TOXIC EFFECTS OF NICKEL NANOPARTICLES AT A SUBACUTE ORAL ADMINISTRATION TO RATS

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Nickel nanoparticles (NiNPs) are used in catalysts for hydrogenation of dietary fats, in cosmetics, insecticides, preparations for theranostics in medicine, and can also expose people occupationally in the metallurgy and mining industry. The adverse effects of long-term oral exposition to low doses of NiNPs are not well understood. **The aim of the study** was to evaluate the toxic effects of NiNPs at their prolonged oral administration to male Wistar rats

Methods

Ten groups of 12 male Wistar rats received during 92 days balanced semi synthetic diet (AIN93G) either without additions (Control group 1), or supplemented with Ni carbonate basic salt (Ni salt) at a dose 0.1;1 and 10 mg/kg b.w. as Ni (groups 2-4), or Ni-NPs preparation 1 (Ni-NP1) (groups 5-7) or Ni-NPs preparation 2 (Ni-NP2) (groups 8 -10) at the same doses respectively. At the end of the feeding period biochemical, immunological and morphological endpoints were studied.

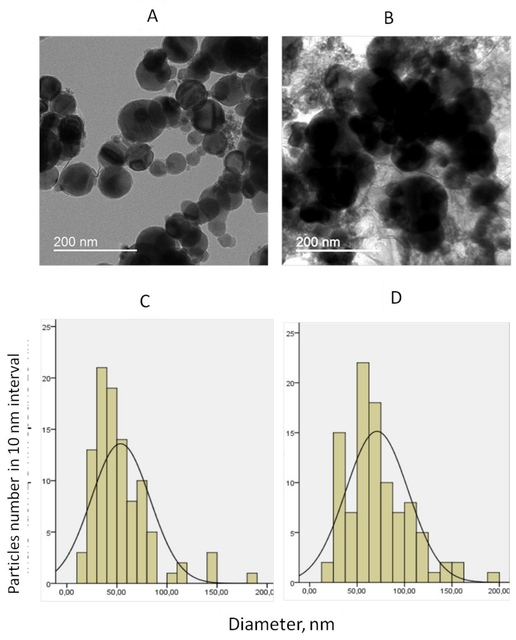

Fig.1. Ni NPs TEM image. Two preparations of NiNPs were used, which contained spherical metal Ni particles with an average diameters of 54 and 71 nm (data obtained by Ph.D. A.G. Masyutin).

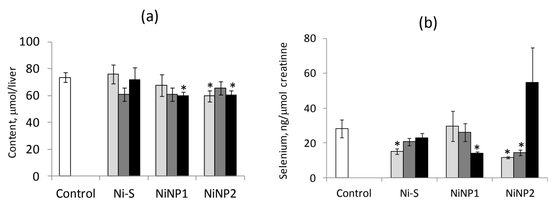

Fig.2. The content of thiol compounds (reduced glutathione) in rat's liver, µmol/organ (a); specific urinary excretion of selenium, ng/µmol creatinine (b); selenium content in blood serum, ng/ml (c). On the y-axis - values (M ± s.e.m/) in the appropriate units. *- difference with the control group is significant , p<0.05, Mann-Whitney U-test. Number of animals in groups: 6 (a): 8 (b, c).

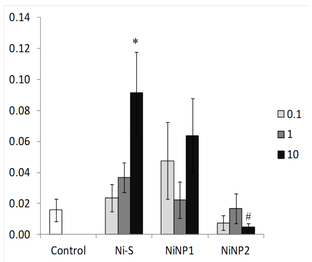

Fig.3. The content (M±s.e.m.) of the fatty acids binding protein, FABP2, in the blood serum of rats .* - the difference with the control group is significant; # - difference with nickel salt group is significant, p<0.05. Mann-Whitney U-test. The number of animals - 8 in each group.

Fig.4. Relative expression of the fibrosis genes *Timp3* (a); *MMp2* (b), *MMp9* (c) and apoptosis factor *Tp53* (d) in rat's liver. On the y-axis - expression (M±s.e.m.) in relative units (see the text of the article). * - difference with the control group is significant; # - difference with nickel salt group is significant, p<0.05, Mann-Whitney U-test. The number of animals - 6 in each group. e-h. Morphology of rat's liver: e) liver of a rat from control group; f) liver of a rat with Ni salt, 10 mg/kg b.w; g) liver of a rat with with Ni-NP1, 10 mg/kg b.w., h) liver of a rat with Ni-NP2, 10 mg/kg b.w; staining with Fuxin-picric acid (van Gieson) magnification ×200.

Table 1. Biochemical endpoints in rat's blood serum (M±s.e.m.)

Endpoint, meas.units.[1]	Control value (1st group)	Dose of Ni, mg/kg b.w.	Values in the exposed groups, Ni forms		
			Salt of Ni	Ni NP1	Ni NP2
Glucose, mmol/l	6.27±0.30	0.1	6.16±0.36	6.35±0.29	5.70±0.19
		1.0	6.13±0.43	6.65±0.25	5.85±0.12
		10.0	6.57±0.27	7.45±0.31*#	6.45±0.14
Triglycerides, mmol/l	1.65±0.17	0.1	1.54±0.32	1.77±0.31	1.04±0.09*
		1.0	1.46±0.18	1.51±0.22	1.36±0.12
		10.0	2.47±0.23*	2.17±0.24	1.24±0.25#
LDL cholesterol mmol/l	0.25±0.02	0.1	0.24±0.01	0.26±0.02	0.26±0.02
		1.0	0.32±0.03*	0.31±0.03	0.20±0.02#
		10.0	0.30±0.02	0.37±0.08*	0.21±0.02
Total protein, g/l	73.6±1.5	0.1	73.2±0.9	76.3±1.2	71.2±0.8
		1.0	76.9±0.9	78.5±0.7	72.8±0.9
		10.0	77.8±1.0	80.5±1.2	72.2±0.7
Albumines, g/l	39.4±0.4	0.1	39.0±0.4	40.5±0.4*	36.6±0.3*
		1.0	39.3±0.4	40.4±0.4	35.8±0.3*
		10.0	40.2±0.4	41.7±0.4*	35.3±0.4*
Globulines, g/l	35.1±0.9	0.1	34.1±0.8	35.9±0.9	34.7±0.7
		1.0	37.6±0.7*	38.1±0.8*	43.5±0.8*#
		10.0	37.7±0.8	38.8±1.0*	43.3±0.6*#
Uric acid, mmol/l	118±7	0.1	107±9	109±5	81±4*#
		1.0	104±4	92±6*	78±4*#
		10.0	115±10	95±5*	83±5*#
AlAT, IU/l	65.5±4.1	0.1	63.3±3.3	51.9±3.6*#	49.3±4.2*#
		1.0	62.9±3.1	64.5±5.2	52.5±3.2*#
		10.0	62.7±3.9	62.3±5.3	55.0±3.2
AsAT, IU/l	157±22	0.1	167±22	139±24	154±14
		1.0	138±24	115±27	148±14
		10.0	103±22	109±25	91±20*

Notes
[1] Number of animals is 12 in groups 1-8; 8 - in groups 9,10
* - difference with the control group is significant , p<0.05, Mann-Whitney U-test.
# - difference with nickel salt group is significant, p<0.05, Mann-Whitney U-test.

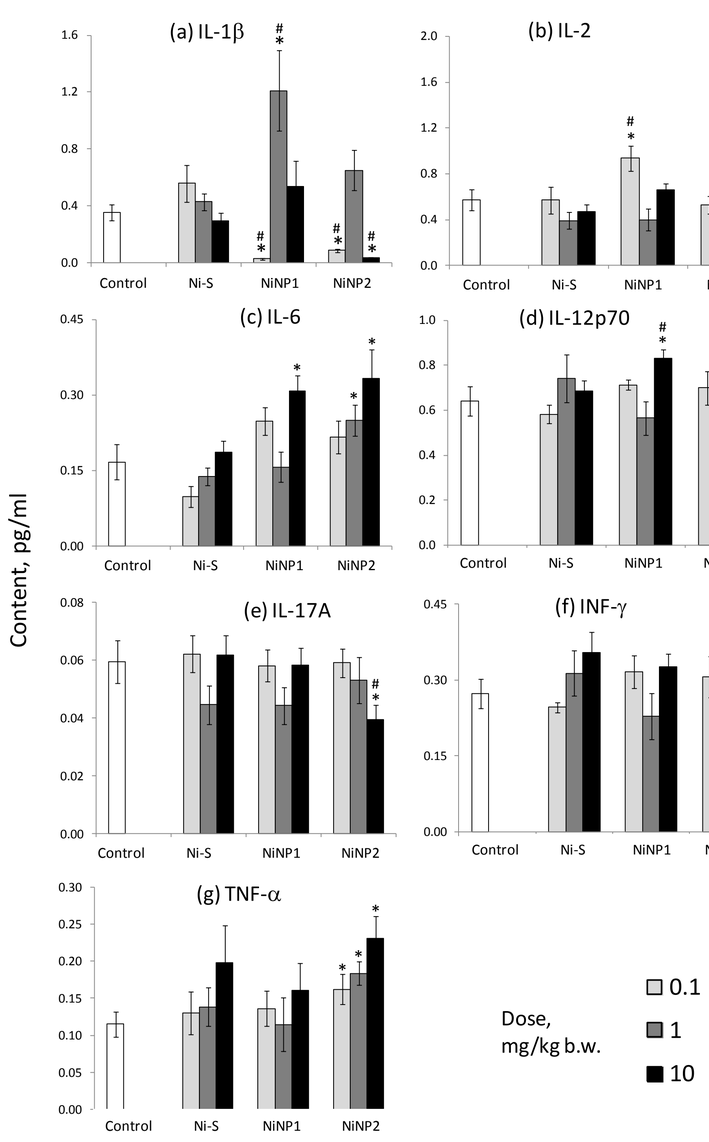

Fig.5 The levels of cytokines in the blood serum of rats: a) IL-1β; b) IL-2; c) IL-6; d) IL-12p70; e) IL-17A; e) INF-γ; g) TNF-α. On the y axis - concentration, pg/ml (M±s.e.m.) * - difference with the control group is significant; # - difference with Ni salt group is significant, p<0.05, Student's T-test and/or Mann's U-test . Number of animals - 8 in each group.

RESULTS

As a result of oral exposure of animals to NiNP1, an increase in glucose, LDL, serum albumin and globulin fraction, decrease of uric acid were noticed (Table 1). The main change produced by Ni salt, included the rise in triglycerides level. Corresponding changes in rats receiving NiNP2, were absent or less pronounced with exception of protein fractions levels. Serum AlAT an AsAT activities in experimental groups stayed mainly within normal range.

The data presented on Fig.2 show, that the stores of reduced glutathione in liver and selenium reserves in rats subjected to NiNPs were significantly depleted, at that said effects of Ni in nanoform were in some cases more pronounced than in Ni-salt exposed animals.

The increase of fatty acids binding protein (FABP2) levels in Ni salt exposed animals (Fig.3) suggested the presence of intestinal mucosal barrier violation in these animals. Corresponding effect was less pronounced in NiNP1 exposed rats and were eventually absent in NiNP2 exposed.

Oral exposure of rats to NiNP1 and NiNP2 resulted in increase of fibrosis and apoptosis markers expression (Fig.4). In rats receiving Ni salt these changes were in some cases less pronounced. Light microscopy study of liver (van Gieson staining) revealed the accumulation of collagen elements in perivascular area of tissue that was somewhat more pronounced in rats receiving Ni salt and especially NiNP1 and NiNP2 than in control.

The study showed the complex nature of blood cytokines response to nickel NPs consumed by rats (Fig.5). The maximum levels of IL-1β were achieved at the intermediate dose of Ni-NP1, IL-2 at the smallest dose of Ni-NP1 and the intermediate dose of Ni-NP2. At highest doses of both types of NPs, elevated levels of the pro-inflammatory cytokines IL-6 and IL-12 were observed, and in the case of Ni-NP2, also INF-γ and TNF-α. The latter was accompanied by a decrease in the levels of IL-1β and IL-17A. Corresponding changes under the influence of an equivalent dose of nickel salt were absent or were less pronounced. The data obtained indicate the formation of a pro-inflammatory profile of cytokines in rats exposed to nickel NPs.

The data on figs 6,7 demonstrate changes in liver and ileal mucosa structure in rats exposed to both Ni salt and two preparations of Ni-NPs. Changes in kidney morphology (data no shown) included glomerular edema and decrease of capsule to glomerulus diameter ratio and were most pronounced in Ni-salt exposed rats.

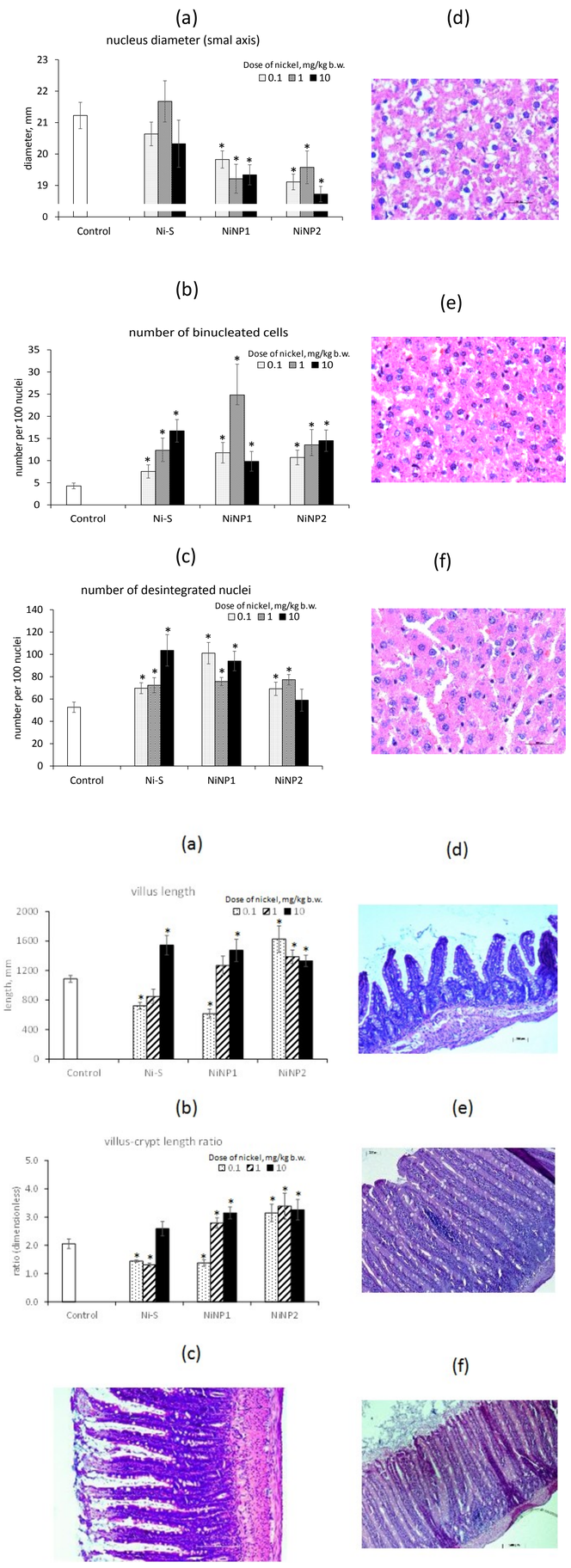

Fig.6. Liver morphology in rats. a) Mean (M±s.e.m.) diameter of nucleus (small axis); b) number of binucleated cells per 100 nuclei; c) number of disintegrated nuclei per 100 normal nuclei. d) liver micrograph of a rat from control group; e) liver of a rat with Ni salt 10 mg/kg b.w.; f) liver of a rat with NiNP1, 1.0 mg/kg b.w. Staining with hematoxylin-eosin (d-e), magnification ×400. * - difference with the control group is significant, p<0.05, Student T-test.

Fig.7. Ileum mucosa morphology in rats. a) mean (M±s.e.m.) length of villi, b) villus to crypt length ratio, c) mucosa of a rat from control group; d) mucosa of a rat with Ni salt, 1.0 mg/kg b.w.; e) mucosa of a rat with Ni salt, 10 mg/kg b.w; f) mucosa of a rat with with NiNP1, 10 mg/kg b.w. Staining with hematoxylin-eosin (d-e), magnification ×100. * - difference with the control group is significant, p<0.05, Student T-test.

CONCLUSION

The severity of the toxic effects of NiNPs depended on their size and, in some cases, were more pronounced at their low or intermediate doses than at the highest one. Some manifestations of the toxic effect of NiNPs, including immunological endpoints (cytokines levels), blood biochemistry, *Timp3* and *Tp53* expression were absent or less pronounced in animals that were subjected to a soluble Ni salt in a metal-equivalent dose. We may conclude that toxic action of NiNPs is mediated in general by Ni++ ions emission from them in biological environment, but in some degree, this may be influenced by kinetic peculiarities of NPs penetration through biological barriers if compared to soluble Ni salt. The estimate for LOAEL of NiNPs is less than 0.1 mg/kg of body weight according to the endpoints studied. This indicates the need to regulate the content of nickel in nanoform in various types of products and the environment.