medicinska revija
medical review

UDK: 615.099:546.48
615.099:547.562.4ž562.1

Ćurčić M. et al. ■ MD-Medical Data 2016;8(1): 017-020

Originalni članci/
Original articles

INFLUENCE OF DECABROMINATED DIPHENYL ETHER ON CADMIUM AS OXIDATIVE STRESS INDUCER IN KIDENEY – SUBACUTE ORAL STUDY IN RATS

UTICAJ DEKABROMOVANOG DIFENIL ETRA NA TOKSIČNOST KADMIJUMA POSREDSTVOM OKSIDATIVNOG STRESA – SUBAKUTNA ORALNA STUDIJA NA PACOVIMA

Correspondence to:

Marijana Ćurčić
University of Belgrade – Faculty of Pharmacy, Department of Toxicology „Akademik Danilo Soldatović“,
Vojvode Stepe 450,
11221 Belgrade, Serbia;
phone: +381 11 3951248;
e-mail: marijana.curcic@pharmacy.bg.ac.rs

Marijana Ćurčić[1], Marko Antunović[2], Maja Džoković[3], Saša Janković[4], Vesna Milovanović[5], Snežana Đorđević[2], Vesna Kilibarda[2], Slavica Vučinić[2], Biljana Antonijević[1]

[1] University of Belgrade – Faculty of Pharmacy, Depertment of Toxicology „Akedamik Danilo Soldatović“, Belgrade
[2] National Poison Control Center, Military Medical Academy, Belgrade
[3] Consultant in analytical biochemistry, Belgrade
[4] Institute of Meat Hygiene and Technology, Belgrade
[5] University Childrens Hospital Tiršova, Belgrade

Key words
cadmium, decabromiated diphenyl ether (BDE-209), oxidative stress

Ključne reči
kadmijum, dekabromovani difenil etar (BDE-209), oksidativni stres

Abstarct

It is known that kidneys are target tissue for Cd toxicity but there is not enough literature data on kidney effects of BDE-209. Moreover, Cd as toxic metal and BDE-209 as organic halogenated pollutant can share similar mechanism of action like, oxidative stress which is already assumed. However, how will Cd act as prooxidant in presence of BDE-209 is still not investigated, therefore, the objective of this study was to assess this BDE-209 influence. Results of this study indicated slight decrease in MDA in rat's kidney homogenates after subacute exposure to Cd and/or BDE-209, while SOD activity was increased and content of –SH groups decreased. Based on dose-response assessment, BDE-209 did not influence Cd toxicity mediated by chosen oxidative stress parameters, namely the derived $CEDL^5$ values were the same for single Cd and for Cd given in combination with the BDE-209.

In conclusion, dynamic of oxidative process has influence on the parameters levels. Co-exposure with polybrominated organic pollutant did not influence Cd effects on oxidative stress parameters. Having in mind that this is pilot study, performed with only one dose of BDE-209, further investigations are necessary, including changes in dose ranges, duration of exposure etc. Moreover, results contributes to the issue of mixture toxicology even BDE-209 did not influenced oxidative stress as mechanism of Cd toxicity.

INTRODUCTION

Cadmium (Cd) is widely distributed in the environment, either naturally or as a result of human activities, while decabromiated diphenyl ether (BDE-209) is mainly released from industrial processes or products containing this flame retardant[1-7]. Humans are exposed to a of Cd and BDE-209 mainly by inhalation or ingestion, which is confirmed by their presence in human tissues[1-2,6-8] which can result in toxic effects that are difficult to be identified based on information on single substances. It is known that kidneys are target tissue for Cd toxicity but there is not enough literature data on kidney effects of BDE-209. Moreover, Cd as toxic metal and BDE-209 as organic halogenated pollutant can share similar mechanism of action like, oxidative stress which is already assumed[9-10]. However, how will Cd act as prooxidant in presence of BDE-209 is still not investigated, therefore, the objective of this study was to assess this BDE-209 influence.

METERIALS AND METHODS

Male *Wistar* rats (weighing 220g) were kept in cages, under controlled conditions, at the air humidity of 40-60%, temperature of 20-24°C and under dark:light cycles 12h:12h. Food and water were available *ad libitum*. Study was done in accordance with the Law on animal welfare (Off. Gazette RS. No. 41/09) and decision of Ethical Committee of Military Medical Academy No. 9667-1/2011 - Ethical Committee, Military Medical Academy, Belgrade, Serbia.

Animals (6 to 8 per group) were given by gavage, single oral dose of Cd, BDE-209 or their three combinations during 28 days. Control groups were treated with water and dimethyl sulfoxide (DMSO). Three groups of rats were treated with 2.5, 7.5 or 15 mg Cd/kg/day. These doses were chosen on the bases of literature data exposure[2,11-13]. One group of rats was treated with 1000 mg BDE-209/kg/bw/day[1,14]. Furthermore, three groups of animals were treated with different doses of Cd in combination with dose of BDE-209. Purity of used chemicals was: BDE-209 (98%) (Sigma-Aldrich, St. Louis, MA, USA), $CdCl_2$ ($CdCl_2$xH2O, 99.99%) (Merck, Darmstadt, Germany) and (DMSO, 99.9%) (Sigma-Aldrich, St. Louis, MA, USA).

During the subacute exposure, water and food intake were recorded weekly, while body weight, clinical signs of toxicity and behaviour were recorded daily.

Kidneys were taken after the saccrifiing the animals at the end of experiment, and part of kidney tissue homogenised for oxidative stress parameters analysis like we described in Curcic et al. (2015)[15]. Oxidative stress determination in kidney homogenates was based on: malondialdehiyde (MDA) concentration, superoxide dismutase (SOD) activity, -SH groups content and morphological changes in the kidneys. The level of MDA in liver homogenates was based on its reaction with thiobarbituric acid under acidic condition for 15 min at 95 °C. In this reaction light yellow to pink colour complexes were formed depending on the concentration of MDA in tissue homogenates. Intensity of colour was measured at 523 nm and 600 nm. Activity of SOD, EC 1.15.1.1. in homogenates was measured using the method of Misra and Fridovich (1972)[16], The total content of -SH groups in liver homogenates was determined by the Ellman's method (1959)[17].

Statistically significant differences among data ($p < 0.05$) were tested using analysis of variance (ANOVA) followed by post hoc Tukey test (Statistica 7.0). Effect assessment i.e. dose dependent influnce on mesured parameters was done by PROAST software (Bilthoven, Netherland). Calculated Banchmark dose (CED in sofware) and its lower confidence limit (CEDL[5]) of 5% for the impact of 5% on paramether implied dose dependent manner, but only when ratio between CED and CEDL[5] is lower than 10[18].

RESULTS AND DUSCUSSION

Results on MDA concentration imply that BDE-209 in dose of 1000 mg/kg/day did not have impact on this parameter. Calculated two CEDL[5] of 1.008 mg Cd/kg/day for Cd given alone or in combination with BDE-209 are the same and confirm this statment (Table 1). Even contradictory, the role of oxidaticve stress in Cd toxicity is evident[19-20]. First observations on this issue came from early 80ties and are related with Cd impact on normal mitohondrial function. It was also shown that Cd can cause lipid peroxidation in tissues, and that oxidative stress is probably one of the most important machanisms of its toxicity including carcinogenicity[21]. On the other hand, there are still many doubts on oxidative stress induced by Cd as mechanism of toxicity, paricularly since Cd does not produce reactive species directly[22]. Suppotive evidences are: Cd in short time period start induces free radicals production, including superoxide radical, hidroxyl radical, hydrogen peroxide as wel as free lipid radicals. Increase of lipid peroxidation is proven in lungs, brain and liver[23], as well as blood[11] only few hours after exposure. Probably explantion for Cd induced oxidative damage could be inhibition of antioxidative defence system, mostly by decreasing glutathion concentration, or by interactions with the Fe, resulting in Fenton rection and free radicals production[11,22]. Results of this study on Cd are in accordance with the literature. Additionaly, even in vitro proven as poten prooxidative agent[24], BDE-209, did not influenced Cd induced MDA production (Figure 1, Table 1). Based on results from Table 1, after effects assessment and confirmation of dose dependent manner, type of interaction between Cd and BDE-209 could be considered as additivity.

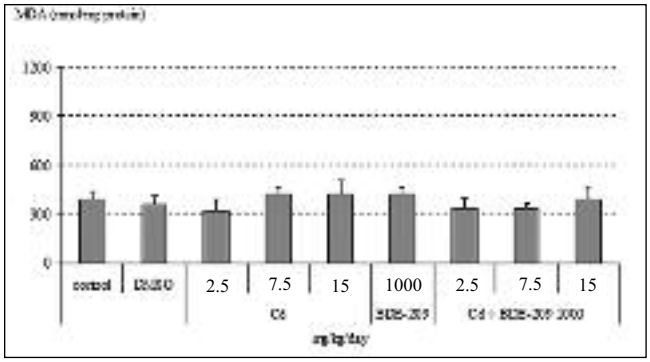

Figure 1. *MDA concentration in Wistar rats kidney homogenates peroraly exposed to Cd and/or 1000 mg BDE-209/kg/day during 28 days*

One way ANOVA and post-hoc LSD test, $p < 0.05$: a – vs. DMSO group; b – vs. group receiving BDE-209; c vs. groups receiving same dose of Cd.

Observed decrease of SOD activity after increase of dose of Cd and/or BDE-209 in our study are contradictory with results from other studies where exposure was much longer[2,12]. It could be explained by application of different Cd doses, and differentin exposure duration. Decrease in SOD activity after Cd application observed in this experiment (Figure 2) could be explained by exhausting protective mechanisms or by up regulation of antioxidative enzimes activities. Dose–dependent manner was not conformed, therefor nether effect on SOD activity for the dose ranges (Table 1).

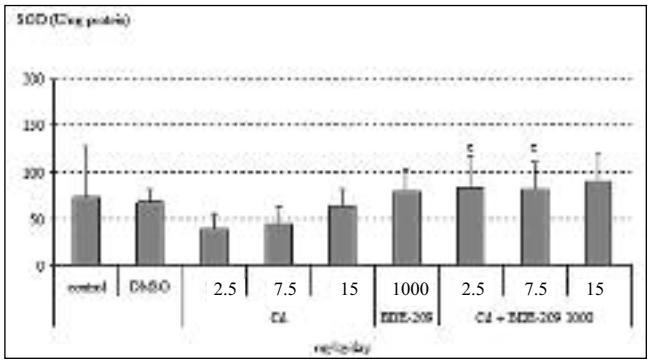

Figure 2. *SOD activity in Wistar rats kidney homogenates peroraly exposed to Cd and/or 1000 mg BDE-209/kg/day during 28 days*

One way ANOVA and post-hoc LSD test, $p < 0.05$: a – vs. DMSO group; b – vs. group receiving BDE-209; c – vs. groups receiving same dose of Cd.

Important place in explanation of Cd toxicity mediated by oxidative stress take proteins and peptides reach in –SH groups, particularly methalotioneins (MT). Cadmium induces sintheis of MT, which play important role in trasport, deposition and protection of Cd toxic effects[20]. In this experiment, increased concentrations of –SH groups were observed aftre exosure to Cd or to the combination of Cd and BDE-209. In our previous experiment we observed in vitro prooxidative effect of BDE-209, higher than one caused by Cd [24,25] which could be explanation for increase of –SH groups as addaptive response. For Cd is known that reacts with –SH groups, which could be explanation for decrease in this parameter, however it can damage protein structure, particularly by produced very toxic hydroxil radical[25]. Literature data when rats were exposed to Cd by drinking water during 6, 12 or 24 weeks (3,17 - 4,28 mg/kg/day)[26] shown similar results like ones ones observed in our study.

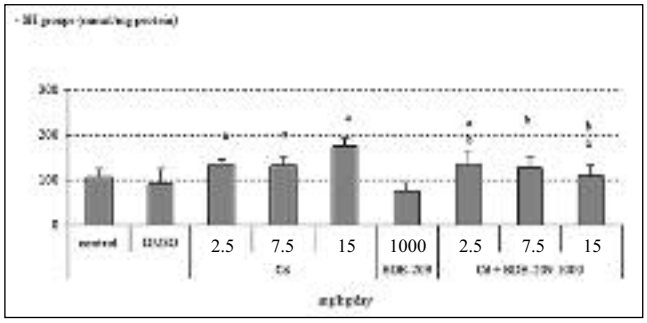

Figure 3. *_SH groups concentration in Wistar rats kidney homogenates peroraly exposed to Cd and/or 1000 mg BDE-209/kg/day during 28 days*

One way ANOVA and post-hoc LSD test, $p < 0.05$: a – vs. DMSO group; b – vs. group receiving BDE-209; c – vs. groups receiving same dose of Cd.

Table 1. *Benchmark doses for Cd and Cd with BDE-209 as covariance for the effects on MDA concentration, SOD activity and -SH groups content in kidney homogenates*

Effect on kidney		**Cd (mg/kg/day)**	**Cd (mg/kg/day) - BDE209 covariance**
		0	1000
MDA	CED	1.43	1.43
(nmol/mg protein)	CEDL5	1.008	1.008
	model	(+) E2	(+) E2
SOD activity	CED	/	/
(U/mg protein)	CEDL5	/	/
	model	(-)	(-)
-SH groups	CED	2.84	2.84
(nmol/mg protein)	CEDL5	1.839	1.839
	model	(+) E2	(+) E2

CED –*Benchmark* doza; CEDL5 – statistical lower confidence limit of Benchmerk dose that cause chage of 5% in effect; (-) no dose dependent effect; (+) dose dependent effect; En (n = 1, 2, 3, 4 ili 5) type of model dose – response, given in details in Slob (2002).

Observation from studies show that Cd after acute exposure inhibits enzymes of antioxidative protection i.e. SOD, and also decrease antioxidative capacity of tissue cells because of glutation depletion and –SH groups inactivation[11,20,25]. In study of Mladenovic et al. (2010)[20] dose of 2.5 mg Cd/kg caused after 24 hours significant increase of MDA, almost four folds higher SOD activity, since –SH groups content stayed at the control level. After prolonged exposure for 12 weeks Haouem and El Hani (2013)[27] showed that dose od Cd od 1.2 µg/kg/day and 132 µg/kg/day caused significant decrease in MDA while SOD and –SH groups were at the control levels.

CONCLUSION

Dynamic of oxidative process has influence on the parameters levels. Co-exposure with polybrominated organic pollutant did not influence Cd effects on oxidative stress parameters. Having in mind that this is pilot study, performed with only one dose of BDE-209, further investigations are necessary, including changes in dose ranges, duration of exposure etc. Moreover, results contributes to the issue of mixture toxicology even BDE-209 did not influenced oxidative stress as mechanism of Cd toxicity.

ACKNOWLEDGEMENT

This work was partly supported by the Ministry of Education, Science and Technological Development of Serbia (Project III 46009).

Sažetak

Poznato je da su bubrezi ciljni organ toksičnosti kadmijuma, ali je mnogo manje literaturnih podataka o toksičnim efektima BDE-209 na bubrege. Kadmijum, kao toksičan metal i BDE-209, kao organska halogenovana zagađujuća supstanca mogu imati sličan mehanizam dejstva posredovan oksidativnim stresom. Međutim, kako će Cd da deluje kao prooksidant u prisustvu BDE-209 još uvek nije proučavano, te je cilj ovog rada bio da se proceni uticaj BDE-209 na oksidativni stres posredovan kadmijumom. Rezultati ovog istraživanja ukazuju na blagi pad koncentracije MDA u homogenatima bubrega pacova nakon subakutne ekspozicije Cd i/ili BDE-209, dok se aktivnost SOD povećava i sadržaj -SH grupa smanjuje. Na osnovu procene odnosa doza-odgovor, BDE-209 nije imao uticaja na toksičnost Cd izazvanu posredstvom oksidativnog stresa (posmatrajući odabrane parametre). Potvrda je dobijena i iz izračunatih donjih granica pouzdanosti doze od 5% - $CEDL^5$ jer su vrednosti su bile iste za sam Cd i Cd primenjen u kombinaciji sa BDE-209. Može se zaključiti da dinamika oksidativnih procesa ima uticaj na vrednosti parametara oksidativnog stresa, da stoga istovremena ekspozicija Cd i polibromovanom organskom polutantu nije promenila uticaj samog Cd na parametre oksidativnog stresa. Imajući u vidu da je ovo pilot studija, izvršena sa samo jednom dozom BDE-209, potrebna su dalja istraživanja, uključujući i promene opsega doza, trajanje ekspozicije itd. Pored toga, rezultati ove studije doprinose tumačenju toksikologija smeša, iako BDE-209 nije imao uticaja na oksidativni stres kao mehanizam Cd toksičnosti za date uslove ekspozicije.

REFERENCES

1. ATSDR, 2004. Toxicological profile for polybrominated biphenyls and polybrominated diphanyl ethers. Agency for Toxic Substances and Disease Registry. 2004.

2. ATSDR, 2012. Toxicological profile for cadmium. Agency for Toxic Substances and Disease Registry. 2012.

3. Birnbaum LS, Staskal DF. Brominated flame retardants: cause for concern? Environ Health Perspect. 2004;112(1):9-17.

4. Darnerud PO, Eriksen GS, Johannesson T, Larsen PB, Viluksela M. Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. Environ Health Perspect. 2001; 109(Suppl 1): 49-68.

5. de Wit CA. An overview of brominated flame retardants in the environment. Chemosphere. 2002; 46(5): 583–624.

6. EFSA, 2011. Use of BMDS and PROAST software packages by EFSA Scientific Panels and Units for applying the Benchmark Dose (BMD) approach in risk assessment. EN-113. 2011;1-190.

7. EFSA, 2012. Scientific report of EFSA cadmium dietary exposure in the European population. 2012;10(1):1–37.

8. Roosens L, Cornelis C, D'Hollander W, Bervoets L, Reynders H, Van Campenhout K et al. Exposure of the Flemish population to brominated flame retardants: model and risk assessment. Environ Int. 2010; 36(4): 368–76.

9. Matović, V., Buha, A., Bulat, Z., Đukić-Ćosić, D., Miljković, M., Ivanišević, J. and Kotur-Stevuljević, J. Route-dependent effects of cadmium/cadmium and magnesium acute treatment on parameters of oxidative stress in rat liver. Food and chemical toxicology, 2012; 50(3): 552-557.

10. Milovanovic V, Buha A, Matovic V, Curcic M, Vucinic S, Nakano T, Antonijevic B. Oxidative stress and renal toxicity after subacute exposure to decabrominated diphenyl ether in Wistar rats. Environmental Science and Pollution Research. 2015; 16: 1-8.

11. Matović, V., Đukić-Ćosić, D., Buha, A. and Bulat, Z., 2013. Route, dose and duration of exposure to cadmium-relevance to oxidative stress induction. Peroxidases: Biochemical Characteristics, Functions and Potential Applications. Eds: Bogaert L, Coppens N, Nova Science Publisher, New York, pp.159-175.

12. Brzóska MM, Rogalska J, Kupraszewicz E. The involvement of oxidative stress in the mechanisms of damaging cadmium action in bone tissue: a study in a rat model of moderate and relatively high human exposure. Toxicology and applied pharmacology. 2011; 250(3): 327-35.

13. Piłat-Marcinkiewicz B, Sawicki B, Brzoska M, Moniuszko-Jakoniuk J. Effect of chronic administration of cadmium on the rat thyroid: radioimmunological and immunohistochemical studies. Folia Histochem Cytobiol. 2001; 40(2): 189–90.

14. Van der Ven L, van de Kuil T, Leonards PE, Slob W, Canton RF, Germer S et al. A 28-day oral dose toxicity study in Wistar rats enhanced to detect endocrine effects of decabromodiphenyl ether (decaBDE). Toxicol lett. 2008: 179(1): 6-14.

15. Ćurčić M, Tanasković S, Stanković S, Janković S, Antunović M, Đorđević S, Kilibarda V, Vučinić S, Antonijević B. Relationship of hepatotoxicity and the target tissue dose of decabrominated diphenyl ether in subacutely exposed Wistar rats. Vojnosanitetski pregled. 2015; 72(5): 405-13.

16. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247(10): 3170–5.

17. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82(1): 70–7.

18. Slob W. Dose-response modeling of continuous endpoints. Toxicol Sci. 2002; 66(2): 298–312.

19. Dong W, Simeonova PP, Gallucci R, Matheson J, Flood L, Wang S et al. Toxic metals stimulate inflammatory cytokines in hepatocytes through oxidative stress mechanisms. Toxicol Appl Pharmacol. 1998;151(2):359–66.

20. Mladenovic D, Radosavljevic T, Ninkovic M, Vucevic D, Sljivancanin T, Grbatinic I et al. Possible role of oxidative stress in acute cadmium hepatotoxicity in rats. Acta Vet (Beogr). 2010; 60(5-6): 449–59.

21. Nordberg GF. Historical perspectives on cadmium toxicology. Toxicol Appl Pharmacol. 2009; 238(3): 192–200.

22. Liu J, Qu W, Kadiiska MB. Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol Appl Pharmacol. 2009; 238(3): 09–14.

23. Manca D, Ricard AC, Trottier B, Chevalier G. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology. 1991;67(3):303–23.

24. Curcic M, Durgo K, Kopjar N, Ancic M, Vucinic S, Antonijevic B. Cadmium and decabrominated diphenyl ether mixture: in vitro evaluation of cytotoxic, prooxidative and genotoxic effects. Environmental toxicology and pharmacology. 2014; 30;38(2): 663-71.

25. Rani A, Kumar A, Lal A, Pant M. Cellular mechanisms of cadmium-induced toxicity: a review. International journal of environmental health research. 2014; 24(4): 378-99.

26. Brzoska MM, Moniuszko-Jakoniuk J, Pilat-Marcinkiewicz B, Sawicki B. Liver and Kidney Function and Histology in Rats Exposed To Cadmium and Ethanol. Alcohol Alcohol. 2003; 38(1): 2–10.

27. Haouem S, El Hani A. Effect of cadmium on lipid peroxidation and on some antioxidants in the liver, kidneys and testes of rats given diet containing cadmium-polluted radish bulbs. Journal of toxicologic pathology. 2013; 26(4): 359.

■ The paper was received on 01.03.2016. / Accepted on 09.03.2016.