Ind J Clin Biochem (Jan-Mar 2016) 31(1):93–98
DOI 10.1007/s12291-015-0503-3

ORIGINAL ARTICLE

Regional Distribution of Copper, Zinc and Iron in Brain of Wistar Rat Model for Non-Wilsonian Brain Copper Toxicosis

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Received: 17 March 2015 / Accepted: 12 April 2015 / Published online: 28 April 2015

Abstract In previous studies, we have reported first in vivo evidence of copper deposition in the choroid plexus, cognitive impairments, astrocytes swelling (Alzheimer type II cells) and astrogliosis (increase in number of astrocytes), and degenerated neurons coupled with significant increase in the hippocampus copper and zinc content in copper-intoxicated Wistar rats. Nonetheless, hippocampus iron levels were not affected by chronic copper-intoxication. Notwithstanding information on distribution of copper, zinc and iron status in different regions of brain due to chronic copper exposure remains fragmentary. In continuation with our previous study, the aim of this study was to investigate the effects of intraperitoneally injected copper lactate (0.15 mg Cu/100 g body weight) daily for 90 days on copper, zinc and iron levels in different regions of the brain using atomic absorption spectrophotometry. Copper-intoxicated group showed significantly increased cortex, cerebellum and striatum copper content (76, 46.8 and 80.7 % increase, respectively) compared to control group. However, non-significant changes were observed for the zinc and iron content in cortex, cerebellum and striatum due to chronic copper exposure. In conclusion, the current study demonstrates that chronic copper toxicity causes differential copper buildup in cortex, cerebellum and striatum region of central nervous system of male Wistar rats; signifying the critical requirement to discretely evaluate the effect of copper neurotoxicity in different brain regions, and ensuing neuropathological and cognitive dysfunctions.

Keywords Copper intoxication · Iron · Zinc · Cognition · Neurodegeneration

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Introduction

Copper ($_{29}Cu^{63.5}$) is the third-most abundant transition metal in the brain. Owing to its redox activity, Cu serves as a cofactor for key metabolic enzymes that mediate various cellular processes, including neurotransmitter biosynthesis (dopamine β hydroxylase), mitochondrial energy generation (cytochrome *c* oxidase), free radical detoxification (Cu/Zn superoxide dismutase) and iron homeostasis (ceruloplasmin) [1, 2]. There is uneven distribution of Cu in different regions of the brain with variations reported in different age groups [3], and species to species [4]. Average neural Cu concentrations are the order of 0.1 mM. [1].

Brain is particularly sensitive to free radical damage and oxidative stress because of the high levels of polyunsaturated lipids that are component of neuronal cell membranes [5]. The abundance of Cu in the brain and its strong redox activity necessitates tight control over Cu homeostasis to evade oxidative injury to the central nervous system (CNS) by uncontrolled Cu-elicited Haber–Weiss and Fenton type reactions. Cells have therefore developed various protection mechanisms in event of drastic increase in Cu concentration. The incorporation of Cu into metallothionein (MT) is an example of cells defense mechanisms to protect cell structure from Cu toxicity and to prevent oxidative damage [6].

Imbalance in Cu, zinc (Zn) and iron (Fe) homeostasis may lead to initiation and/or progression of several

neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis and Huntington's disease [7–10]. Cu, Zn and Fe play paramount roles in neurodegeneration by affecting protein structure (misfolding) and oxidative stress [10]. There is mounting proof that Cu dyshomeostasis in AD patients leads to oxidative stress and neurodegeneration [11] subsequently resulting in memory deficits. Central cholinergic system plays a significant role in learning, memory, and cognition in both animals and humans [12].

Cu content in the brain is subdivided further into tightly bound Cu and labile Cu pools. Labile brain Cu stores have been demonstrated in the soma of cerebellar granular and cortical pyramidal neurons, as well as in neuropil within the cerebral cortex, cerebellum, hippocampus and spinal cord [13]. Fujiwara et al. [14] have shown no significant effect of Cu-intoxication (1 ppm $CuSO_4$ dissolved in drinking water for 6 weeks) on memory functions and Cu levels in different brain regions of Wistar rats [14]. However, Leiva et al. [15] have shown that Cu-intoxicated Wistar rats (1 mg/kg $CuSO_4$ dissolved in saline by intraperitoneal (i.p.) route for 30 days) exhibited 16.71 and 14.24 times greater Cu accumulation than that found in control saline treated animals in the visual cortex and dorsal hippocampus, respectively coupled with suppression of long term potentiation (LTP) but without significantly altering the learning and memory in the Morris water maze (MWM) test [15]. Chronic Cu-intoxication has been shown to induce memory deficits and/or neurotoxicity with increase in Cu content of the brain, albeit, by use of genetically compromised animals [16], diet supplemented with high cholesterol [17], CuO nanoparticles [18] or by bilateral common carotid artery occlusion [19] in experimental animals. However, these studies have not reported Cu, Zn and Fe concentrations in different brain regions of Cu-intoxicated animals. Long Evans cinnamon (LEC) rats, an animal model of Wilson's disease (WD), rarely exhibits neurological abnormalities and increased brain Cu content [14, 20, 21]. Interestingly, Terwel et al. have reported memory deficits with neuroinflammation in 12 month old toxic milk mice, an another animal model of WD and demonstrated that Cu content was increased in striatum, hippocampus and cerebellum but unaltered in cortex [22]; underlining, the critical requirement to evaluate Cu along with Fe and Zn content and ensuing neurological manifestations in these brain regions in other animal models of Cu toxicosis.

Earlier, we have reported a Wistar rat model for non-Wilsonian brain Cu toxicosis; documenting first in vivo evidence of memory deficits in conjunction with Cu deposition in the choroid plexus, decreased serum AChE activity, astrocytes swelling (Alzheimer type II cells) accompanied by astrogliosis (increase in number of astrocytes), degenerated neurons in cerebral cortex, and augmented levels of Cu and Zn in the hippocampus of chronically Cu-intoxicated male Wistar rats. In addition, liver sections of chronic Cu-intoxicated rats showed grade 1 Cu-associated protein and grade 4 Cu deposition by Shikata's orcein stain and rhodanine stain, respectively [23]. Importantly, MT has been confirmed as the biochemical counterpart of orcein positive material [24]. Further, chronic Cu toxicity resulted in massive increase in serum non-ceruloplasmin bound Cu coupled with strong correlation between serum Cu and non-ceruloplasmin bound Cu, and grade 1 hemosiderin deposition in Kupffer cells without altering hepatic and hippocampus Fe levels in male Wistar rats [25].

The widespread distribution of Cu, Zn and Fe prerequisite for normal CNS functioning, coupled with many links between Cu, Zn and Fe dyshomeostasis and neurodegenerative diseases, have impelled curiosity in studying Cu, Zn and Fe content in striatum, hippocampus, cerebellum and cortex in chronic Cu-intoxicated rats. In this study, we have reported the effects of chronic Cu-intoxication on Cu, Zn and Fe levels in different regions of the brain with relation to development of neurodegeneration and neurobehavioral impairments in Wistar rat model for non-Wilsonian brain Cu toxicosis.

Materials and Methods

Chemicals

Copper chloride, Cu, Zn and Fe standard solutions assigned for atomic absorption spectrophotometry (AAS) (Sigma-Aldrich Co., Germany), and lactic acid (Qualigens fine chemicals) were purchased. All the other reagents and chemicals used in this study were of analytical grade.

Animals and Experimental Design

Male Wistar rats in the weight range of 60–80 g were procured from the institute animal house, PGIMER, Chandigarh, India. All the rats were housed in the polypropylene cages (one animal per cage), kept in well ventilated rooms maintained at 22 ± 2 °C. The rats were fed standard rat chow and water ad libitum. Institutional Animal Ethical Committee (IAEC-161) consent was taken and IAEC guidelines were strictly followed for all the animal experimentation. Two groups of male Wistar rats, each consisting of eight animals were used as follows:

Control group intraperitoneal (i.p.) injection of isotonic sodium chloride solution daily for the period of 90 days.

For ethical reasons, group receiving sodium lactate solution was not kept as it has been reported previously that it does not alter any vital biochemical parameters and Cu levels in various tissues in Wistar rats [23, 26].

Cu-intoxication group: i.p. injection of Cu lactate solution (0.15 mg Cu/100 g. B.W.) daily for the period of 90 days [23].

Collection and Preservation of Tissue Samples

All the animals were sacrificed at the end of 99th day of the study under ether anesthesia after completion of neurobehavioral studies [23], and brains were dissected into four discrete regions (cortex, cerebellum, striatum and hippocampus) by the method of Glowinski and Iversen [27]. Brain autopsies samples for Cu, Zn and Fe measurement were cut with clean stainless steel scalpel blades. All the samples were stored at −80 °C till further processing. All the glassware used in Cu, Zn and Fe estimation were soaked in concentrated HCl for 24 h and rinsed several times in double distilled water (DDW) before use.

Measurement of Copper, Zinc and Iron Concentration

The concentrations of Cu, Zn and Fe were measured by a flame (an air-acetylene burner) atomic absorption spectrophotometry (AAS) (AAnalyst 400, Perkin Elmer) [23]. For tissue samples, approximately 10–50 mg of tissue was digested with 1:1 perchloric acid and nitric acid in hot air oven and diluted five times with 10 mM HNO_3 before analysis by AAS. The instrument was calibrated using the Sigma standards of Cu, Zn and Fe. Bovine liver standard reference material SRM1577 obtained from National Bureau of Standard (NBS, Washington, DC) was used as standard reference material for quality control. Each sample was analyzed in triplicate and reported as mean, and the concentration of Cu, Zn and Fe were derived by comparing absorption with the calibration curve. The Cu, Zn and Fe estimations were carried out in a blinded experiment.

Statistical Analysis

All values were expressed as the mean ± standard error of the mean (SEM) of eight animals in each group. Unpaired Student's *t* test and Mann–Whitney rank sum test were used for analysis of the data, and values with $p < 0.05$ were considered statistically significant. All the calculations were carried out by the SigmaStat computer software program.

Results

Effects of Chronic Copper-Intoxication on Cortex, Cerebellum and Striatum Copper Content

Tissue samples obtained from different regions of brain were digested as described earlier and read at 324.75 nm by AAS for quantification of Cu in the tissues. AAS studies demonstrated significant increase in the cortex, cerebellum and striatum Cu content in Cu-intoxicated group compared to control group ($p < 0.01$) (Table 1). Previously, we have reported around 73 % increase in hippocampus Cu content of Cu-intoxicated animals compared to control animals [23]. Interestingly, brain Cu was affected differently in different brain regions. Cu-intoxicated group showed significant increase in cortex, cerebellum and striatum Cu content (76, 46.8 and 80.7 % increase, respectively) compared to control group (Table 1). Maximum increase in Cu content upon chronic Cu-intoxication was found in the striatum followed by cortex and hippocampus, and least in cerebellum.

Effects of Chronic Copper-Intoxication on Cortex, Cerebellum and Striatum Zinc Content

Previously, we have reported around 77.1 % increase in hippocampus Zn content of Cu-intoxicated animals compared to control animals [23]. In contrast, cerebral cortex, cerebellum and striatum Zn content were comparable between control and Cu-intoxicated group ($p > 0.05$) (Table 1).

Effects of Chronic Copper-Intoxication on Cortex, Cerebellum and Striatum Iron Content

AAS studies demonstrated non-significant changes in the cortex, cerebellum and striatum Fe content in Cu-intoxicated group compared to control group ($p > 0.05$) (Table 1). Similarly, we have documented comparable hippocampus Fe content between Cu-intoxicated and control animals [25].

Discussion

The present study documents biochemical basis of chronic Cu-intoxication elicited neurodegeneration and cognitive waning fraternized with significant increase in cortex, cerebellum and striatum Cu content (Table 1) in Wistar rat model for non-Wilsonian brain Cu toxicosis. The precise mechanisms of Cu induced neurotoxicity remains equivocal. Astrocytes has been proposed as the Cu depots of the brain [28] and their involvement in learning and memory consolidation is gaining momentum [29]. Keeping in consideration the pivotal role of astrocytes in many aspects of brain homeostasis, including Cu homeostasis, glutamate and K^+ ion uptake, spatial buffering, water excretion from brain and defense against oxidative stress; astrocytes has been hypothesized to play a key role in the biochemical and cellular pathology of several neurodegenerative

Table 1 Metal content in different brain regions of rats exposed to chronic Cu toxicity (90 days)

Brain region	Copper content (μg Cu/g of wet tissue weight)		Zinc content (μg Zn/g of wet tissue weight)		Iron content (μg Fe/g of wet tissue weight)	
	Control	Cu-intoxicated	Control	Cu-intoxicated	Control	Cu-intoxicated
Cortex	2.2 ± 0.06	9.19 ± 0.54**	23.3 ± 0.91	21.97 ± 1.58[#]	24.87 ± 0.11	24.23 ± 0.12[#]
Striatum	2.18 ± 0.07	11.31 ± 0.67**	25.34 ± 0.67	25.11 ± 1.3[#]	25.14 ± 0.07	25.31 ± 0.12[#]
Cerebellum	2.69 ± 0.08	5.07 ± 0.11**	32.93 ± 0.12	34.17 ± 2.6[#]	30.13 ± 0.16	30.58 ± 0.19[#]

Values are expressed as mean ± SEM (n = 8/group)

** $p < 0.01$; statistical significance with respect to controls

[#] Not significant

diseases [28]. Cu-intoxication has been shown to increase Cu content of visual cortex and dorsal hippocampus in Wistar rats along with LTP suppression [15]. Terwel et al. demonstrated significant Cu accumulation in the hippocampus, striatum and cerebellum of 12 month old toxic milk mice with differential inflammatory response in the different regions of brain depending on the extent of Cu accumulation. Remarkably, inflammatory changes seemed to be restricted to microglial cells in the striatum and to astrocytes in the hippocampus. Under certain conditions, astrogliosis can be more prominent than microgliosis supported by the fact that astrocytes behave as a syncytium [22]. In animal models of AD, astrogliosis seemed to be more prevalent compared to microgliosis. As astrocytes assist neuronal metabolism, astrocytes are expected to respond to altered neuronal functioning. For example in tauopathy models, predominantly neuronal functioning is affected but astrogliosis is more apparent and microgliosis is almost undetectable [22, 30].

The previously documented histopathological changes in the cerebral cortex like degenerated neurons showing pyknotic nuclei and dense eosinophilic cytoplasm, swelling of astrocytes (Alzheimer type II cells) accompanied by astrogliosis in the cerebral cortex of Cu-intoxicated animals reported in our previous study [23] can be explained on the basis of increased accumulation of Cu in the cortex. Nevertheless, no obvious neuronal loss in the striatum, hippocampus and cerebellum was observed, which suggests a more subtle type of damage in these brain regions despite Cu accumulation or astrogliosis and microgliosis may be sub threshold in these brain regions. Motor behavior and memory in the Cu-intoxicated group were compromised in accordance with Cu deposition found in the striatum and hippocampus, respectively. It is quite possible that increased Cu deposition in the hippocampus impairs synaptic transmission accounting for the compromised capability to acquire a spatial memory task in MWM test. This augurs well with the observation that chronic Cu loading in rats or mice impairs LTP in the hippocampus [31]. However, unlike choroid plexus which stained positively with rhodanine stain, the other brain regions failed to show Cu deposition by rhodanine stain [23], despite increased Cu build up in cortex, hippocampus, striatum and cerebellum. This observation can be explained by the fact that rhodanine stain did not stain positively with tissue sections having Cu concentrations below 0.9 mmol/kg (≈57 μg/g dry weight) [32] and reported conversion factor for calculating wet weight to dry weight of tissues is 3.18 [33]. In addition, no amyloid deposition was seen in any brain region despite the fact that amyloid precursor protein (APP) is a Cu binding protein.

Cu, Zn and Fe are indispensable trace elements responsible for functioning of several enzymes and proteins; however, excessive intracellular accumulation of these elements results in cytotoxicity. In the brain, Zn in its ionic form (Zn^{2+}) binds to metalloproteins and is highly enriched at many but not all glutamatergic nerve terminals. Zn^{2+} is synaptically released during neuronal activity. In particular, oxidative stress causes intracellular mobilization of Zn^{2+}. The mobilization of intracellular Zn^{2+} under oxidative stress conditions may provide the relationship between apoptotic and necrotic pathways. Zn and Cu homeostasis are closely regulated and are vital for brain physiology. Dysregulated metal homeostasis occurs in several neurodegenerative disorders. Increased content of Cu and Zn are found in amyloid plaques from AD brains, and in the cerebrospinal fluid (CSF) of AD and PD patients. Both increased and decreased Zn levels are found in hippocampal, CSF and serum of AD patients compared to controls [9, 10]. Zn^{2+} is a potent neurotoxin participating in variety of conditions involved in excitotoxicity, including epilepsy, ischemia, and brain trauma. Zn^{2+} cation also promotes neuronal as well as glial death in vitro and in vivo. Comprehensive in vivo studies have demonstrated transsynaptic movement of Zn^{2+} plays a role in neuronal cell death linked to transient global ischemia. Further,

Zn^{2+} can play a key role in the development/or progression of AD. Zn^{2+} is an important component of amyloid plaques, and recent reports have suggested that brain Zn^{2+} dyshomeostasis causes synaptic discrepancies and cognitive deficits as observed in AD [34].

Fe is a redox active metal found in two main oxidation states, Fe^{2+} and Fe^{3+}. Despite the vast research to understand the brain Fe homeostasis, however it is still ambiguous how the brain regulates homeostasis, storage and flux of Fe into astrocytes, neurons, oligodendroglia and glial cells [9, 10]. Like Cu and Zn, Fe content vary in different regions of brain with highest Fe concentrations usually found in the substantia nigra and globus pallidus. These high brain Fe concentrations can be attributed predominantly to the quick rate of oxidative metabolism necessary to maintain synaptic transmission, axonal transport, and ionic membrane gradients [10]. Cu and Fe content in the brain seems to increase with advancing age. AD and PD patients have higher Fe levels than those expected by normal aging but the exact physiological and molecular mechanisms fraternized with such Fe accumulation are still obscure. Cu, Zn and Fe dyshomeostasis mechanisms is not simply associated with increased or toxicological exposure to these metals, but rather it is determined by breakdown of metal homeostasis mechanisms [9]. Discrepancy of brain Fe homeostasis regulation can also be caused due to disrupted expression of brain Fe metabolism proteins induced by non-genetic factors. These currently unknown factors might unsettle normal control mechanisms of protein expression, and lead to brain Fe dyshomeostasis, and then induce oxidative stress and neuronal cell death in few neurodegenerative diseases. Brain Fe buildup occurs slowly over time with concomitant increases in ferritin levels. Augmented brain Fe content may induce neuronal cell damage even after it has become bound to ferritin. The possible explanation for this Fe induced neuronal cell damage may be because of the fact that Fe can be released in its ferrous form under the acidic conditions present in extracellular fluid and through interaction with components viz. excess ascorbate and superoxide radicals. Excess Fe load leads to huge increase in the chelatable free Fe pool which is too large to be sequestered by ferritin within cells. Fe toxicity is mediated by Fenton chemistry, which affects the mitochondrial inner membrane respiratory complexes [9].

Conclusions

In conclusion, in the present study it was found that Cu content in striatum, cortex and cerebellum increased significantly due to chronic Cu-intoxication in Wistar rat model for non-Wilsonian brain Cu toxicosis. However, more studies on neuroinflammatory markers and molecular mechanisms for neuronal apoptosis due to buildup of Cu in different regions of brain together with interaction between neurons, astrocytes, microglia, oligodendroglia and endothelial cells in the CNS Cu homeostasis are warranted to elucidate exact mechanisms of Cu induced cognitive deficits and neurodegeneration.

Acknowledgments The authors acknowledge the financial assistance provided by Indian Council of medical Research (I.C.M.R., New Delhi) as JRF to Mr. Amit Pal [3/1/3/JRF-2009/MPD-13 (11279)]. Special thanks to Dr. J. K. Pradhan of Jaypee University, Solan for providing facilities and guidance to carry out tissue Cu, Zn and Fe estimations for this study. Authors also acknowledge the support of Mr. Rakesh Mohindra for statistics.

Conflict of interest The authors report no declarations of interest.

Ethical standard Institutional Animal Ethical Committee of P.G.I.M.E.R., Chandigarh (IAEC no. 161) consent was taken and IAEC guidelines were strictly followed for all the animal experimentation.

References

1. Que EL, Domaille DW, Chang CJ. Metals in neurobiology: probing their chemistry and biology with molecular imaging. Chem Rev. 2008;108(5):1517–49. doi:10.1021/cr078203u.
2. Tapiero H, Townsend DM, Tew KD. Trace elements in human physiology and pathology. Copper. Biomed Pharmacother. 2003;57(9):386–98.
3. Prohaska JR, Wells WW. Copper deficiency in the developing rat brain: a possible model for Menkes' steely-hair disease. J Neurochem. 1974;23(1):91–8.
4. Lutsenko S, Bhattacharjee A. Hubbard AL Copper handling machinery of the brain. Metallomics. 2010;2(9):596–608. doi:10.1039/c0mt00006j.
5. Ozcelik D, Uzun H. Copper intoxication; antioxidant defenses and oxidative damage in rat brain. Biol Trace Elem Res. 2009;127(1):45–52. doi:10.1007/s12011-008-8219-3.
6. Sturniolo GC, Mestriner C, Irato P, Albergoni V, Longo G, D'Inca R. Zinc therapy increases duodenal concentrations of metallothionein and iron in Wilson's disease patients. Am J Gastroenterol. 1999;94(2):334–8. doi:10.1111/j.1572-0241.1999.851_w.x.
7. Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem. 2010;345(1–2):91–104. doi:10.1007/s11010-010-0563-x.
8. Zheng W, Monnot AD. Regulation of brain iron and copper homeostasis by brain barrier systems: implication in neurodegenerative diseases. Pharmacol Ther. 2012;133(2):177–88. doi:10.1016/j.pharmthera.2011.10.006.
9. Kozlowski H, Janicka-Klos A, Brasun J, Gaggelli E, Valensin D, Valensin G. Copper, iron, and zinc ions homeostasis and their role in neurodegenerative disorders (metal uptake, transport, distribution and regulation). Coord Chem Rev. 2009;253(21–22):2665–85. doi:10.1016/j.ccr.2009.05.011.
10. Kozlowski H, Luczkowski M, Remelli M, Valensin D, Valensin D. Copper, zinc and iron in neurodegenerative diseases (Alzheimer's, Parkinson's and prion diseases). Coord Chem Rev. 2012;256(19-20):2129–41. doi:10.1016/j.ccr.2012.03.013.
11. Rossi L, Squitti R, Calabrese L, Rotilio G, Rossini PM. Alteration of peripheral markers of copper homeostasis in Alzheimer's

disease patients: implications in aetiology and therapy. J Nutr Health Aging. 2007;11(5):408–17.
12. Overstreet DH. Behavioural plasticity and the cholinergic system. Prog Neuropsychopharmacol Biol Psychiatr. 1984;8(1):133–51.
13. Palm R, Wahlstrom G. Hallmans G Age related changes in weight and the concentrations of zinc and copper in the brain of the adult rat. Lab Anim. 1990;24(3):240–5.
14. Fujiwara N, Iso H, Kitanaka N, Kitanaka J, Eguchi H, Ookawara T, Ozawa K, Shimoda S, Yoshihara D, Takemura M, Suzuki K. Effects of copper metabolism on neurological functions in Wistar and Wilson's disease model rats. Biochem Biophys Res Commun. 2006;349(3):1079–86. doi:10.1016/j.bbrc.2006.08.139.
15. Leiva J, Palestini M, Infante C, Goldschmidt A, Motles E. Copper suppresses hippocampus LTP in the rat, but does not alter learning or memory in the morris water maze. Brain Res. 2009;1256:69–75. doi:10.1016/j.brainres.2008.12.041.
16. Sparks DL, Schreurs BG. Trace amounts of copper in water induce beta-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. Proc Natl Acad Sci USA. 2003;100(19):11065–9. doi:10.1073/pnas.1832769100.
17. Lu J, Zheng YL, Wu DM, Sun DX, Shan Q, Fan SH. Trace amounts of copper induce neurotoxicity in the cholesterol-fed mice through apoptosis. FEBS Lett. 2006;580(28–29):6730–40. doi:10.1016/j.febslet.2006.10.072.
18. An L, Liu S, Yang Z, Zhang T. Cognitive impairment in rats induced by nano-CuO and its possible mechanisms. Toxicol Lett. 2012;213(2):220–7. doi:10.1016/j.toxlet.2012.07.007.
19. Mao X, Ye J, Zhou S, Pi R, Dou J, Zang L, Chen X, Chao X, Li W, Liu M, Liu P. The effects of chronic copper exposure on the amyloid protein metabolisim associated genes' expression in chronic cerebral hypoperfused rats. Neurosci Lett. 2012;518(1):14–8. doi:10.1016/j.neulet.2012.04.030.
20. Vonk WI, Wijmenga C, van de Sluis B. Relevance of animal models for understanding mammalian copper homeostasis. Am J Clin Nutr. 2008;88(3):840S–5S.
21. Wu J, Forbes JR, Chen HS, Cox DW. The LEC rat has a deletion in the copper transporting ATPase gene homologous to the Wilson disease gene. Nat Genet. 1994;7(4):541–5. doi:10.1038/ng0894-541.
22. Terwel D, Loschmann YN, Schmidt HH, Scholer HR, Cantz T, Heneka MT. Neuroinflammatory and behavioural changes in the Atp7B mutant mouse model of Wilson's disease. J Neurochem. 2011;118(1):105–12. doi:10.1111/j.1471-4159.2011.07278.x.
23. Pal A, Badyal RK, Vasishta RK, Attri SV, Thapa BR, Prasad R. biochemical, histological, and memory impairment effects of chronic copper toxicity: a model for non-wilsonian brain copper toxicosis in wistar rat. Biol Trace Elem Res. 2013;153(1–3):257–68. doi:10.1007/s12011-013-9665-0.
24. Evans J, Newman SP, Sherlock S. Observations on copper associated protein in childhood liver disease. Gut. 1980;21(11):970–6.
25. Pal A, Kumar Vasishta R, Prasad R. Hepatic and hippocampus iron status is not altered in response to increased serum ceruloplasmin and serum "Free" copper in Wistar rat model for non-Wilsonian brain copper toxicosis. Biol Trace Elem Res. 2013;154(3):403–11. doi:10.1007/s12011-013-9753-1.
26. Narasaki M. Laboratory and histological similarities between Wilson's disease and rats with copper toxicity. Acta Med Okayama. 1980;34(2):81–90.
27. Glowinski J, Iversen LL. Regional studies of catecholamines in the rat brain. I. The disposition of [3H]norepinephrine, [3H]-dopamine and [3H]dopa in various regions of the brain. J Neurochem. 1966;13(8):655–69.
28. Tiffany-Castiglioni E, Hong S, Qian Y. Copper handling by astrocytes: insights into neurodegenerative diseases. Int J Dev Neurosci. 2011;29(8):811–8. doi:10.1016/j.ijdevneu.2011.09.004.
29. Gibbs ME, Hutchinson D, Hertz L. Astrocytic involvement in learning and memory consolidation. Neurosci Biobehav Rev. 2008;32(5):927–44. doi:10.1016/j.neubiorev.2008.02.001.
30. Schindowski K, Bretteville A, Leroy K, Begard S, Brion JP, Hamdane M, Buee L. Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional synapses in a novel mutated tau transgenic mouse without any motor deficits. Am J Pathol. 2006;169(2):599–616. doi:10.2353/ajpath.2006.060002.
31. Goldschmith A, Infante C, Leiva J, Motles E, Palestini M. Interference of chronically ingested copper in long-term potentiation (LTP) of rat hippocampus. Brain Res. 2005;1056(2):176–82. doi:10.1016/j.brainres.2005.07.030.
32. Jain S, Scheuer PJ, Archer B, Newman SP, Sherlock S. Histological demonstration of copper and copper-associated protein in chronic liver diseases. J Clin Pathol. 1978;31(8):784–90.
33. Zatta P, Frank A. Copper deficiency and neurological disorders in man and animals. Brain Res Rev. 2007;54(1):19–33. doi:10.1016/j.brainresrev.2006.10.001.
34. Sensi SL, Paoletti P, Koh JY, Aizenman E, Bush AI, Hershfinkel M. The neurophysiology and pathology of brain zinc. J Neurosci. 2011;31(45):16076–85. doi:10.1523/JNEUROSCI.3454-11.2011.