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Role of liver fatty acid binding protein in hepatocellular injury: Effect of CrPic treatment

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ABSTRACT

This study was designed to investigate the molecular mechanisms of chromium picolinate (CrPic, Fig. 1) hepatoprotective activity from alloxan-induced hepatic injury. Diabetes is induced by alloxan-treatment concurrently with the hepatic injury in mice. In this study, we investigate the protective effect of CrPic treatment in hepatic injury and the signal role of liver fatty acid binding protein in early hepatocellular injury diagnostics. In this study, alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) levels in the alloxan group were higher 71% and 50%, respectively, than those of the control group (ALT: 14.51 ± 0.74; AST: 22.60 ± 0.69). The AST and ALT levels in CrPic group were of minimal difference compared to the control groups. Here, CrPic exhibited amelioration alloxan induced oxidative stress in mouse livers. A significant increase in liver fatty acid-binding protein (L-FABP) was observed, which indicates increased fatty acid utilization in liver tissue [1]. In this study, the mRNA levels of L-FABP increased in both the control (1.1 fold) and CrPic (0.78 fold) groups compared the alloxan group. These findings suggest that hepatic injury may be prevented by CrPic, and is a potential target for use in the treatment of early hepatic injury.

1. Introduction

Chromium (Cr) was accepted as an essential element for over 30 years [2]. Recently, a study by Kristin R et al. [3] reported that Cr should no longer be considered an essential element. However, Cr may still influence the metabolism of biological macromolecules, such as nucleic acids, proteins, carbohydrates, and fats [4,5]. Trivalent chromium (Cr^{3+}) is essential for proper insulin function, is required for normal protein, fat, and carbohydrate metabolism, and is a recognized dietary supplement [6]. In fact, Cr^{3+}supplements have been widely used as an alternative therapy for Type 2 diabetes mellitus (T2DM). Some studies suggest that Cr supplementation lowered blood glucose levels, glycosylated hemoglobin, and oxidative stress in diabetic animals and humans [7–10]. Moreover, Cr^{3+} is still considered an essential nutrient, and has been shown to lower oxidative stress as well as improve glucose and lipid metabolism in mammals [7]. It has been proposed that Cr supplementation increases the amount of a chromium-containing oligopeptides present in insulin-sensitive cells that bind to the insulin receptor, thus markedly increasing insulin activity stimulated by tyrosine kinase phosphorylation of the insulin receptor's substrate-1 and the glucose transporter, Glucose transporter 4 (GLUT4) [7]. In our studies, hepatic injury was induced via oxidation and lipid accumulation after alloxan-treatment. The experimental groups that received chromium picolinate (CrPic) supplements, then given alloxan dosing, demonstrated few changes of antioxidant enzymes in CrPic group. Moreover, oxidative induced injury was reduced, lipid membrane integrity was increased, and lipid peroxidation products did not accumulate in the group receiving Cr^{3+} supplements [11]. However, some studies have reported that a mild oxidative stress was observed in the liver and brain of CrPic-supplemented normal rats [12]. These conflicting results have created questions regarding CrPic and redox states in the body. One study demonstrated the beneficial effects CrPic had against microvascular complications. In that study, 10 weeks of continuous CrPic treatment significantly decreased metabolic risk factors, as evidenced in histopathology of the liver, kidney, and pancreas [13]. Mammalian cells have evolved elaborate protection mechanisms against toxic effects of electrophilic metabolites, such as reactive oxygen species (ROS) [14]. Lipid peroxidation in cell membranes is a classic perturbation of cellular

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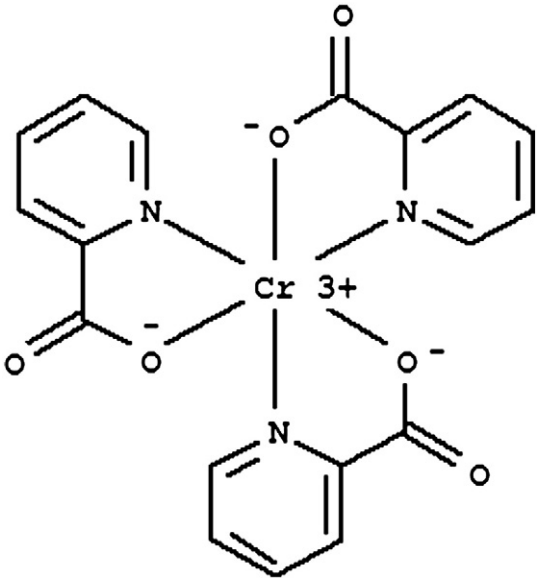

Fig. 1. Chemical structure of CrPic.

homeostasis [15]. The lipid peroxides were protected against by Cr^{3+}, in the rat liver cells [16]. Metal ions play an important role in biological systems, and without their presence in trace or ultratrace amounts many essential cofactors in numerous biochemical reactions would be lacking efficient catalytic activity [17]. However, essential metals become toxic to cells when their concentrations surpass homeostatic levels [18]. Another deleterious effect on cells is elevated levels of oxidative stress which impairs cellular glucose metabolism via a variety of mechanisms, including redox imbalance and insulin resistance [19]. One study reported mild oxidative stress in the liver and brain of CrPic-supplemented normal rats [12].

The recommended dietary Cr^{3+} intake range in adults is 50–200 μg/day [20]. Human studies have primarily used 1000 μg Cr^{3+}/day [21]. Assuming an average 70 kg body weight, this would translate to an intake of nearly 15 μg Cr^{3+}/kg body weight [22]. In 2001, the National Academy of Sciences established an adequate intake (AI) of chromium of 35 μg/day for men and 25 μg/day for women [23].

Various Cr^{3+} compounds have been used as nutritional supplements, weight-loss agents, and muscle-development agents in humans and as feed additives in domestic animals [7]. Actually, it is difficult for healthy individuals to develop Cr deficiency. However, the certain people may still lack chromium. Strong evidence showed that the lack of chromium intake leads to the multiple manifestations associated with the metabolic syndrome such as diabetes and obesity conditions [24,25]. These metabolic-related disturbances are ameliorated with Cr^{3+} supplementation [26]. Pregnancy may increase urinary Cr loss making pregnant women susceptible to Cr deficiency [27]. Morris et al. examining Cr excretion during pregnancy found that persistently elevated excretion in otherwise normal pregnancies seemed to be associated with a significant reduction of insulin sensitivity [28]. For this reason, they speculated that enhanced Cr excretion had the potential to put mother at increased risk of developing gestational or postpartum diabetes. Supplemental Cr might be beneficial in mediating deficiency states and their metabolic consequences.

L-FABP is a 14.4 kDa cytosolic protein belonging to a family of proteins that are products of a single gene family [29]. The L-FABP has a similar expression pattern in all vertebrates and is highly expressed in the liver [30]. One study reported that L-FABP is required for hepatocyte mitotic activity [31]. This protein exhibits a marked affinity for long-chain fatty acids and is considered to play a significant role in cellular uptake and intracellular targeting of fatty acids [1,32]. In the transport and metabolism of long-chain fatty acids during fatty acid overload, a FABP plays a crucial role in the activity of peroxisomal enzymes [33]. Fatty acid overload has been shown to result in uncontrolled diabetes mellitus, which is accompanied by an increased capacity for fatty acid oxidation by mitochondrial, peroxisomal and microsomal pathways in the liver [34].

In this study, the effects of L-FABP and CrPic were examined on hepatic injury. Our findings showed that the hepatic injury can be ameliorated through CrPic-treatment in alloxan mice. This indicates that CrPic may have health care and/or pharmaceutical applications. These results also indicate the L-FABP has potential as a biomarker for early diagnosis of hepatic injury.

2. Materials and methods

2.1. Experimental materials

2.1.1. Reagents

CrPic and alloxan were obtained from Sigma-Aldrich Co. LLC.

2.1.2. Ethics statement

All the procedures involving animals were approved by the institutional ethics committee of the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences, in compliance with the Experimental Animal Regulations of the National Science and Technology Commission, China. This experiment was conducted under animal use permit SYXK jing 2009-0004.

2.1.3. Animal protocol

ICR (Institute of Cancer Research) mice were purchased from the Institute of Zoology, Chinese Academy of Medical Sciences (Beijing China). The mice were housed at 24–26 °C, 50–60% humidity, and in 12 h light/dark cycles. They were fed a standard diet and water ad libitum. After 2 weeks of acclimation, 30 mice were divided into 3 groups of 10, and all subjects weighed 20 ± 2 g. Animals in group 1 (control) were given tap water intragastrically until the end of this

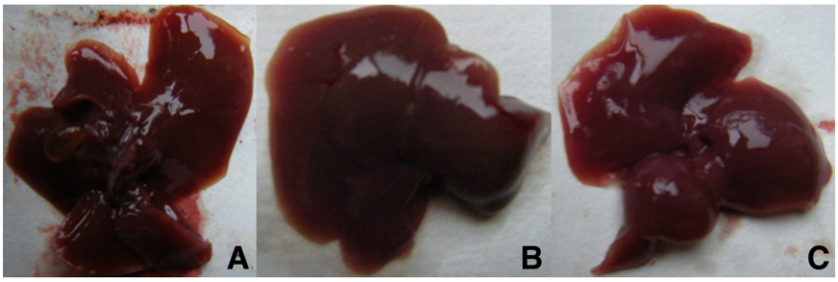

Fig. 2. Pictures of livers. A is a picture of a control mouse liver. Control mice (the normal mice) were treated with tap water intragastrically; B is a picture of an alloxan mouse liver. Alloxan mice were given tap water intragastrically for 34 days, then they were treated with alloxan (40 mg/kg) intraperitoneally on the last 6 days; C is a picture of a CrPic mouse liver. CrPic mice were treated with CrPic (Cr^{3+} 40 μg/kg bm/day) intragastrically for 34 days, then they were given alloxan (40 mg/kg) intraperitoneally on the last 6 days.