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Effects of chronic chromium picolinate treatment in uninephrectomized rat

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Abstract

Chromium picolinate [Cr(pic)3] is a nutritional supplement that is advocated as an adjuvant therapy for impaired glucose tolerance/type 2 diabetes because it improves glucose homeostasis. Because renal dysfunction is a major complication of type 2 diabetes, the potential impact of Cr(pic)3 on kidney function requires due consideration. This investigation takes added importance because the kidney is not only the principal route of elimination for chromium but also an organ that preferentially accumulates it. To avoid the confounding influence of chronic hyperglycemia, and its associated complications, we used the unilaterally nephrectomized (UNX) rat that shows impaired kidney function with age. We tested the hypothesis that chronic treatment of the UNX rat with Cr(pic)3 exacerbates the age-related decline in renal function. Accordingly, UNX rats were fed a diet lacking (eg, control; n = 5) or containing 5 mg/kg of Cr(pic)3 (n = 7) for 60 days. The treatment did not affect glucose tolerance as reflected by lack of any effect on changes in blood glucose concentration during glucose tolerance testing. Although nonfasting blood glucose concentrations were similar between the 2 groups, plasma insulin concentration was lower in the Cr(pic)3-treated group ($P < .05$), suggesting improved insulin sensitivity. Body weight, blood pressure, heart rate, daily food and fluid consumption, daily urinary fluid and electrolyte excretions, urine osmolality, and daily protein excretion were similar between the 2 groups before and during Cr(pic)3 treatment. Although renal excretory responses to acute administration of a 5% isotonic saline volume load were similar between the 2 groups, the Cr(pic)3-treated group displayed a more robust ability to excrete a 10% isotonic saline volume load, an effect primarily related to reduced tubular reabsorption of the filtered fluid and sodium loads. In conclusion, chronic Cr(pic)3 did not adversely affect renal function. Rather, the treatment improved the ability of the animal to dispose of an acute isotonic saline volume load, suggesting preservation of renal function in the UNX rat.

1. Introduction

The manufacture and sale of nutritional supplements is a multibillion dollar industry with various formulations of trivalent chromium contributing significantly to this market [1]. The interest in chromium supplementation stems from earlier animal studies that indicated an essential role for trivalent chromium in carbohydrate metabolism [2-4]. Subsequent observations that patients on total parenteral nutrition also develop a deficit in carbohydrate metabolism, which can be alleviated with trivalent chromium, established the essential role of trivalent chromium in human diet as well [5-8]. Because dietary chromium is poorly absorbed (about 0.5%-2%), there has been a surge of interest in use of bioavailable formulations of trivalent chromium (eg, chromium picolinate [Cr(pic)3]; ~5% bioavailable) as adjunctive therapy for impaired glucose tolerance/ type 2 diabetes [9-11]. Indeed, Cr(pic)3 ranked second in sales, after calcium supplements, with a market value of over half a billion dollar in the year 2000 [1]. Thus, chromium is the second leading inorganic element sold in the market place.

Clearly, rational and effective therapeutic strategy should minimize potential adverse effects while enhancing therapeutic efficacy of any drug or nutritional supplement. With regards to Cr(pic)3, several anecdotal case reports of individual subjects suggest renal dysfunction may be a common side effect of this nutritional supplement although a cause-and-effect relationship has not been established [10,12-15]. Awareness of the potential for adverse effects has increased after the demonstration of a clastogenic (ie, chromosomal breakage) effect of Cr(pic)3 in vitro [16-18].

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However, to our knowledge, adverse renal effects of Cr(pic)3 have not been verified in placebo-controlled studies using human subjects. Indeed, it has been suggested that the clastogenic potential may not be a major threat in vivo because the majority of Cr(pic)3 is distributed into the cytosol rather than the nucleus or the mitochondria [9,19]. On the other hand, the assertion that trivalent chromium supplementation is safe and effective has primarily relied on results of studies which have used normal animals without coexistence of renal dysfunction [10,11,20]. More importantly, these studies have rarely focused on the chronic renal functional effects of Cr(pic)3. Because the kidney is the principal route of elimination for Cr(pic)3 and an organ in which it accumulates [9,10,19,21], the investigation of the impact of Cr(pic)3 on a dysfunctional kidney, independent of chronic hyperglycemia and its associated complications, is warranted.

We have previously shown that removal of one kidney at 4 weeks of age leads to impaired renal function, causing a significant reduction in the ability of the animal to dispose of a saline volume load as the animal reaches 6 months of age [22,23]. It is noteworthy that renal adaptation to loss of one kidney is dependent on the age of the animal at the time of unilateral nephrectomy (UNX; [24]). In other ongoing studies, we have observed a more marked, and accelerated, deterioration of renal function in rats that have undergone UNX at about 10, rather than 4 [22,23], weeks of age. Thus, we examined the chronic impact of Cr(pic)3 on renal function of rats in whom the right kidney was removed at 10 weeks of age. Accordingly, we tested the hypothesis that chronic treatment of the UNX rat with Cr(pic)3 exacerbates the age-related decline in renal function.

2. Materials and methods

Male Wistar-Kyoto rats (9-week-old) were obtained from Harlan Laboratories (Indianapolis, Ind). All rats were maintained at constant humidity (60% ± 5%), temperature (24°C ± 1°C), and light cycle (06:00 AM to 06:00 PM). Unless otherwise indicated, animals had free access to food and water.

One week after arrival, the right kidney was removed from each rat under pentobarbital anesthesia (50-60 mg/kg, IP) and postoperative analgesia (1 mg/kg butorphanol, SC) [22,23,25]. After 2 weeks to allow for compensatory renal hypertrophy, the animals were subdivided and either remained on the standard rat diet (Harlan Teklad diet no. 8604; n = 5) or were switched to the standard diet that was supplemented with 5 mg/kg of Cr(pic)3 (Harlan Teklad diet no. 03326; n = 7); the diet contained 5 mg of Cr(pic)3 rather than 5 mg/kg of Cr as Cr(pic)3. It is noteworthy that others have used diets containing more than 100 mg/kg of Cr(pic)3 [20]. We used the 5 mg/kg of Cr(pic)3 diet because, based on measured daily intake of ~20 g food, this diet provides Cr(pic)3 to the animal in doses that are closer to those consumed by human subjects [9]. Hemodynamic parameters (tail cuff; IITC/Life Science Instruments, Woodland Hills, Calif) and daily metabolic data were collected before as well as 14, 28, and 60 days after initiation of Cr(pic)3 treatment. For daily metabolic data, the animals were housed individually in metabolic cages. After a period of 3 days of acclimation, 2 consecutive 24-hour urine samples were collected and daily consumption of food and fluid measured. Each rat underwent a glucose tolerance test at about 8 weeks after initiation of dietary Cr(pic)3 supplementation and before renal function studies. For glucose tolerance testing, after an overnight fast, each rat was injected with a glucose load (2 g/kg, IP). Blood samples were obtained after a 1- to 2-mm cut at the end of the tail; a drop of blood sample was placed directly on a test strip and glucose concentration measured with a glucometer [23,25]. Nonfasting blood samples were collected for determination of blood glucose concentration and plasma insulin concentrations by radioimmunoassay (ICN Biomedicals, Costa Mesa, Calif; [26]).

For renal function studies in the conscious animal, each rat was preimplanted with femoral vessels catheters and a bladder cannula under pentobarbital anesthesia (50-60 mg/kg, IP) followed by postoperative analgesia (1 mg/kg butorphanol, SC). Two days after the surgical procedure, each rat was placed in an environmental conditioning unit to which the animal was acclimated for several hours, 5 days before renal function studies. The arterial and venous catheters were flushed with isotonic saline containing 5 U/mL of heparin.

To determine the glomerular filtration rate (GFR), each rat received an intravenous injection of 0.2 mL of isotonic saline containing a priming dose of 2 μCi of ^{3}H-inulin (Dupont Co, Boston, Mass), followed immediately by a 20 μL/min intravenous infusion of isotonic saline containing 2.5 μCi/mL of ^{3}H-inulin [22,23,25]. After a 45-minute equilibration period, which is sufficient to achieve a steady-state radioactive level in the plasma, a 30-minute urine sample was collected for determination of baseline excretory parameters. A 30-minute volume expansion (equivalent to 5% of the animal's body weight) was then initiated with isotonic saline containing ^{3}H-inulin [22,23,25]. This was followed by infusion of isotonic saline containing ^{3}H-inulin at the rate of 20 μL/min for the remainder of the experiment. Urine samples were collected at 15, 30, 60, and 90 minutes after initiation of the saline volume load. At the midpoint of each urinary collection period, 0.2 mL of arterial blood sample was collected for determination of plasma radioactivity and electrolyte composition; the blood sample was replaced with an equal volume of isotonic saline. The next day, the infusion protocol was repeated with an isotonic saline volume load which was equivalent to 10% of the animal's body weight and administered over 30 minutes. At the conclusion of renal function studies the animals killed with pentobarbital (50 mg/kg, IV); the weight of the remaining kidney was determined for each animal.

Sodium and potassium were measured by flame photometry and used to calculate sodium and potassium