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Original Article

Changes in Catecholamines and Acetylcholinesterase Levels of Crebellum, Mid-brain and Brain Cortex in Chromium Treated Rats

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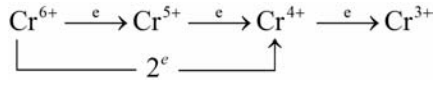
Abstract

The short and long term effects of chromium toxicity on brain catecholamines and acetylcholinesterase levels were investigated. Rats were injected daily with varying amounts of chromium. The short term (2 h) administration of chromium (8 mmol/kg) reduced catecholamines level of cerebellum, mid-brain and brain-cortex by 22.8, 19.4 and 21.2% respectively. Acetylcholinesterase activity was also reduced by 36.1, 29.0 and 26.7%. Administration of 38 μmol/kg chromium for 15, to 60 days, reduced catecholamine levels of cerebellum (8.3-32.8%), midbrain (4.5-20.3%) and brain cortex (6.1-21.3%) respectively. Acetylcholinesterase activity of cerebellum, mid-brain and brain cortex was reduced by 9.4-27.1, 6.8-22.6 and 7.2-24.9 percent respectively. It might be concluded that brain disturbances in chromium treated rat occurred through the reduction in catecholamines and acetylcholinesterase levels

Keywords: Chromium; catecholamine; acetylcholinesterase; neurological disease.

Introduction

Chromium (Cr) is ubiquitous in the environment occurring naturally in soils, rocks and living organisms. Chromium exists primarily in two forms, trivalent Cr (III) and hexavalent Cr (VI), with the latter primarily produced by anthropogenic source (1). Chromium (III) is an essential ultratrace element and plays an important role in the biological system production of insulin (2). This element is also produced by many different industries including welding chrome plating, chrome pigmenting, leather tanning, wood preserving, and in ferrochrome industry (3). Occupational exposure to Cr (III) and Cr (VI) by inhalation depends upon the job function and industry (4). Chromium enters the air and soil mostly in the chromium (III) and chromium (IV) forms (5). In the air, chromium compounds are presented mostly as fine dust particles which eventually settle over land and water (6). The chemistry of chromium is very interesting and complicated. The inter-conversion of chromium (III) and chromium (IV) is controlled by several factors including the presence and concentrations of chromium species, oxidizing and reducing agents, the electrochemical potentials of the oxidation and reduction reactions, acid-base reactions, complex forming agents, and so on. The reduction of Cr under physiological conditions is illustrated by the following equation.



It is important to note that trivalent chromium is the most stable from of chromium which can be produce by Cr (VI) by a number of reductants (8-10) including vitamin C, reduced glutathione (GSH) and cysteine. The initial step

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Table 3. Long term effects of chromium on the catecholamine s levels of cerebellum, mid -brain and brain-cortex.

Parts of brain	Group	Catecholamines level (ng/mg protein)		
		15(days)	30 (days)	60 (days)
Cerebellum	Controls	185.1 ± 3.8	184.7 ± 4.9	184.1 ± 7.5
	Cr-treated	169.6 ± 4.3*	158.3 ± 4.6*	123.7 ± 7.5*
Mid-brain	Controls	120.1 ± 6.1	119.1 ± 7.1	115.2 ± 6.1
	Cr-treated	114.6 ± 4.3	109.5 ± 6.7*	91.81 ± 8.31
Brain-cortex	Controls	70.6 ± 3.9	68.2 ± 5.6	67.1 ± 2.9
	Cr-treated	66.2 ± 2.8*	61.1 ± 3.9	52.8 ± 4.2*

Rats were injected with (38 mole/kg) of chromium for 15, 30 and 60 days. Animals were killed at the end of experimental times. Catecholamines levels were derermined in different regions of the brain the number of rats were as mentioned for table 1. Data are presented as mean±SE. *Indicates statistically significant at P<0.05.

involves a two electron reduction to Cr (IV) followed by one electron reduction to Cr (III), in the presence of intracellular reductants. This can produce a number of diseases including bone and renal diseases (10, 11).

Patients with chronic renal failure have a slightly higher mean serum chromium concentration than normal subjects. Whereas, patients on hemodialysis have mean serum chromium concentration over 20 times higher than normal individuals (12). It has been reported that high serum chromium concentration is a result of dialysis treatment and not of renal failure (12) since transplanted patients have serum chromium levels similar to those of chronic renal failure. Thus, the restoration of normal kidney function by transplantation leads to a drop in serum chromium concentration to almost normal levels (14). The source of chromium is from the hemodialysis concentrate and also dialysis apparatus but not from the water supply as previously reported for aluminum toxicity in these patients (15-16).

The increased body burden of chromium in hemodialysis patients appears, therefore, to be confined to plasma compartment where it binds to serum transferrin (17). Serum human transferrin is a -glycoprotein with a molecular weight of approximately 80 KD and it is the major iron carrier protein in the plasma (18). Due to physiochemical similarities with iron a number of other elements including Mn (19), Zn (20), Cd (21) and Indium (22) bind to this protein in the plasma.

Chromium transfers across dialysis membrane and therefore binds to serum transferrin (17). It has been reported that this binding activity may lead to the disturbances of iron metabolism (23). Morris et al found that the concentration of transferrin in patients with Alzheimer's disease is much higher than normal subjects (24). Therefore, the interaction of chromium with transferrin may disturb brain function.

Therefore, the major aim of the present study was to investigate the short and long term effects of chromium on the level of rat brain catecholamines and acetylcholinesterase activity.

Experimental

Animals

Male wistar rats weighing (100-150) gram were purchased from Pasteur Institute (Tehran, Iran) and maintained in animal house until the desired weight (200-220) gram was attained. All rats were fed with Food and water under standard condition. Four rats each served as experimental and controls for each individual studied. The indicated dose of chromium as chromium chloride was dissolved in saline and injected intraperitoneally to experimental

Table 4. Long term effects of chromium on the acetylcholin esterase activity of cerebellum, mid -brain and brain-cortex.

Parts of brain	Group	Acetylcholinesterase (mol enzyme/mg protein/min)		
		15(days)	30 (days)	60 (days)
Cerebellum	Controls	55.6 ± 8.2	53.7 ± 5.7	52.4 ± 9.4
	Cr-treated	48.6 ± 6.2*	44.5 ± 2.6*	38.3 ± 2.6*
Mid-brain	Controls	62.8 ± 5.7	62.4 ± 4.8	61.3 ± 6.3
	Cr-treated	58.56 ± 5.6	54.1 ± 1.5*	47.5 ± 7.5
Brain-cortex	Controls	31.1 ± 3.8	30.2 ± 5.8	30.1 ± 3.7
	Cr-treated	28.8 ± 2.7	26.3 ± 2.3	22.5 ± 1.9

mole enzyme/mg protein/min

Animals were treated as mentioned in the legend for table 3. Acetyl cholinesterase activity was determined as mentioned in the Materials and Meth ods. Data are expressed as mean± SEM. *Indicates statistically sig nificant at P<0.05.

Table 1. Chromium determination in rat serum

Chromium dose (mg/kg)	Days of injection	Serum chromium (ng/L)	
		Control	Treament
2	15	4.6 ± 0.13	19.7 ± 0.3*
2	30	3.7 ± 2.0	28.8 ± 1.2*
4	60	4.2 ± 0.5	39.8 ± 0.4*

Rats were injected with 38 mole/kg of chrom ium as chromium chloride for 15, 30 and 60 days. Animals were killed and sera were collected in pre -acid washed tube for chromium determination. Serum chromium was determined as mentioned in methods and materials. Data are presented as mean ± SE. The numbe r of animal in each group was 4. Each blood sample was read for chromium determination 5 times by Atomic absorption. *Indicates statistically significant at P<0.05.

groups. Controls were injected only with saline. The onset and duration of each injection series are given in the tables. Animals were killed by decapitation. Brains were carefully removed and dissected in to cerebellum, mid-brain and brain cortex. Catecholamines levels of each section were determined according to the method described by Messripour and Haddady. Brain was homogenized in acidic pH, centrifuged and the catecholamines fraction was separated using Al2O3 and measured by using spectrofluorimetry technique. Lowry's method was used for protein determination (26). Blood samples were collected and sera were stored in pre-acid washed tubes for chromium determination.

Acetylcholinesterase activity of brain fractions was measured using the method of metanitrophenol (27).

Chromium determinations were carried out using a Perkin-Elmer (HG-Ao600) flameless atomic absorption spectrophotometry as reported for aluminum determinations (28).

Chemicals

All chemicals were reagent grade and were obtained from Sigma Chemical Company (Germany). Deionized water was used throughout this project. Statistical analysis was done using student's t-test.

Results and Discussion

Prior to study, the baseline serum chromium concentrations of experimental and untreated control animals were determined (Table 1). Daily administration of chromium as CrCl3 led to the significant elevation of serum Cr after 15 to 60 days of injection P<0.05. Administration of a single dose of 8 mmol/kg of chromium in two hours reduced catecholamines levels of cerebellum (22.8%), Mid-brain (19.4%) and Brain-cortex (21.2%) in comparison to untreated chromium controls (Table 2).Daily dose (38 mol/kg) of chromium for 15, 30 and 60 days reduced catecholamines levels of cerebellum by 8.3, 14.3 and 32.8%, midbrain by 4.5, 8.6 and 20.3% and brain-cortex by 6.1, 10.4 and 21.3% respectively (Table 3).

The short and long term effects of chromium on different regions of rat brain acetylcholinesterase activity were studied next.

A single dose of 8 mmole/kg of chromium after two hours reduced acetylcholinesterase activity of cerebellum (36.1%). Mid-brain (29.0%) and brain cortex by 26.7% rrespectively table 2.

Administration of 38 mol/kg of chromium daily for 15, 30 and 60 days reduced cerebellum acetylcholinesterase activity by 9.4, 17.2 and 27.1%, mid-brain by 6.8, 13.4 and 22.6% and brain cortex by 7.2, 12.8 and 24.9% respectively (Table 4).

Published studies from various countries have documented significantly raised serum and whole blood chromium concentrations in

Table 2. Short term effects of chromium on the catecholamines content and acetylcholinesterase activity of rat cerebellum mid -brain and brain-cortex.

Treatment	Catecholamines ng/mg protein	Acetylcholinesterase mole enzyme/mg protein/ min
Cerebellum: c ontrols	183.1 ± 4.7	34.3 ± 8.2
Cr	133.9 ± 4.2*	34.7 ± 4.6*
Mid-brain: controls	121.1 ± 7.2	6.1 ± 5.2
Cr	97.5 ± 4.3*	43.3 ± 4.9*
Brain-cortex: controls	69.3 ± 4.1	30.1 ± 4.8
Cr	54.6 ± 2.1*	22.1 ± 2.7*

mole enzyme/mg protein/min

A single dose of chromium (8 mmol e/kg) was injected to rat. Animals were killed by decapitation after 3 hours. The cerebellum, mid-brain and brain cortex content of catecholamine was determined according to the mthods and materials. Data are expressed as the mean±SEM. Four rats we re in each group. *Indicates statistically significant at P<0.05.

dialysis patients (29-30). Chromium is widely used in many metal alloys and contaminations of the dialysis fluid during manufacturing process leads to the transfer of chromium into systemic circulation during the dialysis process (29-31).

The probable mechanism by which chromium causes neurological disease is still a matter of discussion. Previously, it has been reported that transferrin which is an iron carrier protein is also responsible for the transportation of chromium into the circulation and it has high affinity for chromium and transferrin receptors on the lumen of brain capillaries (24) which may be able to mediate the uptake of chromium in the brain.

Data which have been presented in this manuscript show that a single dose of chromium caused an approximate parallel reduction in the levels of catecholamines and acetylcholinesterase activity of various part of brain. When lower doses of chromium (2 mg/kg) were administered for 15 to 60 days, significant reductions in the levels of catecholamines content and acetylcholinesterase activity were seen particularly in 60 days of chromium administration since both catecholamines and acetylcholinesterase are necessary for biochemical function of brain and their reduction may be due to the interference of high level of chromium with the synthesis of any specific enzymes which may be responsible for the production of catecholamines and acetylcholinesterase. Alternatively chromium (VI)-containing compounds after reduction to chromium (III), interfere with DNA synthesis in the treated cells (32). Chromium treatment rapidly inhibits DNA replication and secondarily blocks RNA and protein synthesis (32), which seems to be possibly related to the depletion of intracellular nucleotide triphosphates (adenylate and guanylate) pools resulting from the formation of Cr (III) dependent coordinate complexes with desoxynucleotid three phosphate (dNTP). This might be considered for the reduction in the production of acetylcholinesterase and also those enzymes which are involved in the biochemical pathways of catecholamine.

It has been already reported by this laboratory and others that aluminum administration to rats significantly reduces catecholamines content of cerebellum, mid-brain and brain cortex (34). Due to the chemical similarities between aluminum and chromium, both metals may follow the same processes in the brain for the disturbances of brain function. It has been also reported that when aluminum salts are administered to experimental animals, a slow progressive encephalopathy characterized by neurofibrillar degeneration occurs (35). However, the exact mechanism by which chromium interferes with brain function and causes neurological disorders is not fully clear and similar to aluminum more investigation should be done to elucidate this speculation.

References

(1) Stocker B J. Chromium absorption, safety and toxicity. *J. Trace elements Experimental Medicine* (1999) 12: 163-169

(2) Anderson R A. Nutritional role of chromium. *Sci. Total Environ.* (1981) 17: 13-19

(3) Fishbein L. Source, transport and alteration of metal compounds and overview. Arsenic, beryllium, cadmium, chromium and nickel. *Environ. Health Perspct.* (1981) 40: 143-65

(4) Flora S D, Serra B D and Zanacchi P. Genotoxicity of chromium compounds. A. Review. *Mutat. Res.* (1990) 238: 99-172

(5) Pankow J F. Analysis for chromium traces in aquatic ecosystem. A study of Cr (II) and Cr (VI) in the Susquehanna River Baisen New York and Pennsylvania. *Sci. Total Environ.* (1977) 7: 17-25

(6) Baron D, Palmer C D and Stamely I. Identification of two iron chromate precipitates in a chromium contamitant soil. *Sci. Technol.* (1996) 30: 964-968

(7) Beaubien S, Nriagu J, Blowes D and Lawson G. Chromium speciation and distribution in the Greate Lakes. Environ. *Sci. Technol.* (1994) 28: 730-7388

(8) Steans D M, Kennedy K D, Courtney P H, Giangrande L S and Phieffer K E. Reduction of chromium (VI) by ascorbate lead to chromium-DNA binding and DNA streand breaks in vitro. *Biochemistry* (1995) 34: 910-919

(9) Standeven A M and Wetterhahn K E. Ascorbate is the principle reductant of chromium (VI) in rat liver and kidney ultrafiltrates. *Carcinogens* (1991) 12: 1733-1734

(10) Ning I and Grant M N. Chromium (VI) induced cytotoxicty to osteoblast-derived cells. *Toxicol.* (1999) 13: 879-887

(11) Malecka J, Greszczak W, Zukowska E A, Jendryczko A and Baczynski R. Concentration of chromium in blood serum of patients with chronic renal failure. *Pol.*

Arch. Med. Wewn. (1995) 93: 25-31

(12) Henderson I, Leung A and Halls D. Hyperchromia in chronic dialysis patients. *Proc. EDTA. ERA* (1985) 22: 270-275

(13) Swen A, Derita M V, Michellis M F, Anderson R and Preuss H. Effect of chromium supplement on hemodialysis. *Neph.* (1996) 7: 1465-1470

(14) Leung A T, Henderson L, Kennedy A C, Halls D J and Fell GS. Chromium transfer studies in hemodialysis and continous ambulatory peritoneal dialysis. In: Taylor A. (ed.) *Aluminum and other Trace Elements in Renal Ddisease.* Bailliere Tindall, London (1986) 332-336

(15) Salgado R A and Rodriguez Bitahan J B. Blood levels of chromium in diabetics and non-diabetics hemodialysis patients. *Transplantation Proceeding* (1996) 28: 3382-3384

(16) Haese D, Couttenye P C, Lamberts M, Elseviers L V, Goodman M M, Schrooten W G, Cabera I and De-Broe W E. Aluminium, iron, cadmium, chromium, magnesium, strontium and calcium content in bone of end-stage renal failure. *Clin. Chem.* (1999) 45: 1548

(17) Moshtaghie A A and Ani M. Comparative binding study of aluminum and chromium to human transferrin effect of iron. *Biol. Trace Element Res.* (1992) 32: 39-46

(18) Brock J H. Transferrin. In: Harison PM. (ed.) *Metaloproteins.* Vol. II. McMillan, London (1985) 183-262

(19) Moshtaghie A A, Badii A and Hasanzadeh T. Role of ceruloplasmin and ethamalamine in Manganase binding to serum apotranferrin. *Iranian J. Sci. Technol.* (1997) 21: 157-168

(20) Moshtaghie A A and Badii A. Comparative binding studies of Zinc and Iron to serum human transferrin. *Iranian J. Sci. Technol.*(1996) 20: 177-188

(21) Moshtaghie A A, Taghikhani M and Sandughchin M. Cadmium interaction with iron metabolism. *Clin. Chem. Enzyme Comm.* (1997) 7: 307-316

(22) Moshtaghie A A and Ghaffari M. Identification of amino acids involved in indium binding to serum human apotransferrin. *Iranian Biomed. J.* (2001) 5: 149-153

(23) Ani M and Moshtaghie A A. The effect of chromium on parameters related to iron metabolism. *Biol. Trace Element. Res.* (1992) 32: 57-64

(24) Morris C M, Court J A and Moshtaghie A A. Transferrin and transferrin receptors in normal brain and in Alzheimer's disease. *Biochem. Soc. Transaction* (1985) 15: 891-892

(25) Messripour M and Haddady H. Effect of ascorbic acid administration on copper-induced changes in rat brain hypothalamic catecholamine contents. *Acta Neurol. Scand.* (1988) 77: 481-5

(26) Lowry O H, Rosenbrough N Y, Farr A L and Randall R Y. Protein measurement with folin reagent. *J. Biol. Chem.* (1951) 193: 265-75

(27) Moshtaghie A A, Rahimi S and Messripour M. Aluminum administration on acetylcholinesterase activity of different regions of rat brain. *Medical J. of Islamic Academic of Science* (1999) 12: 105-108

(28) Moshtaghie A A, Saundughchin M, Badii A and Azani M. Development of a method for aluminum determination in serum and dialysis fluid by flameless atomic absorption with graphite furnace. *Med. J. I.R.I.* (1995) 9: 233-237

(29) Rudolf E. A review of findings on chromium toxcicity. *Acta Medica Hsadec-kralove*. (1997) 41: 55-65

(30) Schiffi H, Weidmann P, Weiss M and Massry S G. Dialysis treatment of acute chromium intoxication and comparative efficacy of peritoneal versus hemodialysis in chromium removal. *Min. Elec. Met.* (1982) 7: 28-35

(31) Thompson M M, Sterens B Y, Humphery T Y and Adoins R C. Comparison of trace elements in peritoneal dialysis, hemodialysis and uremia. *Kidney Int.* (1983) 23: 9-14

(32) Wilks M F, Kwizera E N and Bach P H. Assessment of heavy metal nephrotoxicity in vitro using isolated rat glomeruli and proximal tubular fragmerts. *Ren. Physiol. Biochem.* (1990) 13: 275-84

(33) Biandi V, Toso R D, Debetto P, Levis A G, Luciani S, Majone F and Tamino G. Mechanism of chromium toxicity in mammalian cell culture. *Toxicol.* (1980) 17: 219-224

(34) O`Brien T S O, Ceryak S and Patierno S R. Complexities of chromium carcinogenesis. Role of cellular response, repair and recovery mechanisms. *Mutat. Res.* (2003) 533: 3-36

(35) Moshtaghie A A, Rahimi S and Messripour M. Changes in catecholamine levels of cerbellum, mid-brain and brain cortex in aluminium intoxified rats. *Indian J. Pharmacol.* (1996) 28: 244-248

(36) Crapper DR and Tomoko GJ. Neuronal correlates of an encephalopathy associated with aluminum neurofibrillary degeneration. *Brain Res.* 97: 253-264

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