Available online at www.sciencedirect.com
ScienceDirect
Nutrition Research 28 (2008) 714 – 721
Nutrition Research
www.nrjournal.com

Dietary inositol hexakisphosphate, but not *myo*-inositol, clearly improves hypercholesterolemia in rats fed casein-type amino acid mixtures and 1,1,1-trichloro-2,2-*bis* (*p*-chlorophenyl) ethane

Yukako Okazaki*, Tetsuyuki Katayama[1]

Department of Human Life Sciences Education, Graduate School of Education, Hiroshima University, Higashi-Hiroshima 739-8524, Japan

Received 27 April 2008; revised 20 June 2008; accepted 23 July 2008

Abstract

We have previously shown that dietary inositol hexakisphosphate (IP6) and *myo*-inositol prevent fatty liver in rats fed a casein-based diet containing 1,1,1-trichloro-2,2-*bis* (*p*-chlorophenyl) ethane (DDT). This study was performed to examine the comparative effects of dietary equimolar amounts of sodium IP6 (1.02%) and *myo*-inositol (0.2%) on the development of DDT-induced fatty liver and hypercholesterolemia in rats fed 20% casein-type amino acid mixtures designed to exclude a possible *myo*-inositol contaminant in casein. Thirty-six male Wistar rats were divided into 6 groups of 6 rats each for: a control group, *myo*-inositol–supplemented group, IP6-supplemented group, DDT-treated group, DDT + *myo*-inositol–supplemented group, and a DDT + IP6–supplemented group. Dietary IP6 clearly suppressed the rises in serum concentrations of cholesterol and phospholipids because of DDT feeding, but *myo*-inositol had no significant influence on such elevations. Dietary IP6, but not *myo*-inositol, caused significant body weight gain with or without DDT intake. Supplemental IP6 and *myo*-inositol significantly increased hepatic-free *myo*-inositol regardless of DDT intake and prevented fatty liver in rats fed DDT. In conclusion, dietary IP6 and *myo*-inositol exert similar effects on DDT-induced fatty liver and *myo*-inositol status but distinct effects on DDT-induced hypercholesterolemia and growth rate in rats fed casein-type amino acid mixtures.

Keywords: Inositol hexakisphosphate (IP6); *myo*-inositol; Hepatic lipids; Hepatic free *myo*-inositol level; Phosphatidylinositol; DDT; Rat

Abbreviations: DDT, 1,1,1-trichloro-2,2-*bis* (*p*-chlorophenyl) ethane; HPLC, high-performance liquid chromatography; IP6, Inositol hexakisphosphate; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; G6PD, glucose 6-phosphate dehydrogenase; ME, malic enzyme; NADH, reduced nicotinamide adenine dinucleotide.

1. Introduction

Inositol hexakisphosphate (IP6, phytate) is an abundant constituent of plants, comprising 1.5% to 6.4% by weight of whole grains, cereals, legumes, nuts, and oil seeds [1,2]. Because IP6 is postulated to impede the bioavailability of minerals, it historically has been considered to be an antinutrient, whereas *myo*-inositol is considered a vitamin-like substance [3,4]. Interest in IP6 and *myo*-inositol as nutrients was initially stimulated by the work of Woolley [5], who reported on alopecia in young mice. However, little attention has been paid to the similarity in nutritional or physiologic functions between IP6 and *myo*-inositol since then. The anticancer effects of IP6 have been shown in a series of reports by Shamsuddin et al [6,7]. They have suggested that the most consistent and best anticancer

* Corresponding author. Department of Human Life Studies, Faculty of Human Life Sciences, Fuji Women's University, Ishikari, Hokkaido 061-3204, Japan. Tel.: +81 133 74 7498; fax: +81 133 74 8344.
E-mail address: yokazaki@fujijoshi.ac.jp (Y. Okazaki).
[1] Present address: Tsuno Rice Fine Chemicals Co. Ltd., Wakayama 649-7194, Japan.

0271-5317/$ – see front matter
doi:10.1016/j.nutres.2008.07.003

results were obtained from the combination of IP6 plus *myo*-inositol [8].

Administration of xenobiotics such as 1,1,1-trichloro-2,2-*bis* (*p*-chlorophenyl) ethane (DDT) or dietary sucrose cause hypercholesterolemia and fatty liver [9-11]. Recently, we demonstrated that dietary equimolar amounts of IP6 and *myo*-inositol similarly reduced hepatic lipids and the lipogenic enzyme activities in rats fed DDT or a high-sucrose diet [9-11]. Moreover, we have shown that supplemental *myo*-inositol elevated the hepatic-free *myo*-inositol level and recovered the reduced phosphatidylinositol (PI)/phosphatidylcholine (PC) ratio induced by DDT intake [12]. Therefore, we postulated that IP6 also affects hepatic *myo*-inositol status in DDT-fed rats.

Some researchers reported that dietary IP6 exerts cholesterol-lowering effects [13,14]. But our previous results showed that the intake of IP6 or *myo*-inositol did not affect the rise in serum cholesterol levels induced by DDT feeding [9,10]. The recommended source of protein in AIN-93 diet for laboratory rodents is milk casein, with a purity of less than 85% [15]. In addition, it is well-known that milk contains *myo*-inositol [16]. We opined that dietary casein may be contaminated with *myo*-inositol and that the contaminant could affect serum cholesterol level and *myo*-inositol status in rats fed DDT. Thus, our present study was conducted to examine the comparative effects of dietary IP6 and *myo*-inositol on DDT-induced increases in serum cholesterol and accumulation of hepatic lipids and hepatic *myo*-inositol status in rats fed casein-type amino acid mixtures.

2. Methods and materials

2.1. Chemicals

1,1,1-Trichloro-2,2-*bis* (*p*-chlorophenyl) ethane was purchased from Tokyo-kasei Ind, Ltd (Tokyo, Japan). Inositol hexakisphosphate and *myo*-inositol were obtained from Tsuno Rice Fine Chemicals Co, Ltd (Wakayama, Japan). Amino acids, Triacylglycerol E-Test Wako, Cholesterol E-Test Wako, and Phospholipid-Test Wako were purchased from Wako Pure Chemicals (Osaka, Japan). Silica high-performance thin-layer chromatography plate was purchased from Merck KGaA (Darmstadt, Germany). High-performance liquid chromatography (HPLC) column and 0.45μm Millipore filters were purchased from Waters (Milford, Mass). High-performance liquid chromatography internal standard of *epi*-inositol was a gift from Hokko Chemical industry Co, Ltd (Kanagawa, Japan). All other chemicals were analytical grade.

2.2. Animals and experimental diets

Male rats of Wistar strain, weighing 57 to 70 g (3 weeks of age), were obtained from Hiroshima Laboratory Animal Center (Hiroshima, Japan). They were housed individually in stainless steel wire-mesh cages in a temperature-controlled room (24 ± 1°C) with a 12-hour light-dark cycle (lights on 8 AM to 8 PM). After feeding a stock diet (MF, Oriental Yeast Co, Ltd, Tokyo, Japan) for 3 days, the rats were divided into 6 groups of 6 rats each. Compositions of experimental diets are listed in Table 1. Rats were fed control diet, *myo*-inositol–supplemented diet (0.2 g/100 g of diet), IP6-supplemented diet (1.02 g/100 g of diet), DDT-containing diet (0.07 g/100 g of diet), DDT-containing diet supplemented with *myo*-inositol, and DDT-containing diet supplemented with IP6. DDT, *myo*-inositol, and sodium IP6 were supplemented at the expense of sucrose. Composition of the amino acid mixture simulating casein is shown in Table 2 [15]. Because we decided to add L-cystine at 3 g/kg as the supplement to the AIN 93G rodent diet (MF, Oriental Yeast Co Ltd, Tokyo, Japan), the composition included L-cystine in addition to the diet [15]. The animals had free access to the experimental diets and water for 2 weeks. Food intake and body weight were measured every day.

At the end of the feeding period, 12 hours after the last feeding, the rats were euthanized by withdrawing blood from abdominal aorta under diethyl ether anesthesia. Serum was separated by centrifugation at 3000*g* for 10 minutes. After collection of blood, the whole liver was immediately removed and weighed. A portion of the liver was stored at −80°C until analyzed for free *myo*-inositol. The remaining liver was homogenized in 4 volumes of 0.14 mol/L KCl. An aliquot of the homogenate was used for lipid analysis. Another aliquot of the homogenate was centrifuged at 10 000*g* for 10 minutes, followed by recentrifugation of the supernatant at 105 000*g* for 60 minutes. The resulting supernatant was stored at −80°C until needed for assays of lipogenic enzymes. The experimental protocol was performed according to the “Guide for the Care and Use of Laboratory Animals” established by Hiroshima University and the Animal Care Committee of Hiroshima University, Japan. Feeding of 0.07% DDT for 2 weeks had no apparent

Table 1
Composition of the experimental diets

Ingredients (g/kg diet)	Control	DDT
Amino acid mixture [a]	200	200
Sucrose	503	502.3
Potato starch	150	150
Corn oil	50	50
Cellulose powder	50	50
Mineral mixture [b]	35	35
Vitamin mixture [b]	10	10
Choline bitartrate	2	2
DDT [c]	–	0.7

[a] The content was based on amino acid composition of casein [15]. The amino acid composition was explained in further detail in Table 2.

[b] Mineral and vitamin mixtures for AIN-93G rodent diet.

[c] DDT purchased from Tokyo-kasei Ind, Ltd, was supplemented at the level of 0.7 g/kg diet. *myo*-Inositol and sodium IP6 supplied by Tsuno Rice Fine Chemicals Co, Ltd, were added at the levels of 2 g/kg of diet and 10.2g/kg of diet, respectively. The additions of them were made at the expense of sucrose.