Bull. Environ. Contam. Toxicol. (2000) 64:611-616

DOI: 10.1007/s001280000047

Bulletin of Environmental Contamination and Toxicology

Genotoxicity of Lanthanum (III) and Gadolinium (III) in Human Peripheral Blood Lymphocytes

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Received: 2 August 1999/Accepted: 26 January 2000

With the widespread use of rare earth (RE) in industry and agriculture, release of RE into the environment and exposure to humans have increased significantly over the past three decades. It is estimated that coal-fired electric power plants released approximately 1840 tons each of cerium, lanthanum, and neodymium into the air within the European Community in 1985 (Goetz et al. 1982). In China, RE compounds are applied mainly in agriculture as microelement fertilizers, and this use is estimated to have spread over about 16-20× 10^6 ha of agricultural lands by 1995 (Tu et al. 1994). In recent years, as the largest potential source of exposure to humans, industrial applications of RE have attracted considerable attentions, especially gadolinium, samarium, and neodymium chelate complexes used in Magnetic Resonance Imaging contrast agents (Rocklage et al. 1991; Bondemark et al. 1994; Donohue et al. 1995).

Whether these long-time applications of RE could induce adverse effects on human health is little known, however, epidemiological data suggest this possibility. RE exposure and absorption levels of RE-refinery workers and farmers living in RE mining areas markedly increase (Brune et al. 1980; Lu et al. 1995). Symptoms of pneumoconiosis and arteriosclerosis have been observed in RE highly exposed groups in some RE-enriched or polluted areas (Husain et al. 1980; Sabbioni et al. 1982; Zhu et al. 1997).

RE elements generally are considered to be low toxicity in acute, sub-acute, and chronic tests with experimental animals or cultured mammalian cells, and this toxicity is mostly dose-related (Das et al. 1988; Seishiro and Kazuo 1996). However, genotoxicity tests of RE have not given definite results with Ames, chromosomal aberration, and micronucleus tests. Results of these tests are contradictory in that, RE have tested as non-mutagenic (Schroeder and Mitchener 1971; Ji and Cui 1988) mutagenic (Das et al. 1988; Ji and Zhang 1995; Huang et al. 1996; Yang et al. 1998) anti-mutagenic (Cui et al. 1992; Gu et al. 1994a, 1994b), and even anti-tumor (Xiao et al. 1997).

As indices of genotoxic insult, formation of single stranded DNA breaks (SSB) and unscheduled DNA synthesis (UDS) have relatively high sensitivity to xenobiotic exposure (Hasspieler et al. 1995). However, whether RE can induce formation of

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SSB and UDS has not been reported previously. In this study we report the genotoxicity of Lanthanum (III) and Gadolinium (III) in primary cultures of human peripheral lymphocytes as measured in induction of micronucleus, SSB, and UDS. La (III) and Gd (III) are selected as representatives of the groups of light and heavy RE because they are the most common elements of RE and have wide applications nowadays.

MATERIALS AND METHODS

La (III), Gd (III) stock solutions were prepared from $La(NO_3)_3 \cdot nH_2O$, $Gd(NO_3)_3 \cdot nH_2O$ (Shanghai Yuelong Nonferrous Metal Ltd., purity $\geq$ 99.9%) which were dissolved with double-distilled water. Before tests began, concentrations of RE stock solutions were determined, then a series of logarithmic concentrations of fresh test solutions were prepared by diluting stock solutions. Double-distilled water was used as the negative control.

Human peripheral blood of a healthy male adult was obtained from the Nanjing Redcross Blood Bank, China. The procedure of lymphocyte isolation was essentially as described by Albertini et al. (1982). Number of viable lymphocytes per ml was determined by trypan blue exclusion using a Neubauer haemocytometer. Cell viability was more than 95% and the yield averaged 12×10^6 lymphocytes per 10 ml fresh whole blood. Culture medium was RPMI 1640 (Gibco) pH=7.2 supplemented with Hepes (30 mM, Sigma), penicillin (100 IU / ml), streptomycin (100 µg / ml), sodium pyruvate (2 mM), and 10% heat-inactivated fetal calf serum (Gibco).

The micronucleus test was carried out with whole blood cultures. 0.3 ml of Heparinised blood was added in 4.7 ml complete RPMI 1640 medium with phytohemagglutinin (PHA, 15 µg / ml). After incubation for 24hr at 37 °C, 0.1 ml of RE test solution was added and the cultures were exposed for 48 hr. Blood was harvested, fixed, and smears prepared as Xue et al. (1992) described. Triplicate cultures were used. Blind-coded slides were examined, 2,000 lymphocytes per slide were scored, the number of micronuclei was recorded under a Nikon optic microscope, and micronucleus frequency (‰) was calculated.

Cytotoxicity of La and Gd to human primary peripheral lymphocytes were determined by 24 hr acute assay. To each well of 24-well tissue culture plates (Nunc), 1 ml of complete medium containing appropriate concentrations of RE (0.0015, 0.005, 0.015, 0.05, 0.15, 0.5 mM) and 10^6 cells were added. After incubation at 37 °C for 24 hr, cells in each well were counted and the median lethal concentration (LC50) was calculated using the Trimmed Spearman-Karber method (Hamiliton et al., 1977). The concentrations of 1/2, 1/4, 1/8, 1/16 LC50 were employed as treatments in following DNA damage assays and a minimum of quadruplicates in each treatment were performed.

A procedure for analysis of single-strand DNA breaks described by van Loon et al.

(1993) was adapted: (i) fluorescence dye was substituted by radioactively labeled ^{3}H-TdR. Lymphocytes were incubated in complete media containing PHA and ^{3}H-TdR (0.5 µCi / ml) for 48 hr at 37 °C to label DNA, then media were discarded and cells exposed in medium containing RE for 1.5 hr. (ii) RE was removed by washing cells with 3 ml of ice-cold CMF-PBS containing 2 mM EGTA before alkaline filter elution (Blazka and Shaikh, 1991). (iii) elution fractions and air-dried filters were combined with scintillation cocktail (0.5% PPO, 0.015% POPOP in 2:1 toluene:Triton X-100) and analyzed by liquid scintillation counting. Data were presented as F-values corresponding to radioactivity in elution fraction (single strand) divided by the sum of radioactivity in elution and filter fractions.

In UDS assay, lymphocytes were maintained in RPMI 1640 medium containing 0.5% heat-inactivated fetal calf serum at 37 °C for 48 hr to minimize scheduled DNA synthesis. 10 mM hydroxyurea (Sigma) was added and the cells were incubated for 1 hr to inhibit residual scheduled DNA synthesis. Medium was discarded and cells resuspended in complete medium containing RE, ^{3}H-TdR (0.5 µCi / ml), hydroxyurea, and PHA. After incubation for 4.5 hr, cells were collected onto nitrocellulose filters (0.45 µm), and washed in succession with 3 ml of ice-cold CMF-PBS containing 2 mM EGTA, 10 ml of cold 5% TCA, and 5 ml of 95% ethanol. Filters were air-dried, combined with scintillation cocktail and measured by liquid scintillation analysis. An increase in the incorporation of ^{3}H-TdR over background levels indicated the induction of UDS (Hasspieler et al. 1995).

Data were presented as mean ± standard error, and examined using one-way ANOVA and Turkey's multiple comparison.

RESULTS AND DISCUSSIONS

Table 1. Mean ± SE micronucleus frequencies of human peripheral blood lymphocytes at different RE treatment concentrations.

Concentration	Micronucleus frequency (‰)	
(mM)	La (III)	Gd (III)
0.016	1.27 ± 0.20	1.24 ± 0.22
0.040	1.50 ± 0.24	1.55 ± 0.25
0.100	1.61 ± 0.25	1.41 ± 0.16
0.250	2.60 ± 0.23	2.76 ± 0.39
0.625	5.42 ± 0.58	5.83 ± 0.39
control	1.36 ± 0.18	

Statistics analysis of Table 1 shows that there is significant difference of micronuclei frequency among treated and control groups (La, F=49.26, p<0.01; Gd, F=79.13, p<0.01), and that micronuclei frequency at concentrations of 0.25, 0.625 mM of La and Gd increased in comparison with other groups and a dose-response relationship was observed (p<0.05). This result suggests possible mutagenic effect of RE at these two treatment concentrations.

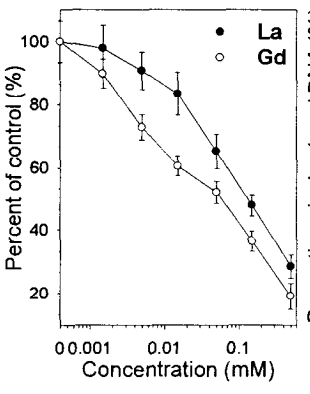

Figure 1. Cytotoxicity of La and Gd in human primary peripheral lymphocytes after 24-hr exposure. Data are presented as percentage of untreated control group (100% ± SE).

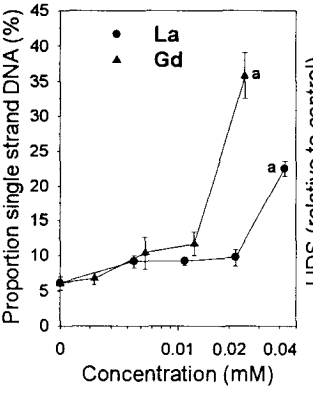

Figure 2. Responses of human primary peripheral lymphocytes to La and Gd in alkaline filter elution assay for DNA single-strand breaks. [a] significant difference from other groups at p=0.05.

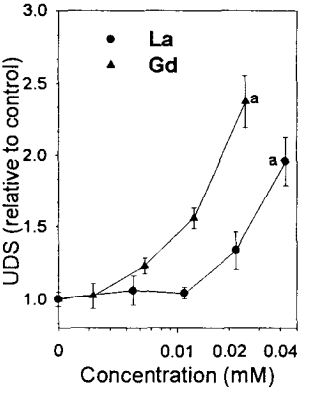

Figure 3. Responses of human primary peripheral lymphocytes to La and Gd in liquid scintillation-based assay for unscheduled DNA synthesis (UDS). [a] significant difference from other groups at p=0.05.

In 24-hr acute toxicity test of RE with primary cultures of blood lymphocytes, LC50 were La 0.108 mM (95% CI: 0.093-0.117 mM) and Gd 0.063 mM (95% CI: 0.059-0.066 mM), respectively (Fig. 1). They are not comparable to that of 20-hr LC50 with primary cultures of pulmonary macrophages, 0.052 mM for lathanum chloride, probably because those cytotoxicity studies were in culture medium without serum (Palmer et al. 1987).

Responses of lymphocytes to RE in DNA damage assays for SSB and UDS are illustrated in Fig. 2 and 3, respectively. Significant differences among treated and untreated groups were both observed in SSB (La, $F=44.64$, $p<0.01$; Gd, $F=37.04$, $p<0.01$) and UDS (La, $F=6.48$, $p<0.05$; Gd, $F=19.12$, $p<0.01$). Since SSB result from mis-repair of DNA and UDS is indicative of repair activity of DNA lesions, the co-occurrence of UDS with SSB is to be expected. Lymphocytes responded to the treatments of RE with induction of SSB and UDS at both the concentrations of 1/2 LC50 ($p<0.05$). The co-occurrence of SSB and UDS formation implies that there is the presence of damaged DNA in RE-treated cells.

Damaged DNA produced by exposure of cells to mutagens may be either lethal or repaired by DNA repair enzymes. However, replication errors in the recovery period are likely to be directly involved in inducing mutation. Therefore, it is useful for measuring DNA damage and repair to evaluate the possible mutagenic effects of chemicals (Cleaver 1984). It should be noted that this study demonstrates SSB and UDS formations at concentrations that do not increase the frequency of micronuclei, which indicates potential mutagenicity of RE at these low treated concentrations.

Micronucleus test detects the formation of micronuclei originated from chromosome break (Schimd 1976), and the DNA damage assays in this study measure the amount of single strand DNA breaks and the amount of subsequent damaged DNA repair distinct from the consequences of semi-conservative replication of DNA (Swenberg and Petzold 1979). Therefore, the results of this study suggest possible DNA cleavage by RE in human primary peripheral blood lymphocytes. It has been reported that lanthanide metal ions and their complexes can catalyze non-enzymic sequence-selective hydrolytic scission of isolated linear DNA and RNA under mild physiological conditions (Komiyama M. et al. 1994; Yashiro M et al. 1996). However, the molecular approaches of DNA cleaving by exposure of cells to RE are still unknown, further studying is desirable for the DNA cleavage on cellular level.

Acknowledgments. This research was sponsored by the National Natural Science Foundation of China, grant NO. 29677006.

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