This may explain the lower L/M ratio's found in the clinically negative challenges of our study.

In the present study 5 patients with clinically positive challenges after placebo pretreatment had clinically negative challenges after DSCG pretreatment and an equal number had negative challenges after placebo pretreatment, but positive challenge after DSCG pretreatment. Thus, pretreatment with 2 doses of 100 mg DSCG did not reduce the number of clinically positive challenges in this group of cow's milk allergic children. In other studies as reviewed by Sogn (1) and Cavagni (7) conflicting results on the beneficial effects are reported with respect to the effective dose and duration of pretreatment. Falth-Magnusson's study shows a beneficial effect after pretreatment for 2 weeks with 200 mg DSCG daily with respect to clinical reactions to cow's milk, as well as IP measured with PEG (8). In another study by Andre et al. (22), a single dose of 300 mg of DSCG prevented clinical symptoms and the increase of IP as reflected by the L/M ratio in most of the patients. However, in other studies, a protective effect on clinical symptoms, if any, was found only if 1600 mg of DSCG daily was given. In the current study DSCG did not prevent clinical symptoms, but it did reduce the allergen induced changes in IP, as measured with the SAT, between reactors and non-reactors.

The increased IP in reactors reflects an alteration of the functional integrity of the small bowel. Two observations support the notion that this alteration is probably secondary to food allergic reactions and not a primary characteristic of children having clinically positive challenges. Firstly, the prechallenge L/M_{baseline} in reactors was not increased compared to non-reactors or healthy controls. In fact, it was higher in non-reactors versus controls, and significantly higher versus reactors ($p = 0.015$). This indicates that in the latter the L/M ratio was increased, although not significant versus controls, for other reasons, for example intercurrent viral infections. Secondly, DSCG pretreatment reduced the differences in IP between reactors and non-reactors during cow's milk challenges, while not decreasing the number of clinically positive challenges. In these children, two doses of DSCG probably decreased the local intestinal response but not the distant, extraintestinal reactions.

Neither age, level of IgE antibodies to cow's milk, total IgE, eosinophils nor the L/M_{baseline} was significantly different in reactors after DSCG pretreatment compared to non-reactors. This is consistent with other studies as reviewed by Sogn (1) and Cavagni (7). The prechallenge level of eosinophils was higher in reactors after placebo pretreatment than in nonreactors. Thus, eosinophils might play a role in the pathophysiology of food allergic reactions. However, the range is large and therefore the level of eosinophils can not be used as an indicator of a positive challenge in an individual patient. Perhaps eosinophil markers will be more useful in the future for this purpose.

The SAT is a suitable, easy to perform and non-invasive measurement of IP in children. Although the SAT is not specific for food allergy in children, measuring it before and during food challenge might be an additional objective parameter of the response to food challenges.

This study shows that cow's milk allergy is a complex phenomenon: extraintestinal reactions may be (partially) independent of the local intestinal response. Short term treatment with 2×100 mg DSCG reduced the allergen-induced increase in permeability, but did not influence the extraintestinal reactions. Long-term treatment with DSCG may suppress the local intestinal response for a prolonged period and reduce the uptake of antigenic materials. Further studies of long-term treatment with DSCG before cow's milk challenge should elucidate their influence on intestinal permeability (SAT), development of extraintestinal reactions and their possible relationship.

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