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CATALASE ACTIVITY AND MALONDIALDEHYDE CONCENTRATION IN THE BRAIN TISSUE OF RATS TREATED WITH CARBON TETRACHLORIDE

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Carbon tetrachloride (CCl_4) is a potent oxidative agent, used in animal models for the induction of liver and neuronal damage. In this study, we tracked the changes in the concentration of malondialdehyde (MDA) and the activity of catalase (CAT) in the brain tissue of Wistar rats exposed to CCl_4. The animals were divided into two groups of six rats each. The control group was treated with vehicle olive oil (10 ml/kg) and the experimental group included CCl_4-treated animals (1 ml/kg). The level of oxidative stress was determined in a 10% homogenate of whole encephalitic mass (WEM). The levels of MDA in the experimental group were significantly increased ($p = 0.0009$), while CAT activity was significantly decreased ($p = 0.0143$) in the CCl_4-treated group compared to the control group. The results confirmed the theory about the CCl_4-induced oxidative damage on the brain tissue in rats and may be a basis for further research related to potentially protective substances in this animal model.

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Key words: *carbon tetrachloride, brain, oxidative stress, malondialdehyde, catalase*

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Introduction

The oxygen is essential for regular brain activity, hence the human brain uses around 20% of its total basal consumption (1-3). However, the harmful effects of oxygen are also described, throughout the term of oxidative stress (4). Brain, and especially neurons, appear to be particularly vulnerable to the effects of oxidative and nitrosative stress. Free radicals are components of the processes of neuroinflammation and neurodegeneration, hence they are involved in the pathogenesis of Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Huntington's disease and multiple sclerosis (4). Under physiological conditions, there is a balance between oxygen products generated by mitochondria – free radicals and their elimination, which is the function of the antioxidant system (5, 6). Under pathological conditions, there is a decrease in adenosine triphosphate (ATP) synthesis and antioxidants and increasing of free radicals, such as reactive oxygen and nitrogen species (ROS and RNS), which results in oxidative stress (7). Oxidative stress is a consequence of the alteration in the balance between oxygen products called pro-oxidants (ROS and RNS) and cellular antioxidant substances, in favor of the former, which can lead to potential damage (5, 6). Antioxidant capacities can be endogenous or exogenous. Endogenous antioxidant system is determined by enzymatic and non-enzymatic defenses, precursors and cofactors of antioxidants (8). Enzymatic antioxidant system includes following enzymes: superoxide dismutase (SOD), catalase, (5, 6), glutathione-S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), NADH-dehydrogenase and NADPH-dehydrogenase (6) and peroxiredoxins (8).

One of the potential inductors of oxidative stress is exogenous substance carbon tetrachloride (CCl_4), which is a noninflammable, colorless liquid with an extreme destructive capacity of damaging cells. (9). This industrial solvent is a potent hepatotoxic agent, mostly used to induce hepatotoxic effects in animals, for the purposes of experiment (6), but it also has systemic effects, including brain damage (10), as well as kidneys, lungs, muscles, testis damage (11-14), through the action of free radicals (10). The toxicity of CCl_4 is mostly related to

its lipophilicity, which mostly leads to distribution and deposition in the liver and brain (15, 16). Toxic effects of CCl_4 involves lipid peroxidation, mediated by the free radicals that are generated during its metabolism by cytochrome P450 (16). The most important products of CCl_4 are trichloromethyl (CCl_3) and trichloromethyl peroxy ($OOCCl_3$) radicals (17). Acute toxic doses of CCl_4 are responsible for hepatocellular necrosis and degeneration, but hepatic failure is not so frequent when the liver capacities for regeneration are preserved (18). Nevertheless, CCl_4-induced oxidative damage is currently an approved experimental animal model for central nervous system damage, because CCl_4-induced hepatic dysfunction may lead to neurotoxicity as well, mainly via oxidative stress (10). When it comes to the brain, this tissue is more sensitive to the harmful effects of CCl_4, because of the following facts: being rich in polyunsaturated fatty acids (PUFA), more oxygen utilization and low amount of antioxidants combined with high levels of non-hem iron (6).

While there are numerous known facts about hepatotoxic effects of CCl_4, research about its influence on the brain is incomplete.

The aim

Our study aimed to determine the extent of changes in levels of malondialdehyde (MDA) and activity of catalase (CAT) in the brain tissue of rats, after administration of CCl_4.

Materials and methods

Chemicals

Chemicals were of analytical grade and were purchased from Sigma–Aldrich, Sr. Louis, USA. Drug solutions were made on the day of the experiment.

Animals and housing

Experiments were performed using male Wistar rats, weighing 250-300 g. They were maintained in plastic cages in groups of 6, at 22 ± 2 ^{O}C, relative humidity 60%, with 12/12 h light/dark cycle and had free access to food and water. Animals were obtained from the Vivarium of the Institute of Biomedical Research, Faculty of Medicine, University of Niš, Serbia. Experimental procedures were conducted following the declaration of Helsinki and European Community guidelines for the ethical handling of laboratory animals (EU Directive of 2010; 2010/63/ EU) and with the consent of the National Ethics Committee. All efforts were made to reduce the number of laboratory animals used and to minimize the pain or distress encountered by animals in this experiment.

Experimental design

All the animals were separated into two equal groups, each consisting of 6 rats. The control group comprised animals that were administered only with vehicle (olive oil) in a dose of 10 ml/kg, while the experimental group included CCl_4-treated animals. Dose of 1 ml/kg CCl_4 was given to rats via intraperitoneal injection, 24 h before sacrificing. During the experiment, the animals were sacrificed by an overdose of ketamine, and the whole encephalitic mass - WEM (composed of the removed brain tissue without the cerebellum and brainstem) and cerebellar tissue were dissected, washed in PBS, stored at -80 ^{O}C until 10% homogenates were prepared for later biochemical analysis.

Determination of malondialdehyde (MDA) concentrations

Malondialdehyde (MDA) is a prooxidant marker and one of lipid peroxidation end products (20). Concentrations of MDA were measured to determine the extent of lipid peroxidation in WEM, by using a thiobarbituric acid reactive substance (TBARS) and measuring the absorbance spectrophotometrically (21). This method is based on the reaction between thiobarbituric acid and MDA under increased heat and under low pH. The absorbance of the reaction mixture was recorded at 532 nm and values were expressed in nmol/mg proteins.

Determination of catalase (CAT) activity

Activity of antioxidant marker – catalase (20) was determined by the method which Goth with coworkers previously described (22). This method includes the formation of a yellow complex between ammonium molybdate and remaining H_2O_2. Results were obtained by spectrophotometric measuring and the absorbance was determined at 405 nm and the enzyme activity was expressed as U/g of WEM tissue proteins.

Protein estimation

Protein content was measured and determined by the Lowry's method, using bovine serum albumin standard curve (19).

Statistical analysis

Calculated results are presented as mean values ± SD. Data comparison was done by applying Students t-test and the difference was considered significant when $p < 0.05$. Statistical calculations were conducted using the Statistical Package for Social Sciences (SPSS) version 13.0.

Results

In our study, we examined values of CCl_4–induced oxidative stress, by measuring the values of its markers MDA, as the end product of lipid peroxidation and CAT, as the antioxidant enzyme. Levels of MDA ($p = 0.0009$) were significantly increased, as well as the activity of CAT ($p = 0.0143$) (Figures 1, 2).

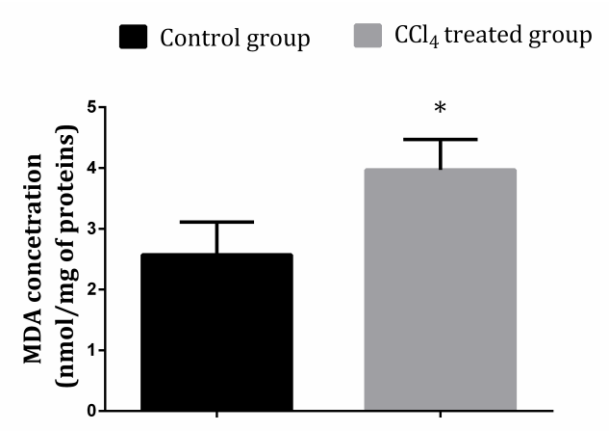

Figure 1. MDA concentration in rats' WEM, after exposure to CCl_4, in the experimental group, compared with the control group. Values are expressed in terms of nmol/mg protein, as mean ± SD (n = 6). The significance of differences among groups is determined by Student's t-test. * p = 0.0009 compared to the control group of rats.

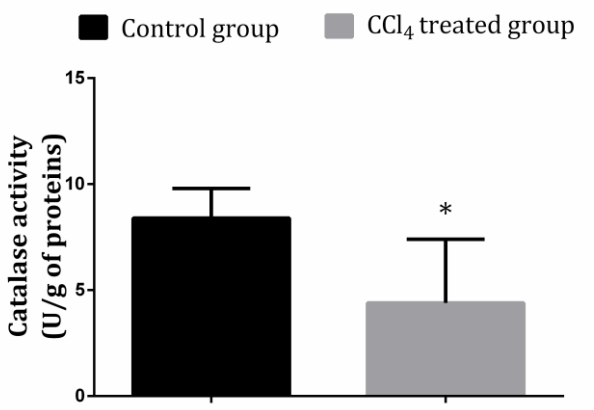

Figure 2. Catalase activity in rats' WEM, after exposure to CCl_4, in the experimental group, compared with the control group. Values are expressed in terms of U/g protein, as mean ± SD (n = 6). The significance of differences among groups is determined by Student's t-test * p = 0.0143 compared to the control group of rats.

Discussion

Our research has shown that neurotoxicity induced by CCl_4 is a result of combined two effects: oxidative stress generated by free radicals of CCl_4 and decreased activity of antioxidant defenses (6). In general, the most important free radicals are ROS and RNS and the antioxidant system includes superoxide dismutase (SOD), CAT, (5, 6) reduced glutathione (GSH) and glutathione peroxidase (GPx) (6). Reactive oxygen species are important in normal metabolism, although, in excess, they are known to induce apoptosis, cellular injury or necrosis (2, 7).

Free radical CCl_3 is the leading factor of lipid peroxidation in different cells/organelles membranes. Hence, measuring markers of lipid peroxidation (MDA in this case) point at the consequences of the harmful effects of CCl_4 radicals (6). That is the reason for being a marker of lipid peroxidation in tissues and the signal for the existing oxidative stress. It is also responsible for cellular changes, such as dehydration, deformity or even cell death (9). One study on mice also showed elevated levels of MDA in the brain (23). Increased level of oxidative stress in the hippocampus of mice treated with CCl_4 can be explained by activation of one type of cytochrome P450 – CYP2E1 (18).

Catalase is an antioxidant enzyme located in peroxisomes, but minor concentration is found in the rest of the cell, precisely in cytoplasm and mitochondria. This enzyme performs the conversion of hydrogen peroxide (H_2O_2) into oxygen and water and cofactor of this process may be iron or manganese. Its role is most important when the levels of H_2O_2

are higher, while when the levels are lower, their removal is mainly related to the activity of peroxiredoxins (PRX) (8). A couple of previous research articles showed the same, statistically significant reduction in CAT activity and an increase of MDA level in the brain tissue of rats exposed to CCl_4 (14, 24-26). The increased MDA level in the brain suggests enhanced peroxidation leading to tissue damage and failure of the catalase, as an antioxidant enzyme, to prevent the excessive production of free radicals.

Despite the fact that more studies describe significant hepatotoxicity of CCl_4, some researches, such as the one by Ritesh and co-workers (6) showed that neurotoxicity, induced by CCl_4 is even more manifested, than hepatotoxicity caused by the same single dose. The reason for this may lie in richer lipid content of the brain tissue which represents a better surrounding for lipid peroxidation. This especially applies to white matter, because it contains lower PUFA in its myelin sheath, than is the case with grey matter (6). Additionally, there have been some explanations about decreased activities of antioxidants that suggest that the enzymes are inactivated by lipid peroxides, which leads to a deficiency of antioxidant defenses and further tissue damage. However, this process is also happening in reverse, because decreased activities of antioxidant enzymes, such as CAT and SOD may lead to superoxides' overproduction within the cell, which then causes even more lipid peroxidation. The high sensitivity of the brain can also be described by a higher level of metabolism and a naturally lower level of antioxidants' concentrations. This specifically is referred to CAT activity and GSH concentrations (9).

Conclusion

Our results showed a significant elevation of oxidant forces and a decrease in antioxidant defenses which confirms the suggested theory related to the toxic effects of CCl_4 and a generation of free oxygen radicals that could cause damage to the brain tissue in rats. This knowledge may be a basis for some future research about potentially protective effect of different molecules, not only against the action of CCl_4 but also towards some other harmful factors with similar consequences on brain tissue.

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References

1. Weickenmeier J, Kurt M, Ozkaya E, de Rooij R, Ovaert TC, Ehman RL, et al. Brain stiffens post mortem. J Mech Behav Biomed Mater 2018 Aug 1;84:88-98. [CrossRef] [PubMed]
2. Chen JJ. Functional MRI of brain physiology in aging and neurodegenerative diseases. Neuroimage 2019 Feb 15;187:209-25. [CrossRef] [PubMed]
3. Goyal MS, Vlassenko AG, Blazey TM, Su Y, Couture LE, Durbin TJ, et al. Loss of brain aerobic glycolysis in normal human aging. Cell Metab 2017 Aug 1;26(2): 353-60. [CrossRef] [PubMed]
4. Steinert R. J, Chernova T, Forsythe D. I. Nitric oxide signaling in brain, function, dysfunction, and dementia. Neuroscientist 2010;16(4):435-52. [CrossRef] [PubMed]
5. Etienne I, Magalhães LV, Cardoso SA, de Freitas RB, de Oliveira GP, Palotás A, et al. Oxidative stress markers in cognitively intact patients with diabetic neuropathy. Brain Res Bull 2019 Jun 5. [CrossRef] [PubMed]
6. Ritesh KR, Suganya A, Dileepkumar HV, Rajashekar Y, Shivanandappa T. A single acute hepatotoxic dose of CCl_4 causes oxidative stress in the rat brain. Toxicol Rep 2015 Jan 1;2:891-5. [CrossRef] [PubMed]
7. Coimbra-Costa D, Alva N, Duran M, Carbonell T, Rama R. Oxidative stress and apoptosis after acute respiratory hypoxia and reoxygenation in rat brain. Redox Biol 2017 Aug 1;12:216-25. [CrossRef] [PubMed]
8. Kim H. G, Kim E. J, Rhie S. J, Yoon S. The role of oxidative stress in neurodegenerative diseases. Exp Neurobiol 2015;15:325-8. [CrossRef]
9. M Al-Olayan E, F El-Khadragy M, A Omer S, TM Shata M, B Kassab R, E Abdel Moneim A. The beneficial effect of cape gooseberry juice on carbon tetrachloride-induced neuronal damage. CNS Neurol Disord Drug Targets 2016 Apr 1;15(3):344-50. [CrossRef] [PubMed]
10. Naseem M, Parvez S. Hesperidin restores experimentally induced neurotoxicity in Wistar rats. Toxicol Mech Methods 2014 Oct 1;24(7):512-9. [CrossRef] [PubMed]
11. Suzuki K, Nakagawa K, Yamamoto T, Miyazawa T, Kimura F, Kamei M, et al. Carbon tetrachloride-induced hepatic and renal damages in rat: inhibitory effects of cacao polyphenol. Biosci Biotechnol Biochem 2015 Oct 3;79(10):1669-75. [CrossRef] [PubMed]
12. Ganie SA, Haq E, Hamid A, Qurishi Y, Mahmood Z, Zargar BA, et al. Carbon tetrachloride induced kidney and lung tissue damages and antioxidant activities of the aqueous rhizome extract of Podophyllum hexandrum. BMC Complement Altern Med 2011 Dec;11(1): 17. [CrossRef] [PubMed]
13. Lilić LM, Toskić D, Stefanović RŽ, Mekić BB, Ilić IR, Stojanović NM. The role of mast cells in carbon tetrachloride induced rat skeletal muscle tissue damage. Acta med Median 2019;58(2):11-5. [CrossRef]
14. Elsawy H, Badr GM, Sedky A, Abdallah BM, Alzahrani AM, Abdel-Moneim AM. Rutin ameliorates carbon tetrachloride (CCl_4)-induced hepatorenal toxicity and hypogonadism in male rats. PeerJ. 2019 May 29;7: e7011. [CrossRef] [PubMed]
15. Coballase-Urrutia E, Cárdenas-Rodríguez N, González-García MC, Núñez-Ramírez E, Floriano-Sánchez E, González-Trujano ME, et al. Biochemical and molecular modulation of CCl4-induced peripheral and central damage by Tilia americana var. mexicana extracts. Saudi Pharm J 2017 Mar 1;25(3):319-31. [CrossRef] [PubMed]
16. Ismail A, Salem A, Eassawy M. Modulation of gamma-irradiation and carbon tetrachloride induced oxidative stress in the brain of female rats by flaxseed oil. J Photochem Photobiol B Biol 2016;9:91-9. [CrossRef] [PubMed]
17. Zaib S, Rashid Khan M. Protective effect of *Cucurbita pepo* fruit peel against CCl_4 induced neurotoxicity in rat. Pak J Pharm Sci 2014;27(6):1967-73. [PubMed]
18. Altinoz E, Erdemli ME, Gul M, Aksungur Z, Gul S, Bag HG, et al. Neuroprotection against CCl_4 induced brain damage with crocin in Wistar rats. Biotech Histochem 2018;8:623-31. [CrossRef] [PubMed]
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75. [PubMed]
20. Karumuri SB, Singh H, Naqvi S, Mishra A, Flora SJ. Impact of chronic low dose exposure of monocrotophos in rat brain: Oxidative/nitrosative stress, neuronal changes and cholinesterase activity. Toxicol Rep 2019 Nov 14. [CrossRef] [PubMed]
21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979 Jun 1;95(2):351-8. [CrossRef] [PubMed]
22. Goth L. A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta 1991 Feb 15;196(2-3):143-51. [CrossRef] [PubMed]
23. Shaalan S, Radwan OK, Saleh H. Role of Wheat Germ Oil against Carbon Tetrachloride Induced Neurotoxicity in Brain Tissues of Adult Male Mice. Egypt. J Exp Biol (Zoo) 2017 Jun;174(5542):1-8. [CrossRef]
24. Ojo OA, Ojo AB, Ajiboye B, Akintayo C, Oyinloye BE, Ojo AA. Carbon Tetrachloride-induced oxidative stress in Wistar rat brain: Neurocurative potential of Ficus-asperifolia (Miq). J Chem Pharm Sci 2016;9(3): 1334-8.
25. Simeonova R, Vitcheva V, Kondeva-Burdina M, Popov G, Shkondrov A, Manov V, et al. Alcesefoliside protects against oxidative brain injury in rats. Rev Bras Farmacogn 2019 Mar 1;29(2):221-7. [CrossRef]
26. Jayakumar T, Sakthivel M, Thomas PA, Geraldine P. *Pleurotus ostreatus*, an oyster mushroom, decreases the oxidative stress induced by carbon tetrachloride in rat kidneys, heart and brain. Chem Biol Interact 2008; 176:108-20. [CrossRef] [PubMed]

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AKTIVNOST KATALAZE I KONCENTRACIJA MALONDIALDEHIDA U MOŽDANOM TKIVU PACOVA TRETIRANIH UGLJEN-TETRAHLORIDOM

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Ugljen-tetrahlorid (CCl_4) je snažno hepatotoksično oksidaciono sredstvo, koje se koristi u animalnim modelima indukcije oštećenja jetre i nervnog tkiva. U ovom istraživanju, pratili smo promene koncentracije malondialdehida (MDA) i aktivnost katalaze (CAT) u moždanom tkivu Wistar pacova, koji su bili izloženi dejstvu CCl_4. Životinje su bile podeljene u dve grupe od po šest pacova. Pacovi iz kontrolne grupe tretirani su maslinovim uljem (10 ml/kg), dok je pacovima iz eksperimentalne grupe apliciran CCl_4 (1 ml/kg). Nivo oksidativnog stresa određen je u desetoprocentnom homogenatu celokupne encefalične mase. Nivoi MDA kod pacova eksperimentalne grupe bili su statistički značajno povećani ($p = 0,0009$), dok je aktivnost CAT signifikantno smanjena ($p = 0,0143$) kod pacova iz eksperimentalne grupe u poređenju sa pacovima iz kontrolne grupe. Rezultati su potvrdili teoriju o oksidativnom oštećenju moždanog tkiva ugljen-tetrahloridom kod pacova i mogu biti osnova za dodatna istraživanja potencijalno protektivnih supstanci u ovom animalnom modelu.

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Ključne reči: *ugljen-tetrahlorid, mozak, oksidativni stres, malondialdehid, katalaza*