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Looking for Biological Protectors against Adverse Health Effects of Some Nanoparticles that Can Pollute Workplace and Ambient Air (A Summary of Authors' Experimental Results)

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Abstract

Especially high health risks associated with impacts of metallic nanoparticles (Me-NPs) and their presence in the workplace and ambient air of not only the nano-industry but also of some long-existing traditional technologies make it necessary, along with keeping respective dangerous exposures as low as possible, to look for ways of increasing the organism's resistance to them. Based on theoretical premises of such beneficial interference with toxicokinetics and toxicodynamics of Me-NPs developed by our research team and on understanding general and specific key mechanisms of different Me-NPs' toxic action, we proposed several bioprotective complexes (BPCs) comprising mainly pectin, some vitamins, glutamate, glycine, N-acetylcysteine, omega-3 PUFA, and different essential trace elements. Results of our animal experiments with different Me-NPs showed that, against the background of such BPCs' oral administration, the integral and specific toxicity of Me-NPs and even their genotoxicity can be markedly attenuated. Therefore we would recommend to further develop this vector of nano-toxicological research.

Keywords

Nanoparticles, Toxic metals, Bioptotectors

1. Background

Nanoparticles (NPs) of elemental metals and some metalloids, and even more so

of their oxides are of special interest in the framework of health risk assessment and risk management problems. Along with engineered metallic NPs (Me-NPs), there usually exists a substantial nanoscale fraction of condensation aerosols generated as byproducts of many long existing and new technologies (steel and nonferrous metallurgies, arc-welding, laser metals treatment, etc) and thus polluting both workroom and ambient air in respective industries and adjacent areas. Other fractions of such aerosols are 8 usually presented mostly be submicron particles with dimensions above 100 nm. Examples illustrating these statements are given in Figure 1 and Figure 2.

In such industries air is, as a rule, polluted by multi-component mixtures of chemically different particles of similar or dissimilar geometry. For instance, in arc-welding and alloyed steel making one usually finds different combinations of iron, chromium, nickel, manganese and silicon oxides, while in crude copper smelting and copper refining-those of copper, lead, cadmium, zinc, and arsenic oxides.

In the vast nano-toxicological literature of the last decade, studies concerned with the assessment of Me-NP toxicity are quite numerous. We can refer, for example, to several works devoted to the same Me-NPs that were the subject-matter of our own studies: silver [1]-[25], gold [26]-[38], copper and copper oxide [25] [39]-[48], nickel oxide [43] [49]-[53], manganese oxides [54] [55] [56], zink oxide [57]-[61], lead oxide [62] [63], silicon dioxide ([64]-[71], and a

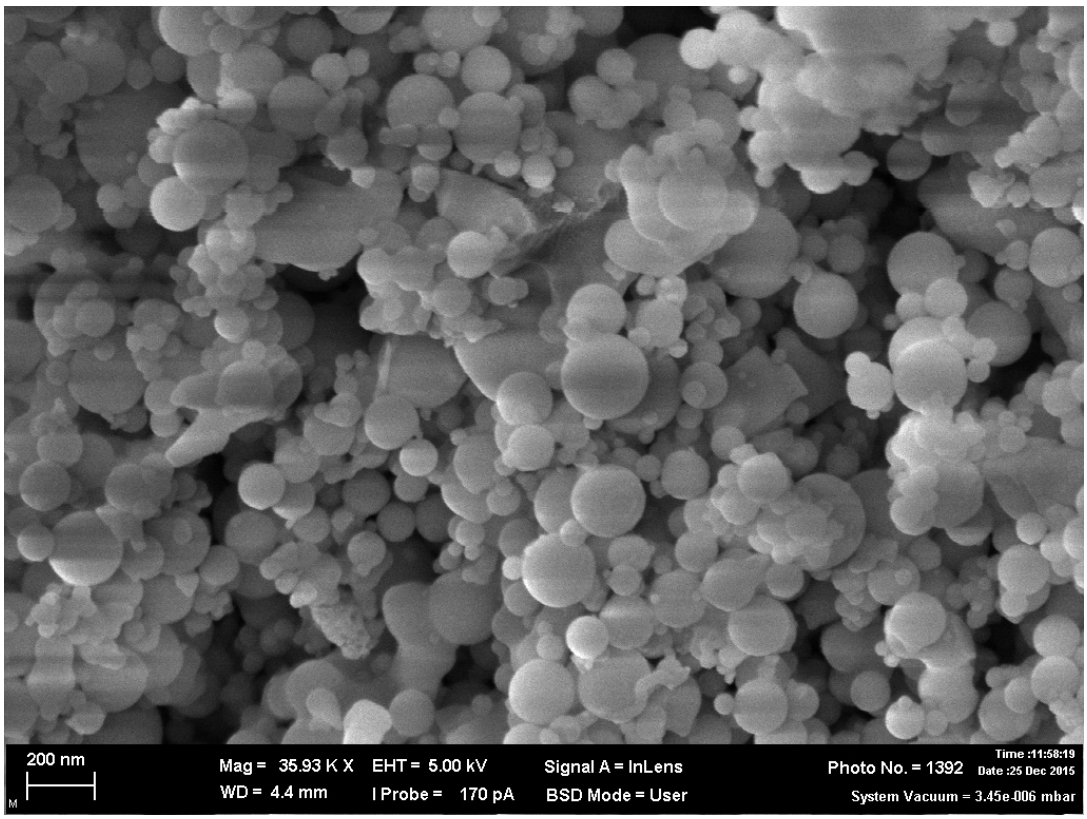


Figure 1. Particles of SiO_2 collected in the flue gas duct from the hood over a silicon smelting ore-thermal furnace (scanning electron microscopy, magnification ×35,930).

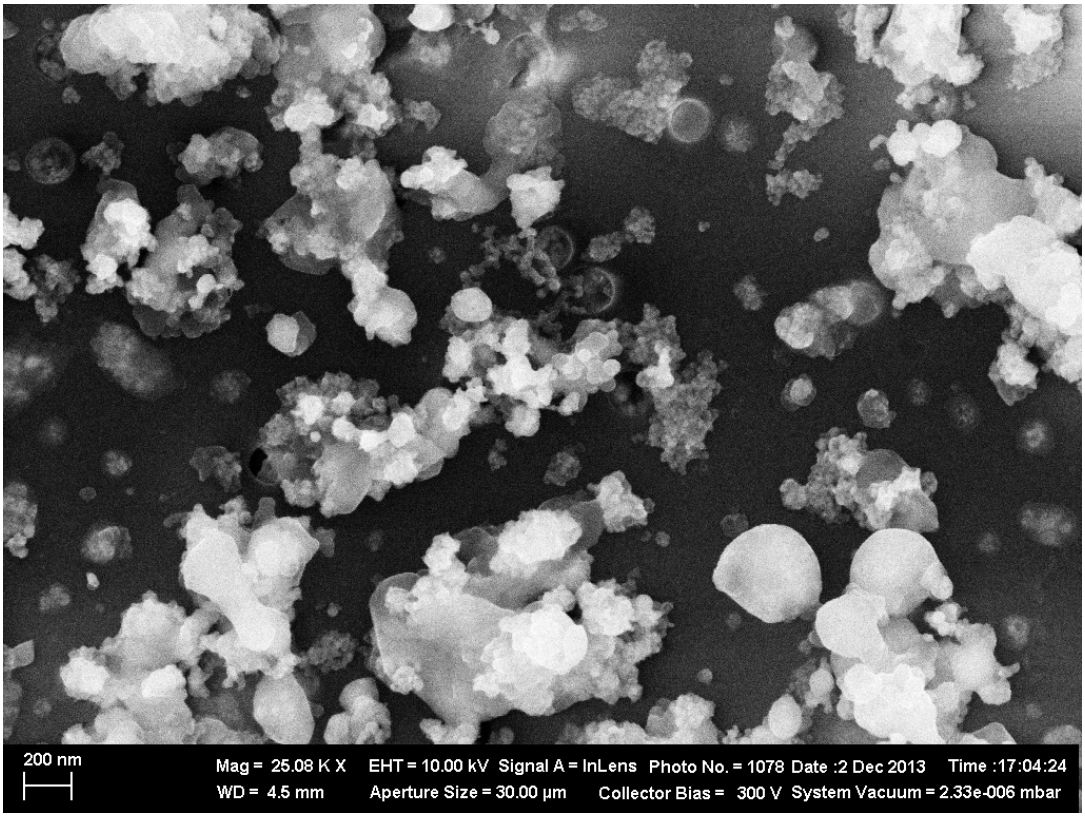


Figure 2. Particles sampled from copper smelter workplace air (SEM, magnification ×25,080).

lot of others). However, the prevailing majority of these researchers assessed adverse effects of Me-NPs *in vitro* on stable cell lines. This approach features a number of well-known advantages relating, in particular, to analysis of primary toxicity mechanisms on cellular and sub-cellular levels. At the same time, any extrapolation of the results of these experiments to the organism level and even the organ-systemic level is associated with a number of uncertainties and assumptions. Moreover, some important aspects (in particular, organism level toxicokinetics, relationships between doses and systemic responses, the functioning and efficiency of supracellular self-regulatory and protective mechanisms, etc.) can generally be addressed only through experiments on the whole mammalian organism. In such experiments carried out during 2010-2016 we [72]-[87] demonstrated that Mt-NPs should be considered one of the most dangerous occupational and environmental hazards due to their especially high toxicity and virtually obligatory genotoxicity.

2. Some Theoretical Premises of Biological Protection

Beyond any doubt, the most protectively effective way to manage occupational and environmental health risks associated with any hazardous impact would be to decrease the latter to a level exerting no observed adverse effects. However, due to the especially high toxicity and virtually obligatory genotoxicity of Me-NPs, their NOAELs and respective permissible exposures to Me-NPs proposed so far are so much lower than those for chemically analogous Me-MPs (e.g. [75]

[88] [89]) that they are hardly practicable in reality. We therefore decided to try and make the same goal attainable going from the other end, namely to enhance the natural resistance to the adverse health effects of Me-NPs [86]. This idea of possibly efficient "biological protection" against nano-toxicity was based on our long-term experience of successful bio-protection of the animal and human organism against various other toxicants, including some mineral microparticles [78] [80] [90].

The organism-level mechanisms of what we designate as bio-protection or bio-prophylaxis are schematically presented in the flow-chart (**Figure 3**). Although it has already been discussed by us in this Journal [80], we reckon it useful to be briefly presented here.

In general terms, we the mammalian organism can be protected against occupational or environmental toxic impacts using:

1) bio-protectors aimed primarily at increasing the effectiveness of the natural mechanisms of bio-transformation and/or elimination of toxics, and thus, at reducing the inner dose of a harmful substance retained in the organism and especially in the target organs (designated in our chart as "toxicokinetic effects");

2) bio-protectors aimed at enhancing the functional reserves at all levels of the organism affected by a toxic substance; at increasing the effectiveness of repair and compensatory processes; and at employing physiological and toxicological antagonisms (designated in the chart collectively as "toxicodynamic effects").

However, these two modes of action are usually interrelated and interdependent, as it is schematically shown with reciprocally directed arrows. Indeed, by reducing the retention of a toxic substance in the organism and especially in target organs, a bio-protector inhibits the development of a pathological process (thus, a bio-protector of a primarily toxicokinetic type of action produces a beneficial toxicodynamic effect). On the other hand, primary enhancement of resistance to the damaging impact of a toxic on the cells and organs that control the processes of its elimination or biotransformation (pulmonary macrophages, liver, kidneys) maintains the effectiveness of these processes and, thus, reduces the retention of this toxic in the organism (so we see a beneficial toxicokinetic effect of a toxicodynamic bio-protector). Such bilateral interdependence of toxicokinetic and toxicodynamic effects is pronounced to a varying degree in response to the action of different harmful substances but, on the whole, can be

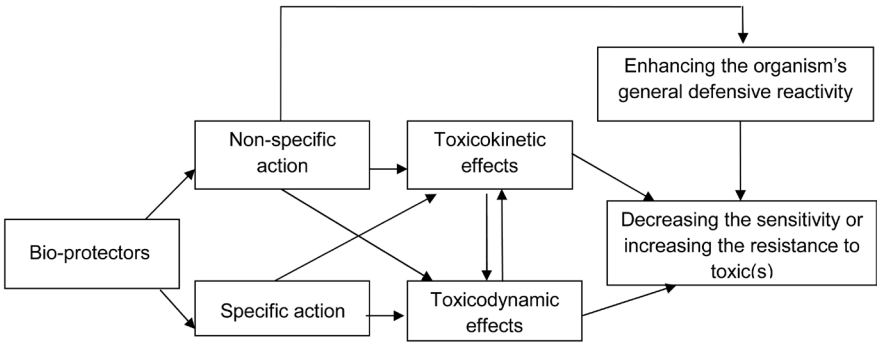


Figure 3. Schematic presentation of anti-toxic biological prophylaxis.

considered as a consistent pattern.

The flow-chart shows also that both toxicokinetic and toxicodynamic bio-protectors can be:

- more or less specific with regard to a particular toxic or a particular range of toxics if bioprotection interferes with the mechanisms of toxicokinetics and toxicodynamics pertaining just to these toxics or to a class of similarly acting ones;
- predominantly non-specific, if their effect is realized through such integral responses at the organism level as Selye's general adaptation syndrome or a related but still distinct concept of "non-specifically enhanced resistance" developed by the school of late Nikolay Lazarev, an outstanding Russian toxicologist and pharmacologist.

However, one and the same bio-protector may, in different cases. either render a largely specific effect or help the organism mainly as an agent enhancing its nonspecific defenses and thus decreasing its sensitivity or increasing its resistance to harmful exposures (see respective boxes and links in the same chart).

In our experiments, bio-protectors employing mechanisms that are not fully identical proved to be most effective when administered in combinations which we call "bio-protective complexes", or BPCs [78] [80] [90].

As concerns bio-protection against the adverse effects of *metallic nanoparticles*, we have so far chosen, based on the above formulated theoretical premises, and experimentally tested four BPCs protecting from nano-silver [78], nano-copper oxide [82], and combinations of NiO-NP + Mn_3O_4-NP [83] [84] [85] and PbO-NP + CuO-NP + ZnO-NP [91]. Being different in some important details depending on specific toxicodynamic and toxicokinetic mechanisms underlying the toxic action of different metals, the compositions of all those BPCs still has much in common. The most important components in all our experiments were:

1) Glutamate used by us, based on lung positive experience, as an effective cell membrane stabilizer acting through the intensification of ATP synthesis under exposure to the damaging effect of various cytotoxic particles (e.g. [92]) and, at the same time, as one of the precursors of glutathione. The latter, in its turn, serves a powerful cell protector against oxidative stress which is regarded as one of the key primary mechanisms underlying high cytotoxicity and genotoxicity of virtually all metallic NPs [93]. In addition to these non-specific and almost universal bio-protective effects of glutamate, it may more specifically increase resistance to the neurotoxicity of manganese, lead and some other Me-NPs due to its major role in the transmission of excitatory signals in the mammalian central nervous system and thus its involvement in most aspects of normal brain functioning. It is known, for instance, that manganese impairs the expression and function of the main glutamate transporters in astrocytes [94] and that lead interferes with glutamate release in the hippocampus [95]. It stands to reason that additional glutamate supply to the brain would partly compensate for these adverse effects of the respective Me-NPs.

2) The other two glutathione precursors: glycine and cysteine (the latter in a

highly active and metabolically well available form of N-acetylcysteine). The reasons for including these amino-acids into our BPCs were both theoretical (taking into consideration the above-mentioned general important role played by oxidative stress as a mechanism of Me-NP toxicity) and experimental as there were data of other researchers demonstrating that glutathione deficiency potentiates some specific metal toxicities-e.g. manganese-induced damage to the rat striatum and brainstem [96].

3) Other agents of the organism's anti-oxidant system: vitamins A, E, and C, and selenium (on the same theoretical grounds).

4) Omega-3 polyunsaturated fatty acids (PUFA) whose intracellular derivatives-eicosanoids-activate DNA replication and thus play an important part in its repair. Meanwhile the DNA fragmentation was found to be a common effect of all Me-NPs studied by us up to now.

5) Iodine, taking into consideration the well-known disturbances of the thyroid function caused by lead, manganese and some other metallic intoxications.

6) Trace elements when they are known to be antagonists of the specific metal that form Me-NPs under study.

7) Pectin enterosorbent as an agent that hinders the re-absorption of toxic metals excreted into the intestines with bile (which, again, is of special importance for metals that are excreted predominantly by this route (e.g. manganese and copper) or released within the GIT by Me-NPs translocated there after deposition in the airways.

In all the studies we have found that, as was expected, the *in vivo* cyto = and organo-systemic toxicity as well as genotoxicity of Me-NPs could be noticeably attenuated against the background administration of adequately composed BPCs or by the pre-medication with them. It should be stressed that we never claimed to be the first who have shown a possibility of inhibiting some metallic nanoparticles' toxicity with the help of this or that agent targeted at a certain mechanism of such toxicity. However, other researchers would demonstrate this possibility in experiments *in vitro* as a rule and used it as evidence of the importance of this mechanism (e.g. [10]) rather than as the foundation of a holistic bio-protective system. In the meantime, the goal of our mechanistically substantiated approach is just such a system ("biological prophylaxis" in our terminology), and as far as we know, we were the first indeed who began to investigate the effects of bio-protectors against metallic nanoparticles in animal experiments *in vivo*.

3. Examples of Results Obtained in Our Experiments

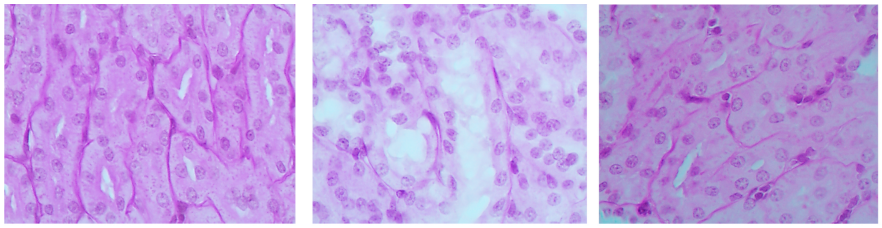
We have demonstrated the high beneficial efficacy of anti-MeNP bioprotection in our experiments using a lot of indices, but in this self-overview we illustrate this efficacy but with some typical examples.

Thus, virtually all Me-NPs studied by us prove to be markedly nephrotoxic, causing, in particular, significant damage to the epithelial cells of proximal convoluted renal tubules. Histological preparations of kidneys in rats repeatedly exposed intraperitoneally to these nanoparticles during 6 - 7 weeks revealed partial

destruction of the brush border and marked degenerative and necrotic changes in these cells up to their disappearance, while rats exposed to the same nanoparticles against background BPC administration demonstrated absence or marked alleviation of such tubular damage. Figure 4 presents as a typical example microscopic picture of kidneys from rats exposed to a combination of NiO-NPs and Mn_3O_4-NPs with or without concomitant oral administration of a BPC comprising pectin, glutamate, glycine, N-acetylcysteine, vitamins A, C, E, selenium, iodide and omega-3 PUFA.

Table 1 provides respective morphometric results obtained in this experiment [83] and quite similar results of an earlier experiment involving copper oxide nanoparticles [81] [82] against which we tested a BPC of a similar composition plus vitamin B12 and biotic doses of iron, zinc, molybdenum and manganese.

Another well established adverse effect of virtually all Me-NPs is damage to the organs rich in RES cells, in particular, to the spleen and liver, which accumulate them more avidly than any other organs. Indeed, in both of these organs we observed an explicit pathology the type of which virtually did not depend on



(a) (b) (c)

Figure 4. (a) Kidney of a control rat (proximal convoluted tubules with an intact brush border). (b) Kidney of a rat exposed to NiO-NPs + Mn_3O_4-NPs (marked degenerative and necrobiotic changes in tubular epithelial cells up to their disappearance; partial destruction of the brush border). (c) Kidney of a rat similarly exposed against background administration of a BPC. Periodic Acid Schiff (PAS) stain, magnification ×400.

Table 1. Some morphometric indices for tubular epithelium damage in the kidneys of rats after repeated intraperitoneal injections of some metallic oxides nanoparticles with or without background oral administration of a BPC (X ± s.e.)

Groups of rats given	Brush border loss (% lengthwise)	Epithelial desquamation (% lengthwise)
NiO nanoparticles + Mn_3O_4 nanoparticles		
Water (control)	5.44 ± 0.90	0.00 ± 0.00
Nanoparticles	12.33 ± 2.30*	2.43 ± 1.00*
Nanoparticles + BPC	7.08 ± 1.70	0.00 ± 0.00$^+$
CuO nanoparticles		
Water (control)	5.39 ± 0.42	0.33 ± 0.13
Nanoparticles	8.36 ± 0.76*	1.16 ± 0.38*
Nanoparticles + BPC	5.98 ± 0.46$^+$	0.98 ± 0.35

Note: *statistically significant difference from the control group; $^+$from the group given nanoparticles without the BPC ($p < 0.05$ by Student's t-test).

the chemical nature of the Me-NPs, and this pathology was also alleviated by background BPCs administration as exemplified in Table 2 by the results of an experiment with Ag-NPs [78].

A more specific adverse effect characterizing the toxicity of Mn_3O_4-NPs (acting either with or without NiO-NPs) and CuO-NPs was marked damage to some specialized structures of the brain (to the striatum and the hippocampus especially). In both cases, this damage was also significantly attenuated by the respective BPCs [81] [83]. Examples are given in Figure 5 and Table 3.

Table 2. Some morphometric indices of the cell structure of liver and spleen in rats exposed to Ag-NPs with or without background BPC[1] administration($x \pm$ s.e.).

Index	Rats injected with water (control)	Rats injected with nanoparticles	Rats injected with nanoparticles and administered a BPC
Liver			
Akaryotic hepatocytes per 100 cells	17.6 ± 0.6	18.5 ± 1.3	13.0 ± 1.0*+
Kupffer cells per 100 liver cells	16.5 ± 0.5	25.0 ± 0.8*	20.0 ± 0.6*+
Average particle load of Kupffer cells, score#	0	0.91 ± 0.7	0.51 ± 0.09+
Spleen			
White to red pulp ratio†	0.59 ± 0.036	0.37 ± 0.035*	0.59 ± 0.086+

Note: *statistically significant difference from the control group; +from the group given nanoparticles without the BPC ($p < 0.05$ by Student's t-test); #the particle burden of a cell is visually estimated as a score of points from 0 to 4. The weighted average index is calculated allowing for the percentage ratio between cells given different scores (the total number of scored cells = 100); †Measured with the help of a planimetric grid.

Table 3. Some morphometric indices for the state of rat's brain after repeated intraperitoneal injections of NiO and Mn_3O_4 nanoparticles with or without background oral administration of a BPC (X ± s.e.).

Golgi neurons (%%)	Rats injected with water (control)	Rats injected with nanoparticles	Rats injected with nanoparticles and administered BPC
Nucleus caudatus			
Without a nucleolus	30.50 ± 2.77	60.30 ± 2.26*	37.15 ± 2.89+
With a distinct centrally located nucleolus	25.12 ± 1.16	12.35 ± 0.95*	23.28 ± 1.09+
Hippocampus (CA 1)			
Without a nucleolus	30.50 ± 2.30	70.40 ± 3.75*	41.30 ± 2.14*+
With a distinct centrally located nucleolus	46.4 ± 2.92	11.0 ± 1.13*	30.5 ± 1.96*+

Note: *statistically significant difference from the control group; +from the group given nanoparticles without the BPC ($p < 0.05$ by Student's t-test).

[1]In this case, the BPC comprised pectin, glutamate, glycine, N-acetylcysteine, vitamins A, C, E, selenium, copper, calcium and omega-3 PUFA.

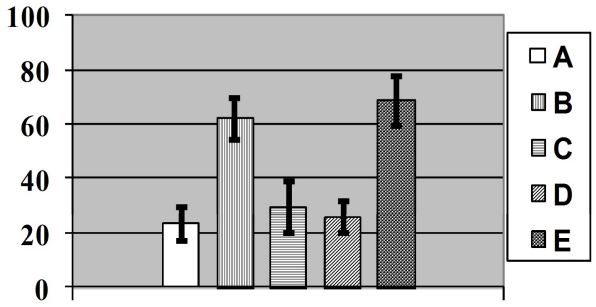


Figure 5. Number of cells without a nucleolus per 100 Golgi cells in nucleus caudatus of rats exposed (A) to water (Control); (B) to water suspension of CuO nanoparticles; (C) to the same against the background of bioprotective complex (BPC) administrations; (D) to the BPC only; (E) to water suspension of Cu/Cu_2O submicron particles (Average values with 95% CI). Differences are statistically significant between (B) and (A), (E) and (A), and (C) and (B) ($p < 0.05$ by Student's t-test).

Still another metal-specific outcome of a subchronic Me-NP intoxication was an increased reticulocytes percentage under the impact of PbO-NPs (24.7‰ ± 2.7‰ against 10.2‰ ± 1.4‰ in control rats, $P < 0.05$). This effect was even more pronounced under a combined impact of PbO-NPs + CuO-NPs + ZnO-NPs (29.7‰ ± 3.2‰) but was significantly attenuated (18.00‰ ± 1.6‰, $p < 0.05$) under the same impact against background administration of a BPC[2]. Similar attenuation (although statistically non-significant) was observed in respect to the decrease in the hemoglobin level and to the increase in the δ-ALA urine concentration [91].

To illustrate the efficacy of bioprotection against non-specific systemic toxic effects of Me-NPs, we may once again provide some results of the experiment involving nickel oxide in combination with manganese oxide nanoparticles. Table 4 presents the values of those indices for which the difference between the groups exposed to these Me-NPs with *vs.* without BPC administration proved statistically significant, but there were even more indices in the protected group that lost their statistically significant distinction from the control values. In general, the group exposed to the Me-NPs combination without protection had a statistically significant adverse deviation from the control value in 25 out of the 50 functional and biochemical indices for the organism's status, whereas only one index (decrease in the number of head dips into holes) was observed to have such deviation in the group so exposed along with background BPC administration [83]. This table also demonstrates that the BPC, which significantly attenuated the adverse effects caused by nanoparticles, had by itself no effect on the respective indices. This is quite typical of all our experiments.

It should also be stressed that significant attenuation in the toxic effects was not necessarily associated with a decrease in the target organ's burden of toxic

[2]Apple pectin, glutamate, glycine, *N*-acetylcysteine, vitamins A, C, D3, E, selenium, omega-3 rich PUFA, calcium, iodide, iron supplements

Table 4. Some functional indices for the condition of rat after repeated intraperitoneal injections of NiO and Mn_3O_4 nanoparticles and/or oral administration of a BPC (X ± s.e.).

Index	Groups given:			
	Water (control)	Nanoparticles	Nanoparticles and BPC	BPC
Leukocytes, 10^3/μl	4.3 ± 0.4	6.1 ± 0.5*	5.7 ± 0.6$^+$	4.3 ± 0.4
Bilirubin in blood serum, μmol/L	2.02 ± 0.40	1.15 ± 0.10*	1.5 ± 0.1$^+$	1.7 ± 0.1
Albumin in blood serum, g/L	46.6 ± 0.8	38.6 ± 0.8*	41.8 ± 1.1$^+$	47.3 ± 1.2
Diuresis, ml	32.7 ± 1.8	17.9 ± 2.9*	30.2 ± 2.7$^+$	31.2 ± 4.5
Urine relative density	1.017 ± 0.001	1.023 ± 0.001*	1.019 ± 0.001$^+$	1.019 ± 0.001
Creatinine in urine, mmol/L	1.09 ± 0.10	1.8 ± 0.20*	1.2 ± 0.1$^+$	1.2 ± 0.1
δ-ALA in urine, μmol/day	0.23 ± 0.07	0.54 ± 0.13	0.22 ± 0.02$^+$	0.25 ± 0.08

Note: *statistically significant difference from the control group; + from the group given NiO-NPs + Mn_3O_4-NPs (without the BPC) ($p < 0.05$ by Student's t-test with Bonferroni correction).

metal, although this beneficial toxicokinetic effect of the BPCs was also observed in some experiments, as illustrated by **Table 5** [82]. In the experiment with a combined exposure to NiO-NPs plus Mn_3O_4-NPs [83], BPC administration significantly decreased the retention of nickel, though not of manganese, in the liver, spleen and brain. Under exposure to Ag-NPs, the retention of silver in the liver, spleen and kidneys over the control levels was very significant but did not depend at all on BPC administration [78]. We believe that this seeming inconsistency is due to the predominance of toxicodynamic bio-protection mechanisms over toxicokinetic ones.

All of the above-described results demonstrate the attenuating effects of the bioprotectors on the subchronic systemic toxicity of Me-NPs. Meanwhile, it was similarly demonstrated that the same bioprotectors also beneficially influenced the immediate pulmonary response to the deposition of NPs in the lower airways. To this end, we carried out two experiments with BPC premedication during 4 weeks before the instillation of NiO-NPs + Mn_3O_4-NPs [79] or PbO-NPs + CuO-NPs + ZnO-NPs [97] and assessed this response by total and differential cell counts and by some biochemical BALF indices. In both experiments, as demonstrated by **Table 6**, we observed the usual prevalence of neutrophil leukocyte (NL) recruitment over that of alveolar macrophages (AMs), which is the most characteristic feature of the immediate pulmonary reaction to an impact of cytotoxic particles, including all Me-NPs studied by us up until now. In both experiments, the increase in the BALF NL count and NL/AM ratio over the respective control values was significantly lower in rats exposed to the same Me-NPs after a premedication with BPCs.

Taking the experiment with PbO-NPs + CuO-NPs + ZnO-NPs as an example,

Table 5. Copper content of some organs (mcg/g of dry-frozen tissue) in rats after repeated intraperitoneal injections of copper oxide nanoparticles and/or oral administration of a BPC ($x \pm$ s.e.).

Group of rats given	Kidneys	Liver	Spleen	Brain
Water (control)	42.4 ± 2.9	12.2 ± 2.4	22.5 ± 2.1	18.9 ± 0.7
CuO-NPs	62.5 ± 7.1*	28.8 ± 6.3*	24.2 ± 1.5	21.5 ± 1.7
CuO-NPs and BPC	59.4 ± 10.0	22.1 ± 3.5*	18.0 ± 2.5^{+}	18.8 ± 1.4
BPC	50.4 ± 5.6	10.6 ± 0.3	25.3 ± 2.2	20.8 ± 1.5

Note: *statistically significant difference from the control group; $^{+}$from the group given nanoparticles without the BPC ($p < 0.05$ by Student's t-test).

Table 6. Influence of bioprotective premedication on the cell counts in the bronchoalveolar lavage fluid (BALF) of rats exposed to different metallic nanoparticles (x ± s.e.)

Exposure to:	Number of cells ×10^6			NL/AM count ratio
	total	neutrophil leukocytes (NL)	alveolar macrophages (AM)	
24 hours after the intratracheal instillation of NiO-NPs and Mn_3O_4 + NPs				
Me-NPs	9.6 ± 1.6*	7.17 ± 1.24*	2.3 ± 0.43	3.44 ± 0.62*
Me-NPs after 4 weeks BPC administration	5.7 ± 1.49	3.36 ± 1.38*$^{+}$	2.3 ± 0.29	1.46 ± 0.54*$^{+}$
Water after 4 weeks BPC administration	3.8 ± 0.75	0.67 ± 0.21	3.09 ± 0.64	0.23 ± 0.07
Water (control)	3.8 ± 0.9	0.34 ± 0.12	3.4 ± 0.86	0.12 ± 0.05
24 hours after the intratracheal instillation of PbO-NPs + CuO-NPs + ZnO-NPs				
Me-NPs	7.93 ± 0.62*	5.86 ± 1.52*	2.07 ± 0.21	2.83 ± 0.77
Me-NPs after 4 weeks BPC administration	3.30 ± 0.53*$^{+}$	1.47 ± 0.36^{+}*	1.82 ± 0.40	1.11 ± 0.30^{+}
Water after 4 weeks BPC administration	2.18 ± 0.41	1.20 ± 0.35*	0.98 ± 0.25	1.59 ± 0.55*
Water (control)	1.40 ± 0.07	0.094 ± 0.029	1.30 ± 0.07	0.075 ± 0.024

Note: *statistically significant difference from the control group; $^{+}$from the group given nanoparticles without the BPC ($p < 0.05$ by Student's t-test).

Table 7 demonstrates that all biochemical BALF indices for AM damage (such as the release of lysosomal enzymes) or for inflammation with increased vascular permeability (increased albumin content) were also lower in rats exposed after BPC premedication. Although the intergroup difference for each index is not significant statistically, the probability of a unidirectional chance difference between the groups in all 4 indices is <0.1 (0.0625)

In the same context of pulmonary anti-NP protection, of interest are also

Table 7. Influence of bioprotective premedication on the biochemistry of the bronchoalveolar lavage fluid (BALF) of rats exposed i.t. to a Me-NP combination (x ± s.e.).

Indices	Exposure to			
	Water (control)	Me-NPs	Me-NPs after 4 weeks BPC administration	Water after 4 weeks BPC administration
Albumin, g/L	1.90 ± 0.08	2.50 ± 0.16*	2.20 ± 0.06*	1.99 ± 0.05
Amylase, IU/L	6.56 ± 1.20	49.09 ± 15.46*	27.75 ± 7.86*	9.81 ± 1.47
***γ*-Glutamyl transpeptidase, IU/L**	1.01 ± 0.52	4.02 ± 0.93*	3.64 ± 1.10	1.15 ± 0.43
Lactate dehydrogenase, IU/L	54.60 ± 10.74	91.10 ± 18.96	57.36 ± 6.59	36.20 ± 7.14

Note: *statistically significant difference from the control group ($p < 0.05$ by Student's t-test).

some data obtained by us in a chronic inhalation experiment with iron oxide nano-aerosol (Sutunkova *et al*., 2016). Airborne Fe_2O_3-NPs with a mean diameter of 14 ± 4 nm obtained by sparking from 99.99% pure iron rods were fed during 4 months, 5 times a week, 4 hrs per day into a nose-only exposure chamber for rats, while an analogous chamber was used for sham exposures. The mean (±s.e.) concentration of Fe_2O_3-NPs was equal to 1.21 ± 0.17 mg/m^3. When being out of the chambers, half of the animals were given to drink 1.5% sodium glutamate solution (which is an obligatory component of all our BPCs) instead of water. It had been repeatedly demonstrated that drinking this solution increased dramatically organism's resistance to the cytotoxicity, pulmonotoxicity and fibrogenicity of inhaled quartz dust and even decreased respective indices in control rats as well (e.g. [92]).

As follows from the results of this experiment (**Table 8**), glutamate proved to be an effective bioprotector against inhaled Fe_2O_3-NPs even if administered alone. Its protective efficacy was demonstrated in the same rats also when assessing the influence of iron oxide inhalation, with or without drinking the glutamate solution, by the activity of well known marker enzymes in the BALF. Thus, for lactate dehydrogenase activity, the average values (x ± s.e.) were 33.80 ± 2.78 IU in control (sham exposed) rats and 43.00 ± 7.39 IU in those inhaling NPs, while the same inhalation exposure with glutamate drinking provided only 26.40 ± 2.96 IU ($p < 0.05$). For γ-glutamyl transpeptidase, the respective values were 4.08 ± 0.28, 6.09 ± 0.87 and 4.02 ± 0.44 IU ($p < 0.05$).

Along with protective action of the tested BPCs on cell and organ-systemic levels, it was demonstrated also on sub-cellular and molecular ones.

In rats exposed to repeated IP injection of CuO-NPs, PbO-NPsand/or ZnO-NPs [91], transmission electron microscopy of liver, spleen, kidney, myocardium, brain, thymus and testicle tissues revealed uniform ultrastructural changes, the most frequent being vacuolisation of the cytoplasm with concentric membranous inclusions in it, demyelinizations of nervous fibres in the brain and

Table 8. Influence of glutamate solution drinking on the cell counts in the bronchoalveolar lavage fluid (BALF) of rats chronically exposed to Fe_2O_3-NPs in the inhaled air (x ± s.e.).

Exposure	Number of cells ×10⁶			NL/AM count ratio
	Total	Neutrophil leukocytes (NL)	Alveolar macrophages (AM)	
Sham (drinking water)	2.16 ± 0.22	0.22 ± 0.05	1.93 ± 0.19	0.12 ± 0.02
Sham (drinking glutamate)	1.52 ± 0.13*	0.11 ± 0.02	1.40 ± 0.13	0.09 ± 0.05
Fe_2O_3-NPs (drinking water)	1.96 ± 0.18	0.43 ± 0.07*	1.51 ± 0.18	0.32 ± 0.07*
Fe_2O_3-NPs (drinking glutamate)	1.76 ± 1.53	0.24 ± 0.04⁺	1.52 ± 0.09	0.15 ± 0.02⁺

Note: *statistically significant difference from the control group; ⁺from the group inhaling nanoparticles without the glutamate drink ($p < 0.05$ by Student's t-test).

especially damage to mitochondria with partial or complete loss of cristae (Figure 6 and Figure 7).

Using the percentage of damaged mitochondria as a semi-quantitative measure of this effect, we introduced the following scale: 0—0%, 1—to 30%, 2—over 30% to 70%, 3—over 70%. Considering not only the morpho-functional similarity of mitochondria in different cells of a given animal organism but also the repeatability of damage to the organelles in all organs and in all groups, it seemed admissible to sum up the scores across a given exposure group irrespective of organ. In this way, the mitochondrial toxicity of the triple NP combination (CuO-NP + PbO-NP + ZnO-NP) was assessed by 14 points, while the same combination against background BPC administration gave a minimal total score of 6, whereas in the control group it was equal to 2, all the differences being statistically significant ($p < 0.05$ by the Friedman rank test and the Kruskal-Wallis test).

One of the most important effects of subchronic exposures to the studied Me-NPs on the molecular level was the DNA fragmentation. All the BPCs tested by us up till now significantly attenuated this most adverse effect. An example pertaining to the experiment with Ag-NP [78] is given by Table 9.

4. Conclusions

Highly adverse effects of metallic nanoparticles on all levels from molecular to organ-systemic can be markedly attenuated by background administration of or premedication by adequately composed combinations of some bioactive agents in innocuous doses. We therefore believe that, along with decreasing exposures to nanoparticles, enhancing the organism's resistance to their adverse effects with the help of such bio-protectors can be an efficient auxiliary tool of health risk management.

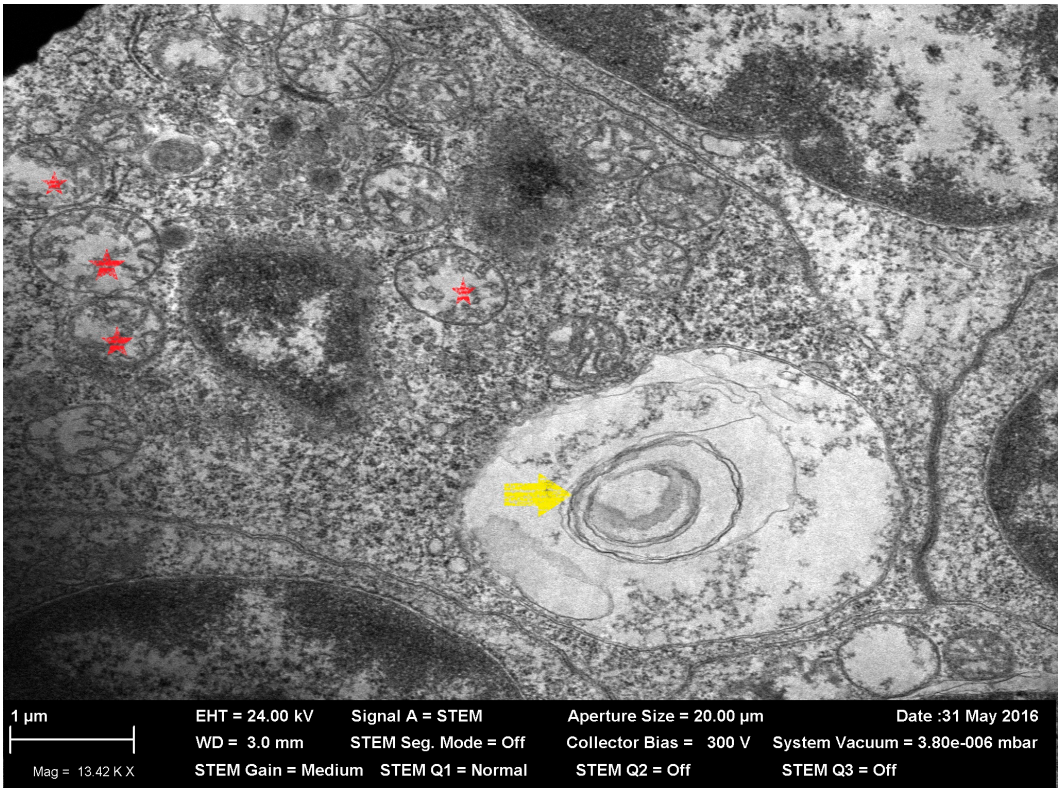


Figure 6. Concentric membranous formation and cytoplasmic vacuolization (arrow), and marked damage to mytochondria (asterisks) in a spleen cell from a rat exposed to ZnO-NPs. TEM, magnification ×13,420.

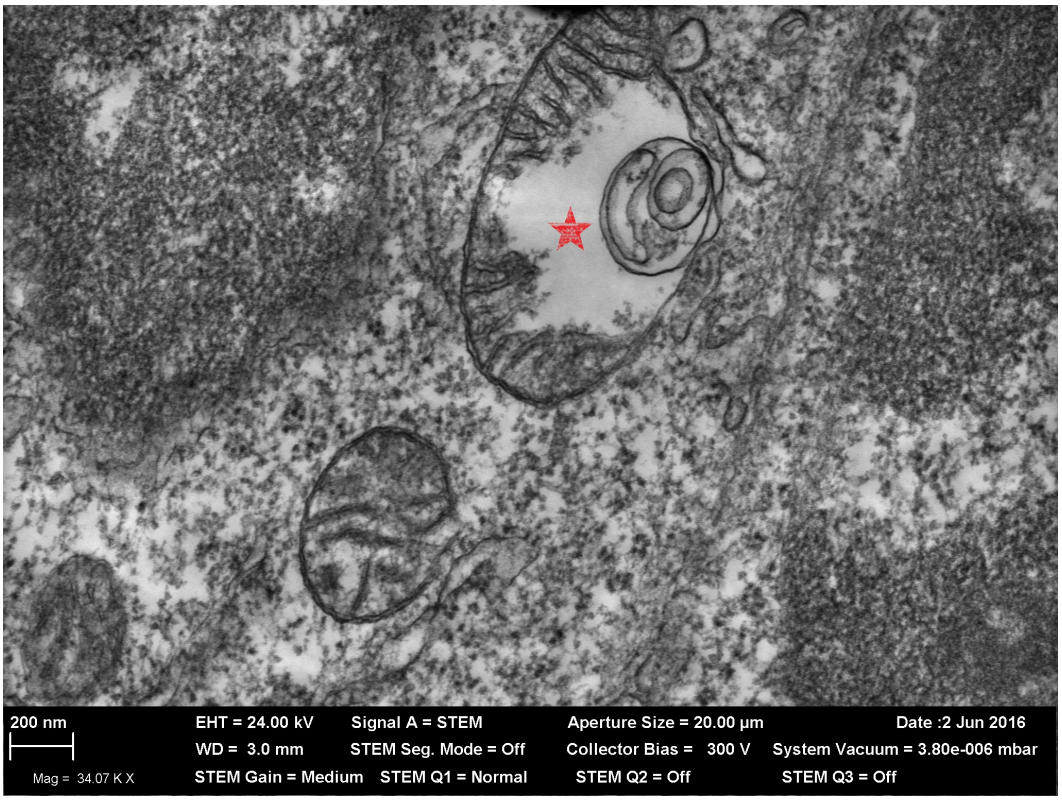


Figure 7. A partially destroyed mitochondrion (marked by asterisk) in a thymus cell of a rat exposed to PbO-NPs and ZnO-NPs. TEM, magnification ×34,070.

Table 9. Coefficients of the genomic DNA fragmentation in rats exposed to subchronic administration of silver nanoparticles with or without BPC based on the results of RAPD test (X ± s.e).

Group of given:	Tissues				
	Liver	Bone marrow	Spleen	Kidney	Nucleated cells of blood
Water (controls)	0.40 ± 0.001	0.39 ± 0.003	0.38 ± 0.002	0.39 ± 0.003	0.38 ± 0.001
Nano-particles	0.46 ± 0.002*	0.46 ± 0.032*	0.46 ± 0.001*	0.42 ± 0.008*	0.41 ± 0.012*
Nanoparticles and BPC	0.41 ± 0.011^{+}	0.37 ± 0.003^{*+}	0.42 ± 0.003^{*+}	0.40 ± 0.006^{*+}	0.39 ± 0.007

Note: *statistically significant difference from the control group; $^{+}$from the group given nanoparticles without the BPC ($p < 0.05$ by Student's t-test.

Our previous positive experience in organizing first a selective and then a large-scale "biological prophylaxis" of adverse health effects of many other toxicants makes us expect that it would be no less practicable and effective in the field of nanotoxicology as well.

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