

The Neuroprotective Effect of L-Cysteine Towards Cadmium or Nickel Neurotoxicity on Adult Rat Brain Antioxidant Status and Acetylcholinesterase Activity

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Abstract

Background: Cadmium (Cd) and nickel (Ni), as heavy metals, are the major environmental and industrial contaminant ions which can exert serious oxidative and neurotoxic effects. The aim of this study was to investigate the potential effects of the antioxidant property of L-cysteine (Cys) on the adult rat brain total antioxidant status (TAS) and acetylcholinesterase (AChE) activity induced by Cd or Ni administration.

Materials and Methods: Thirty-six male Sprague-Dawley rats were divided into six groups: A (saline-treated negative control), B (Cys-positive control, 7 mg/kg), C (Cd, 3CdSO₄. 8H₂O, 1 mg/kg), D (Ni, NiCl₂, 13 mg/kg), E (Cd+Cys) and F (Ni+Cys). All rats were treated once daily for 7 days with intraperitoneal injections of the tested compounds. Rats were killed by decapitation and mentioned parameters were measured spectrophotometrically.

Results: The rat brain AChE activity was significantly increased by Cd, Ni and Cys ($P < 0.001$ vs control for all), while it was adjusted into control levels by the co-administration of Cys with Cd ($P < 0.001$ vs control, $P < 0.05$ vs Cys) or with Ni (non-significant vs control, $P < 0.001$ vs Cys). Moreover, the treatment with Cd or Ni alone was exhibited a significant reduction in brain TAS ($P < 0.001$ and $P < 0.01$ respectively vs control) that was statistically significant reversed near to control by Cys co-administration ($P < 0.05$ vs Cd or Ni); Cys group alone had mild effect on TAS.

Conclusion: The exposure to Cd in vivo causes a more statistically significant decrease in the rat brain TAS and an increase in AChE activity than the exposure to Ni. Both effects can be, significantly reversed into the control levels by Cys co-administration but Cys could be considered more neuroprotective agent against chronic exposure to Cd than Ni regarding the above parameters.

Keywords: Cadmium, Nickel, L-Cysteine, Acetylcholinesterase, Antioxidants

Introduction

Many heavy metals, including cadmium (Cd) and nickel (Ni) are widely distributed, posing occupational and environmental exposure risks which may result in adverse health effects. Exposure to these metals can occur through contact with contaminated soil, air, water, and food, or by absorption through the skin as a result of manufacturing, pharmaceutical, or industrial processes or environmental contamination⁽¹⁾. Exposure to Cd even in low levels exerts its toxic effects not only on kidneys, liver and testis but also on the central nervous system (CNS) due to long biological half-life (15–30 years in man)

and low rate of body excretion⁽²⁾. The acute Cd toxicity may lead to brain intracellular accumulation, cellular dysfunction and lethal cerebral oedema⁽³⁾; moreover, Cd may influence the synaptic neurotransmission in the brain of rats and brain antioxidant status^(4,5).

Ni is also used extensively in many industrial and consumer products such as stainless steel, magnets, coins, and alloys⁽⁶⁾. The one of adverse health effects of Ni is to induce oxidative stress, through the inhibition of antioxidant enzymes and damaging DNA through the inhibition of repair enzymes and deregulate cell proliferation^(7,8).

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L-Cysteine (Cys) is an antioxidant agent that is believed to form a relatively stable chelator-metal complex with metals such as Cd and Ni, assisting in the excretion of the latter and resulting in a decrease of tissue Cd or Ni concentrations⁽⁹⁾.

Regarding neurotransmission enzymes, acetylcholinesterase (AChE) is a very important enzyme for cholinergic neurotransmission with strong indications of non-cholinergic functions (co-released with dopamine from dopaminergic neurones)^(10, 11), and on cell survival⁽¹²⁾. However, areas of higher AChE expression generally correlate with brain regions that degenerate early in Alzheimer's disease and Parkinsonism^(13, 14).

Current study was aimed, firstly, to investigate the effects of subacute Cd or Ni administration (either alone or in combination with Cys) on brain AChE, activity and brain total antioxidant status (TAS) in adult rats, secondly, to evaluate the interaction between Cd or Ni and L-Cys on the above parameters (Bliss independence analysis).

Materials and Methods

Animals.

Thirty six male Sprague-Dawley adult rats (10-12 weeks old, weighing 223 ± 18 g) were used in all experiments. The rats were purchased by National Centre for Drug Research and Quality Control, Baghdad, Iraq and were housed six in a cage, at a constant room temperature ($22 \pm 1^\circ$) under a 12- hr light:dark cycle. Food and water were provided *ad libitum*. Animals were cared for in accordance with the principles for the care, use and protection of experimental animals as the international guidelines of laboratory animals' care and the ethical guidelines for the investigations on experimental animals (International Society for Applied Ethology)⁽¹⁵⁾.

Cadmium, Nickel and L-Cysteine administration.

Rats were divided into six groups (n= 6 at each group), as follows: (A) control (saline-treated- negative control), (B) Cys (7 mg/kg body weight-positive control), (C) Cd (1 mg/kg body weight, as $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$), (D) Ni (13 mg/kg as NiCl_2), (E) Cd+Cys (on the aforementioned doses), and (F) Ni+Cys (on the aforementioned doses). All rats received intraperitoneal daily injections for 7 days. No behavioral or physiological effects were observed over this period of administration.

Tissue preparation.

The animals were sacrificed by decapitation 1 hr after the last injection and their whole brains were rapidly removed (and stored at -70° until use). The tissue was homogenized in 10 volume ice-cold medium containing 50 mM Tris-HCl, pH 7.4, and 300 mM sucrose, using an ice-chilled glass homogenizing vessel at 900 r.p.m. (4–5 strokes). Then, the homogenate sample was centrifuged at $1000 \times g$ for 10 min to remove nuclei and debris^(16, 17). In the resulting supernatant, the protein content was determined according to the method of Lowry *et al.*⁽¹⁸⁾ and then the enzyme activities were measured.

Determination of brain AChE activity.

AChE activity was determined by the hydrolysis of acetylthiocholine. The incubation mixture (1 ml) contained 50 mM Tris-HCl, pH 8, 240 mM sucrose and 120 mM NaCl. The protein concentration of the incubation mixture was 80–100 $\mu\text{g/ml}$. The reaction was initiated after addition of 0.03 ml of 5,5'-dithionitrobenzoic acid (DTNB) and 0.05 ml of acetylthiocholine iodide, which was used as substrate. The final concentrations of DTNB and substrate were 0.125 and 0.5 mM, respectively. The reaction followed spectrophotometrically by the increase of absorbance (ΔOD) at 412 nm⁽¹⁹⁾.

Determination of the brain total antioxidant status (TAS).

TAS was measured in each homogenized rat brain sample spectrophotometrically by a commercially available kit (Randox

Laboratories Ltd., Crumlin, UK; cat. no. NX2332). ABTS (2,2'-azino-di-(3-ethylbenzthiazoline sulfonate)) was incubated with a peroxidase (metmyoglobin) and H_2O_2 in order to produce the radical cation ABTS. The latter had a relatively stable blue-green colour, which was measured at 600 nm⁽¹⁶⁾.

Analysis of data

The data of all groups are expressed as the mean \pm SD and were analysed statistically using One-way analysis of variance (ANOVA) followed by multiple comparisons with Bonferroni's list honest significant difference methods. The significance level for all analyses was set at a $P < 0.05$. All analyses were performed by GraphPad Prism 5.3 for Windows (GraphPad Software, San Diego, CA, USA).

Bliss independence interaction analysis

Bliss independence is described by the equation $I_{IND} = I_A + I_B - I_A \times I_B$ for a certain combination of (x) concentration of drug A and (y) concentration of drug B where I_A : value of AChE or TAS at (x) concentration of drug A alone, I_B : value of AChE or TAS at (y) concentration of drug B alone and I_{IND} : expected value of AChE or TAS of a noninteractive (independent) theoretical combination of (x) concentration of drug A with (y) concentration of drug B. Because, value of AChE or TAS (I) is equals to 1-E. Bliss equation can be transformed to $E_{IND} = E_A \times E_B$ where E_A and E_B are difference in value of AChE or TAS of each drug A and B, respectively.

The difference ($\Delta I = E_{IND} - E_{OBS}$) between the expected values E_{IND} , and the experimentally observed values E_{OBS} , describes the interaction of each combination of the concentrations of these two drugs. If ΔE is > 0 ($E_{OBS} < E_{IND}$), hence, Bliss synergy is concluded for that particular combination. If ΔE is < 0 ($E_{OBS} > E_{IND}$), hence, Bliss antagonism is concluded for that particular combination. In any other case, the conclusion is Bliss independence. For each combination, its

statistical significance was assessed by Student's t test and the interaction was assessed as described above⁽²⁰⁾.

Results

Effects of Cd or Ni on the brain AChE adult rat modulated by Cys

The rat brain AChE activity was significantly increased by Cd, Ni and Cys ($P < 0.001$ vs control), while it was tried to adjust to control levels by the co-administration of Cys with Cd ($P < 0.001$ vs control, $P < 0.05$ vs Cys) or with Ni (non-significant vs control, $P < 0.001$ vs Cys) (Figure 1).

Effects of Cd or Ni on the brain TAS adult rat modulated by Cys

The activity of TAS exhibited significantly decreasing by Cd and Ni ($P < 0.001$ vs control) while not affected by Cys. The co-administration of Cys with Cd or Ni was adjusted TAS levels to control values ($P < 0.001$ with Cd vs Cys only) (Figure 2).

Analysis of antagonistic effect between Cys and Cd or Ni according to Bliss independence analysis

According to the AChE data, the combination of Cys with Cd or Ni exerted little antagonistic effects (-4 to -13%) in the tested concentrations while according to TAS data showed good antagonistic effect (-75 to -92%) (Table 1).

Discussion

Cd and Ni are widely spread environmental contaminants that exerts varied toxicity including neurotoxicity that increases lipid peroxidation provoking oxidative stress⁽²¹⁾; moreover, they may modulate brain cholinergic mechanisms, neural excitability and metabolic energy production. Although, increasing in free radical production after oral administration of Cd was observed and was reversed by concomitant administration of antioxidants^(22, 23). Decreased values of TAS reflect the increase of brain free radical production, whereas increased TAS values show the

decrease of free radical production and the protective antioxidant effect on the brain of the administered substance ^(24, 25), moreover, the increases of AChE activity

provoke enhanced acetylcholine (ACh) hydrolysis and choline reuptake, since brain AChE activity is an important regulator of the behavioral processes ⁽²⁶⁾.

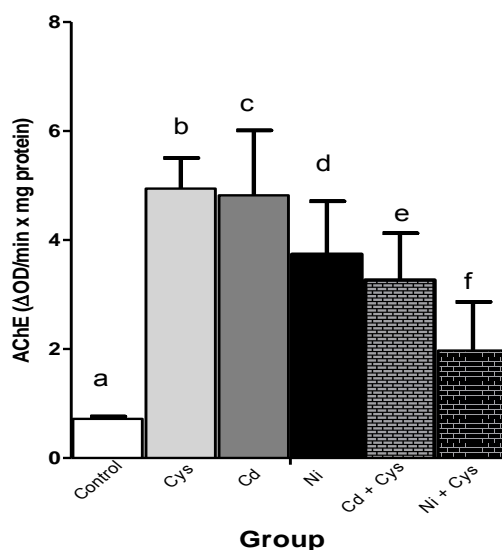


Figure 1. Effects of Cadmium (Cd) and Nickel (Ni) on the brain Acetylcholinesterase (AChE) of adult rats and its modulation by L-cysteine (Cys) coadministration. *Statistical Analysis:* no statistically significant: a/f, b/c, b/d, c/d, d/e, e/f; P<0.05: b/e, d/f; P<0.001: a/b, a/c, a/d, a/e, b/f, c/f. Each value indicates mean ± SD.

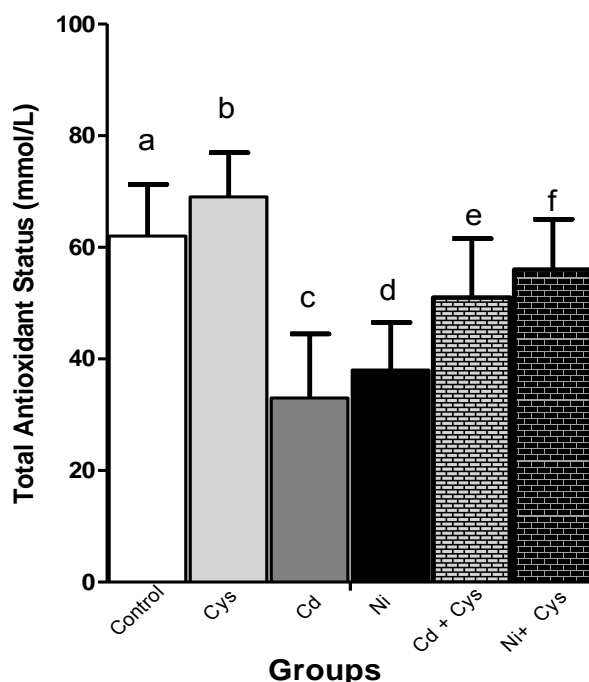


Figure 2. Effects of Cadmium (Cd) and Nickel (Ni) on the brain Total Antioxidant Status (TAS) of adult rats and its modulation by L-cysteine (Cys) coadministration. *Statistical Analysis:* no statistically significant: a/b, a/e, a/f, b/f, c/d, d/e, e/f; P<0.05: b/c, d/f, c/e; P<0.01: a/d, c/f; P<0.001: a/c, b/d, b/e. Each value indicates mean ± SD.

Table 1. Bliss independence interaction analysis of Cysteine with Cadmium or Nickel combination according to acetylcholinesterase (AChE) activity and total antioxidant status (TAS).

Cysteine (7 mg/kg)	Cadmium (1 mg/ kg)			Nickel (13 mg/ kg)		
	E _{OBS}	E _{IND}	ΔE	E _{OBS}	E _{IND}	ΔE
AChE	3.267±0.86	14.078±0.32	-10.811*	1.971±0.89	9.816±0.35	-7.845*
TAS	51±10.45	215±10	-164 [#]	56±8.99	254±12	-198 [#]

*statistically significant p<0.05

#statistically significant p<0.001

Because Cys did not cause a significant increase in the rat brain TAS by itself, the partially corrected Cd or Ni -induced TAS decrease by Cys co-administration could be due to the chelating properties of Cys (assisting to the biological inactivation and/or excretion of Cd)^(27, 9). It should be noted that Cd or Ni brain concentrations have not been related to the extent of lipid peroxidation^(21, 23) or to the oxidative stress biomarkers' alterations observed in certain animal brain regions after exposure to heavy metals, and that such alterations might be reversible⁽⁹⁾. However, Cys (at least under the examined experimental conditions) was not proved sufficiently efficient to neutralize oxidative stress. The co-administration of Cd or Ni and Cys was, on the other hand, insufficiently efficient in order to maintain AChE into the control levels that might be due to Cd or Ni -induced increase of AChE activity has been associated with high brain Cd or Ni accumulation^(22, 28) and L-Cysteine in particular (either alone or before Cd or Ni administration) increased rat brain AChE activity, a fact that is well related to total antioxidant status value increases compared to those induced by heavy metals, these observations could at least in part be explained by the fact that free radical production can decrease brain AChE activity⁽¹⁶⁾. Therefore, Cys is an effective therapy of heavy metals toxicity involves both metal chelating and antioxidant actions that the protective action to membrane damage induced by them and prevent protein damage.

In conclusion, the exposure to Cd in vivo caused a more statistically significant decrease in the rat brain TAS and an increase in AChE activity than the

exposure to Ni. Both effects can be, significantly reversed into the control levels by Cys co-administration but TAS could be considered more precise parameter to investigate the antioxidant effect of Cys against subacute exposure to Cd or Ni regarding the above parameters.

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