

Intestinal uptake of ovalbumin in malabsorption and food allergy in relation to serum IgG antibody and orally administered sodium cromoglycate

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(Received 9 October 1978; accepted for publication 13 November 1978)

Summary

Forty-two children with malabsorption disorders, one third of whom were considered to have an atopic constitution were given ovalbumin (OA) orally and the serum concentrations were determined by RIA technique after 2 and 8 hr. Most patients showed peak concentrations 2 hours after OA administration but the concentrations varied widely. The determinations were shown to be influenced by antibodies directed against OA. The serum levels of IgG-antibodies to OA, as estimated by a sepharose protein-A technique, were shown to decrease significantly after administration of OA, possibly indicating the formation of immune complexes. High antibody levels were found in children with coeliac disease and in addition in children who had an atopic constitution. A moderate serum concentration of OA in such cases might be an effect of blocking antibodies and does not exclude the possibility of an increased absorption. The uptake of OA was also studied in six patients with food allergy before and after sodium cromoglycate treatment. A beneficial effect on symptoms at the challenge and also a decreased serum concentration of OA after sodium cromoglycate treatment were found ($0.05 < P < 0.1$). The results support the view that SCG affects the absorption of food antigens. The OA-absorption test, including estimations of both OA and antibodies to OA is considered a useful model for studies of immunological mechanisms involved in food allergy and gastrointestinal disorders.

Introduction

The intestinal barrier is not completely impermeable to macromolecules. Several studies suggest that food antigens can cross the mucosa and enter the circulation (Schloss & Worther, 1916, Ratner & Gruehl, 1934). Permeability of the gut has been shown to be greater in infancy and food proteins are absorbed intact in sufficient quantities for stimulation of the immune system, with the appearance of specific antibodies in the intestinal secretions and serum (Rothberg, 1969). There is also an

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increased permeability in gastrointestinal disorders as shown by Gruskay & Cooke, (1955) in children recovering from diarrhoea. This may also be true for malabsorption disorders and food allergy in childhood. In such cases the antibody titres to foods are often elevated although they have been shown to correlate poorly with clinical symptoms (Foucard, 1973, Peterson & Good, 1963). However, it is to be emphasised that symptoms are more likely to occur in the presence of antibodies to foods if the respective food antigen is absorbed and enters the circulation.

A beneficial effect on symptoms in food allergy by administered sodium cromoglycate (SCG) has been reported (Freier & Berger, 1973, Dannaeus, Foucard & Johansson, 1977). It is suggested that the drug decreases or prevents an allergen-triggered release of mediators from sensitized mast cells in the intestine, thereby possibly reducing the gut permeability for food allergens.

In the present study, children with malabsorption, atopy and food allergy were challenged with orally administered ovalbumin (OA), which is measurable in serum by RIA-technique at very low concentrations.

The aim of the study was to estimate the absorption of orally administered ovalbumin and to study the influence on the serum concentrations of OA of serum antibodies directed against ovalbumin. In addition, the effects of orally given SCG on absorption of ovalbumin and on symptoms in children with food allergy were studied.

Materials and methods

Forty two children, aged 8 months to 5 years, (mean 22 months) were investigated for malabsorption because of prolonged diarrhoea and/or poor weight gain at the Department of Paediatrics, University Hospital, Uppsala. The children had the following diagnoses: coeliac disease (29%), lactose intolerance (19%), unspecified malabsorption (29%), insufficient weight gain (23%). In addition, 5 food allergic children, aged 2–6 years and the mother of one were challenged without and with SCG. All had a weakly positive RAST to eggwhite and a slight sensitivity to egg. All children had eczema and in addition two children had gastrointestinal disturbances. The mother had a mild form of Crohn's disease and diffuse joint symptoms. The children with malabsorption were divided into two groups. Group I was considered to have abnormal and Group II normal laboratory findings at the ovalbumin absorption test. To be considered an abnormal OA absorption test, at least one of the following criteria was fulfilled: (1) A serum concentration of OA exceeding 50 µg/l at 2 hr after OA-administration. (2) A serum concentration of OA at 8 hr exceeding that at 2 hr or 3. A high serum concentration of antibodies to OA (greater than 10% of a reference).

Symptoms and laboratory findings of the both groups are listed in Table 1.

The peroral loading test was performed after an egg-free diet period of at least 12 hr. After an overnight fast they were given orally 100 mg/kg ovalbumin (O.A.) dissolved in saline 100 mg/ml, blood samples were drawn before and after 2 and 8 hr (Fig. 1). After the collection of the 2 hr samples the children were given their meals at the same timepoints with strict avoidance of egg during the test period.

In the six patients with food allergy the OA-absorption tests were repeated after one week of sodium cromoglycate treatment SCG was administered orally, 400 mg daily in 4 doses. The last dose was given within two hr before the test. In this group, the eight hr sample was only obtained from two patients.

Commercially available OA (once crystallized, Miles Laboratories Ltd.) was used

both *in vivo* and *in vitro*. For immunization of a rabbit a five times crystallized OA preparation was used.

The serum concentration of OA was estimated by a direct, sandwich-type of radioimmunoassay. The IgG-fraction of a specific rabbit-anti-OA serum was coupled to cyanogen bromide activated cellulose paper discs (Ceska & Lundkvist, 1972). The discs were incubated with 100 μ l undiluted serum at room temperature for 3 hr and were then washed three times with saline containing 0.1% Tween. Affinity chromatography purified I^{125} -labelled anti-OA, (100 μ l of approx. 80,000 ct/min in 0.05 M phosphate buffer pH 7.4) with 0.01% normal rabbit gamma globulin was added and

Table 1. Symptoms and laboratory findings in patients with abnormal (Group I) and normal (Group II) OA-absorption tests

Group	Weight less than 2 s.d. of the mean for the age		Diarrhoea		Abnormal xylose absorption		Abnormal lactose absorption		Abnormal biopsy	
	n	%	n	%	n	%	n	%	n	%
I (n=22)	13/22	59	9/22	40	6/9	67	6/9	67	7/18	39
II (n=20)	4/20	20	14/20	70	3/16	19	9/17	53	3/11	27

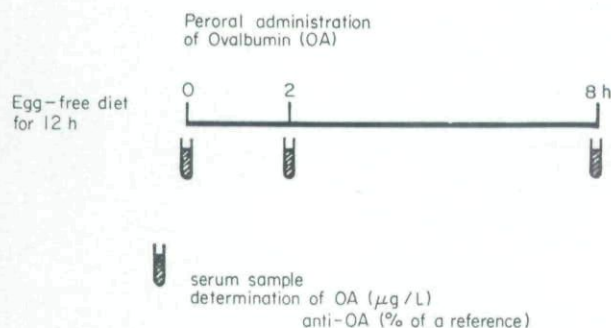


Fig. 1. Design of the OA absorption test.

the discs were incubated overnight. The discs were then washed three times and the radioactivity was counted in a gammacounter. The reference OA-preparation used was diluted in a human serum without detectable amounts of IgG-antibodies to OA in order to maintain a similar protein concentration in the reference and the sample. The technique was considered capable of measuring OA concentrations exceeding 1 μ g/l.

The serum concentration of antibodies to OA was measured by a method based on the binding of IgG to staphylococcal protein-A coated sepharose using I^{125} labelled OA (Johansson *et al.*, 1976) (once crystallized, Miles Laboratories Ltd).

The anti-OA concentrations were expressed as a percentage (%) of a reference serum. The samples were generally diluted 1:10 and when necessary further diluted in a serum lacking antibodies to OA.

Results

Children investigated for malabsorption

Thirty-four out of forty-two children had a measurable serum concentration of OA after oral OA administration. Most cases had a peak concentration after 2 hr and still had measurable concentrations after 8 hr. Antibodies to OA were detected in most sera and the levels showed a significant decrease during the 8 hr observation period ($P < 0.01$) (Table 2).

Table 2. The serum concentration of ovalbumin (OA) and anti-ovalbumin (anti-OA) before and 2 and 8 hr after peroral ovalbumin administration. The results are given as geometric means and the geometric mean $\pm$ 1 s.d.

Hr after OA administration	OA ($\mu\text{g/l}$)	Anti-OA % of a reference
0	< 1.0	10.5 1.5-73.4
2	10.0 1.6-64.1	7.3 1.1-49.9
8	5.5 1.5-21.0	6.7 1.0-45.1

Table 3. The influence of the levels of antibody to OA in serum on the serum concentrations of OA. High anti-OA titre: 10% of a reference. Low anti-OA titres: 10% of a reference. Geometric means and the geometric mean $\pm$ 1 s.d.

Anti-OA levels	No. of patients	OA-concentration $\mu\text{g/l}$	
		After 3 hr	After 8 hr
High	21	3.8 (0.9-15.7) ($P < 0.001$)	3.3 (1.0-11.3) ($P < 0.01$)
Low	21	26.1 (4.5-150.3)	9.3 (2.7-31.8)

Children with high levels of antibodies (greater than 10% of a reference) had significantly lower serum concentrations at both 2 and 8 hr than children with low levels, indicating that the antibodies had a blocking capacity (Table 3).

In children with abnormal OA-absorption tests (Group I), there was a higher frequency of coeliac disease (36%) than in Group II (20%). These children also had a high frequency of atopy (55%) including eczema, urticaria, asthma and/or atopic disease in the family (Table 4). Figure 2 shows that the levels of serum antibodies to OA in both coeliac and atopic children were significantly higher than in other children investigated for malabsorption ($P < 0.05$ and $P < 0.02$). Children with coeliac disease

had high mean concentrations of OA despite high levels of anti-OA but the levels varied widely. Two patients even lacked measurable amounts of OA.

In children with atopy the mean serum concentration after 2 hr was moderate and most patients had high levels of anti-OA. Six atopic children lacked measurable amounts of OA and several children had higher serum concentrations of OA after 8

Table 4. Serum IgE concentration and RAST results to egg in patients investigated for malabsorption.

	IgE ku/l geometric mean $\pm$ s.d.	Positive RAST to egg (%)
Coeliac disease ($n=12$)	5.4 (2.2-13.1)	17
Atopy ($n=17$)	30.3 (3.3-278)	47
Other cases investigated for malabsorption ($n=13$)	5.1 (1.1-22.5)	8

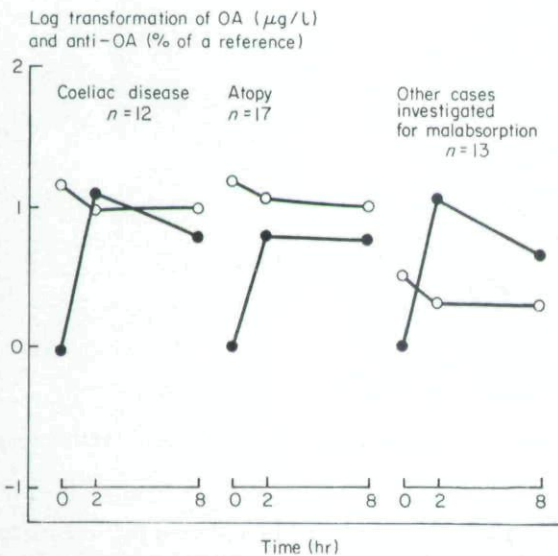


Fig. 2. Serum concentrations of ovalbumin (OA) (●) and antibodies to ovalbumin (anti-OA) (○) in children investigated for malabsorption. The results are expressed as geometric means.

than after 2 hr. Eight out of twelve such cases were atopic. One patient with a high level of anti-OA (66% of a reference) had a measurable amount of OA only in the 8 hr sample. This child, who developed diarrhoea 4 hr after OA administration, had a measurable serum concentration of $36 \mu\text{g/l}$ and a level of anti-OA lower than half of the initial level, in the 8 hr sample. The child had a family history of allergic disease, but the serum IgE concentration was low (1 ku/l) and RAST to egg was negative.

Patients with food allergy before and after treatment with sodium cromoglycate

Four out of six patients, the mother of one child included, developed symptoms such as urticaria (one), increased itching (one) diarrhoea (two) and pain and swelling of the joints (one) after OA administration. Three of them and one additional child without symptoms had detectable amounts of OA in serum (geometric mean 7.1, range 1.1–70 $\mu\text{g/l}$) after 2 hr. All patients had detectable serum antibodies to OA (geometric mean 11.1, range 2.0–82% of a reference). The two children who lacked detectable amount of OA in the serum had high levels of antibodies to OA (82 and 23% of a reference, respectively).

When the test was repeated after treatment for one week with orally given SCG, only one of the six patients had symptoms after OA intake. Urticaria reappeared in the patient who had urticaria also after the first test. However, the reaction was considered weaker. After SCG treatment the serum concentration of OA in the 2 hr sample was lower in three out of the four patients who had a measurable uptake ($0.05 < P < 0.1$) (Fig. 3). No detectable amounts of OA were found in the sera of the two patients who

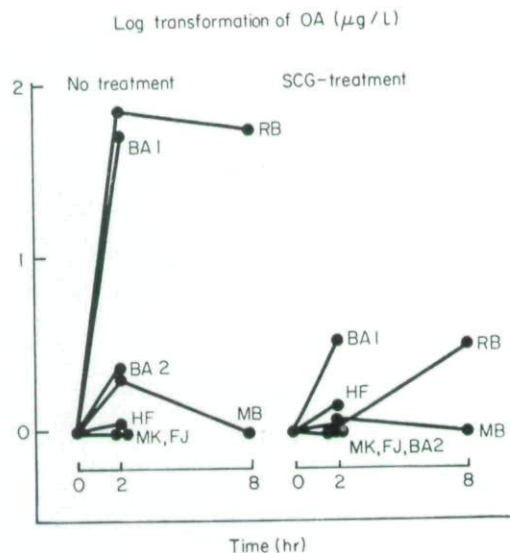


Fig. 3. Serum concentrations of ovalbumin (OA) in patients with egg-sensitivity before and after treatment with sodium cromoglycate (SCG).

lacked detectable OA at the first test. One patient (BA) participated in the test twice and on both occasions the concentrations of OA were lower after SCG treatment than without treatment.

At the second test (after SCG) the levels of antibodies to OA had increased in comparison to the initial levels, indicating that an antigenic stimulation might have occurred. However, the difference was not statistically significant.

Discussion

In the group exhibiting abnormal OA absorption tests (Group I) there was a preponderance of children with severe gastrointestinal disturbances, including eight out of twelve with coeliac disease, and of children with an atopic constitution.

There was a high frequency of atopy as seen from elevated serum IgE levels and a high frequency of positive egg-RASTs among the patients with abnormal OA absorption tests and atopy might be a common and often overlooked cause of gastrointestinal disturbances in infancy.

Most children exhibited peak concentrations of OA 2 hr after intake. The results of the OA determinations were, however, shown to be influenced by the presence of blocking antibodies and in several patients with high antibody levels no OA could be demonstrated. This is probably explained by the high levels of antibodies with a blocking capacity in the circulation. Such antibodies in the intestinal secretions and in the gut wall could also limit the quantity of antigen that can penetrate the mucosa and enter the circulation (Walker, Isselbacher & Block, 1972).

In food allergy the permeability of the gut for food allergens is thought to be a critical factor. The beneficial effect by SCG on symptoms of food allergy is not fully understood. This study showed a beneficial effect on symptoms in slightly egg-sensitive patients after oral challenge with OA. In addition, less OA was detectable in the circulation. The amount of ovalbumin given corresponded approximately to the content in one egg. The results support the view that SCG affects the absorption of food antigens. This effect might be explained by a decreased release of mediators from sensitized mast cells in the intestinal mucosa thereby reducing the permeability of the gut for macromolecules.

From this study we conclude that ovalbumin is not an ideal test-substance for studies of gut permeability because of interaction with antibodies directed against OA which are frequent in food allergy and gastrointestinal disorders.

However an OA absorption test, including determination of both OA and antibodies to OA, is considered a useful model for studies of the physiological handling of food antigens and of mechanisms involved in food sensitivity (Inganäs, & Johansson, 1978). The interaction between antigen and antibody suggests formation of immune complexes, which in certain circumstances can initiate activation of the complement system or cause deposits in the tissues, mechanisms which are likely to contribute to adverse reactions to foods (Delire, Cambiaso & Masson, 1978).

Acknowledgments

This work was supported in part by Fisons Ltd and the Swedish Medical Research Council grant No 16P-4910. Fisons Pharmaceuticals supplied the sodium cromoglycate.

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