Food and Chemical Toxicology 48 (2010) 89–92

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Genotoxicity assessment of chromium(III) propionate complex in the rat model using the comet assay

Halina Staniek [a], Magdalena Kostrzewska-Poczekaj [b], Magdalena Arndt [b], Krzysztof Szyfter [b], Zbigniew Krejpcio [a,*]

[a] Department of Hygiene and Human Nutrition, Poznan University of Life Sciences, 31 Wojska Polskiego Street, 60-624 Poznan, Poland
[b] Department of Environmental Mutagenesis, Institute of Human Genetics, Polish Academy of Sciences, 32 Strzeszynska Street, 60-479 Poznan, Poland

ARTICLE INFO

Article history:
Received 24 July 2009
Accepted 11 September 2009

Keywords:
Comet assay
Genotoxicity
Chromium(III) propionate complex
Cr3

ABSTRACT

The aim of the study was to assess genotoxicity of a chromium(III) propionate complex in rat's peripheral blood lymphocytes by the comet assay. The study was carried out on 18 12-weeks old female Wistar rats that were divided into three equal groups (six animals each): control (0), control-Cr(VI) and Cr(III)-tested rat fed *ad libitum* a basal diet and the diet supplemented either with 10 mg Cr(VI)/kg diet (given as $K_2Cr_2O_7$, equivalent of 1 mg/kg body mass/day) or 1000 mg Cr(III)/kg diet (given as $[Cr_3O(O_2CCH_2CH_3)6(H_2O)_3]NO_3$), equivalent of 100 mg Cr/kg body mass/day) for 4 weeks. High doses of supplementary Cr(III) were found to not affect body mass gain, feeding efficiency ratio and internal organ masses. Treatment of rats with the Cr(III) propionate complex, in contrast to Cr(VI), did not affect significantly the comet assay results in lymphocytes, which suggests that the compound does not exert genotoxic effects in rats.

1. Introduction

Trivalent chromium (Cr^{3+}) is considered to be essential for normal carbohydrate lipid and protein metabolism. It is important for the normal function of insulin binding to insulin receptor sites. Determining accurate dietary chromium intakes is difficult because its content in foods is very small and can be altered during food processing (Food and Nutrition Board, Institute of Medicine, 2002). Recently, the National Academy of Sciences of the United States has established an Adequate Intake (AI) of Cr based on estimated mean intakes at 35 μg/day for men and 25 μg/day for women (National Research Council, 2002).

Currently the most popular nutritional supplement is chromium(III) tris(picolinate)/Cr(pic)$_3$/. In the past decade chromium-containing supplements have become very popular. Cr(pic)$_3$-containing products generated nearly $500 million in retail sales in the year 2000 with the supplement being second only to calcium supplements among mineral supplements (Hepburn and Vincent, 2003). However, chromium picolinate is not approved as a food additive nor listed as Generally Recognized as Safe (GRAS) by the FDA (Whittaker et al., 2005). The FDA has established a Reference Daily Intake (RDI) for chromium of 120 μg (21 CFR 101.9, 2004) to assist consumers in understanding the nutritional significance of the levels of this nutrient in the context of the total daily diet. Additionally, it has been demonstrated to cleave DNA at physiologically-relevant concentrations (Speetjens et al., 1999). Therefore there is a need for searching a new efficient and safer chemical form of this microelement. A number of other chromium-containing compounds have been proposed as substitutes for Cr(pic)$_3$. Of particular interest are low-molecular-weight chromium binding substance (LMWCr), also known as chromodulin, and its biomimetic analogue – $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ cation, also known as the trinuclear Cr(III) complex with propionic acid (CrProp) or Cr3. CrProp activates the insulin-dependent tyrosine protein kinase activity of insulin receptor in a fashion almost identical to that of chromodulin while a variety of the other chromic complexes have in contrast been found to be ineffective or inhibit kinase activity (Davis and Vincent, 1997; Davis et al., 1997). CrProp has a striking effect on plasma triglycerides, total cholesterol, LDL cholesterol, glucose concentrations and insulin levels in Sprague–Dawley rats after 24 weeks of administration of trimer at a level 0–1000 μg Cr/kg body mass (Clodfelder et al., 2005). For these reasons CrProp has been proposed as a potential therapeutic agent (United States Patent 7405313) (Shute et al., 2001; Shute and Vincent, 2002; Clodfelder et al., 2005). Previous studies have shown that CrProp has greater bioavailability than other organic and inorganic forms (Pickering et al., 2004; Rhodes et al., 2005; Kuryl et al., 2006). CrProp at nutritional supplement levels is absorbed *in vivo* with greater than 60% efficiency; and at pharmacological levels, it is absorbed with greater than 40% efficiency, an order of magnitude greater than that of $CrCl_3$, Cr nicotinate, or Cr picolinate, currently marketed nutritional supplements. The difference in degree of

* Corresponding author. Fax: +48 618487332.
E-mail address: zkre@up.poznan.pl (Z. Krejpcio).

0278-6915/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2009.09.020

absorption is readily explained by the stability and solubility of the cation (Clodfelder et al., 2004).

A number of studies have examined the genotoxicity of chromium supplements. Recent reports suggest that the coordinated ligands play an important role in the toxic behavior of chromium(III) compounds (Vijayalakshmi et al., 2000). Recently, the comet assay has become a useful tool for early genotoxicity testing of new pharmaceutical drug candidates because it is rapid and simple to perform and requires only minute amounts of test substances (Tice et al., 2000; Hartmann et al., 2003). Since there are still not enough data concerning the safety of CrProp, the purpose of this study was to evaluate the genotoxic potential of this compound as the extent of DNA degradation measured by the alkaline comet assay.

2. Materials and methods

2.1. Animals and diets

Eighteen female Wistar rats (12-weeks old) were obtained from the Licensed Laboratory Animals Breeding Center (Poznan, Poland). They were divided into three groups of (six rats each): control (0), control-Cr(VI) and tested-Cr(III), in such a way that the mean body mass were similar in each group (~196 g per animal). These groups were allowed to feed *ad libitum* on a commercial diet for maintenance of adult rodents (*Labofeed H*) or on the diet either supplemented with 10 mg Cr(VI)/kg diet (given as $K_2Cr_2O_7$, equivalent of 1 mg Cr/kg body mass/day) or 1000 mg Cr(III)/kg diet (given as $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]NO_3$, equivalent of 100 mg Cr/kg body mass/day) for 4 weeks. The rats were housed in single cages, under controlled temperature, photoperiod and air humidity (19–22 °C, 12-h light/dark cycle, 55–60% of ambient air humidity). Deionized water was available *ad libitum*. Feed intake was measured daily; body mass gains were monitored weekly. At the end of the study after 12 h starvation, rats were sacrificed by carbon dioxide asphyxiation, blood was collected into Li-heparinized tubes, and tissue samples (liver, kidneys, heart, spleen, pancreas, ovaries) were harvested and weighed. The experimental protocol was approved by The Animal Bioethics Committee of Poznan, Poland (No. 48/2005).

2.2. Chemicals

Chromium(III) propionate cation (CrProp) in the form of its nitrate salt (chemical formula $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+(NO_3)^-$ was synthesized in the laboratory of Department of Product Ecology, Poznan University of Economics, according to the method described previously by Earnshaw et al. (1966). The contents of elemental Cr (20.5%) was determined by the AAS method (spectrometer AAS-3 with BC correction, Zeiss, Germany). The authenticity and physicochemical characteristics of CrProp were determined as previously described by this laboratory (Wieloch et al., 2007).

$K_2Cr_2O_7$ (reagent grade, 35.35% Cr) was purchased from P.O.CH, Poland.

Others chemicals were of analytical grade and purchased from Serva, normal melting point agarose, Triton X-100 and 4′,6-diamidino-2-phenylindol·2HCl (DAPI); Sigma, dimethyl sulfoxide (DMSO) and tris; Bio-Rad, and low melting point agarose; IITD Poland, RPMI 1640 medium without L-glutamine; and Aqua-Medica Poland, Gradisol L.

2.3. Peripheral blood lymphocytes: isolation and treatment

Rat peripheral blood lymphocytes (PBL) were obtained from 10 individuals. The cells were separated by the standard method. The cells were suspended in the RPMI 1640 medium without L-glutamine and centrifuged over Gradisol L at 1200 rpm for 15 min. Next, centrifugation was performed twice at 700 rpm for 8 min.

2.4. Alkaline comet assay

The alkaline comet assay was conducted as described by Jaroszyński et al. (1999) and Jaroszyński and Szyfter (1999). Briefly, the PBL suspension (30 μl) was mixed with 70 μl of 1% low melting point agarose in the RPMI 1640 medium at 37 °C. The mixture was pipetted onto microscope slides previously pre-coated with a layer of 1% normal agarose. The slides were immersed in lysis solution (2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris, 1% of freshly added Triton X-100, pH 10) for 1 h to remove proteins.

The slides were then placed in a horizontal electrophoretic tank in cold buffer (4 °C, 3 M NaOH, 1 mM Na_2EDTA, pH 13) for 40 min to allow DNA unwinding. The electrophoresis was carried out in the same solution for 30 min (at 300 mA, 0.56 V/cm). Afterwards electrophoresis slides were removed from the tank, immersed in neutralization buffer (0.4 M Tris, pH 7.5), and stained with DAPI (2 μg/ml in distilled water).

2.5. Image analysis

Slides were examined with an Axiophot fluorescence microscope (Opton, Germany) with IMAC-CCD S30 camera and ISIS 3 v 2.00 image analysis system (MetaSystems Hard- and Software, Altlussheim, Germany). The spontaneous strand breaks were measured as total comet length (increase in DNA migration). Average values were calculated for 50 comets per slide. Slides were prepared in duplicate.

2.6. Statistical analysis

All results are presented as means ± standard deviation. Significance of differences of means were calculated using the one-way ANOVA and Tukey's *t*-test; means were considered statistically different if $p < 0.05$. All calculations were made using the STATISTICA (ver. 7.0) program.

3. Results and discussion

The effects of Cr(VI) and Cr(III) exposure on overall nutritional indices are presented in Tables 1 and 2. Average feed intake was similar in all experimental groups; however, body mass gain was significantly lower (by 30%) in the Cr(VI) treated group, in comparison with the CrProp treated group and the control (0) group. Feeding efficiency ratio expressed in gram of body mass gain per 100 g diet, which demonstrates how well feed is turned into body mass, was insignificantly lower in the potassium dichromate exposed rats, in comparison with the control (0) and the CrProp treated rats. The Cr(VI)-treated rats had significantly lower spleen and pancreas masses (by 30.6% and 54.5%, respectively) while possessing increased heart mass (by 65.2%), as compared to the control group, whereas CrProp did not affect inner organs masses of rats (Table 2). Dietary supplementation with CrProp given in dosages of 1000 mg Cr/kg of diet (ca. 100 mg Cr/kg body mass/day) for 4 weeks did not produce signs of toxicity. Histological analyses did not show deleterious changes in liver and kidney tissue (data not shown). The extents of DNA degradation in peripheral blood lymphocytes measured by the comet assay are shown in Table 3 and Figs. 1–3. The mean comet length obtained from lymphocytes of rats exposed to Cr(VI) (1 mg Cr/kg body mass/day) was significantly longer (by 27%) in comparison with that of the control group

Table 1
The effect of dietary chromate and CrProp supplementation on nutritional indices in rats (mean ± SD).

Index*	Control (0) group	Control-Cr(VI) group	Cr(III)-treated group
Feed intake (g/day/rat)	17.6 ± 0.5	17.7 ± 0.63	18.5 ± 0.5
Body mass gain (g/28 days)	9.5 ± 3.0[b]	7.5 ± 2.7[a]	10.7 ± 2.8[b]
Feeding efficiency ratio (g/body mass/100 g of diet)	1.90 ± 0.97	1.51 ± 0.92	2.11 ± 0.69

* Different letter superscripts indicate a statistically significant difference at $p < 0.05$.

Table 2
Body and organ mass of rats (mean ± SD).

Index*	Control (0) group	Control-Cr(VI) group	Cr(III)-treated group
Final body mass (g)	206 ± 9	204 ± 7	212 ± 16
Liver mass (g)	5.686 ± 0.238	5.634 ± 0.432	5.469 ± 0.118
Kidney mass (g)	1.441 ± 0.078	1.540 ± 0.136	1.495 ± 0.090
Spleen mass (g)	0.640 ± 0.048[b]	0.444 ± 0.039[a]	0.652 ± 0.026[b]
Heart mass (g)	0.414 ± 0.037[a]	0.684 ± 0.038[b]	0.413 ± 0.039[a]
Pancreas mass (g)	1.762 ± 0.061[b]	0.801 ± 0.082[a]	1.755 ± 0.011[b]
Ovaries mass (g)	0.089 ± 0.029	0.112 ± 0.023	0.085 ± 0.007

* Different letter superscripts indicate a statistically significant difference at $p < 0.05$.