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Mutation Research 585 (2005) 86–95
Genetic Toxicology and Environmental Mutagenesis
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Lack of mutagenicity of chromium picolinate in the hypoxanthine phosphoribosyltransferase gene mutation assay in Chinese hamster ovary cells☆

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Received 9 February 2005; received in revised form 4 April 2005; accepted 14 April 2005
Available online 10 May 2005

Abstract

Chromium picolinate (CrPic, Chromax®) is a dietary supplement that is stable and more bioavailable than other commercially available forms of chromium. Chromium supplementation is known to enhance the action of insulin, particularly in insulin resistance and type 2 diabetes mellitus. A previous study reported that CrPic produced increases in mutations of the hypoxanthine phosphoribosyltransferase (*Hprt*) gene in Chinese hamster ovary (CHO) cell mutation tests. This study, however, evaluated CrPic produced by the testing laboratory and used an atypical 48 h exposure period for this test system. The current study evaluated the mutagenic potential of the most widely utilized commercial form of CrPic in CHO/Hprt mutation tests following International Conference on Harmonisation (ICH) Guidelines (±S9 metabolic activation with a 5 h exposure) in addition to repeating the test with a 48 h exposure period −S9 activation. CrPic was suspended in dimethyl sulfoxide (DMSO) up to a concentration of 50 mg/mL; exposures were conducted under conditions in which precipitate was not evident and under conditions in which some precipitate of CrPic was visually evident in the cell culture medium at the highest concentrations (500 μg/mL). The concentrations evaluated for mutagenicity ranged from 15.6 to 500 μg/mL (+S9 and −S9) for the 5 h exposure and 31.3–500 μg/mL for the 48 h exposure (−S9). Only a slight degree of cytotoxicity was seen in the standard tests up to the limit of solubility in the medium. Toxicity, i.e., cloning efficiency ≤ 50% of the solvent control, but no mutagenic increases were observed at 500 μg/mL following a 48 h exposure period. The results of these studies showed that CrPic was non-mutagenic in two independent CHO/Hprt assays and in an assay using a 48 h exposure period.
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Keywords: Chromium picolinate; Chromax®; Chinese hamster ovary cells; Hypoxanthine phosphoribosyltransferase; Hprt mutation assay

☆ Paper presented as a poster at 35th Annual Meeting from the Environmental Mutagen Society, October 2–6, 2004, Pittsburgh, Environ. Mol. Mutagen. 44, 227 (Abstract 163).

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doi:10.1016/j.mrgentox.2005.04.001

1. Introduction

Chromium occurs in different oxidation states from −2 to +6 and the oxidation state can significantly affect its chemical and biological effects [1]. Trivalent chromium (Cr^{3+}) is a biologically important form of chromium that is an essential element required for normal carbohydrate, lipid and protein metabolism in humans and animals [1,2]. Some Cr^{3+} salts and complexes have been shown to produce beneficial health effects on enhancing normal insulin function and improving carbohydrate and lipid metabolism as discussed below. In contrast, hexavalent chromium compounds are potentially toxic for humans and have been reported to produce adverse health effects, mutations and cancer in animals and humans [3]. Cr^{3+} is found in microgram quantities in fruits, vegetables and grain products [1,4] and dietary intake ranges from 25 to 33 μg/day of trivalent chromium from food sources [5,6]. Chromium picolinate (CrPic, Chromax®) is a stable complex of a Cr^{3+} salt and picolinic acid that is produced by a proprietary method by Nutrition 21 Inc., Purchase, NY. Picolinic acid, a pyridine compound, structurally similar to nicotinic acid, is produced normally in the body both as a metabolite of tryptophan and synthesized via the kynurenine pathway [7]. Urinary excretion of picolinic acid conjugate by individuals not consuming picolinate-containing supplements is approximately 20 mg/day [8], indicating that a typical daily dose of up to 3.2 mg of CrPic (providing 400 μg of Cr^{3+} and 2.8 mg of picolinic acid) would not significantly affect normal body levels of picolinic acid.

For the past 2 decades, extensive research has been conducted on the effects of supplemental Cr^{3+} as CrPic on glucose metabolism in diabetes mellitus (DM). These reports have shown that CrPic reduces symptoms associated with diabetes including hyperglycemia [1,9,10], hyperinsulinemia [9–12], dyslipidemia [9,13,14], depression [15–17], polycystic ovary syndrome [18], atherosclerosis [19], pre-diabetes [20], type 2 DM [1,21–25], gestational diabetes [22] and corticosteroid-induced diabetes [24,25]. Studies in animals [26–31] and humans [32–35] indicate that CrPic is safe for consumption. These studies reported no adverse effects on liver or kidney function or other clinical findings.

Genotoxicity studies conducted by National Toxicology Program (NTP) reported that CrPic is not mutagenic in the Ames test in strains TA1535, TA97, TA98, TA100, TA102 or TA104 without activation or with 10 or 30% concentration of hamster or rat liver S9 homogenate metabolic activation mixtures [36]. CrPic monohydrate was not clastogenic in rat bone marrow cells following gavage doses of CrPic suspended in corn oil at doses up to 2500 mg/kg body weight [36]. No positive increases in micronuclei in polychromatic or normochromatic erythrocytes in peripheral blood were observed following dosing by dietary inclusion for 90 days at doses up to 50,000 ppm (equivalent to a calculated dose of approximately 7500 mg/kg) [36]. In 1995 and 2002, Stearns et al. [37,38] reported that samples of CrPic prepared in their laboratory and tested in acetone suspension were mutagenic and clastogenic to Chinese hamster ovary (CHO)–AA8 cells and that the coordination of Cr^{3+} with picolinic acid may be more genotoxic than other Cr^{3+} formulations. The U.K. Food Standards Agency Committee on Mutagenesis (COM) [39] noted that "the results were from a single, unusually long, treatment time of 48 h. The chromium picolinate used had been synthesized by the testing laboratory, and there was uncertainty regarding the nature and quantities of impurities in test material used." The positive genotoxicity findings engendered significant health concerns in the U.K. and the Food Standards Agency advised against consumption of CrPic [40]. In 2003, the U.K. COM [39] requested Nutrition 21 to repeat the CHO/Hprt and chromosome aberration tests in CHO cells with a commercially available form of CrPic (Chromax), using test protocols that complied with International Conference on Harmonisation (ICH) Guidelines [41,42]. Results from CHO/Hprt tests conducted by BioReliance, Rockville, MD, that followed ICH testing guidelines, are reported here that also included an evaluation of mutagenic effects from an extended 48 h exposure to CrPic, reported by Stearns et al. [38,39] to result in increases in mutations of the *Hprt* gene in CHO AA8 cells. Absence of clastogenic effects in CHO cells treated with Chromax CrPic were presented previously [43] and a manuscript summarizing those results is in preparation.

2. Materials and methods

2.1. Materials

The CHO–K1 cells were obtained from the American Type Culture Collection, Manassas, VA. Aroclor

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