Journal of Trace Elements in Medicine and Biology 29 (2015) 136–144

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.de/jtemb

CLINICAL STUDIES

Hypoglycemic activity and acute oral toxicity of chromium methionine complexes in mice

Hai-yan Tang[a,b,c], Qing-gui Xiao[a,b,*], Hong-bin Xu[a,b], Yi Zhang[a,b]

[a] National Engineering Laboratory for Hydrometallurgical Cleaner Production Technology, Institute of Process Engineering, Beijing 100190, China
[b] Key Laboratory of Green Process and Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China
[c] University of Chinese Academy of Sciences, Beijing 100190, China

ARTICLE INFO

Article history:
Received 6 March 2014
Accepted 4 July 2014

Keywords:
Chromium methionine
Hypoglycemic activity
Acute oral toxicity
Mice

ABSTRACT

The hypoglycemic activity of chromium methionine (CrMet) in alloxan-induced diabetic (AID) mice was investigated and compared with those of chromium trichloride hexahydrate ($CrCl_3 \cdot 6H_2O$) and chromium nicotinate (CrNic) through a 15-day feeding experiment. The acute oral toxicity of CrMet was also investigated in ICR (Institute for Cancer Research) mice by a single oral gavage. The anti-diabetic activity of CrMet was explored in detail from the aspects of body weight (BW), blood glucose, triglyceride, total cholesterol, liver glycogen levels, aspartate transaminase (AST) and alanine transaminase (ALT) levels. The obtained results showed that CrMet had beneficial effects on glucose and lipid metabolism, and might possess hepatoprotective efficacy for diabetes. Daily treatment with 500 and 1000 μg Cr/kg BW of CrMet in AID mice for 15 days indicated that this low-molecular-weight organic chromium complex had better bioavailability and more beneficial effects on diabetics than $CrCl_3 \cdot 6H_2O$. CrMet also had advantage over CrNic in the control of AST and ALT activities. Acute toxicity studies revealed that CrMet had low toxicity potential and relatively high safety margins in mice with the LD_{50} value higher than 10.0 g/kg BW. These findings suggest that CrMet might be of potential value in the therapy and protection of diabetes.

Introduction

Diabetes mellitus (DM) is one of the most common chronic diseases caused by defects in insulin secretion, insulin action or both in nearly all countries [1], and continues to increase in numbers and significance. Globally, the number of adults with diabetes was estimated at 366 million for 2011 and is expected to rise to 552 million in 2030 [2]. This fact has promoted researchers to investigate the effects of agents with anti-hyperglycemic activity [3]. It has been noted that trivalent chromium (Cr(III)) has hypoglycemic activity [4] and plays a vital role in proper carbohydrate, lipid and protein metabolism [5–7]. Laboratory and clinical trials have demonstrated that administration with Cr(III) can lower blood glucose (BG) levels in diabetics [8–10]. It has also been indicated that certain forms of Cr(III) had the capability to overcome insulin resistance and ameliorate diabetes [6,11–13].

Lots of Cr(III) products including inorganic chromium and organically bound chromium have been synthesized and applied in the relative fields. Compared to inorganic forms, organic ones are easier to be utilized by mammals and have higher biological activity [14,15], so a variety of organic chromium products have been developed and used in feed, food and pharmaceutical industries. Among them, the most popular form is chromium(III) picolinate (CrPic). But the nutritional function of CrPic is still controversial because of conflicting clinical data [16–18]. Therefore, the preparation of organic chromium(III) complexes with natural ligands and less or non-toxicity is of great interest [19–21].

L-Methionine (L-Met) is a naturally occurring and essential amino acid and is indispensable for normal growth of mammals. L-Met is also a precursor amino acid for glutathione which protects the cells from oxidative damage and plays vital role as an antioxidant [22–24]. The coordination compound of L-Met and Cr(III) has been used in feed, food and pharmaceutical industries on the market currently [25]. CrMet has been approved as a feed additive for growing–finishing pigs by Ministry of Agriculture of the People's Republic of China (Notification Number 2045).

The published researches on the biological functions of CrMet mainly focused on its effects on improving growth and reproductive performance, and ameliorating metabolism in farm animals

* Corresponding author at: National Engineering Laboratory for Hydrometallurgical Cleaner Production Technology, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China. Tel.: +86 10 82544808; fax: +86 10 82544808.
E-mail address: qgxiao@ipe.ac.cn (Q.-g. Xiao).

http://dx.doi.org/10.1016/j.jtemb.2014.07.001
0946-672X/

including cows [26–36], pigs [37–40] and chickens [41–44]. The studies in farm animal models showed that the administration of CrMet could effectively enhance glucose, insulin and lipid metabolism, improve the growth and reproductive performances. Ghiasi et al. [3] investigated the effects of CrMet on laboratory animals and the results indicated that CrMet supplementation could be effective for lowering insulin resistant index, fasting insulin, glucose and triglyceride (TG) in insulin resistant rats.

However, no research has evaluated the hypoglycemic activity and acute oral toxicity of CrMet. Approximately, in all studies that observed positive effects of CrMet, elemental Cr(III) was fed between 1 and 1200 μg/kg [26–44]. Moreover, there is no sufficient information about appropriate dose of CrMet administration and effects of its over dose feeding in laboratory animals with DM [3]. Therefore, the objective of this research was to evaluate the anti-diabetic properties of relative high dose (500 and 1000 μg Cr/kg) of CrMet and determine the acute oral toxicity of CrMet in mice.

Materials and methods

Chemicals and materials

Alloxan and metformin hydrochloride (MH) were purchased from Aladdin Industrial Inc. (Shanghai, China) and Sino-American Shanghai Squibb Pharmaceuticals Ltd. (Shanghai, China), respectively. Chromium nicotinate (CrNic) was of feedstuff grade and obtained from Shanghai Huating Chemicals Factory Co., Ltd. (Shanghai, China). L-Met of 99% purity was biochemical reagent. All other chemicals and reagents purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China) were of analytical grade and used without any further treatment. Normal saline was used to prepare stock solutions of Cr(III) complex for all experiments unless otherwise mentioned.

CrMet was prepared and characterized according to the previous study [45]. $CrCl_3{\cdot}6H_2O$ (0.01 mol) and aqueous ethanol (20 mL) were added into a three-neck flask equipped with reflux condenser, thermometer, dropping funnel, and magnetic stirrer. Then the solution was heated to 81.1 °C in a water bath. Meanwhile a mixture of L-Met (0.0431 mol), NaOH (0.0431 mol), and aqueous ethanol (20 mL) was stirred at the same temperature until the solids were dissolved; then the mixture was added into the three-neck flask dropwise. After it was agitated and reacted for 127 min, the reaction mixture was cooled to room temperature. The solid product was filtered off, washed with water, and dried at 100 °C in an oven for further use.

Experimental animals

ICR (Institute for Cancer Research) mice aged 6–7 weeks with 23 ± 1 g of body weight (BW) were selected from Vital River Laboratories (VRL) (the license number SCXK (JING) 2011-0011) for all the experiments. For the anti-diabetic activity study and acute oral toxicity tests were selected 90 and 20 mice, respectively. For both trials, the ratio of female to male was selected to be 50:50. All the animals were kept and maintained under the standard guidelines and housed in the VRL-approved animal facility in rooms (the license number SYXK (JING) 2012-0024). All the animals were kept and maintained under the conditions of temperature (24 ± 1 °C), humidity (45 ± 5%) and 12-h photoperiod. The mice were allowed free access to food (standard pellet diet) and drinking water. They were treated in conformity with the animal ethical and animal welfare standards. The experimental protocol for this study was approved by the Animal Care and Use Committee of Vital River Laboratories, Beijing, China. The mice were housed 3 days for adjustment to the environment after arrival before use.

Table 1
The grouping design and doses of chromium methionine (CrMet), $CrCl_3{\cdot}6H_2O$, chromium nicotinate (CrNic), metformin hydrochloride (MH, positive control) and carrier (control) on alloxan-induced diabetic (AID) and normal mice.

Groups	Intervention	Dose	Cr(III) content
Normal control group	None	0.5 mL normal saline	–
Diabetic control group	AID	0.5 mL normal saline	–
$CrCl_3{\cdot}6H_2O$-treated group	AID	5.2 mg $CrCl_3{\cdot}6H_2O$/kg BW	1000 μg/kg BW
CrNic-treated group	AID	8.1 mg CrNic/kg BW	1000 μg/kg BW
Low dose CrMet-treated group	AID	4.8 mg CrMet/kg BW	500 μg/kg BW
High dose CrMet-treated group	AID	9.6 mg CrMet/kg BW	1000 μg/kg BW
Positive control group	AID	500 mg MH/kg BW	–

Anti-hyperglycemic activity study

Diabetes in mice was induced according to the method of Wu et al. [19]. 80 mice were injected with alloxan at the dose of 240 mg/kg BW through intraperitoneal injection. Ten control mice were injected with normal saline. The mice were starved for a period of 12 h prior to treatment, but with free access to drinking water. The blood was collected at the tail vein of mice. BG levels were determined with one-touch glucometer (ARKRAY Factory, Inc., Japan) after the injection for 5 days. The BG levels >11.1 mmol/L in the mice were taken as successful induction of diabetes (the number was 60).

Ten normal mice (five each sex) were selected as normal control group. Sixty alloxan-induced diabetic (AID) mice were randomly divided into six diabetic groups with ten mice each (five each sex). All the mice were allowed free access to standard solid diet and drinking water. $CrCl_3{\cdot}6H_2O$ and CrNic were used as Cr(III) controls, and MH was used as positive control. The grouping and dose designs of all the test substances are shown in Table 1. The drugs were administered by oral gavage to both the normal and AID mice once daily for a period of 15 days.

CrMet was administrated at doses of 500 and 1000 μg Cr/kg BW as dose lower than these has been reported to be partially effective in diabetic animals [25]. It has been observed that supplementation at these and at higher doses has no significant toxic effect [3]. In comparison with CrMet, $CrCl_3{\cdot}6H_2O$ and CrNic were supplemented at a dose of 1000 μg Cr/kg BW. This dose of $CrCl_3{\cdot}6H_2O$ and CrNic were also reported to be efficacious in diabetics and had no significant harmful effect [20,46,47].

Biochemical assays

The BW levels of the mice were determined on day 0 before the experiment and every 5 days thereafter. The BG levels of the mice were measured prior to the administration and on the 15th day (4-h starvation after the last administration), respectively. After the determination of BG levels, all the mice were killed by carbon dioxide euthanasia. The blood samples of all mice were obtained through cardiac puncture, and then all the mice were dissected in order to harvest their livers which were washed with pre-cooling saline, dried and then weighed. Blood serum was separated by centrifuging at 3000 rpm for 15 min at room temperature. The serum