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Review Article

Chromium(III) complexes

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CONTENTS

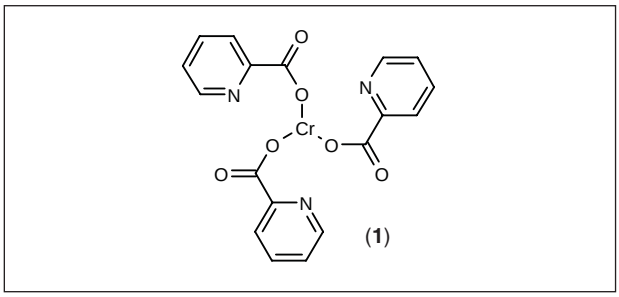
Abstract

While chromium picolinate ([Cr(pic)$_3$]) and the other currently marketed chromium(III) supplements are best known by the general public as weight loss and muscle building agents, the supplements have no effect on body composition. At pharmacological levels, they can have beneficial effects on type 2 diabetic subjects (lowering serum glucose, insulin and/or cholesterol levels). The beneficial effects probably arise from each compound serving as a source of Cr and not from any inherent activity from the intact compound. Deleterious effects, of particular concern at pharmacological doses, presently appear unique to [Cr(pic)$_3$] compared to other Cr supplements, while the beneficial effects have been observed with other Cr sources. The poor solubility of [Cr(pic)$_3$] and chromium polynicotinate limits their absorption; the chromic center of chromium chloride ($CrCl_3$) can chelate with large biomolecules and can form hydroxo-bridged oligomers (especially at the basic pH of the intestines), limiting its absorption. However, inorganic chromium salts, such as $CrCl_3$, are precursors to synthesizing chromium polynicotinate and [Cr(pic)$_3$] and significantly less expensive (even when differences in absorption are considered) and appear to fail to generate the deleterious effects. These salts and perhaps other organic Cr complexes (if they have substantial value-added benefit without deleterious effects) may have a future in the treatment of symptoms of adult-onset diabetes and related conditions.

Introduction

The element chromium (Cr) is generally assumed to be an essential trace element; the evidence is strongly supportive but not definitive (1). The only potential well-characterized cases of Cr deficiency arise from 5 subjects on total parenteral nutrition (TPN), before TPN was supplemented with Cr (2). The Food and Nutrition Board of the National Academy of Sciences (U.S.) established in 2001 that the daily adequate intake (AI) of Cr was 35 μg for adult males and 25 μg for adult females (3). [AI is the recommended intake value based on approximations or estimates of nutrient intake by healthy people who are assumed to have an adequate diet.] Anderson and Kozlovsky (4) in 1985 reported the Cr content of self-selected diets of 32 American men and women. The average daily Cr intake for men and women was 33 and 25 μg, respectively. Diets ranged in content from 13-48 μg, with the mean Cr intake per 1000 cal being ~15 μg. Other studies have shown that humans consuming 35 μg of Cr daily are Cr sufficient (5); thus, humans consuming a reasonable diet are not likely to be Cr deficient and should receive little if any benefit from dietary Cr supplementation.

The best candidate for a biologically active form of Cr is the oligopeptide chromodulin, formerly known as low-molecular-weight Cr binding substance (6, 7). Chromodulin is a naturally occurring oligopeptide composed of glycine, cysteine, aspartate and glutamate with the carboxylates comprising more than half of the total amino acid residues. Chromodulin has been proposed to function as part of a unique autoamplification system for insulin signaling. In this mechanism, apochromodulin is stored in insulin-sensitive cells. In response to increases in blood insulin concentrations, insulin binds to its receptor bringing about a conformation change that results in the autophosphorylation of tyrosine residues on the internal side of the receptor. This transforms the receptor into an active kinase and transmits the signal from insulin into the cell. In response to insulin, Cr, stored in the blood plasma bound to the iron transport protein transferrin, is moved from the blood into cells via endocytosis. The Cr flux results in loading of apochromodulin with Cr. The holochromodulin then binds to the insulin receptor, presumably assisting to maintain the receptor in its active conformation, amplifying its kinase activity. When insulin



(**1**)

signaling is to be turned off, a drop in blood insulin levels facilitates relaxation of the conformation of the receptor, and the holochromodulin is excreted from the cell into the blood. Ultimately chromodulin is excreted in the urine. The studies on which the mechanism is based suggest an essential relationship between Cr and proper glucose metabolism, probably associated with insulin action (6, 7). As insulin, in combination with other hormones, is responsible for regulating fat storage and protein synthesis, changes in levels of insulin action could in theory bring about changes in body composition.

This review will focus on the three most widely used forms of Cr(III) in nutritional supplements: chromium picolinate ($[Cr(pic)_3]$), chromium polynicotinate and chromium chloride ($CrCl_3$).

Synthesis

The synthesis of chromium picolinate ($[Cr(pic)_3]$) (**1**) was first described by Ley and Ficken in 1917 (8). In this procedure, the pinkish red complex is prepared by the reaction of a Cr(III) source and picolinic acid in water. Numerous procedures have subsequently been developed (1). However, the original procedure and variations on it provide the complex as a red crystalline solid in good yield and purity.

The reaction of two or three equivalents of nicotinic acid (Hnic) with an aqueous solution of chromic ions at elevated temperatures yields blue solutions from which blue solids of chromium polynicotinate precipitate at pHs of 2 or greater (9). These solids have an apparent formula of $Cr(nic)_2(H_2O)_3(OH)$, whereas the blue solutions have been proposed to contain the cation $[Cr(nic)_2(H_2O)_4]^+$. Thus, the composition and structure of chromium polynicotinate is less well-defined than that of $[Cr(pic)_3]$.

The commercially available form of chromium(III) chloride, $CrCl_3$, is actually *trans*-dichlorotetraaquochromium(III) chloride dihydrate, *trans*-$[CrCl_2(H_2O)_4]Cl{\cdot}2H_2O$. The compound is prepared by reaction of HCl gas with Cr(III) aquo complexes (10).

Description

$[Cr(pic)_3]$ in the procedure of Ley and Ficken is obtained as red crystals of the monohydrate. The three dimensional structure of the complex has been determined by X-ray crystallography; this technique clearly reveals the red monomer to be the meridinal isomer (11). The complex has limited solubility in water (~0.6 μM) as might be expected from its lack of net electric charge. The lipophilicity of $[Cr(pic)_3]$ has been measured and found to be surprisingly small despite the complex being neutral (12). The limited solubility of $[Cr(pic)_3]$ has complicated characterization by spectroscopic and electrochemical techniques, although the complex recently has been characterized by a variety of techniques including infrared, Raman, electronic and nuclear magnetic resonance spectroscopies (9, 12, 13) and by cyclic voltammetry (13).

$Cr(nic)_2(H_2O)_3(OH)$ is insoluble in water. Spectroscopic studies indicate the nicotinate ligands are bound to the chromic centers through the carboxylate oxygen atoms, not the pyridine nitrogen atoms (9). The nitrogens are probably protonated, such that the formula may be $Cr(Hnic)_2(OH)_3(H_2O)$. The insolubility suggests that the material may be a polymer.

$CrCl_3$ occurs as a dark green, crystalline solid and is deliquescent and extremely soluble in water. In aqueous solution, the compound is susceptible to hydrolysis, producing hydroxo-bridged oligomers. The addition of hydroxide to aqueous solutions of the compound results in the precipitation of the blue solid $Cr(OH)_3{\cdot}(H_2O)_x$. In the solid state, the compound exists as chains of $[CrCl_2(H_2O)_4^+$ cations linked by hydrogen-bonded cages of chloride anions and water molecules (14).

Pharmacokinetics and metabolism

In humans, dietary Cr is absorbed with an efficiency of 0.4-2% (4). In rats, Olin *et al.* (15) and Anderson *et al.* (16) have shown that $CrCl_3$, Cr polynicotinate and $[Cr(pic)_3]$ are absorbed to similar extents (0.5-1.3% of a gavaged dose of 0.14 or 0.15 μg Cr, respectively, after 24 h). The time dependence of the absorption of all 3 compounds is very similar (15). Thus, these common nutritional supplements are not efficiency absorbed. The low absorption of Cr polynicotinate and $[Cr(pic)_3]$ is probably due to their limited solubility. $CrCl_3$ is not stable in the presence of chelating biomolecules with which the Cr forms complexes and is particularly unstable under basic conditions as in the intestines; the $[CrCl_2(H_2O)_4]^+$ cation is susceptible to hydrolysis in water, resulting in the formation of hydroxo-bridged oligomers. Not all Cr(III) complexes are so poorly absorbed. For example, a trinuclear Cr(III) complex, which protects the Cr(III) centers from forming complexes with biomolecules and from oligomerization and which is very soluble in water, is absorbed with an efficiency of 40-60% within 24 h of gavage administration (17).

To generate deleterious effects, $[Cr(pic)_3]$ needs to enter cells intact and remain intact long enough to produce a significant quantity of reactive oxygen species or degrade releasing picolinate. Recently Vincent *et al.* have

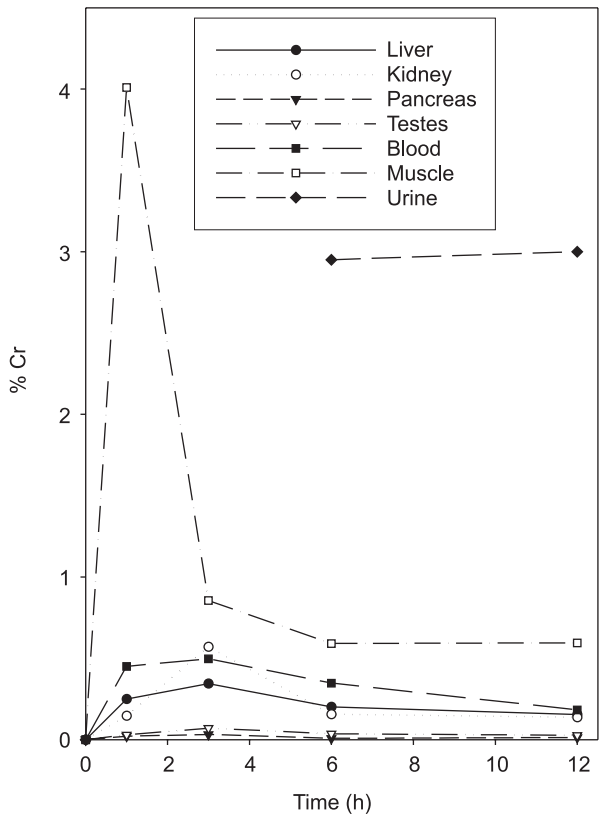


Fig. 1. Distribution of absorbed chromium from gavage administration of chromium picolinate (2.7 nmoles) to rats (150-170 g) as a function of time. Presented as Cr in tissue or fluid divided by total absorbed Cr x 100% (Data from ref. 15).

shown that $[Cr(pic)_3]$ is able to rapidly pass from the bloodstream and enter cells intact, although the lifetime of the complex in cells is short (18, 19). For enough of the compound to be present in tissues to be observed in these studies, the supplement was administered intravenously. However, the appearance and distribution of Cr in tissues from the intravenous injections of $[Cr(pic)_3]$ is very similar to that when $[Cr(pic)_3]$ is administered orally (Fig. 1) (15). In hepatocytes, Cr from $[Cr(pic)_3]$ first appears in the nucleus and mitochondria, then the cytosol, and finally the lysosomes and microsomes; the complex has no propensity to bind to DNA, however (20). Kelley *et al*. have found that hepatocyte microsomes can catalytically modify the picolinate ligands, which would result in Cr release (21).

Toxicology

While Cr(III) complexes may have beneficial effects on diabetic and atypical depression subjects at levels of ≥400 μg/day (~13 times the AI) (see below), significant potential for deleterious effects exists for at least one of these supplements at these levels. In 1995, Wetterhahn *et al.* (22) reported that $[Cr(pic)_3]$ generated chromosome damage in a Chinese hamster ovary (CHO) cell model; in contrast, $CrCl_3$ and chromium polynicotinate did not generate this damage. Similar damage has also been demonstrated in murine macrophages (23), and another study using the same cell line observed oxidative damage (24). Chromium nicotinate also gave rise to damage but not to the same extent as the picolinate complex. Continuing work with the CHO model has observed mitochondrial damage and apoptosis generated by $[Cr(pic)_3]$ (25) and found that the supplement is mutagenic (26). The effects have been postulated to arise from the released picolinate ligand (22, 25) or from reactive oxygen species catalytically generated by the intact complex (26-28). Physiologically relevant concentrations of Cr as $[Cr(pic)_3]$ (*e.g.*, 120 nM) and of biological reducing agents such as ascorbic acid and thiols have been shown to result in catalytic production of reactive oxygen species (27, 28). Most forms of Cr do not generate such species in the absence of a strong oxidant such as peroxide. Hence, these studies are consistent with investigations that demonstrated that mutagenic forms of Cr(III) possessed chelating ligands containing imine nitrogens (*e.g.*, 2,2'-bipyridine, phenanthroline and Schiff bases) coordinated to the metal and generated reactive oxygen species (29). Chromium compounds lacking imine ligands lack the DNA cleaving activity in the presence of biological reducing agents (30). Alternatively, neutral $[Cr(pic)_3]$ could serve as a vehicle to transport picolinate to cells. [Chromium polynicotinate also possesses imine nitrogen-containing ligands; however, the nitrogens do not coordinate to the chromium center and, thus, do not alter its redox potential.]

Isolated incidents of deleterious effects of $[Cr(pic)_3]$ supplementation and one incident for chromium nicotinate have been reported (31-38). The nature of these incidents makes their significance difficult to ascertain. In contrast, Anderson *et al*. (39) fed rats diets containing up to 100 mg Cr as $[Cr(pic)_3]$ per kg diet for 24 weeks; no acute toxic effects, as well as no effects on body mass or composition or on plasma glucose or insulin levels, were observed. However, potential effects of oxidative damage were not investigated. No toxic effects of $[Cr(pic)_3]$ supplementation were noted in any of the studies covered by the review articles mentioned below which combined hundreds of monitored subjects (1, 40). No study has yet to examine the effects, positive or negative, of long-term (more than 1 year) use of $[Cr(pic)_3]$.

Human and animal studies looking for DNA damage and oxidative damage have started to appear. No effect on 5-hydroxymethyl uracil levels has been observed in 10 obese women given 400 μg Cr as $[Cr(pic)_3]$ per day for 8 weeks (41); unfortunately, the obese subjects started the study with elevated 5-hydroxymethyl uracil levels as a result of their condition which the authors indicated could have affected their results. In contrast, Vincent *et al*. (20) have found that rats given $[Cr(pic)_3]$ (4 μg Cr) daily by intravenous injection for 60 days have elevated levels of urinary 8-hydroxydeoxyguanosine and peroxidized lipids, establishing an upper level required for *in vivo* DNA

damage. Both direct DNA oxidation and indirect DNA damage via lipid peroxidation resulting from [$Cr(pic)_3$] administration provide potential pathways for the chromosome damage (22) and more recently mutations (26) seen in cell culture studies. When given orally to rats in quantities equivalent to humans taking commercial supplements, [$Cr(pic)_3$] has been reported to cause oxidative damage (42). Potentially deleterious *in vivo* effects of [$Cr(pic)_3$] have recently been examined by O'Donnell *et al.* using *Drosophila melanogaster* (43). [$Cr(pic)_3$], but not $CrCl_3$, at levels of 260 μg Cr/kg food (approximately equivalent to that received by a human taking daily [$Cr(pic)_3$] supplement containing 200 μg Cr) or less was found to lower the success rate of pupation and eclosion and to arrest development of pupae in a concentration-dependent fashion. X-linked lethal analysis indicated that the supplement greatly enhances the rate of appearance of lethal mutations and dominant female sterility.

In March 2003, the Expert Group on Vitamins and Minerals (44) determined that [$Cr(pic)_3$] was a potential carcinogen and requested that the health supplement industry voluntarily withdraw chromium picolinate-containing products, while also consulting on a ban on the use and sale of [$Cr(pic)_3$] in Great Britain. Currently, the U.S. FDA, working with the National Academy of Sciences, is studying the potential regulation of chromium picolinate.

Clinical studies

Healthy Individuals

Between 1989 and 2003, several investigators have examined the effects of Cr(III) complexes on body mass and composition and on blood plasma parameters such as glucose and insulin concentrations (45-72). The results of these studies have recently been the subject of several meta-analyses and reviews. Unfortunately, the results of the initial reports have failed to be supported. The most recent major review stated "the results of well-designed and performed studies indicate that [$Cr(pic)_3$] supplementation has no effect on body composition when given up to 1000 μg Cr as [$Cr(pic)_3$] daily, regardless of whether an exercise programme is involved or not" (40). Other reviews have come to similar conclusions (73-78). Meta-analyses by Nissan and Sharp (79) and Pittler (80) reenforce these conclusions. A meta-analysis by Althius *et al*. (81) indicates that these supplements have no effects on plasma glucose and insulin levels, a conclusion also drawn by a recent review (1). Thus, current evidence suggests that supplementation of the diet of healthy individuals with Cr(III) complexes has no efficacy.

Type 2 diabetes

Type 2 diabetes (82) and pregnancy (83) are examples of conditions leading to increased urinary Cr loss. This increased urinary Cr loss could potentially lead with time to a decrease in Cr status, although this is yet to be proven. Additionally, because these individuals have lowered insulin sensitivity and could potentially benefit from increased Cr loading of chromodulin resulting in increased insulin signaling, administration of pharmacological amounts of Cr(III) might be beneficial. Yet, a meta-analysis of studies with diabetic subjects revealed that "A study of 155 diabetic subjects...showed that chromium reduced glucose and insulin concentrations; the combined data from...the other studies did not" (81). The positive placebo-controlled study was performed by Anderson *et al.* (84) with 155 subjects in China and is the largest study reported using diabetic subjects. In the study, subjects received 0, 200 or 1000 μg Cr as [$Cr(pic)_3$] daily for 4 months. At the higher Cr amount, subjects had reduced fasting serum glucose, insulin, HbA1c and total cholesterol and lower 2-h insulin and glucose concentrations after a glucose challenge. A similar conclusion to the meta-analysis has been drawn in a recent review; the consensus of double-blind, placebo-controlled crossover studies of the effects of chromium supplementation for 6-16 weeks on type 2 diabetic subjects was that Cr supplementation had no effect (1). However, a subsequent double-blind, crossover study on type 2 diabetic subjects in India indicated that Cr supplementation (400 μg Cr as [$Cr(pic)_3$]/day) for 12 weeks lowered serum insulin and glucose levels (85). Anderson (86, 87) has reviewed studies on the effects of Cr supplementation of type 2 diabetic subjects and found that the amount of Cr used may be important; only studies using ≤ 200 μg/day of Cr reported no effects, leading him to postulate that larger quantities of Cr may have beneficial effects in subjects with type 2 diabetes. However, subsequent studies using 400 μg/day of Cr have failed to observe beneficial effects in type 2 diabetic subjects (88).

Significantly, the potential beneficial effects of Cr supplements when given at the higher doses for diabetic subjects are supported by studies on model rats. A trinuclear Cr(III) propionate complex (which uniquely mimics the ability of chromodulin to activate insulin receptor kinase activity *in vitro*) has beneficial effects (lower fasting plasma total and LDL cholesterol, triglycerides and insulin levels) on Zucker obese rats, models for the early stages of type 2 diabetes (89, 90); also, in this case, significant effects were also noted in healthy rats. Cefalu *et al*. (91) have observed beneficial effects on insulin sensitivity in type 2 diabetes model rats with cardiovascular disease, but not healthy rats, from [$Cr(pic)_3$] administration (18 μg/kg/day, equivalent to 540 μg for a 60 kg human). Rat studies using [$Cr(pic)_3$] and $CrCl_3$ (up to 100 mg Cr/kg diet for 24 weeks) observed no effects on fasting serum glucose, cholesterol or triglycerides levels (39). Thus, while rat studies with the popular Cr supplements fail to generate effects in healthy animals (similar to human studies), large, pharmacological doses of Cr appear to have beneficial effects on type 2 diabetic animals.

Additionally, one study has appeared on the effects of Cr on gestational diabetes (92). Women received 0, 4 or

8 μg Cr as [Cr(pic)$_3$]/kg body mass daily; both Cr groups had significant lower plasma insulin and glucose levels. If the results of this single study are reproduced by additional studies, this could have significant implications for the treatment of this condition. Recently, studies on the effects of chromium supplemention on steroid-induced diabetes have generated interesting results (93, 94), including improvements in plasma glucose levels. These studies still need to be followed up by larger investigations, yet they are also supported by the results of a recent study with rats treated with dexamethasone (95). Treated rats receiving a very large dosage of ~4 mg Cr daily as [Cr(pic)$_3$] had lower fasting serum insulin levels and lower insulin, triglycerides and glucose areas in glucose or insulin challenges than controls.

Depression

Recently a potentiating effect of [Cr(pic)$_3$] on antidepressant pharmacotherapy for dysthymic disorder has been reported (96-98). The studies have been very small, and larger scale studies are required before further conclusions can be drawn. However, the initial results are quite suggestive. [Cr(pic)$_3$] has been reported to lower cortisol response to 5-hydroxytryptophan precursor (99), which may be related. These reports in combination with the isolated report of [Cr(pic)$_3$] possibly causing perceptual and motor changes may potentially be of concern in terms of healthy subjects consuming the supplement. Picolinic acid/picolinate is a natural catabolite of the amino acid tryptophan, generated as an end product in the kynurenine pathway in the body (100). Molecules generated along this pathway tend to have neurological effects (101). This raises concerns about [Cr(pic)$_3$] in the body as the [Cr(pic)$_3$] being neutral might cross the blood-brain barrier and should deliver and release 3 picolinates (or its degradation products) per Cr. Safety concerns regarding picolinic acid have arisen several times (102-106), leading to suggestions that picolinic acid by itself should not be used as a dietary supplement. Thus, effects on perceptual and motor function and dysthymic disorder could potentially be related to picolinate released in the body; this is an area that requires more investigation.

Acknowledgements

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