The Effect of Dietary \( \alpha \)-Linolenic Acid in the Rat on Fatty Acid Profiles of Immunocompetent Cell Populations

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ABSTRACT

Analysis of diet-induced fatty acid changes in the major phospholipids of various immune cell populations has not been previously documented, particularly modifications induced by dietary \( \alpha \)-linolenic acid. Rats were fed purified diets containing either 10% corn oil (CO), 10% linseed oil (LO) or 10% soybean oil-linseed mixture (SL) for 8 weeks. The \( \alpha \)-linolenic to linoleic acid ratios of the diets were 1:32, 1:1 and 3:1, respectively. Fatty acid analysis of cell populations isolated from the spleen, thymus, thoracic cavity and peripheral blood phospholipids showed increases in \( \omega3 \) fatty acids accompanied by decreases in the \( \omega6 \) fatty acids when diets high in \( \alpha \)-linolenic to linoleic acid ratios were fed. The extent of change observed was dependent on the magnitude of the \( \alpha \)-linolenic to linoleic acid ratio. Both magnitude of change and the specific fatty acids altered varied with the cell population examined.

Lipids 18:737-742, 1983.

INTRODUCTION

The essentiality of \( \alpha \)-linolenic acid is currently being reevaluated. In the case of \( \alpha \)-linolenic acid, it is believed that one of its essential functions lies in its ability to alter the metabolism of other fatty acids, notably linoleic acid, via a preference of the \( \Delta6 \) desaturase for the \( \omega3 \) series fatty acid. Such a competitive inhibition can lead to alterations in the capacity of tissues for synthesis of prostaglandins (PG) and lipoxygenase products. Recently, we have shown with the rat that, the higher the level of \( \alpha \)-linolenic relative to linoleic acid, the more PG synthesizing capacity is reduced (1). As would be expected, the higher linolenic acid diets lead to lower levels of \( \omega6 \) fatty acids, notably arachidonic (20:4\( \omega6 \)), and increased \( \omega3 \) fatty acids, notably timnodonic (20:5\( \omega3 \)). Among the tissues studied were those of the immune system. We have now selected the immune system as a model for illustrating the modulating effect of \( \alpha \)-linolenic acid on PG synthesis. As a prelude to studies on the functional activities of immunocompetent cells, we studied the effects of various dietary levels of linolenic to linoleic acids on their major phospholipid fatty acid profiles.

MATERIALS AND METHODS

Animals and Diets

Male weanling Sprague-Dawley rats (Holtzman Co., Madison, WI) (50-60 g) were maintained on one of three purified diets, each adequate in all nutrients, for 8 weeks. The diets varied in the type of fat fed, i.e., 10% of the diet by weight contained either corn oil (CO), soybean-linseed mixture (SL) or linseed oil (LO). Diet composition was previously reported in full detail by this laboratory (1). All animals were individually housed in polypropylene cages with SanicelR bedding. A diurnal light cycle of 12 hr was maintained and food and water were available ad libitum. Body weights were recorded once a week throughout the feeding trial. The SL and LO diets were prepared fresh every day and all diets and oils were periodically checked for deterioration by determination of the peroxide number (2).

Cell Isolation

At the end of the feeding trial, the rats were lightly anesthetized with ether and bled by heart puncture. The blood was drawn into 2 ml of 10 mg/ml heparin-phosphate-buffered saline (pH 7.4) solution. The spleen and thymus were quickly excised and individually placed in 10 ml of ice-cold medium RPMI 1640 with L-glutamine (Grand Island Biological Company (GIBCO), Grand Island, NY) and 25 mM 4(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffer (pH 7.4). The cells were obtained by perfusion of the tissue with RPMI 1640. The cells were pelleted and washed twice by centrifugation at 400 \( \times \) g for 10 min. Red blood cells were removed from the splenocytes by resuspending the cell pellet in 0.83% ammonium chloride for 10 min at 25 C. After centrifugation at 400 \( \times \) g for 10 min, both

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the thymocyte and splenocyte pellets were stored under nitrogen at -20°C for lipid analysis. Trypan blue exclusion was used to demonstrate 95-99% cell viability after the isolation procedures.

The heparinized blood was added to 3 times its volume of RPMI 1640. The blood-RPMI mixture was layered over sterile lymphocyte separation medium (LSM) (Litton Bionetics, Inc., Kensington, MD) which has a density of 1.077-1.080 and centrifuged at 400 x g for 35 min. The peripheral lymphocytes at the interface between plasma-RPMI and LSM were removed. The isolated lymphocytes were washed in sterile RPMI 1640 medium, by successive centrifugations at 400 x g for 10 min. Assessment by light microscopy showed the peripheral lymphocytes to be 97-99% pure and >95% viable as determined by Trypan blue exclusion. At this point, the cells were pelleted and stored at -20°C under nitrogen for lipid analysis.

Mast cells were isolated as described by Sullivan et al. (3). Mast cells were harvested and purified using a mast cell medium (MCM) containing 150 mM NaCl, 3.7 mM KCl, 3.0 mM Na2HPO4, 3.5 mM KH2PO4, 0.9 mM CaCl2, 5.6 mM dextrose, 0.1% bovine serum albumin (BSA) (w/v), 0.1% gelatin (w/v) and heparin, 10 units/ml, pH 6.8. Thirty ml of ice-cold MCM was injected into the thoracic cavity of anesthetized rats, the area was gently massaged and the cell suspension was recovered by aspiration with a siliconized pasteur capillary pipette. The suspension was centrifuged at 50 x g for 7 min, the erythrocytes were lysed with 0.83% ammonium chloride and the cell suspensions from 4 rats were pooled. After centrifugation at 50 x g for 7 min, the pooled cell pellet was resuspended in 2 ml of MCM and layered onto 4 ml of a 38% BSA in MCM solution (w/v) in a 50-ml polycarbonate centrifuge tube. The cells settled for 25 min at 25°C and then were centrifuged at 450 x g for 20 min at 25°C. The MCM layer and the cells at the interface were removed by aspiration. The interface was washed twice with 4 ml MCM. Ten vol of MCM were then added to the BSA layer and the cells were collected by centrifugation at 50 x g for 10 min at 25°C. These cells were frozen for lipid analysis. Wright's differential staining and toluidine blue staining (4) showed the final cell pellet to be an essentially pure mast cell population.

Lipid Analysis

Lipid analysis of the splenocyte, thymocyte, peripheral lymphocyte, and mast cell populations was carried out as previously described (5). After extracting the cell pellet with chloroform/methanol (2:1, v/v), the lipids were separated by two-dimensional thin layer chromatography (TLC) on Silica Gel H (6). The choline glycerophosphatide (PC) and ethanolamine glycerophosphatide (PE) fractions were collected and their derivatized fatty acids analyzed by gas liquid chromatography (GLC).

Statistical Analysis

Analysis of variance for balanced data (ANOVA), general linear model analysis for unbalanced data (GLM) and Student Newman-Keuls' Test (α=0.05) for variability were performed using Statistical Analysis System (SAS) (Cary, NC) computer programs.

RESULTS

The dietary treatments showed no significant effect on rat body weights which were 421 ± 8 g, 422 ± 14 g and 402 ± 11 g (mean ± SEM) for the CO, SL and LO diets, respectively.

Fatty Acid Analysis

The PC and PE fatty acid composition of cells isolated from the spleen were significantly altered by the higher ratio of α-linolenic to linoleic acid. In the PE fraction (Table 1), 18:3ω3 increased from 1.2 ± 0.3% in the CO group to 3.5 ± 0.8% in the LO group. This trend was also seen with 20:5ω3 which increased from nondetectable levels to 6.5 ± 0.3% (p<0.005). Docosahexaenoic acid (22:6ω3) also significantly increased, but to a lesser extent than the other ω3 derivatives. The ω6 fatty acids were shown to decrease on the SL and LO diets. Arachidonic acid significantly decreased from 29.6 ± 1.4% in the CO diet to 21.8 ± 0.7% (p<0.005) in the LO diet and 22:4ω6 decreased from 8.1 ± 0.4% to 1.7 ± 0.3% (p<0.005). The same trends were observed in the PC fatty acid (Table 1) response to the higher 18:3ω3/18:2ω6 diets. Arachidonic acid and 22:4ω6 both significantly decreased. The ω3 derivatives, 20:5ω3, 22:5ω3 and 22:6ω3 all increased significantly when the CO diet was compared to the LO diet.

Due to insufficient sample number, PC results for the thymocyte population are not reported. The changes in thymocyte PE fatty acids observed were similar to those shown by the splenocyte PE fatty acid profile (Table 2). Arachidonic acid from rats fed the CO diet was 43 ± 2.7% and significantly different (p<0.0011) from SL and LO (37.3 ± 0.5%, 26.6 ± 0.9%). Timnodonic acid (20:5ω3) increased from nondetectable levels in the CO group to 7.2 ± 1.2% in the LO group.