Macedonian pharmaceutical bulletin, 68 (Suppl 1) 369 - 370 (2022)
Online ISSN 1857 - 8969
DOI:10.33320/maced.pharm.bull.2022.68.03.178
Short communication

Effects of toxic metal mixtures on hematologic parameters in male Wistar rats after the subchronic exposure

Katarina Baralić, ĐorđeStanivuk, Dragana Javorac, Đurđica Marić, Evica Antonijević Miljaković, Marijana Ćurčić, Danijela Đukić-Ćosić, Zorica Bulat, Aleksandra Buha Djordjevic

University of Belgrade – Faculty of Pharmacy, Department of Toxicology "Akademik Danilo Soldatović", Vojvode Stepe 450, 11221 Belgrade, Serbia

Introduction

Toxic metals are present in the Earth's crust, from which they reach the environment naturally or through various anthropogenic activities. As they are not degradable, they remain in the environment for a very long time, making exposure of living organisms inevitable (Andrade et al., 2017; Rahman et al., 2019). Therefore, contamination of our environment with metals is considered one of the biggest toxicological problems today (Andrade et al., 2017), especially bearing in mind that people are exposed to various mixtures of toxic metals on a daily basis, and not individual chemicals. Adverse effects on various organs after the exposure to many metals individually, are well known and tested (Jaishankar et al., 2014). Nevertheless, effects of their mixtures warrant further toxicological assessment, especially in environmentally relevant concentrations.

The aim of the current subchronic animal study was to investigate hematotoxicity of the mixture of six toxic metals/metalloids (arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), hexavalent chromium (Cr VI), and nickel (Ni)), in doses selected on the basis of previously conducted human biomonitoring study and literature data.

Material and methods

The experiment was carried out on male Wistar rats (*Rattus norvegicus*) weighing 150-200 g. The animals were kept under regular maintained settings (temperature 20-24 °C), light (12 h night: 12 h day), and humidity levels of 35-60%. During the study, they were given free access to food and tap water and were allowed seven days to adjust to their new surroundings and experimental circumstances. All experimental protocols were authorized by the University of Belgrade, Faculty of Pharmacy's Ethical Committee on Animal Experimentation (Ethical permission number: 323-07-11822/2018-05).

Animals (n=20) were randomly divided into 4 experimental groups (3 treated and 1 control), with 5 animals in each group. Aquatic solutions of toxic metal mixtures were orally applied to animals for 90 days in three different doses corresponding to the results of the previously conducted human biomonitoring study in which toxic metals were measured in the blood of the general population (M1 group: median dose; M2 group: 95th percentile exposure; M3 group: Benchmark doses based on the effects on hormone levels).

Blood samples were collected in heparin vacutainers by cardiac puncture. The MYTHIC 22 analyzer (Orphee Medical, Geneva, Switzerland) was used to test hematological parameters in accordance with acceptable laboratory procedures. The following hematological parameters were examined: white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), and platelet count (PLT). RBC, WBC and PLT were determined by flow cytometry and HGB content by standard colorimetric method.

*katarinab@pharmacy.bg.ac.rs

The significance of differences between parameter values in different groups were examined using ANOVA followed by LSD test.

Results and discussion

The applied metal mixtures in the M1 and M3 groups did not have a significant effect on RBC compared to the control, while in the M2 significantly higher values were noted in comparison with the M1 group. There was no effect of the tested mixtures on the WBC. However, based on the obtained median values, a decreasing trend was observed in the treated groups (M1<M2<M3) compared to the control. The most significant effects were observed on PLT. In the M2 and M3 groups, there was a significant increase in PLT in treated rats compared to the control ($P<0.001$), as well as compared to M1 group. Furthermore, it should be noted that elevated PLT compared to the control group occurred in animals that received the highest (M2 group) and lowest dose (M3 group) of the tested mixture, while the effect was absent in the M1 group. This can be explained by the non-monotonic dose response (NMDR). Some metals have been shown to exert this phenomenon at different doses (Gong et al., 2019; O'Doherty et al., 2019). NMDR is characteristic of substances that do not show a linear dose-response relationship or threshold effects (O'Doherty et al., 2019). The HGB content was significantly increased in M2 group compared to M1, similar to the effects of the investigated mixture on RBC, while HCT values were significantly increased in the M2 group ($P<0.05$) compared to the control and M1 group. Overall, most changes were observed in the M2, in almost all the examined hematological parameters (except WBC) which was expected having in mind that, in this group, metal concentrations were the highest.

To the best of our knowledge, the conducted study is one of the first to examine the effects of metal mixtures in doses that mimic the conditions of actual environmental exposure of people. The uptake, distribution, accumulation and toxic effects of metals and metalloids in tissues and organs depend on many factors, such as the physicochemical characteristics of the metal, dose, mode and length of exposure, ability to bind to different ligands in cells and sensitivity of exposed individuals (Whittaker et al., 2011). Nevertheless, the obtained results have indicated that mixtures of toxic metals may lead to certain toxic effects on the hematopoietic system in animals after the subchronic exposure. Accordingly, the potential effects of these metals on the human hematopoietic system might exist, having in mind that the doses used in this study were chosen to reflect the conditions of real-life exposure from the environment.

Conclusion

The results of the study indicate the possibility of toxic effects of the test mixture on the hematopoietic system at doses of individual substances that are considered safe according to studies conducted with individual substances. However, further research is needed to elucidate the effects of long-term exposure to low doses of metal and metalloid mixtures.

Acknowledgement

This research was supported by the Science Fund of the Republic of Serbia, PROMIS, Grant No 6066532, DecodExpo project.

References

Andrade, V.M., Aschner, M., Marreilha Dos Santos, A.P., 2017. Neurotoxicity of metal mixtures. Adv. Neurobiol. 18, 227-265. https://doi.org/10.1007/978-3-319-60189-2_12

Gong, Y., Liu, J., Xue, Y., Zhuang, Z., Qian, S., Zhou, W., Li, X., Qian, J., Ding, G., Sun, Z., 2019. Non-monotonic dose-response effects of arsenic on glucose metabolism. Toxicol. Appl. Pharmacol. 377, 114605. https://doi.org/10.1016/j.taap.2019.114605

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. Interdiscip. Toxicol. 7(2), 60. https://doi.org/10.2478/intox-2014-0009

O'Doherty, C., Keenan, J., Horgan, K., Murphy, R., O'Sullivan, F., Clynes, M., 2019. Copper-induced non-monotonic dose response in Caco-2 cells. In Vitro Cell. Dev. Biol. Animal 55(4), 221-225. https://doi.org/10.1007/s11626-019-00333-8

Rahman, Z., Singh, V.P., 2019. The relative impact of toxic heavy metals (THMs)(arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and lead (Pb)) on the total environment: an overview. Environ. Monit. Assess 191(7), 1-21. https://doi.org/10.1007/s10661-019-7528-7

Whittaker, M.H., Wang, G., Chen, X.Q., Lipsky, M., Smith, D., Gwiazda, R., Fowler, B.A., 2011. Exposure to Pb, Cd, and As mixtures potentiates the production of oxidative stress precursors: 30-day, 90-day, and 180-day drinking water studies in rats. Toxicol. Appl. Pharmacol. 254(2), 154-166. https://doi.org/10.1016/j.taap.2010.10.025