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In vivo toxicity of copper oxide, lead oxide and zinc oxide nanoparticles acting in different combinations and its attenuation with a complex of innocuous bio-protectors

Ilzira A. Minigalieva[a], Boris A. Katsnelson[a,*], Vladimir G. Panov[b], Larisa I. Privalova[a], Anatoly N. Varaksin[b], Vladimir B. Gurvich[a], Marina P. Sutunkova[a], Vladimir Ya. Shur[c], Ekaterina V. Shishkina[c], Irene E. Valamina[d], Ilya V. Zubarev[c], Oleg H. Makeyev[d], Ekaterina Y. Meshtcheryakova[d], Svetlana V. Klinova[a]

[a] *The Medical Research Center for Prophylaxis and Health Protection in Industrial Workers, Ekaterinburg, 620014, Russia*
[b] *Institute of Industrial Ecology, the Urals Branch of the Russian Academy of Sciences, Ekaterinburg, Russia*
[c] *School of Natural Sciences and Mathematics, The Ural Federal University, Ekaterinburg, Russia*
[d] *The Central Research Laboratory of the Ural Medical University, Ekaterinburg, Russia*

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ABSTRACT

Stable suspensions of metal oxide nanoparticles (Me-NPs) obtained by laser ablation of 99.99% pure copper, zinc or lead under a layer of deionized water were used separately, in three binary combinations and a triple combination in two independent experiments on rats. In one of the experiments the rats were instilled with Me-NPs intratracheally (i.t.) (for performing a broncho-alveolar lavage in 24 h to estimate the cytological and biochemical indices of the response of the lower airways), while in the other, Me-NPs were repeatedly injected intraperitoneally (i.p.) 18 times during 6 weeks (for estimating the accumulation of corresponding metals in the blood and their excretion with urine and feces and for assessing subchronic intoxication by a large number of functional and morphological indices). Mathematical description of the results from both experiments with the help of the Response Surface Methodology has shown that, as well as in the case of any other binary toxic combinations previously investigated by us, the response of the organism to a simultaneous exposure to any two of the Me-NPs under study is characterized by complex interactions between all possible types of combined toxicity (additivity, subadditivity or superadditivity of unidirectional action and different variants of opposite effects) depending on which effect it is estimated for as well as on the levels of the effect and dose. With any third Me-NP species acting in the background, the type of combined toxicity displayed by the other two may change significantly (as in the earlier described case of a triple combination of soluble metal salts). It is shown that various harmful effects produced by CuO-NP + ZnO-NP + PbO-NP combination may be substantially attenuated by giving rats per os a complex of innocuous bioactive substances theoretically expected to provide a protective integral and/or metal-specific effect during one month before i.t. instillation or during the entire period of i.p. injections.

1. Introduction

However extensive the advancements and developments in general and specific nanotoxicology have been over the recent decade, noteworthy is a virtually complete lack of studies devoted to comparative and *combined* toxicity of different substances as particles in the nanoscale range (Tong et al., 2015; Minigalieva et al., 2015). Meanwhile, the broader the usage of nanomaterials in various industries, science, and medicine, the higher the probability of human exposure, simultaneous or successive, to the multiple impacts of these materials. Moreover, if we consider not only purposely engineered nanoparticles but also those by-produced in many traditional technologies, it is just multifactor effects rather than isolated potentially hazardous nano-impacts on human health that appear to be a common rule. Specifically, metallic and metal oxide nanoparticles (Me-NPs) generated by arc-welding

* Corresponding author at: 30 Popov Str, Ekaterinburg, 620014, Russia.
E-mail addresses: bkaznelson@etel.ru, bkaznelson@ymrc.ru, honored_sci@yahoo.com (B.A. Katsnelson).

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and alloyed steel metallurgy and contaminating workplace and ambient air usually have a complex chemical composition comprising oxides of iron, manganese, nickel, chrome, vanadium, silicon and other elements. In nonferrous metallurgies, typical are combined exposures to some of the above listed or to some other Me-NPs (e.g. those of PbO, CuO, and ZnO in copper smelting and refining) that pollute the air together with submicron particles of the same metal oxides measuring >100 nm.

Both the chemical identity of these NPs and quantitative relationships between them vary broadly depending on specific technology, on its phase, on the composition of the alloy that is being molten or welded and of welding electrodes, on melting temperature, etc. One of the urgent challenges for the nanotoxicologist is therefore the need to assess not only the comparative toxicities of various Me-NPs but also their combined effects.

In a series of papers (Varaksin et al., 2014; Katsnelson et al., 2014; Minigalieva et al., 2014, 2015; Panov et al., 2015; Katsnelson et al., 2016; Panov and Varaksin, 2016), we have discussed the current situation in the complicated domain of the combined toxicity theory and of its mathematical modeling. The mainstream philosophy dominating in the relevant scientific literature and in some official guidelines or recommendations as well as its controversies have been critically overviewed by us, mainly in the first article (Varaksin et al., 2014). In our own studies described in all those papers we addressed subchronic intoxications induced by repeatedly injecting IP pre-made solutions of some non-particulate inorganic chemicals (binary combinations Pb-Cd, Pb-F, Cr-Ni, Cr-Mn, Ni-Mn; three-factorial combination Cr-Ni-Mn as respective salts). We analyzed our experimental results using different mathematical models based on (a) ANOVA and (b) Mathematical Theory of Experimental Design, which correspond to the well-known paradigms of effect additivity and dose additivity (Loewe additivity), respectively. Now we mostly use the Response Surface Methodology, which generalizes these traditional paradigms (Tallarida, 2001; Euling et al., 2002; Box and Draper, 2007; Myers et al., 2009).

In all cases of binary toxic salt combinations that we have considered so far, the analysis, irrespective of its methodology, has led us to the following principal postulates:

(1) the widely recognized paradigms of effect additivity and dose additivity of combined action are virtually interchangeable and so might be regarded as different methods for modeling combined toxicity rather than as concepts reflecting fundamentally differing processes;
(2) within both paradigms, there exist more than three traditionally recognized types of combined toxicity (additivity, subadditivity and superadditivity), and we have found at least 10 variants of it depending on exactly which effect is considered and what its level is, as well as on dose levels and their ratios.
(3) when one deals with multi-outcome characterization of combined intoxications, both unidirectional (additive, subadditive or superadditive) and oppositely directed action of one and the same pair of toxicants is usually found in respect to even one and the same effect but at different dose or effect levels.

Later on we revealed essentially the same patterns of combined action in two different experiments with NiO-NPs and Mn_3O_4-NPs (Katsnelson et al., 2015c; Minigalieva et al., 2015) which we claim to have been the very first study on the combined toxicology of Me-NPs, although a retrospective analysis of the data obtained in our laboratory earlier has demonstrated the same pattern also for virtually insoluble microparticles of $BaCrO_4$ + MnO_2 (Privalova et al., 2016).

Thus one of the goals of the present study was to try and reproduce these conclusions for three more binary combinations of Me-NPs, namely: CuO-NP + PbO-NPs, CuO-NP + ZnO-NPs and PbO-NP + ZnO-NPs.

As concerns the combined toxicity of *three* metals acting together, we (Katsnelson et al., 2015a) proposed a new health risk-oriented approach based on a consideration whether the addition of a third toxic to the other two leads to the type of binary combined action becoming either more or less adverse (Classes A and B, respectively) or remaining virtually unchanged (Class C). This approach was successfully tested with reference to subchronic intoxication with nickel, chromium (VI) and manganese salts. We revealed some stable patterns of classification fully or partly reproduced when considering, one by one, various metals as the third component of the combination and found that the classification appeared to be inherently consistent for the absolute majority of outcomes. Again, this approach was to be tried for analyzing the three-factorial Me-NP toxicity as well, and to this end we investigated a CuO-NP + PbO-NP +ZnO-NP combination in addition to the above mentioned binary ones.

Lastly, the research considered in this paper looked for some means to render the exposed organism more resistant to the Me-NP combined toxicity. The principles of such “biological prophylaxis” against different occupational and environmental intoxications, its theoretical premises and numerous examples of its practical realization have been published by us repeatedly over several decades, including in review articles (Katsnelson et al., 2008a, 2008b, 2014). It was but natural to explore the potential of such preventive strategy in the field of nanotoxicology as well (Katsnelson et al., 2015b). Thus we proved that both the systemic toxicity and the *in vivo* genotoxicity of Ag-NPs (Katsnelson et al., 2013) and of CuO-NPs (Privalova et al., 2014a,b), as well as of the Mn_3O_4-NP + NiO-NP combination (Minigalieva et al., 2015), were markedly attenuated with background oral administration of multi-component bioprotective complexes (BPC). These BPCs comprised pectin, multivitamin-multimineral preparations, some amino acids, and omega-3 PUFA. All the studies demonstrated that these BPCs protect against various adverse effects of subchronic toxicity. We also found (Katsnelson et al., 2014) that in rats that were given glutamate, glycine, *N*-acetyl cysteine, iodide and a Se-containing multivitamin preparation orally during 4 weeks *before* a single-shot intratracheal (i.t.) instillation of NiO-NPs + Mn_3O_4-NPs, the latter exposure evoked significantly weaker neutrophil leukocyte recruitment into the lower airways than in rats so exposed without any pretreatment. In this paper, we demonstrate the preventive efficacy of the bio-protective complexes, always administered orally either alongside repeated i.p. injections or before a single i.t. instillation of a triple combination of Me-NPs (CuO-NP + PbO-NP + ZnO-NP).

2. Materials and methods

2.1. Nanoparticles

The suspensions of NPs were produced by laser ablation of metal targets (99.99% purity, 1-mm thick) placed on the bottom of a glass vessel with 5–30 mL of deionized water (Fig. 2.1). The laser system used was an Fmark-20RL (LTC, Russia) device based on an Yb fiber laser with a wavelength of 1080 nm, pulse duration of 100 ns, pulse energy of 1 mJ, and a repetition rate of 21 kHz. The target's surface, cleaned with deionized water in an ultrasonic bath, was irradiated by a spot of laser irradiation with a fixed diameter of 40 μm and a fluence ranging from 15 to 80 J/cm^2. The thickness of the water layer covering the target ranged from 2 to 10 mm. The scanning velocity was about 270 mm/s, and the scanned area ranged from 25 to 300 mm^2. A motor-driven agitator