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Original article

Polyunsaturated fatty acids affect intestinal anaphylactic response in BALB/c mice sensitized with β-lactoglobulin

Les acides gras polyinsaturés affectent la réponse anaphylactique intestinale chez la souris BALB/c sensibilisée à la β-lactoglobuline

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Abstract

Objective. – Polyunsaturated fatty acids (PUFA) have immunomodulatory proprieties and their use in the prevention and treatment of allergy symptoms is proposed as a therapeutic option. The present study investigates the effect of n-3 and n-6 PUFA on intestinal anaphylactic response in mice allergic to β-lactoglobulin (β-lg), a major allergen in cow milk proteins.

Material and methods. – Female BALB/c mice were fed by gavage for 15 days with either fish oil (FO) (n-3 PUFA) or corn oil (CO) (n-6 PUFA) at different concentrations (0.6%, 1%, 1.5% V/W) and were then sensitized with β-lg. To study the local allergic manifestations in the intestine, electrophysiological parameters (short-current circuit, Isc, in $\mu A/cm^2$ and tissue conductance, G, in mS/cm^2) were measured in jejunum segments in an Ussing chamber, while morphological changes were assessed by histological analysis.

Results. – FO at 0.6% V/W significantly decreased Isc ($\mu A/cm^2$) values ($P < 0.05$). All other doses of both oils proved ineffective. The three doses of FO (but not CO) significantly reduced tissue conductance (mS/cm^2) values ($P < 0.05$, $P < 0.001$). Histological analysis showed morphological improvement in all groups with increased villus height ($P < 0.01$, $P < 0.001$).

Conclusion. – Low-dose PUFA supplementation, especially with n-3 PUFA, considerably reduced part of the intestinal damage resulting from sensitization with β-lg in BALB/c mice.

Keywords: n-3 PUFA; n-6 PUFA; β-lactoglobulin; BALB/c; Ussing chamber

Résumé

Objectif. – Les acides gras polyinsaturés (AGPI) ont des propriétés immunomodulatrices, leur utilisation pour la prévention et le traitement des symptômes d'allergie est proposée comme une stratégie thérapeutique. Le présent travail étudie l'effet des AGPI n-3 et n-6 sur la réponse anaphylactique intestinale chez des souris rendues allergiques à la β-lactoglobuline (β-lg), un allergène majeur des protéines du lait de vache.

Matériels et méthodes. – Des souris BALB/c femelles gavées pendant 15 jours avec de l'huile de poisson (FO) (AGPI n-3) ou de l'huile de maïs (CO) (AGPI n-6) à différentes doses : 0,6 %, 1 %, 1,5 % V/Pds sont ensuite sensibilisées avec la β-lg. Pour étudier les manifestations allergiques locales au niveau intestinal, les paramètres électrophysiologiques ; le courant de court circuit Isc ($\mu A/cm^2$) et la conductance tissulaire G (mS/cm^2) ont été mesurés en chambre de *Ussing*, une étude histologique a été aussi réalisée.

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Résultats. – FO à 0,6 % V/Pds a significativement diminué les valeurs de l'Isc ($\mu A/cm^2$) ($p < 0,05$). Les autres doses des deux huiles se sont révélées inefficaces. Les trois doses de FO (mais pas celles de CO) ont significativement diminué la conductance (mS/cm^2) ($p < 0,05$, $p < 0,001$). L'analyse histologique a montré une amélioration morphologique pour tous les groupes avec une augmentation de la hauteur villositaire ($p < 0,01$, $p < 0,001$).
Conclusion. – Les AGPI n-3 à faible dose réduisent considérablement certains des dommages intestinaux induits par la sensibilisation à la β-lg.

Mots clés : AGPI n-3 ; AGPI n-6 ; β-lactoglobuline ; BALB/c ; Chambre de *Ussing*

1. Introduction

n-3 PUFA alpha-linolenic acid (ALA; 18:3n-3) and n-6 PUFA linoleic acid (LA; 18:2, n-6) are essential FA and must be provided by the diet. Via an enzymatic pathway that includes desaturases and elongases, n-6 and n-3 PUFA are converted to biologically active long-chain polyunsaturated fatty acid (LCP-UFA), LA is converted into arachidonic acid (AA; C20:4 n-6) via gamma-linoleic acid (GLA; C18:3 n-6) and di-homo-gamma-linoleic acid (DGLA; C20:3 n-6), while ALA is transformed to eicosapentaenoic acid (EPA; C20:5 n-3) and docosa-hexaenoic acid (DHA; C22:6 n-3).

The literature reports the immunomodulatory proprieties of PUFA [1,2]. These substances can exert potent, sustaining, but opposing effects on immune activity [3]. n-3 PUFA are generally considered anti-inflammatory, while n-6 fatty acids are known to be proinflammatory at high intake levels [4].

Several studies have been conducted to investigate the effects of PUFA on Th2-mediated hypersensitivity, IgE-mediated food allergies and their symptoms [5–8], but to our knowledge their effects on intestinal anaphylactic response secondary to food allergy has not been studied.

Intestinal anaphylaxis can be defined as a dysfunction in fluid and electrolyte regulation with increased vascular permeability, resulting in massive fluid shifts from the intravascular to the extravascular space [9].

To study the local allergic manifestations in the intestine in response to food allergens, an in vitro approach could be adopted starting with systemic sensitization of an animal model with antigen plus adjuvant. This approach uses intestinal segments removed from sensitized animals, mounted in Ussing chambers, and challenged in vitro with antigen delivered to the luminal or the serosal surface of the tissue. The tissue is voltage clamped, allowing the measurement of net active ion transport across it [10].

The typical responses of the intestinal epithelium to allergens are an increase in watery diarrhea due to the stimulation of chloride secretion, and increased inward protein permeability from lumen to blood [11]; indeed, several studies have related increased intestinal Cl- secretion to local allergic reaction [12,13].

Since cow milk protein allergy (CMPA) is one of the most common food allergies among young children [14], we thought it would be of value to investigate a possible preventive effect of n-3 and n-6 PUFA against intestinal anaphylactic response secondary to sensitization with a major bovine serum allergen, β-lactoglobulin (β-lg), in a CMA animal model.

2. Materials and methods

2.1. Reagents

β-lactoglobulin, fish oil (F8020) rich in n-3 PUFA, corn oil (C8627) rich in n-6 PUFA, and all other reagents used in this experiment were purchased from Sigma (France), except for alum [Al (OH)3], and calcium chloride, which were obtained from Merck (France). All materials and instruments for the Ussing chamber were purchased from physiologic instruments (San Diego, CA, USA).

2.2. Animals

BALB/c mice were housed and had mated in the animal facility of nutrition physiology and food safety laboratory-university of Oran1, originally purchased from the Pasteur Institute in Algiers. Animals were kept in a controlled environment at 22 °C with 12-hour light and dark cycles, and they had ad libitum access to a standard diet and water.

All animals were handled in accordance with current Algerian legislation covering animal welfare.

2.3. Experimental design

5-week-old offspring of BALB/c females weighing 20 ± 2 g were divided into 7 groups: 6 experimental groups were given either fish oil (FO) or corn oil (CO) by gavage at different doses, namely 0.6%, 1% or 1.5% volume/weight (v/w), for 15 consecutive days prior to immunization with β-lg. A control group (CL) also received a solution of PBS 1/10 by gavage in a single dose of 0.6% V/W for 15 days and were then immunized with β-lg. Details of the experimental and immunization protocols are shown in (Fig. 1). At the end of the study, the mice were sacrificed and jejunum segments were removed for histological analysis and local anaphylactic study in an Ussing chamber by the measurement of electrophysiological parameters, i.e. short current circuit Isc ($\mu A/cm^2$) and tissue conductance G (mS/cm^2).

2.4. Measurement of intestinal anaphylactic response

The Ussing chamber provides a physiological system for measuring the transportation of ions, nutrients and drugs across various epithelial tissues [15]. A jejunum fragment was mounted in the Ussing chamber according to the protocol described by Grar et al. (2015) [13]. After a stabilization phase, the tissue was challenged with 60 μg/ml of β-lg deposited on the serosal

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