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Molecular analysis of *hprt* mutations induced by chromium picolinate in CHO AA8 cells

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Abstract

Chromium picolinate (CrPic) is a popular dietary supplement, marketed to the public for weight loss, bodybuilding, and control of blood sugar. Recommendations for long-term use at high dosages have led to questions regarding its safety. Previous studies have reported that CrPic can cause chromosomal aberrations and mutations. The purpose of the current work was to compare the mutagenicity of CrPic as a suspension in acetone versus a solution in DMSO, and to characterize the *hprt* mutations induced by CrPic in CHO AA8 cells. Treatments of 2% acetone or 2% DMSO alone produced no significant increase in 6-thioguanine (6-TG)-resistant mutants after 48 h exposures. Mutants resistant to 6-TG were generated by exposing cells for 48 h to 80 μg/cm^2 CrPic in acetone or to 1.0 mM CrPic in DMSO. CrPic in acetone produced an average induced mutation frequency (MF) of 56 per 10^6 surviving cells relative to acetone solvent. CrPic in acetone was 3.5-fold more mutagenic than CrPic in DMSO, which produced an MF of 16.2. Characterization of 61 total mutations in 48 mutants generated from exposure to CrPic in acetone showed that base substitutions comprised 33% of the mutations, with transversions being predominant; deletions made up 62% of the mutations, with one-exon deletions predominating; and 1–4 bp insertions made up 5% of the characterized mutations. CrPic induced a statistically greater number of deletions and a statistically smaller number of base substitutions than have been measured in spontaneously generated mutants. These data confirm previous studies showing that CrPic is mutagenic, and support the contention that further study is needed to verify the safety of CrPic for human consumption.

Keywords: Chromium picolinate; *hprt*; Mutational spectrum; Genotoxicity; Chinese hamster ovary

1. Introduction

Chromium picolinate (CrPic) is a popular dietary supplement, originally advertised to the public as an aid for weight loss and bodybuilding. The use of CrPic as an anabolic agent was originally patented in 1992 [1]. Sales of chromium supplements in the US were reported to be second only to those of calcium supplements in 1999 [2], with sales of chromium-based supplements exceeding $500 million in that year [3]. It is estimated that 10 million Americans take some form of chromium supplement [2], with CrPic being the most popular.

In spite of its marketing success, clinical studies have not supported the efficacy of CrPic for weight loss or muscle building. A recent meta-analysis [4] found only a minor effect, if any, of CrPic on weight loss relative to placebo in 10 randomized, double-blind, placebo-controlled trials [5–14]. The mounting evidence against CrPic's efficacy for weight loss has recently led

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to a shift in focus to another market, namely type II diabetes.

Several studies have shown significant effects for CrPic on serum parameters related to diabetes, with responses proportional to the doses ingested. At a dose of 200 μg CrPic/day for 2 months CrPic supplementation had no affect on glucose control, LDL or HDL levels; however, triglyceride levels were decreased in 28 non-insulin-dependent diabetics [15]. Supplementation with 600 μg CrPic/day decreased fasting glucose levels by 40% in three subjects with steroid-induced diabetes [16]. In the most widely publicized study, 180 subjects were either given placebo, 200 or 1000 μg Cr/day as CrPic for 4 months [17]. Both fasting and 2 h glucose levels, as well as total plasma cholesterol were lowered most significantly for the 1000 μg group. Fasting and 2 h insulin levels decreased significantly with both CrPic doses.

The recommended use of CrPic to treat type II diabetes raises several safety issues. A dose of 1000 μg Cr as CrPic is 18–77 times higher than average daily dietary Cr intakes [18–20]. A recent patent recommends even higher doses of CrPic: 1000–10,000 μg Cr/day, for diabetics [21]. The Cr(III) in CrPic is more bioavailable than dietary Cr, and this metal can accumulate in humans [22,23]. Coordination of Cr by picolinate may alter the redox chemistry of Cr(III) [24], making it more toxic than other forms of Cr(III). Diabetics using CrPic would be ingesting large amounts of the supplement over their lifetimes; therefore, the potential for toxic effects from long-term exposures to high doses of CrPic needs to be considered.

There are a growing number of studies characterizing the cytotoxicity and genotoxicity of CrPic. The first study to question its safety showed that CrPic caused chromosomal aberrations after 24 h exposures in Chinese hamster ovary (CHO) AA8 cells, whereas equivalent doses of chromic chloride and chromium nicotinate were inactive, and free picolinate was clastogenic only above the 1.5 mM concentration [25]. The same doses of CrPic were mutagenic at the *hprt* locus in CHO cells after 48 h exposure, whereas equivalent doses of free picolinate and chromic chloride were not mutagenic [26]. CrPic was also shown to cause mitochondrial damage and apoptosis in the CHO AA8 cell line [27]. Studies funded by the supplement manufacturer did not find evidence of *hprt* mutations [28] or chromosomal aberrations [29]; however, the mutagenicity of CrPic has been independently confirmed in *Drosophila melanogaster* [30] and in the mouse-lymphoma assay [31].

Observations that CrPic can cause direct DNA damage have supported reports of its mutagenicity. An *in vitro* study showed that CrPic caused single strand breaks in isolated plasmid DNA in the presence of ascorbate plus dioxygen, dithiothreitol plus dioxygen, or hydrogen peroxide, presumably by a Fenton-type mechanism where CrPic cycles between the Cr(III), Cr(II) and Cr(IV) oxidation states to generate free radicals [24]. Analysis of CrPic-exposed cells by the alkaline comet assay have shown DNA strand breaks that increased with post-treatment exposure to formamidopyrimidine glycosylase (FPG) and decreased with post-treatment exposure to methyl methanesulfonate (MMS), suggesting the presence of DNA strand breaks, oxidative damage and DNA crosslinks (Lencinas et al., in preparation).

The purpose of the current study was to characterize the *hprt* mutations induced by CrPic in CHO AA8 cells. Results showed that the mutation spectrum was significantly different than that reported for spontaneous mutations in CHO K1-BH4 cells [32]. Determination of the molecular spectrum of mutations will assist in the elucidation of the mechanism underlying the observed CrPic-induced mutations, will be helpful for both predicting and confirming the types of DNA lesions induced by this dietary supplement, and will contribute to evaluation of the safety of this compound for human ingestion.

2. Materials and methods

2.1. Chemicals

Chromium trispicolinate (CAS No. 14639-25-9) was synthesized as previously described [27]. Elemental analysis of CrPic was carried out by inductively coupled plasma optical emission spectroscopy (ICP-OES). Expected % Cr for monohydrate $Cr(Pic)_3 \cdot H_2O$ (MW 436.32) $CrC_{18}H_{14}O_7N_3$ 11.9%; expected % Cr for anhydrous $Cr(Pic)_3$ (MW 418.31) $CrC_{18}H_{12}O_6N_3$ 12.4%; found 12.5 ± 0.6%. The solubility of CrPic in DMSO is ~50 mM. The solubility in aqueous solution is ~0.6 mM at ambient temperature and ~3 mM at 37 °C (data not shown). CrPic was prepared as a 50.0 mM stock solution in DMSO by sonication, or was prepared as an acetone slurry of 22.0 mg CrPic/mL by stirring suspensions overnight with a 0.5 in. stir bar in a sealed 20 mL liquid scintillation vial. Treatments consisted of 200 μL stock solution or solvent in 10.0 mL medium in a 100 mm dish with a 55 cm^2 growth area.

2.2. Cell culture

CHO AA8 cells (American Type Culture Collection, Manassas, VA) were cultured as adherent monolayers as previously described [26]. Cells were treated with 200 μM hypoxanthine, 0.04 μM aminopterin, and 17.5 μM thymidine (HAT; Sigma, St. Louis, MO) for 3 days to eliminate background mutations and recovered for 2 days in medium supplemented with 200 μM hypoxanthine and 17.5 μM thymidine (HT; Sigma).