Cr(III)-*tris*(picolinate) Induced Multi-organ Toxicity on Mice: Microscopy Studies

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Abstract— The present study aims to explore the effects of a dietary Cr(III) supplement, Cr-*tris*–picolinate, on mice exposed to different doses of this compound, on relevant organs related to metabolism, excretion, and immune functions.

Chromium is an essential nutrient involved in carbohydrate and lipid metabolis im mammals. [Cr(pic)$_3$] is a very popular human dietary supplement that provides a bioavailable form of Cr(III) . However, its mechanism of action at the molecular level remains so far not totally understood and a number of toxic endpoints have been attributed to its use. Back to 90´s of the last century some adverse effects provoked by its use began to be recurrently reported, especially with regard to its ability to promote DNA cleavage by a radical mechanism. Investigation of the effects of diets supplementation with [Cr(pic)$_3$] are essential to assess the safety and the concerns over this material.

Clinically healthy male mice were randomly divided into 3 groups, and kept under standard laboratory conditions. Cr(III)-*tris*(picolinate) was orally given in doses of 25 and 50 mg/kg, for 14 days. Control groups were given NaCl (0.9%). After sacrifice, hepatic, renal, and spleen tissue fragments were processed for light microscopy. Histopathological lesions were noted on liver, kidney, and spleen in a dose dependent manner compared with controls. Histological sections of liver displayed some haemorrhagic focus through the tissue being more severe on mice exposed to higher doses of [Cr(pic)$_3$]. Renal parenchyma evidenced rupture of Bowman capsule with consequent release of capillaries and haemorrhage on animals exposed to 25 and 50 mg/kg of [Cr(pic)$_3$], respectively. Spleen microscopy observation of mice exposed to both doses of [Cr(pic)$_3$] revealed altered organization of red and white pulp. These results point to deleterious effects *in vivo* resulting from Cr(III)-tris-picolinate supplementation. Concerns about the use of this chromium(III) dietary compound continue to deserve deeper studies and continuous future research.

Index Terms—Cr(III)-*tris*(picolinate), food supplement, histology, kidney, light microscopy, liver, spleen, toxicity.

1 Introduction

Nutrients are chemical substances found in foods that are necessary for life maintainance. For animals and humans those which the body cannot synthesize are essential nutrients, called "tracers" when needed in very low quantities. Initially, essential nutrients were identified when low intakes resulted in the failure to grow or reproduce, or caused a pathological alteration. Chromium (Cr) has a place in the list of previous elements, and the support for it might be beneficial bioactive is the acceptance that a poor diet in Cr increases the risk for chronic diseases that can be disable and/or lead to a premature dead. Cr has been found beneficial to subjects with varying degrees of glucose intolerance, ranging from hypoglycemia to insulin-dependent diabets, thus participatings in carbohydrate and lipid metabolisms [1]. However, no evidence for Cr(III) role on keeping genomic stability was reported [2]. A relatively high Cr intake (>200 μg/d) apparently is needed to get a beneficial effect on glucose metabolism [3]. Nevertheless, the reduction in the risk of chronic disease should be included in the formulation of dietary reference intakes where sufficient data for efficacy and safety exists [4].

Chromium has been proposed to be an essential element over the last five decades and has since been strongly investigated also due to its dual features, *ie*, essentiality of Cr(III), *versus* toxicity of Cr(VI) [5]. However, as a result of recent research and reanalysis of its status, this element currently should only be considered as potentially pharmacologically active [6], [7] and [8].

There have been a few reports suggesting that some organic forms of Cr, *eg* Cr(III)-*tris*(picolinate), [Cr(pic)$_3$], used as nutritional supplement may originate adverse effects on liver and kidney when consumed in amounts >1000 μg/d for several months. Animal studies have not found similar effects. However, individuals with preexisting renal and liver disease may have an increased risk for adverse effects with high Cr supplementation [9].

Supplementation with Cr has been proposed to result in beneficial responses in mammals with demonstrated glucose intoleranceor insulin insensitivity, including type 2 diabetes, cardiovascular disease, and related conditions. Nevertheless, studies in humans tend to be negative or at best ambiguous [9]. A recent meta-analysis that includes reports of clinical studies since 2007 and comprises improved methodologies has found no significant effectiveness of Cr supplementation [10]. Consequently, at this point the position of the American Diabetes Association (ADA) is "There is insufficient evidence to support the routineuse of micronutrients, such as chromium, to improve glycemiccontrol in people with diabetes" [11]. Nonetheless, recent several clinical studies has reported its usefulness in novel therapeutic targets and in the increase of the understanding of how to optimize chromium use in insulin resistance and type 2 diabetes treatment [12] , [13], [14] and [15].

Chromium(III)-*tris*(picolinate) turned to be used freely in a therapeutic nutritional modality, as [Cr(pic)$_3$] is a worldwide used dietary supplement for humans, and for cattle. But its widespread use is now subjected to controversial about its

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effectiveness and safety limits in what scientific evidence issues are concerned [7] and [9]. Possible risks for added chromium individual long term effects have been highlighted since clastogenic, and mutagenic features have been reported by Stearns and coworkers, [16], [17], although surrounded by a controversial and contradictory multitude of publications on this subject [18], [19], [20], [21] and [22].

In a previous report we have described deleterious effects of [Cr(pic)$_3$] on mice spermatogenesis [23]. In the present study, aiming to explore dose increasing effects of [Cr(pic)$_3$] on the morphology of mice relevant organs we present results of liver, kidney, and spleen obtained by light microscopy.

2 Material and Methods

2.1 Synthesis and Characterization of Cr(III)-*tris*(picolinate)

The nutritional supplement [Cr(pic)$_3$] was synthesized and characterized according to the literature [24]. In order to undoubtly clarify [Cr(pic)$_3$] structure as a mononuclear complex, its composition was tested both by ESI-Mass spectrometry and X-ray powder diffraction analysisl, together with single-crystal structural simulation calculations [25].

Two solutions of [Cr(Pic)$_3$] (in 0.9% NaCl) were prepared for using in *in vivo* studies with concentrations of 25 mg and 50 mg of Cr(III) /kg / mice body weight.

2.2 Animals and Experimental Design

Adult male mice (2 months old) delivered by Harlan (Spain) were maintained in a climate chamber (22 ± 2 °C, photoperiod light/dark 12/12 hours; and 40-60% relative humidity). Animals had access to tap water and apropriated rodent pellet food *ad libitum*. After an acclimatization period of one week animals were weighed and divided into 3 experimental groups.

[Cr(Pic)$_3$] was orally given to mice in doses of 25 and 50 mg/kg, for 14 days. Control groups were given NaCl (0.9%) only. During experiments animals were carefully monitored concerning behavior pattern. After the experimental period, mice were sacrificed under anesthesia. Some relevant organs such as liver, kidneys, and spleen were removed, and weighed.

Animal experiments were conducted agreeing to ethical international guidelines for animal experimentation.

2.3 Microscopy Studies

Small fragments of each organ were fixed in Bouin´s solution, dehydrated in a graded ethanol series, and embedded in paraffin wax. Sections 4-5 μm thick were stained with haematoxylin and eosin, dehydrated, and observed using a microscope (Olympus BX41, Tokyo, Japan) with a digital camera (Olympus CamediaC-5060).

2.4 Statistical Analysis

Values of animal weight and relative weights of organs studied are presented as mean ± standard deviation.

3 Results

A survival rate of 100% and a normal behavior among groups was observed during this study. In addition, no significant changes on macroscopic observation of the organs from [Cr(Pic)$_3$] exposed groups compared with the controls were noted.

The results of body weight and organ weights of animals are presented in Tables 1 and 2, respectively.

Table 1: Body weight (g) of mice throughout the experiment

Groups	Initial Weight	Final Weight
I (Control)	30,61 ± 1.50	33,90 ± 1.00
II (25 mg/Kg of [Cr(pic)$_3$])	30,77± 0,70	32,49 ± 0,57
III (50 mg/Kg of [Cr(pic)$_3$])	30,07 ± 3,23	30,74 ± 1,62

The administration of [Cr(Pic)$_3$] in the two doses did not induce a decrease in the body weight of the animals, whereas the weight of the mice increased in all groups over the effected treatments except for mice of group III, wherein its weight remained constant throughout the treatment.

No differences in organ weights between animals of the different groups were noted (Table 2).

Table 2. Relative weight of the organs through the study.

	Relative Weight of Organs		
Groups	**Liver**	**Kidney**	**Spleen**
I (Control)	4,85 ± 0,37	0,84 ± 0,14	0,22 ± 0,02
II (25 mg/Kg of [Cr(pic)$_3$])	4,41±0,47	0,74±0,04	0,23±0,04
III (50 mg/Kg of [Cr(pic)$_3$])	4,47±0,33	0,70±0,09	0,22±0,06

Control sections of all organs demonstrated normal microscopic features (Figs. 1-3). However microscopic observation of the liver, kidney, and spleen, studied in the present work revealed a considerable damage in a dose dependent manner (Figs 1-3).

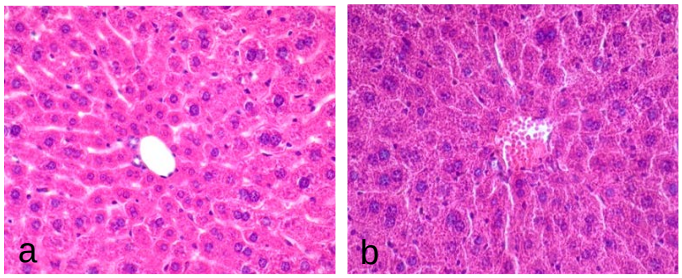

Fig. 1. Microphotographs of hepatic tissues in control (a) and 25 mg/kg Cr-exposed animals (b). HE x 400.

Histological sections of the control group of animals showed normal liver morphology (Fig. 1a). However, histological sections of liver displayed some haemorrhagic focus through the tissue being more severe on mice exposed to higher doses of [Cr(pic)$_3$] (Fig 1b).

Several morphological alterations were observed on kidneys of both doses of Cr-exposed mice compared to controls (Fig. 2a). The renal parenchyma evidenced rupture of Bowman's capsule with consequent release of capillaries, and haemorrhage on animals exposed to 25 and 50 mg/kg of [Cr(pic)$_3$], respectively (Figs 2b, c).

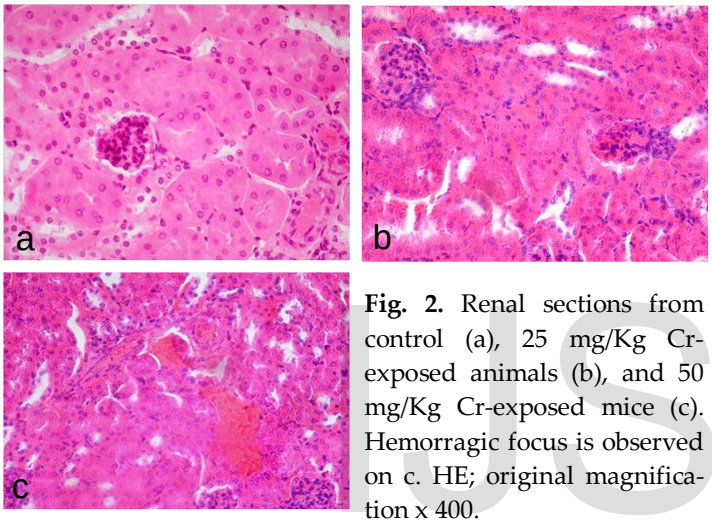

Fig. 2. Renal sections from control (a), 25 mg/Kg Cr-exposed animals (b), and 50 mg/Kg Cr-exposed mice (c). Hemorragic focus is observed on c. HE; original magnification x 400.

Spleen microscopy observation of mice exposed to both doses of [Cr(pic)$_3$] revealed disorganization of red and white pulp, as well as cell depletion compared with control (Fig 3).

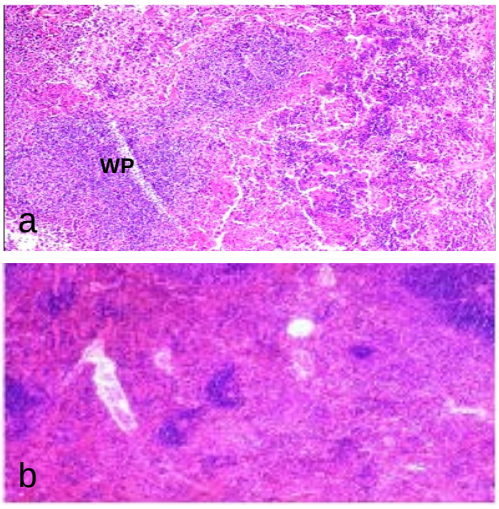

Fig. 3 Representative microphotograph of splenic tissue in control mice (a), evidencing a regular pattern of white pulp (WP), and 25 mg/Kg Cr-exposed animal (b). Disrupted white pulp is noted. HE; original magnification; x 200.

4 Discussion

In the present investigation and during the period of exposure no mortality was noticed. In addition, no differences on the behavior pattern were noted among the three groups. Also no macroscopic changes within the three organs were observed. However, the present work showed evidences morphological changes on the liver, kidney and spleen at both doses of Cr(pic)3 administration (25 and 50 mg/kg), to mice for two weeks. However, supplementation with Cr(III) had no adverse effect on the body weight of mice nor organs weight through the experiments, similarly to a plethora of other research works. Other studies using transmission electron microscopy have shown notorious damage in cells [27] and in other situations [28]. Acute tubular necrosis has also been reported [29] as well as renal failure [30]. Contrarily, eg, chronic studies using unilaterally nephrectomyzed rats treated with this compound referred to the lack of any adverse effects on renal function [31]. Benefits for the remaining kidney with improvement in the renal function were reported by these authors in [Cr(pic)$_3$]-treated rats. Long-term dietary Cr(pic)3 treatment of obese rats reduced oxidative stress and inflammation [32].

5 Conclusions

The present investigation points to some concerns about using food supplements based on [Cr(pic)$_3$] on experimental models as mice. Some contradictory data may remain to be fully elucidated in future work. The microscopic studies above highlight the obvious relevance of this investigation for the knowledge of [Cr(pic)$_3$] on animal models, making a better understanding of these interactions an important research area. Under the installed controversy about the nutritional supplement [Cr(pic)3] effects in vivo, it remains of high pertinence to continue the research in this subject in order to achieve full elucidation of chromium(III)/[Cr(pic)3] roles.

Further studies are in progress in our group to explore the markers of oxidative stress of the lesions reported.

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