

Gastrointestinal permeability in atopic and non-atopic mothers, assessed with different-sized polyethyleneglycols (PEG 400 and PEG 1000)

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Summary

Gastrointestinal permeability was compared in seventy-one women, forty-six allergic and twenty-five non-allergic according to case history, by measuring the 6-hr urinary recovery after an oral load of different-sized polyethyleneglycols (PEG 400 and PEG 1000). Further classification, atopic and non-atopic, was obtained by skin-prick tests to inhalant and food allergens in sixty-four of the seventy-one mothers. No significant differences were observed between allergic and non-allergic women, neither regarding the maximum recovery nor the recovery ratio between a large and a small molecule. The results do not lend support to the hypothesis of a persistently increased gut permeability in atopic subjects, which in turn could possibly imply an increased risk of intrauterine sensitization of the foetus of an atopic mother.

Introduction

Gastrointestinal permeability properties have been assessed by a variety of probes, e.g. ovalbumin [1], mono- and disaccharides [2–4] and different-sized polyethyleneglycols (PEG) [5–9]. Permeability measurements are of particular interest in allergic subjects, since increased permeability to larger molecules might help to induce and/or maintain, for example, food allergy [10]. In fact, increased permeability was observed in atopic subjects with eczema with and without food allergy by Jackson *et al.* [7] but could not be confirmed by others [4, 9]. Leaky mucosal membranes of atopic mothers might increase the possibility that antigenic material could pass to the foetus, and contribute to intrauterine sensitization.

The occurrence of elevated, foetal cord blood IgE levels in some babies is well

established [11] and in a few cases specific cord blood IgE antibodies to allergens, i.e., evidence of intrauterine sensitization has also been described.

The intrauterine life does protect the foetus from the majority of common antigens. However, the maternal and fetal blood contact through placenta constitutes a possible way of antigen exposure. Even in normal subjects, low levels of intact antigen have been demonstrated in blood, e.g. after an oral challenge of cow's milk [12]. Small doses of antigen might impose an even greater risk of sensitization than would be the case with a greater antigen leakage [13].

The aim of the present study was to compare the permeability properties of allergic and non-allergic mothers, participating with their babies in a prospective study of the relation between certain dietary restrictions late during pregnancy and the development of allergy in their babies.

Materials and methods

Patients and allergy tests

All the women participated in a study of the possible effect of maternal avoidance of cow's milk and hen's egg during late pregnancy on the development of atopy in her child.

The study was approved by the Human Research Ethics Committee and informed consent was given by all participants. Forty-six women (age 29.6 ± 4.0 years, mean $\pm$ S.D.), with a case history of allergy (atopic dermatitis, allergic rhinoconjunctivitis, bronchial asthma) and twenty-five women (age 30.8 ± 3.4) with no history of allergy, were enrolled for the study. They showed no clinical signs of malabsorption, were not taking any medicine during the test period, and were all on a normal diet. The PEG test was performed 3 months after delivery. Serum IgE levels were determined (Phadebas IgE PRIST®) in all the women. Skin-prick tests to common allergens (birch, timothy, cat, dog, mite, cladosporium) were performed in duplicate in sixty-four of them with 10 000 BU/ml Spectralgen®/Pharmalgen® (Pharmacia Ltd, Uppsala, Sweden) and to cow's milk and hen's egg white (Dome-Hollister-Stier extract). A weal more than 2 mm in diameter was regarded as positive for inhalants and more than 3 mm in diameter was positive for food allergens.

A positive skin-prick test was found in four out of twenty-two tested women classified as non-allergic. The skin-prick tests were negative in thirteen out of forty-two tested women classified as allergic. Results of the gut permeability studies will therefore be given both with the mothers grouped according to case history only (allergy) and according to the combination of history and skin-prick tests (atopy).

PEG test

After overnight fast and voiding, a liquid probe containing 0.1 g PEG 400 and 2.5 g PEG 1000, covering a molecular weight range from 238 to 1250 dalton (Macrogolum, Apoteksbolaget, Sweden) was taken in a glass of water or lemonade. Another two or three glasses of beverage were allowed during the following hours and a meal was allowed 3 hr after the PEG intake. Urine was collected for 6 hr. None of the mothers had fever or gastroenteritis and the amount and type of stools during the test and possible side effects were registered by the woman.

PEG analysis

The procedure has been described recently [9]. Briefly, the volume of the 6-hr sample was measured, and then 6 ml was treated twice with anionic and cationic resin by end-over-end rotation at 37°C. A sample was freeze-dried overnight, dissolved in methanol/water (42:58), filtered through a 0.45 µm filter and analysed in a high performance liquid chromatograph equipped with a refractive index detector (HPLC, HSRI 931, Tecator, Sweden) and a reversed-phase C-8 column (Lichrosorb RP-8, Fertigkollonnen, Merck AG, Darmstadt, West Germany). A control urine was analysed parallel to the samples and the recovery of each PEG molecular weight species was calculated from the chromatogram peaks [9]. The largest observed percentage recovery of any molecular weight, usually 370 (±44) dalton, in each subject was defined as the maximum recovery.

Results

(a) Intestinal permeability in relation to case history (allergy)

The average maximum recovery of a PEG molecule was $25.1 \pm 0.82\%$ ($n=46$; mean ± S.E.M) in the allergic and $25.7 \pm 1.5\%$ in the non-allergic group. The recovery ratio (ratio between the recovery of a large molecule, 1074 dalton, and of a small molecule, 370 dalton) was 0.19 ± 0.02 , ($n=46$) in the allergic, and 0.19 ± 0.03 ($n=25$) in the non-allergic group. The recovery for each molecular weight species is depicted in Fig. 1a and the recovery ratio in Fig. 2a.

(b) Intestinal permeability in relation to skin-prick test results (atopy)

A case history of allergy and at least one positive skin-prick test was requested for being classified as atopic. For inclusion in the non-atopic group negative history and negative skin-prick tests were mandatory. The average maximum recovery in the atopic group was $25.4 \pm 1.0\%$ ($n=29$) and $26.4 \pm 2.0\%$ ($n=18$) in the non-atopic group. The recovery ratio was 0.18 ± 0.03 ($n=29$) and 0.21 ± 0.03 ($n=18$) respectively. The recovery of each molecular weight species and recovery ratios are shown in Fig. 1b and 2b, respectively.

(c) Intestinal permeability in relation to breast-feeding

No significant differences in intestinal permeability were seen between breast-feeding and non-breast-feeding women, and when the history and skin-prick test results were considered. The maximum recovery was 25.1 ± 0.81 in breast-feeding ($n=60$) and $26.6 \pm 2.3\%$ in non-breast-feeding women ($n=11$). Corresponding values of the recovery ratio were 0.19 ± 0.02 and 0.20 ± 0.05 respectively.

(d) Intestinal permeability in relation to other possible groups characteristics

There was no difference between atopic mothers who, in addition, had an elevated IgE level (≥ 120 kU/l) and mothers without any signs of atopy, i.e. no allergy history, negative skin-prick tests and normal IgE levels.

(e) Individual permeability properties

Four women showed a very low uptake of PEG, i.e. a maximum recovery below 15%. Of these, two patients were non-atopic, one atopic and one allergic. Six women showed a large uptake of PEG, i.e. maximum recovery more than 35%; two of these subjects were allergic and four non-allergic. Four women displayed a recovery ratio greater

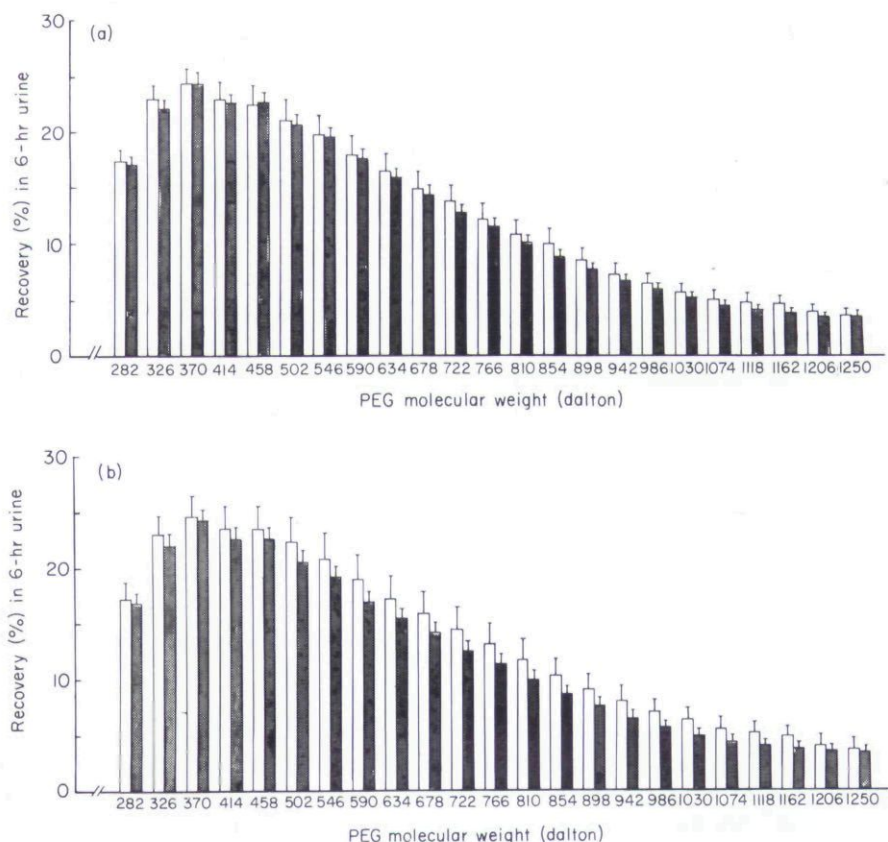


Fig. 1. Intestinal permeability, as assessed by the 6-hr urinary recovery (mean $\pm$ S.E.M.), of differently sized polyethyleneglycols (PEG 400 and PEG 1000) in women regarded as non-allergic (open bars) or allergic (hatched bars) according to case history ((a) $n = 25$ and 46 , respectively), and atopic according to case history confirmed by the skin-prick test ((b) $n = 18$ and 29 , respectively).

than 0.4. One of these was atopic and had a low overall uptake, whereas one atopic, one allergic and one non-atopic woman all were within the normal range of uptake [14, 15]. No side effects were reported, and stools were normal during the permeability tests. The study was performed from August 1983 to July 1984, which would have randomized seasonal effects.

Discussion

Transfer of antigen from maternal food to the foetus presumes the passage through protective barriers. The gastrointestinal mucosal barrier was investigated in this study by the use of different-sized PEGs. No differences were revealed between non-atopic and atopic mothers, neither regarding the maximum recovery, which is assumed to reflect the effective absorptive surface available to small molecules, nor the recovery ratio, suggested to depict the capacity of the mucosal membranes to exclude larger molecules. The observed recovery values in this study, around 24–28%, are in good agreement with previous results in healthy controls [5, 14, 15].

The possibility of increased risk of specific intrauterine sensitization in offsprings of

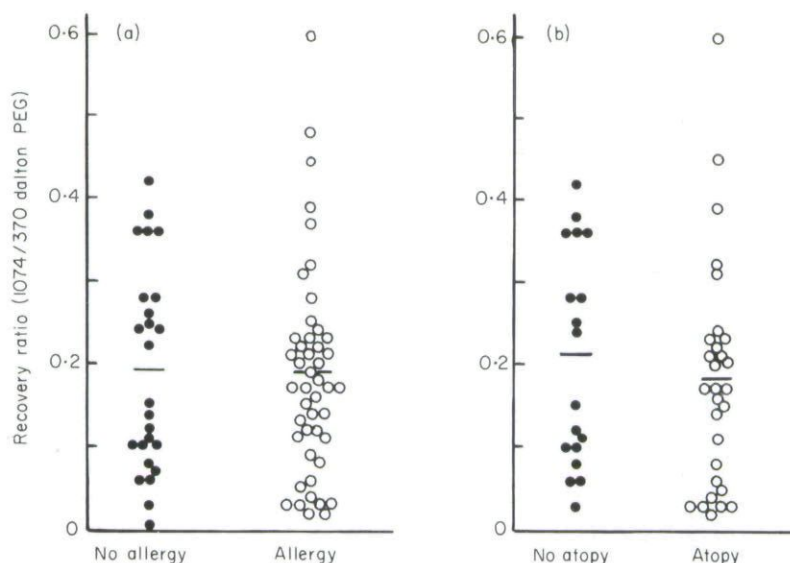


Fig. 2. Selective exclusion of molecules at the intestinal wall, as described by the recovery ratio between 1074 and 370 dalton PEG in women regarded as non-allergic or allergic according to case history (a), and to case history confirmed with skin-prick test (b). The bars indicate mean values, and the number of patients in the groups are the same as in Fig. 1.

atopic mothers cannot be excluded by our findings. Several observations, however, lend support to the relevance of the PEG method. The used PEG mixture with a molecular size range of 238–1250 dalton, was recently found to give a good picture of permeability changes on cow's milk challenge in children with cow's milk allergy (unpubl. observation). Furthermore, recent studies demonstrate that IgE-binding capacity of a cod fish allergen [16] is retained in small fragments, consisting of three homologous tetrapeptides and that allergenicity can be obtained by the synthetic peptide analogues Asp-Glu-Asp-Lys and Asp-Glu-Leu-Lys, i.e. by molecules smaller than 1000 dalton and thus in the molecular size range used by us.

The placenta exerts a potent barrier to protect the foetus against noxious agents. For ethical reasons, the present study was not performed during pregnancy, since nothing is known about the placental handling of PEG. Thus, it is theoretically possible that gut permeability is altered during pregnancy, but not 3 months later. In such case the placental barrier will, eventually, determine to what extent antigens actually reach the foetus. Careful clinical follow-up of these and other babies, whose mothers were on an exclusion diet during pregnancy, will hopefully give an answer to whether or not intrauterine sensitization occurs. However, this study indicates that a general impairment of the gut permeability barrier is probably not a major determinant of foetal sensitization.

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