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RESEARCH ARTICLE

Trivalent Chromium Attenuated Corticosterone Secretion and Actions in Adrenocorticotropic Hormone-Stimulated Rats

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A B S T R A C T

Although mutual interplay between chromium and glucocorticoids exists, there is few data showing the effects of chromium on glucocorticoid secretion and actions. The aim of this study was to investigate the effects of chromium on glucocorticoid secretion and actions in adrenocorticotropic hormone (ACTH)-stimulated Sprague-Dawley rats. Rats were intraperitoneally administrated with ACTH (0.1 mg/kg BW/day) or saline and concomitantly supplemented with chromium (80 μg/kg BW/day) or placebo for 4 weeks. Chromium supplementation decreased the levels of corticosterone, AST, ALT, and ALP in ACTH-stimulated rats. Results of histological examination showed that chromium supplementation improved hypertrophic change in the zona fasciculata and the zona reticularis of adrenal glands and hepatic accumulation of lipid droplets in ACTH-stimulated rats. Chromium administration had an inhibitory effect on the corticosterone secretion by adrenal gland in ACTH-stimulated rats and attenuated the hepatic damage caused by hyperadrenocorticism.

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INTRODUCTION

Spontaneous Cushing's syndrome is an endocrine disorder in mammals, including humans and canines (Kooistra *et al*., 2009; Galac *et al*., 2010). It can be classified into two types, adrenocorticotropic hormone (ACTH)-dependent and ACTH-independent, with over-secretion of glucocorticoids. In addition, central obesity and muscle wasting are alternative prominent clinical signs (Galac and Wilson, 2015). The therapeutic treatments of spontaneous Cushing's syndrome are to control adrenocortical over-activation and ablate or destroy the primary lesion through surgery or medications (Mancini *et al*., 2010). Besides, transsphenoidal hypophysectomy is an alternative option for ACTH-dependent patients (Alexandraki *et al*., 2013).

Trivalent chromium (Cr) is an essential mineral and plays an important role in the hormonal regulation and metabolism (Chen *et al*., 2009[a]; Kumar *et al*., 2015). Cr acts as a cofactor of insulin function to improve glucose and lipid metabolism by increasing insulin receptor expression, insulin binding, and sensitivity of pancreatic ß-cells. Regarding the homeostatic regulation of glucose and lipid, glucocorticoids exert opposite activity as Cr does. Studies showed that Cr supplemented animals suffer less from stress insults due to lower circulating glucocorticoids (Moonsie-Shageer and Mowat, 1993; Sahin *et al*., 2002; Zha *et al*., 2009). On the other hand, evidence also indicates that glucocorticoids cause Cr depletion (Chen *et al*., 2013).

Although mutual interaction between Cr and glucocorticoids exists, there is few data showing the correlation between Cr and glucocorticoid secretion. Thus, the aim of this study was to investigate the effect of Cr on glucocorticoid secretion by ACTH-stimulated rats, an animal of Cushing's disease. Together with relevant studies, we hypothesized that Cr administration could attenuate the secretion of corticosterone by adrenal gland in ACTH-stimulated rats, hence decrease hepatic damage in animals with Cushing's syndrome.

MATERIALS AND METHODS

Animals: Twenty-four male Sprague Dawley (SD) rats (263±8 g) obtained from Biolasco Taiwan Co., Ltd. was housed under a 12:12-hr light-dark cycle and at the constant temperature 21±2°C. All rats had *ad libitum* access to food and water throughout this study. The rats

were divided into four groups: (1) Control group (n=6) receiving placebo supplementation and intraperitoneal injection of saline; (2) Cr group (n=6) receiving Cr supplementation (80 µg/kg BW/day) and intraperitoneal injection of saline; (3) ACTH group (n=6) receiving placebo supplementation intraperitoneal injection of adrenocorticotropic hormone (ACTH) fragment 1-24 (0.1 mg/kg BW) (Sigma Aldrich, USA); (4) Cr with ACTH group (n=6) receiving Cr supplementation (80 µg/kg BW/day) and intraperitoneal injection of ACTH (0.1 mg/kg BW). The BW of all groups were measured and recorded weekly. At the 28th day, all rats were sacrificed for analyses with humanism. The animal study was approved by the Animal Care and Use Committee of National Chung Hsing University, Taiwan, ROC.

Serum preparation: The blood samples were collected from caudal vein at day 0 and day 28, after an overnight fast. The blood samples were then centrifuged at 1000 r/min for 10 min. Following centrifugation, serum from each sample was collected and frozen at -70°C until analysis.

Measurement of serum corticosterone, AST, ALT and ALP: The levels of corticosterone at the day 0 and the day 28 were determined by commercial ELISA kits (USCN, Life Science Inc., USA) and the AST, ALT, and ALP were measured at day 28 by commercial chemistry analyzer (Hitachi 704, JPN).

Histological observation: The adrenal gland and liver were collected for histological examination. All tissues were fixed in 10% formalin and the sections were stained by hematoxylin and eosin (H&E).

Statistical analysis: All data in the experiment were expressed as the mean ± Standard Error Mean (S.E.M.). Differences between groups were tested for statistical significance by Student t-test. P value<0.05 was considered as significant difference.

RESULTS

The results of organ weight and percentage of BW are presented in Table 1. The percentage of adrenal gland weight was higher in ACTH and ACTH with Cr groups when compared with control group (P<0.05). The percentage of muscle weight in Cr and ACTH with Cr groups was higher than control and ACTH groups (P<0.05). However, there was no significant difference in percentage of liver weight and epididymal adipose tissue weight among groups. At day 28, the level of corticosterone elevated in ACTH group and the increase was attenuated in ACTH with Cr group (Table 2). Among the ALT, AST, and ALP, only the level of ALT in ACTH group was significant increment in comparison with the control group (Table 3). The adrenal glands of ACTH groups showed hypertrophic change in the zona fasciculata and the zona reticularis, and the swelling cells were filled with lipid droplets which were prominently apparent than that in other three groups (Fig. 1). Histological examination revealed a very few vacuoles in livers of control, Cr, and ACTH with Cr groups, while there were moderate amount of vacuoles scattered in the hepatocytes of ACTH group (Fig. 2).

DISCUSSION

The percentage of adrenal gland weight in ACTH and ACTH with Cr groups were almost twice as that of control group (P<0.05). There was no significant difference in percentage of adrenal gland weight between the control, Cr, and ACTH groups. However, it is noteworthy that although only slightly, the percentage of adrenal gland weight in ACTH with Cr group was lower than that in ACTH group. It shows that Cr administration had a negligible effect on the percentage of adrenal gland weight in non-ACTH-stimulated rats, but slightly decreased it in ACTH-stimulated rats.

The percentage of liver weight was slightly decreased in ACTH group compared with control group, and the result was inconsistent with some reports that showed glucocorticoids administration might increase the liver weight due to glycogen catabolism in the hepatic cells (Tavoni *et al*., 2013). It might be due to the discrepancy of glucocorticoid levels and action durations between exogenous administration and ACTH-driven endogenous secretion. The percentage of liver weight in Cr and ACTH with Cr groups was higher than control and ACTH groups, in consistence with previous studies demonstrating an increase of hepatic cell volume and liver weight gain by Cr supplementation (Sahin *et al*., 2002; Hematológico, 2006).

The percentage of muscle weight of Cr group and ACTH with Cr group was significantly increased compared with control group and ACTH group, respectively who were consistent with the Cr and insulin action on protein metabolism (Sahin *et al*., 2002). In fact, Cr is an element to muscular development (Vincent and Love, 2012). The percentage of muscle weight of ACTH group was significantly decreased compared to control

Table 1: Weights of organs and percentage of tissue/body weight at day 28 in different groups

Organ weights (g)						
Group	Adrenal gland	Liver	Kidney	Spleen	Lipid	Muscle
Control	0.03±0.0006	11.87±1.15	3.07±0.15	0.62±0.05	2.40±0.32	4.82±0.14
Cr	0.03±0.0007	12.04±0.76	2.90±0.17	0.50±0.05	1.80±0.07	5.02±0.20
ACTH	0.07±0.0042*	9.87±0.35	2.75±0.12	0.67±0.05	2.73±0.18	4.17±0.14
ACTH+Cr	0.06±0.0047*	13.50±0.55	3.08±0.15	0.60±0.02	2.56±0.43	4.72±0.37
Ratio of organ weights/body weights (%)						
Group	Adrenal gland	Liver	Kidney	Spleen	Lipid	Muscle
Control	0.0079±0.0004	2.90±0.25	0.75±0.02	0.15±0.01	0.59±0.07	1.19±0.07
Cr	0.0079±0.0003	3.01±0.17	0.73±0.05	0.12± 0.01	0.45±0.007	1.27±0.08*
ACTH	0.0192±0.019*	2.53±0.07	0.71±0.03	0.17±0.01	0.70±0.05	1.06±0.05*
ACTH+Cr	0.0171±0.017*	3.45±0.15	0.78±0.02	0.15±0.003	0.64±0.09	1.19±0.09

Values (mean±SEM) bearing asterisk in a column differ significantly (P<0.05) compared to control group at day 28.

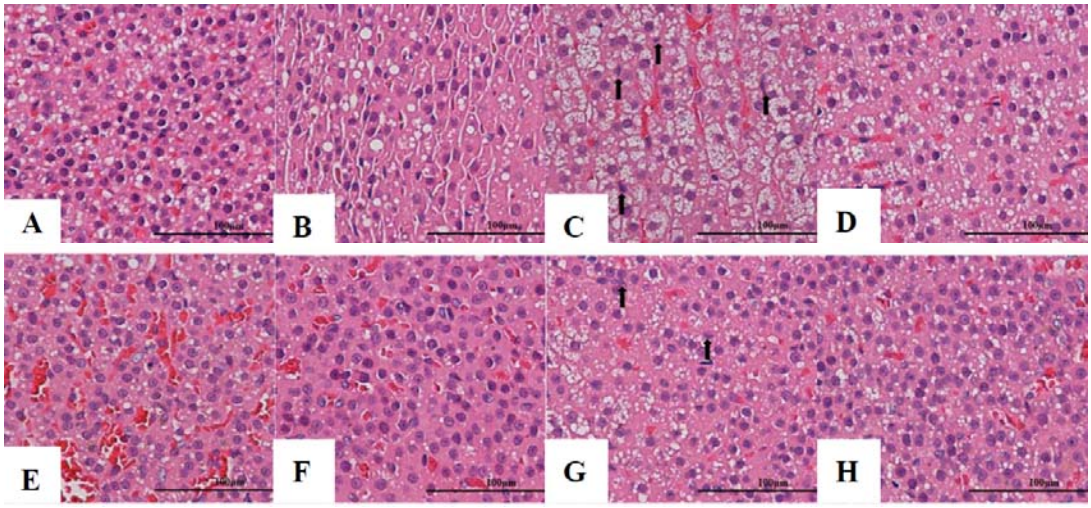

Fig. 1: Histological examination of the zona fasciculata (A-D) and zona reticularis (E-H) in adrenal cortex. H&E staining. Bar=100 μm. A & E: control group, B & F: Cr group, C & G: ACTH group and D & H: ACTH with Cr group. The cells in the zona fasciculate and zona reticularis in ACTH group showed swelling, and the lipid droplets accumulated in the cells were significantly more than other groups. Many mitotic cells (arrows) were seen in the zona fasciculata and zona reticularis in ACTH group.

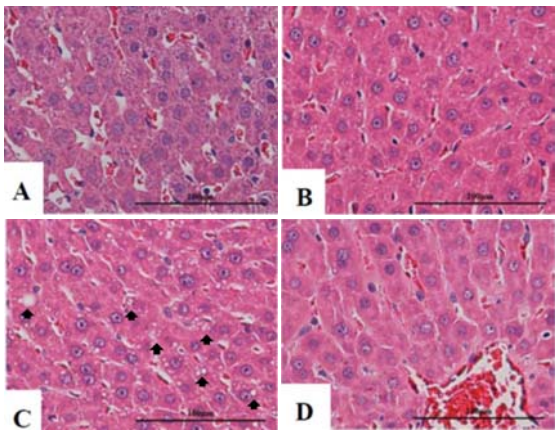

Fig. 2: Moderate hepatic vacuoles (indicated by black arrows) can be seen in C group by histological examination. H&E staining. Bar=100 μm. A: control group, B: Cr group, C: ACTH group and D: ACTH with Cr group.

Table 2: The concentrations of serum corticosterone at day 0 and day 28 in different groups

Corticosterone concentration (ng/mL)		
Group	Day 0	Day 28
Control	11.34±0.90	16.18±0.96
Cr	13.89±0.70	14.86±1.10
ACTH	14.11±0.66	21.15±1.92*
ACTH + Cr	13.02±0.72	15.99±1.41#

Values (mean±SEM) bearing asterisk in a column differ significantly (P<0.05) compared to control group and # to ACTH group at day 28.

Table 3: The level of serum AST, ALT, ALP at day 28 in different groups

Group	AST (u/l)	ALT (u/l)	ALP (u/l)
Control	265.00±22.93	55.67±4.18	166.13±19.96
Cr	235.20±28.44	56.20±6.74	192.80±20.45
ACTH	290.67±28.06	70.67±6.06*	172.57±14.00
ACTH + Cr	243.40±23.17	65.60±3.63	192.20±9.04

Values (mean±SEM) bearing asterisk in a column differ significantly (P<0.05) compared to control group at day 28.

group, which was consistent with the fact that chronic glucocorticoids induce muscle wasting (Castillero *et al*., 2013).

The level of corticosterone in ACTH group was significantly higher than that in control group (P<0.05). Studies showed that Cr supplemented animals, such as rats, cows, and chickens, lowered sensitivity to stress insult-induced injury through reducing glucocorticoids in bloods (Sahin *et al*., 2002; Zha *et al*., 2009). Recently, it has been demonstrated that glucocorticoids reduce the level of Cr in liver, muscle, and fat (Chen *et al*., 2013). Moreover, high levels of Cr inhibit the steroidogenesis in agonist-stimulated adrenocortical cells (Kim *et al*., 2010). Therefore, the reduction of corticosterone in ACTH with Cr group might be a consequence of inhibition of steroidogenesis by Cr.

The AST, ALT and ALP levels were increased in ACTH group which may be primarily that glucocorticoids treatment causes hepatocyte swelling together with glycogen accumulation in the hepatic cells (O'Brien *et al*., 2002). Chen *et al*. (2009[b]) showed the effects of Cr supplementation on the rat model of chronic cholestasis and demonstrated that hepatic damage was attenuated via the antioxidative capacity of Cr. In addition, Cr supplementation prevented progression of NAFLD in KK/HIJ mice (Chen *et al*., 2010). Our results showed that the AST and ALT levels in ACTH with Cr group were lower than ACTH group, which possibly indicated that the hepatic damage caused by glucocorticoids was diminished after Cr treatment. In rats, the sensitivity of ALP to hepatic injury is not as sensitive as ALT and AST (Bai *et al*., 1992; Travlos *et al*., 1996), thus it may explain why the ALP level was increased in ACTH and Cr groups compared with ACTH group.

After administration of ACTH for 28 days, the adrenal glands of ACTH groups showed hypertrophic change in the zona fasciculata and the zona reticularis, and the swelling cells were filled with lipid droplets. Compared to control group, less accumulation of lipid droplets in the cell of the zona fasciculata and the zona reticularis was observed in Cr group. A study proved that high concentration of Cr had an inhibitory effect on the secretion of cortisol and dehydroepiandrosterone sulfate (DHEAs) by agonist-stimulated human adrenocortical cells (Kim *et al*., 2010). The study also showed the dramatically inhibitory effect on the cortisol secretion by Cr which was only on the agonist-stimulated adrenocortical

cells, but minimally on the non-stimulated cells. In the present study, the corticosterone concentration of rats in Cr group was only slightly lower than control group, while the corticosterone concentrations of ACTH with Cr group was significantly lower than ACTH group. The results of corticosterone concentration were in parallel with the histological examination showing lipid droplets in the cell of the zona fasciculata among groups. Those results were similar to previous research (Kim *et al*., 2010), showing the inhibitory effect of Cr was only in the agonist-stimulated adrenocortical cells, but minimally in the non-stimulated cells.

Further studies in elucidating detailed mechanisms of Cr on adrenocortical secretion seem warranted. In control, Cr, and ACTH with Cr group, very few vacuoles were observed via the histological examination in liver, while there were moderate amount of vacuoles scattered in the hepatocytes of ACTH group. The vacuoles in the hepatocytes might be lipid, water, and glycogen, since many studies suggested that administration of glucocorticoids induced glycogen accumulation in hepatocytes (Sepesy *et al*., 2006; Tavoni *et al*., 2013), therefore the vacuoles seen in ACTH group might probably be glycogen. The glycogen accumulation in the hepatocytes might cause the raise of AST, ALT and ALP levels in ACTH group compared with control group, and the levels of AST and ALT decreased in ACTH with Cr group compared to ACTH group might be due to the absence of glycogen accumulation. Glucocorticoids induce glycogen synthesis in liver and enhance proteolysis and lipolysis to provide additional substrates for liver, hence cause glycogen accumulation (Tavoni *et al*., 2013). Since supplementation of Cr decreased the corticosterone concentrations in this study, it might decrease the accumulation of glycogen due to glucocorticoids stimulation as well, this may explained the absence of glycogen accumulation in ACTH with Cr group.

Conclusions: This study demonstrated that supplementation of Cr had an inhibitory effect on the secretion of corticosterone by adrenal gland in ACTH-stimulated rats and attenuated the hepatic damage caused by glucocorticoids excess. However, further studies in elucidating detailed mechanisms of Cr on adrenocortical secretion are still warranted.

Author's contribution: HK and WL conceived and designed the experiment. WL executed the experiment and HW and KC analyzed the sera and tissue samples. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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