

Review Article

Yttrium nitrate induces oxidative stress and cellular damage in peripheral blood

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Abstract: Rare earth elements, including yttrium, which have been used in clinical therapy and magnetic resonance imaging, have also been applied to farmlands in past decades, polluting the environment. This study aimed to investigate the effect of early exposure to yttrium nitrate on the peripheral blood of offspring. Pregnant Sprague-Dawley (SD) rats (10 per dosage group) and their offspring (10 females and 10 males per dosage group, from different dams) were gavaged with different doses of yttrium (0, 2, 8, and 32 mg/kg.bw, respectively) from gestation day 0 to postnatal day 70. The offspring were evaluated using hemograms, the apoptosis/necrosis were evaluated using alkaline comet assays, and the oxidative damage parameters of malondialdehyde (MDA), carbonyl protein (CP) and monoamine oxidase (MAO), and their antioxidative abilities, including total antioxidative capacity (TAOC), and the catalase (CAT) of the offspring's serum were used in a colorimetric determination. Rats fed 2 and 8 mg/kg.bw of yttrium have elevated platelet distribution widths, platelets, mean platelet volumes, and thrombocytocrit; but those fed 32 mg/kg.bw of yttrium had in a decrease in the number of blood cells and lymphocytes. The hemoglobin, neutrophilic granulocyte, and eosinophil counts were all reduced when the rats were fed 8 mg/kg.bw or 32 mg/kg.bw of yttrium. A dose of 32 mg/kg.bw yttrium significantly raised the levels of CP, MAO, and the cellular apoptosis/necrosis rates of the peripheral blood cells, but the TAOC and CAT levels increased. A high dose of yttrium nitrate induced oxidative stress and cellular apoptosis in the peripheral blood, accompanied by an enhanced antioxidant ability.

Keywords: Yttrium, oxidative stress, carbonyl protein, total antioxidative capacity, cellular apoptosis/necrosis

Introduction

Rare earth elements (REEs) include a set of 15 periodic elements of the lanthanide family located at the bottom of the periodic table in the top horizontal row of the f-block elements. They also include scandium and yttrium because they have similar properties to the lanthanide family of elements [1]. Since REEs possess some unique catalyst, magnetic, and optical properties, REEs are widely used in various fields, including medicine and agriculture, etc. For example, REEs have been directly used in humans for cancer, inflammation, and synovitis therapy and for diagnosis by magnetic resonance imaging [2, 3]. Fertilizers containing REEs have been also applied to farmlands in past decades [4]. China is the major producer of REEs, accounting for around 95% of worldwide REE production. However, the lack of effective and necessary regulation in China has

led to some unexpected problems, such as environmental pollution. Moreover, emerging studies have shown that REEs are closely related to human and animal health. Macrophages play an important role in removing foreign materials from the body [5]. REEs can form hydroxyl or polymer gel in the blood, which are easily swallowed by macrophages and deposited in the liver, spleen, bone marrow, and lymph nodes in the reticuloendothelial system [6]. Additionally, several REEs, including La, Ce, Pr and Nd, can pass through the placenta and affect the thymus weight, the humoral immune function, the number of antibodies produced by spleen cells, and cellular immune function. The REEs are predominantly stored in immune or immune-related organs, so the immune function is therefore rather sensitive to REE exposure. One in vitro study showed that REEs modulate immune function in primary immune cells [7]. Another in vivo study showed that a low

dose of REEs can improve immune function in animal models [8]. However, the body's immune system is vulnerable to external factors, especially in the developmental period. A high dose of REE exposure over a long period leads to the inhibition of immune function in rat models [9, 11]. In addition to immune function, hemoglobin (HGB) is also shown to be damaged by CeCl₃ in a rat model [10]. High doses of REEs have been reported to cause oxidative damage in the brain, heart and kidneys [9, 11]. However, the effects of REEs on immune cells and on the hemoglobin of peripheral blood have not yet been systematically studied in terms of neonatal exposure. This work aimed to explore the potential effects of REEs on the peripheral blood.

Materials and methods

Equipment and materials

We obtained a blood cell analyzer (Beckman Coulter Ac.Tdiff2TM, Germany), specialized equipment for comet electrophoresis (Bio-Lab, China), and digital image analysis software Comet A1.0 (Bio-Lab, China). The yttrium oxide (purity >99.99%, white granular solid) was bought from the Beijing Research Institute of Nonferrous Metals and dissolved in nitric acid to form yttrium nitrate.

Animal experiments and dosage

All studies were carried out according to the laboratory management regulations of Beijing regarding animal welfare and experimental ethics and approved by the China National Center for Food Safety Risk Assessment Standing Committee on Ethics in Animal Experimentation (2009028). All experiments were carried out in accordance with all other relevant guidelines and regulations. The present study was conducted according to the Organization for Economic Cooperation and Development (OECD) Guidelines for Developmental Neurotoxicity Study (TG426). The virgin female and male SD rats were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Three different doses of yttrium nitrate (2, 8, and 32 mg/kg.bw, calculated based on yttrium) and a control were administered to the rats by gavage. The dosing began from the initiation of pregnancy (GD 0) and ended at postnatal day (PND) 70.

Sample collection

The offspring were anesthetized with pentobarbital sodium by intraperitoneal injection and sacrificed by bleeding. On PND 70, blood was collected from the abdominal aorta of the rats (10 females and 10 males). A volume of 1 ml of blood (with anticoagulant) was used to perform the hemogram analysis. The peripheral blood lymphocytes were isolated and subjected to the comet assay. Sera isolated from 5 ml of blood (without anticoagulant) were measured for the oxidative stress parameters.

Hematological parameters

On PND 71, following an overnight period (approximately 12 h) of food (but not water) deprivation, each animal was weighed, and then anesthetized with isoflurane. Blood samples were collected from the abdominal aorta of all the animals, prior to exsanguination and necropsy, and subjected to hematology. The hematological parameters from the whole blood were measured using an automatic blood cell counter.

Apoptosis/necrosis analysis by alkaline comet assay

An alkaline comet assay kit was purchased from Huaxing Innovation Biotechnology Co., Ltd, China. Briefly, the cells were mixed with low-melting-point agarose gel and spread on frosted microscopic glass slides. The cells were subsequently lysed (1.25 M NaCl, 0.1% sodium N-lauroyl sarcosine, 50 mM Na-EDTA, 100 mM Tris-HCl, pH=10). The DNA was unwound by first soaking the slides in a strong alkaline buffer (0.6 mM Na-EDTA, 0.18 M NaOH, pH=13) and then electrophoresed in the same buffer (25 V; 400 mA, at 4°C). After staining the cells with GelRed, the comets were visualized using a lighted microscope connected to a digital camera with 510 excitation and 590 emission filters. The images were analyzed using the software Comet A1.0. A total of 50 cells per sample were scored for tail length, tail DNA percent, olive moment, and the ratio of tail/head length.

Oxidative stress parameters

All oxidative stress kits were purchased from Nanjing Jiancheng Co., Ltd, China. The catalase, monoamine oxidase, total antioxidative

Yttrium causes oxidative stress

Table 1. The effect of yttrium on the offspring's hemograms on PND 70 (mean \pm SD, n=20)

Parameters	control	2 mg/kg	8 mg/kg.bw	32 mg/kg.bw
WBC ($\times 10^9/L$)	6.91 \pm 1.95	6.45 \pm 2.34	6.13 \pm 3.21	4.15 \pm 2.89*
RBC ($\times 10^{12}/L$)	7.85 \pm 0.72	7.59 \pm 1.08	7.64 \pm 1.00	7.24 \pm 0.97
HGB (g/L)	154.1 \pm 14.0	146.2 \pm 12.2	142.2 \pm 17.6*	138.6 \pm 1.0*
PDW (%)	8.06 \pm 1.19	9.27 \pm 0.52*	9.20 \pm 0.47*	8.61 \pm 0.80
HCT (%)	44.60 \pm 3.03	44.78 \pm 5.13	43.51 \pm 5.20	40.15 \pm 9.36
PLT ($\times 10^{11}/L$)	10.21 \pm 3.09	12.64 \pm 1.43*	13.25 \pm 1.75*	9.31 \pm 3.61
MPV (fl)	7.64 \pm 0.83	8.15 \pm 0.38*	8.08 \pm 0.39*	7.72 \pm 0.57
PCT (%)	0.75 \pm 0.29	1.03 \pm 0.10*	1.07 \pm 0.15*	0.74 \pm 0.33
NEUT ($\times 10^9/L$)	0.73 \pm 0.40	0.52 \pm 0.22	0.47 \pm 0.24*	0.48 \pm 0.33*
LYMPH ($\times 10^9/L$)	6.35 \pm 2.05	5.72 \pm 2.11	5.46 \pm 2.92	3.65 \pm 2.56*
MONO ($\times 10^9/L$)	0.14 \pm 0.07	0.16 \pm 0.09	0.15 \pm 0.09	0.14 \pm 0.11
EO ($\times 10^5/L$)	8.75 \pm 4.38	5.55 \pm 3.32*	4.45 \pm 1.90*	6.83 \pm 3.82

Note: * $P < 0.05$ vs. the control group. WBC: white blood cells, RBC: red blood cell, HGB: hemoglobin, PDW: platelet distribution widths, HCT hematocrit, MPV: Mean platelet volume, PCT: thrombocytocrit, PLT: Platelets, NEUT: neutrophil count, LYMPH: Lymphocytes, MONO: Monocytes, EO: Eosinophils.

capacity, malondialdehyde, monoamine oxidase, and carbonyl protein were measured according to the manufacturer's instructions.

Statistical analysis

All variables are expressed in the form of the mean plus and minus standard deviation (SD). One-way analyses of variance (ANOVA) were used to compare the differences between groups. Before comparing the differences between two groups, a homogeneity test of variance was carried out first. The least significant difference (LSD) method was used when no significant deviations from homogeneity, including the parameters of white blood cells, red blood cells, hemoglobin, platelet distribution width, hematocrit, neutrophil count, lymphocyte, total antioxidant capacity, malonaldehyde, and carbonyl. Otherwise, we preferred Games-Howell multiple comparisons, including the parameters of monocytes, eosinophils, platelets, mean platelet volumes, thrombocytocrit, catalase, and apoptosis/necrosis ratios. A P value less than 0.05 is considered a significant difference.

Results

The effect of yttrium on the offspring's hemograms

After continuous exposure to yttrium, the offspring were analyzed for their hemograms with a blood analyzer on postnatal day (PND) 70.

The results are shown in **Table 1**. Compared with the control, the rats fed 2 mg/kg.bw and 8 mg/kg.bw of yttrium had higher platelet distribution widths, more platelets, higher mean platelet volumes, and thrombocytocrit ($P < 0.05$). However, the rats fed 32 mg/kg.bw of yttrium had reduced white blood cells and lymph ($P < 0.05$). When the rats were fed with either 8 mg/kg.bw or 32 mg/kg.bw of yttrium, the hemoglobin, neutrophil count, and eosinophils were all decreased ($P < 0.05$). The red blood cells, hematocrit, lymphocytes, and monocytes showed no difference.

Apoptosis/necrosis of karyocytes in the peripheral blood

The karyocyte apoptosis/necrosis was analyzed with the comet assay. The cells with more than 95% tail DNA were considered apoptosis/necrosis, and then the apoptosis/necrosis rate was determined. The result is graphed and shown in **Figure 1**. The apoptosis/necrosis rate (17.63 \pm 4.92) of the 32 mg/kg.bw treatment group was significantly higher than rate of the control (4.21 \pm 0.86) ($P < 0.05$).

The effect of yttrium on oxidative stress

On PND 70, the serum from the offspring was subjected to an oxidative stress parameters analysis. The results are shown in **Table 2**. Compared with the control group, the rats fed 32 mg/kg.bw yttrium had higher levels of catalase, monoamine oxidase, total antioxidant

Yttrium causes oxidative stress

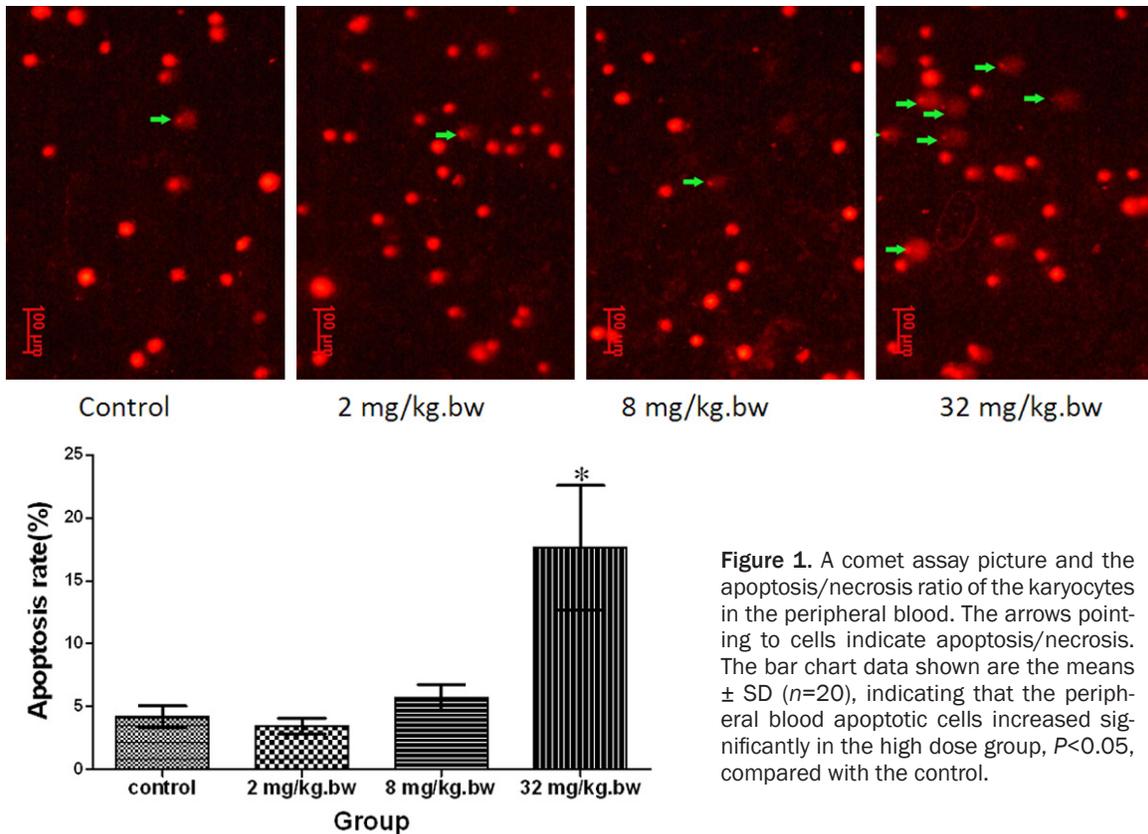


Figure 1. A comet assay picture and the apoptosis/necrosis ratio of the karyocytes in the peripheral blood. The arrows pointing to cells indicate apoptosis/necrosis. The bar chart data shown are the means \pm SD ($n=20$), indicating that the peripheral blood apoptotic cells increased significantly in the high dose group, $P<0.05$, compared with the control.

Table 2. Oxidative stress in the peripheral blood (mean \pm SD, $n=20$)

Parameters (unit)	control	2 mg/kg.bw	8 mg/kg.bw	32 mg/kg.bw
Catalase (U/ml)	0.0 \pm 17.2	4.7 \pm 7.2	8.1 \pm 6.8	28.5 \pm 17.8*
Taoc (nmol/ml)	4.1 \pm 1.9	4.8 \pm 1.6	4.7 \pm 1.3	5.8 \pm 1.4*
MDA (μ mol/l)	5.4 \pm 0.4	5.4 \pm 0.2	5.4 \pm 0.2	5.7 \pm 1.0
Carbonyl (nmol/mg)	5.7 \pm 0.7	6.2 \pm 0.9	6.3 \pm 0.7	6.4 \pm 1.4*

Note: * $P<0.05$ vs. the control group. CAT: catalase, MAO: monoamine oxidase, TAOC: total antioxidant capacity, MDA: malonaldehyde.

capacity, malonaldehyde, and carbonyl protein ($P<0.05$).

Discussion

To optimize the hormetic dose response, a wide dosage range involving multiple samples and experiments is required [12]. To avoid missing the time window of the hormetic response, multiple kinetic (dose-time) experiments will also be required. REEs can induce hormesis in plants [13]. Meanwhile, it is reported that in lactational period exposure, 2 mg/kg of REE treatment resulted in an increase in the level of IgM-PFC/spleen, and 20 mg/kg treatment increased the macrophages' K-value of the carbon clear-

ance rate and expurgational index 8. After 6 months' exposure, 0.534 mg/L of yttrium resulted in an increase in the levels of both IgM and IgG [9]. In the present study, 2 mg/kg.bw increased the levels of PLT, PDW, PCT and MPV, but not of karyocytes, on PND 70, because karyocytes are closely related to immunity [14]. Our results imply that yttrium may not promote immune cell function. Hormetic effects may become toxic with an increase in exposure dosage and time. For instance, after 6 months' exposure to 5340 mg/L of yttrium, the CD3, CD8, IgM, IgG, and CD8/CD4 levels were reduced [9]. In the present study, 32 mg/kg.bw of yttrium treatment led to a reduction in the number of both WBC and lymph, which is con-

sistent with a previous study that found that 20 and 200 mg/kg of REE treatment inhibited the offspring's immunity [15]. All the above studies indicated that long-duration exposure to high doses of REEs led to the inhibition of immune function, especially during the developmental sensitive period. Moreover, our study also demonstrated that 32 mg/kg.bw of yttrium promoted the apoptosis/necrosis of karyocytes in the peripheral blood. The maximum dose in this study was set at 32 mg based on Liu's study which showed that more than 20 mg/kg.bw induced immune-toxicity [15]. The low dose was set at 2 mg/kg.bw according to Zhou's study which reported DNA damage with a dose higher than 2 mg/kg.bw [17].

Oxidative damage is one of the important toxicity mechanisms of REEs. When a rat was fed REEs with a dose higher than 2 mg/kg.bw, DNA damage in the fetal rat was observed [11]. In the present study, both carbonyl protein and MAO were increased when the rats were fed 32 mg/kg.bw of yttrium. Since increases in the carbonyl protein and MAO levels are an important biomarker of oxidative damage [16-18], our results indicated that yttrium caused oxidative stress in the peripheral blood, which is consistent with Zhou's study [11]. Oxidative damage was found not only in the peripheral blood but also in other organs. After the rats were exposed to REEs for 6 months, the renal SOD and CAT activities in the 10.0 and 20.0 mg/kg of the yttrium feeding groups were decreased [19]. CAT can reduce the levels of H₂O₂ and free radicals. In the serum, TAOC consists mostly of non-enzymatic antioxidants and a few enzymes, and a high TAOC implies increased antioxidant capacity [20, 21]. In the present study, the increase of the CAT and TAOC levels in the 32 mg/kg yttrium-fed rats indicates that the anti-oxidative capability is still in the compensatory range.

HGB is sensitive to exogenous poisons. For example, the oxygen affinity of erythrocyte's HGB was influenced by CeCl₃, which showed the secondary structure of hemoglobin change, characterized by a gradual decline in the alpha-helix content, in dose- and feeding time-dependent manners; the 2,3-diphosphoglyceric acid (2,3-DPG) level in the erythrocytes was decreased due to its hydrolysis [7, 13]. In the present study, HGB was also decreased in a dos-

age-dependent manner, which is consistent with the change of oxidative stress and apoptosis/necrosis, implying oxidative stress and apoptosis/necrosis may affect HGB together.

In conclusion, yttrium has a negative impact on offspring rats' hemograms, including PLT, karyocytes and HGB, in a dosage-dependent manner. A high dose of yttrium induces karyocyte apoptosis/necrosis and oxidative stress in the peripheral blood, and it is accompanied by an enhanced antioxidant ability.

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Disclosure of conflict of interest

None.

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Yttrium causes oxidative stress

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