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Communications

Chromium picolinate depressed proliferation and differentiation of 3T3-L1 preadipocytes

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Abstract

This in vitro study investigated the effects of trivalent chromium picolinate (CrPic) on the proliferation and differentiation of preadipocytes. The mouse preadipocyte cell line (3T3-L1) used in this study included control and the 50 ppb CrPic groups. Preadipocyte differentiation results showed that the number of differentiated adipocytes and their triacylglycerol content in the CrPic group is significantly less ($P < .01$) than that in the control group. Transcription factor expression of peroxisome proliferator–activated receptors γ in the CrPic group was substantially depressed ($P < .0001$) compared with that in the control group. Glycerophosphate dehydrogenase activity in the CrPic group was markedly depressed ($P < .0001$) compared with that in the control group; fatty acid synthase activity in the CrPic group was also significantly repressed ($P < 0.0001$). For the proliferation of preadipocytes, the number of cells in the CrPic group was lower than that in the control group ($P < .01$). Results of this study indicated that 50 ppb CrPic significantly depressed preadipocyte proliferation and differentiation.

Keywords: Preadipocytes; Differentiation; Proliferation; Chromium picolinate; PPAR

1. Introduction

Trivalent chromium, recognized as an essential trace element in humans and in domestic animals, has a favorable action on insulin activity [1,2]. As a component of the glucose tolerance factor, trivalent chromium is associated with the metabolism of carbohydrates, lipids, and proteins. Chromium deficiency can result in growth retardation, interfere with blood glucose tolerance, and lead to hyperglycemia, hypercholesteromia, and hyperlipidemia [3]. These syndromes can be alleviated by chromium supplementation [4,5].

Some investigations indicate that chromium picolinate (CrPic) can generate hydroxy radicals and cause damage to cellular DNA [6-9]. However, in 2002, CrPic was affirmed as a GRAS (generally recognized as safe) substance for use in food products to reduce hyperglycemia, stabilize blood glucose levels, increase lean body mass, reduce body fat, and maintain healthy cholesterol level [10,11].

Studies have shown that chromium function improves body composition. A study by Kaats et al [12,13], in which subjects received either a placebo or 200 to 400 μg of CrPic daily, showed that CrPic leads a significant loss of fat mass. Grant et al [14] reported that 400 μg of CrPic added to the diet of subject for 16 weeks caused a significant reduction in body fat and body weight. Domestic animal studies have also indicated that supplementation of 200 to 400 ppb trivalent organic chromium reduced body fat in pigs [15-20].

Other research revealed that CrPic has no effect on body composition in humans [21-26]. A domestic animal study by Mooney and Cromwell [27] showed that supplementation of 200 ppb of chromium from CrPic to barrows was ineffective in altering the composition of the carcass.

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Thus, these conflicting results require further study to determine whether the commonly used level (200 ppb) of chromium supplementation could reduce body fat and to confirm the results in our previous in vivo studies [15-20]. Thus, this study examined the effects of CrPic supplementation on preadipocyte proliferation and differentiation.

2. Methods and materials

2.1. Reagents and materials

The preadipocytes cell line 3T3-L1 (CCRC EO159) was obtained from the Institute of Food Industry of Taiwan. Reagents for 3T3-L1 cell culture including Dulbecco's Modified Eagle's Medium (DMEM), Ham's F-12 medium, HEPES, L-glutamine, sodium bicarbonate, glucose, sodium pyruvate, penicillin and streptomycin, amphotericin B, dexamethasone (DEX), 1-methyl-3-isobutylxanthine (MIX), insulin, transferrin, lecithin, cholesterol, sphingomyelin, Oil Red O, and 0.5% bovine serum albumin were purchased from Sigma chemical Co (St. Louis, Mo, USA). Bovine calf serum (BCS) was acquired from Gibco BRL (Gaithersberg, MD). Chromium picolinate was supplied by Nutrition 21 Inc (Purchase, NY). Peroxisome proliferator–activated receptors γ (PPARγ)-specific rabbit antibody and goat antirabbit IgG horseradish polypeptidase-conjugated second antibody were purchased from Chemicon Inc (Temelula, CA). All other analysis reagents were obtained from Sigma Chemical Co.

2.2. Experiment 1: differentiation

Preadipocyte cell line 3T3-L1 was used in this study. Cell culturing consists of 3 stages of proliferation, induced differentiation, and differentiation. Dulbecco's Modified Eagle's Medium and Ham's F-12 medium (pH 7.2) containing 25 mmol/L HEPES were used in a 1:1 ratio as the basal culture medium, and the following were added: 4 mmol/L L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 1.0 mmol/L sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin, 2.5 μg/mL amphotericin B, and 10% BCS. The culture was maintained at 37°C in 5% carbon dioxide. When the preadipocytes reached 80% confluence, the cells were divided into 2 groups: a control group and a 50 ppb Cr supplementation group (account for the digestibility of CrPic about 25% that equaled to added 200 ppb in vivo). Cr source was an organic CrPic (CrPic made soluble in acetic acid and alcohol).

Cell differentiation was induced by adding 0.1 mmol/L DEX, 0.25 mmol/L MIX, 5 μg/mL insulin, 5 μg/mL transferrin, 1 mL/L lipid mixture (6 mg/mL lecithin, 3 mg/mL cholesterol, 1 mg/mL sphingomyelin), 2% BCS, and 0.5% bovine serum albumin to the medium for 3 days. The differentiation medium used was the same but with DEX and MIX omitted. The medium was changed every 2 days. Cells were then harvested at day 10, placed in liquid nitrogen for 10 minutes, and then placed in an ultrasonic bath for 10 minutes, followed by centrifugation at 1200 × g for 20 minutes. The supernatant was then stored at −80°C for further analysis.

The number of differentiated cells was measured following the procedure described by Brown et al [28]. Cells were washed twice with phosphate-buffered saline (PBS), and 10% formalin and 4% $CaCl_2$ were then added to fix the cells for 1 hour. Cells were then washed twice with ddH_2O and stained with 0.3% Oil Red O (soluble in isopropanol) for 15 minutes at room temperature. Cells were then washed with 20% ethanol and ddH_2O, and finally, the number of stained droplets at 5 fields (scanned by camera) in each sample was counted. Glycerophosphate dehydrogenase (GPDH) activity was measured in a buffer of 50 μL tetraethylammonium (TEAE) solution (pH 7.5) (0.5 mol/L triethanolamine, 10 mmol/L EDTA-2Na, 10 mmol/L 2-mercaptoethanol), 100 μL 5 mmol/L dihydroxyacetone phosphate, and 200 μL 0.5 mmol/L NADH. Enzymatic reaction was started by adding aliquots of 150 μL of cell sample and monitored based on the rate of change in absorbance at 340 nm, 25 °C [29].

Fatty acid synthase (FAS) activity was measured using the method described by Kumar et al [30]. Sample (0.3 mL) was added to the substrate buffer (1.77 mL 0.2 mol/L K_3PO_4, 0.15 mL 20 mmol/L dithiothreitol, 0.18 mL 0.25 mmol/L acetyl-CoA, 0.15 mL 60 mmol/L EDTA-2Na, 0.15 mL 6 mmol/L NADPH, and 0.3 mL 0.39 mmol/L malonyl-CoA) and monitored by 340 nm at 25°C. Protein content was measured using the Lowry method [31]. Triacylglycerol (TG) content in differentiated adipocytes was determined using a kit with a serum autoanalyzer (Roche Cobas Miras, Switzerland).

The PPARγ level was determined via Western blot analysis as described by Ohyama et al [32]. Briefly, cell samples were boiled for 5 minutes before electrophoresis. Equal cellular protein (200 μg) was diluted in a SDS-sample buffer and subjected to sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis. After electrophoresis, the gel was transferred to a polyvinylidine fluoride membrane, and PPARγ was detected using PPARγ-specific rabbit antibody (1:1000). The membrane was then incubated with goat antirabbit IgG horseradish polypeptidase-conjugated second antibody (1:1000). Immunoreactive polypeptides were visualized with the enhanced chemiluminescence sulfate (ECL) detection system. Western blot results were analyzed with computer-assisted image analysis using a charge couple device (CCD) camera and image software.

2.3. Experiment 2: proliferation

The proliferation experiment used a cell seeding 8×10^4 cells in each of 18 flasks. DMEM and Ham's F-12 medium (pH 7.2) (containing 25 mmol/L HEPES) were used in a 1:1 ratio as the culture medium, and 4 mmol/L L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 1.0 mmol/L sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin, and 5% BCS were added. Cells divided into the control (0) and the 50 ppb CrPic groups were incubated for 5 days at 37°C under 5% of carbon dioxide. Cells were