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Effects of Rare Earth Elements on Telomerase Activity and Apoptosis of Human Peripheral Blood Mononuclear Cells

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ABSTRACT

To study the effects of rare earth exposure on human telomerase and apoptosis of mononuclear cells from human peripheral blood (PBMNCs). The blood contents of 15 rare earth elements, including La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, and Y, were measured by inductively coupled plasma–mass spectrometry. Telomeric repeat amplification protocol assay and flow cytometer analysis were carried out to analyze the telomerase activity and apoptosis of PBMNCs, respectively. The total content of rare earth elements in the blood showed significant differences between the exposed group and the control group. The rare earth exposure increased the telomerase activity and the percentages of cells in the S-phase and the G_2/M phase in PBMNCs, but it had no effect on the apoptotic rate of PBMNCs. Under the exposure to lower concentrations of rare earth elements, the telomerase activity of PBMNCs in the exposed group was higher than that of the control group, and there was no effect on the apoptotic rate of PBMNCs, but promoted the diploid DNA replication and increased the percentages of G_2/M- and S-phase cells.

Index Entries: Metals; rare earth; telomerase; apoptosis; peripheral blood.

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INTRODUCTION

China is rich in mineral resources. There has been universal attention to the effects of rare earth elements on human health with its widespread application in industry and agriculture. Up to now, most experiments have used an animal model. There are few studies about the effects of rare earth exposure on humans. The aim of this study is to investigate the effects of rare earth exposure on telomerase and apoptosis of human peripheral blood mononuclear cells (PBMNCs).

METHODS

Materials

1. The peripheral blood (PB) samples of human adults were from the rare earth exposed area and nonexposed area (as the control).
2. The rare earth mining area in Xunwu county of Jiangxi province, the largest ion-absorptive rare earth mine in China, was selected as the investigating mine area. The village is located on the foot of the rare earth mine mountain and was influenced by high rare earth background and rare earth pollutants after exploitation. Another village of Xunwu county 35 km away from the rare earth mine and with its geologic structure, social environment, and population structure comparable to the investigating mine area was selected as the control. In the present study, 30 healthy adults were randomly selected from the investigating mine area as the exposure group and 30 healthy adults were randomly selected from the control area as the control group. The rare earth contents of soil, air, and water detected by spectrometry were higher in the explored mine area than in the control area. Peripheral blood samples were obtained from a total of 60 Chinese individuals ranging from 30 to 50 years of age. The average age of the exposure group was 38.69 ± 8.02 yr and that of the control was 40.45±9.02 years old. Among the healthy volunteers, there is no difference between the ages of two groups. The proportion of male to female in the two groups is 1 : 1. All of the investigating population satisfied the following conditions: born and reside in Xunwu county, live on their own cultivating crop, and have no malignancy, infection, or genetic diseases.
3. Reagents used were Iscove's modified Dulbecco's medium (IMDM) from HyClone (Logan, UT), TRAP-Hybh Kit from Sino-American Biotechnology Co. (Shanghai, China), and Taq DNA polymerase purchased from Wako (Osaka, Japan).

Preparation of Mononuclear Cells

With informed consent, PB was drawn from the rare earth exploring individuals and the control ones. Two milliliters of PB was stored at –20°C for determining the rare earth contents. The mononuclear cells (MNCs) were isolated by Ficoll-Hypaque density-gradient centrifugation from 6 mL heparinized PB, washed three times with IMDM, adjusted the cell concentration to 10^6 cells, and stored in –20°C until use.

Detection of the Rare Earth Contents

The blood contents of 15 rare earth elements, including La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, and Y were determined by inductively coupled plasma–mass spectrometry (ICP-MS; Elmer Elan-5000.PE; USA) and the national standard substance as quality control was used.

Telomeric Repeat Amplification Protocol Assay

Telomeric repeat amplification protocol (TRAP) reactions were carried out using the Telomerase PCR ELISA kit, following the manufacturer's instructions. 10^6 MNCs were centrifuged at 400*g* for 10 min at room temperature and the supernatant carefully removed. The cell pellet was treated with ice-cold lysis buffer for 30 min, centrifuged at 14,000 rpm for 20 min at 4°C, and 2 μL supernatant were transferred to a fresh TRAP tube. At the same time, the negative and positive control tubes were prepared. Two microliters of lysis buffer without cell extract was used for the negative control. The positive control was provided in the kit. To achieve reliable results, the entire assay procedure was performed under nuclease-free conditions. The TRAP assay was performed as follows: 350 μL of TRAP mixture solution and 2 μL of sample were added into TRAP tubes and mixed well. Liquid paraffin was added and the tubes were incubated at 25°C for 30 min. The cDNA was amplified using a PCR thermal cycler (FPROG020; Techne, Cambridge, UK). The PCR reaction was 2 min at 94°C and an additional 30 s at 94°C. Annealing was carried out for 30 s at 48°C, followed by 90 s at 72°C for extension. Thirty-five cycles of PCR were performed and terminated with a final extension for 5 min at 72°C. The hybridization and ELISA procedure was as follows: For the first reaction, after the PCR reaction 100 μL of hybridization reagent B was added into each well of a microtiter plate. Then 50 μL of hybridization reagent A was added to each PCR amplifying product tube, mixed, and 25 μL of the mixture transferred to each wells of the microtiter plate. Then the negative and positive controls were set up and the plate put into the incubator at 37°C for 60 min. For washing the plate, the reaction solution was removed completely and 300 μL of washing buffer was added for 5 s. This procedure was repeated five times. For the second reaction, 100 μL of hybridization reagent C was added to each well. The plates were incubated at 37°C for 15 min and the plates washed as described earlier. To develop the color, 50

μL each of color developing reagents (Chromogen) A and B were added and incubated for color development at 37°C for 10 min. To stop the reaction, stop solution was added and the reaction was stopped. To interpret the results, using a microtiter plate (ELISA, BENLEYMKC, Fenlan) reader, the absorbance of the samples was at measured 450 nm (with a reference wavelength of approx 690 nm) 30 min after addition of the stop solution. Absorbance values was reported as the $A_{459\ nm}$ reading against the blank (reference wavelength A_{690nm}). For the negative control, if the absorbance value of negative control was lower than 0.05, 0.05 is the calculation standard, whereas if the value is higher than 0.05, the detected absorbance value is the calculation standard. Subtract the mean of the absorbance readings of the negative controls from those of the samples. Samples were regarded as telomerase-positive if the difference in absorbance *(A)* was 2.1 times higher than or equal to the absorbance value of negative control.

Apoptosis Assay and Cell Cycle Measurement

One milliliter of cell suspension from PB of the rare earth exposure group and the normal control group were collected and centrifuged for 5 min at 4°C in a Beckman J-6B centrifuge (Hettich, Tuttlingen). For the apoptotic assay, the cell pellet was resuspended; 1×10^6 cells were permeated with 70% ethanol for 30 min at 4°C and stained with ribonuclease A and propidium iodide at 37°C for 60 min. Cell DNA content analysis was carried out using the CellQuest microsolft for the assay and Modeitlt microsolft for analysis (FACSCalibur Flow Cytometer, BD Biosciences, San Diego, CA).

Statistical Analysis

Significant differences were determined by the *t*-test, *t′*-test, and F-test. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The telomere, defined in molecular terms, is the DNA at the ends of the linear eukaryotic chromosomes and usually consists of tandemly repeated simple sequences $(TTAGGG)_n$ and the proteins that bind specifically to those sequences. Telomeres protect natural double-stranded DNA from degradation, fusion, and recombination with chromosome-internal DNA *(1–3).* Telomerase can synthesize telomeric DNA, which is essential to maintain cell proliferation and differentiation. Telomeres provide a solution to the end-replication problem. Because all known polymerases require a primer and synthesize DNA from 5′ to 3′, the 3′ ends of linear DNA pose a problem to the replication machinery *(4).* Telomerase has been thought to be involved in maintaining telomere length stability in

Table 1
Comparison of Rare Earth Contents Between Exposure and Control Groups

Rare earths	Exposure group（n=30）		Control group（n=30）		P
	$\bar{X}$±s (ng/ml)	%	$\bar{X}$±s (ng/ml)	%	
La	1.2685±1.0705	36.55	0.1717±0.2061	17.77	<0.001
Ce	0.5569±0.4729	16.42	0.2017±0.1399	20.88	<0.001
Pr	0.0918±0.1367	2.65	0.0250±0.0162	2.59	<0.05
Nd	0.4055±0.5006	11.68	0.1275±0.1155	13.2	<0.05
Sm	0.1780±0.2517	5.15	0.0750±0.0394	7.77	<0.05
Eu	0.0588±0.1598	1.69	0.0167±0.0107	1.726	>0.05
Gd	0.1646±0.2873	4.74	0.0533±0.0293	5.52	<0.05
Tb	0.0383±0.0664	1.1	0.1330±0.0490	1.38	<0.05
Dy	0.1203±0.0881	3.47	0.3830±0.0190	3.97	<0.001
Ho	0.0227±0.0267	0.65	0.0158±0.0090	0.46	>0.05
Er	0.0482±0.0330	1.39	0.0367±0.0107	3.8	>0.05
Tm	0.0427±0.1757	1.23	0.0150±0.0067	1.55	>0.05
Yb	0.0782±0.0815	2.25	0.0517±0.0225	5.35	<0.05
Lu	0.0273±0.0518	0.79	0.0150±0.0798	1.55	>0.05
Y	0.3479±0.3422	10.02	0.1092±0.0601	11.3	<0.001
Total	3.4704±2.2674	100	0.9658±0.5357	100	<0.001

germline and most cancer cells, but not in normal cells. However, in the present study, it was demonstrated that telomerase activity was detectable at low levels in normal human MNCs; MNCs include T-lymphocytes (70%), monocytes (20%), B-lymphocytes (10%), and hematopoietic progenitor cells (1%) *(5).* Monocytes express no telomerase activity. B-Lymphocytes have lower levels of telomerase activity than T-lymphocytes *(6)* so PBMNC telomerase activity detected by TRAP is the main telomerase activity of T-lymphocytes.

We detected 60 PB samples including 30 healthy adult volunteers and 30 rare earth exposure ones. The total contents of rare earth in the blood of exposure and control group were 3.4704 ± 2.2674 ng/mL and 0.9658 ± 0.5357 ng/mL, respectively, showing a significant difference between the two groups. In the blood contents of 15 rare earth exposure elements, La, Ce, Dy, Y, Pr, Nd, Sm, Gd, and Yb in the exposure group were significantly higher than those in the control group (Table 1).

Telomerase activity in PBMNCs of the exposure group is higher than that in the control group (p<0.05). There were 11 out of 30 adults showing telomerase activity in the exposure group and 5 out of 30 adults in the control group. The average age of the exposure group was 38.69 ± 8.02 yr and that of the control was 40.45 ± 9.02 yr. There was no difference between the ages of two groups (p>0.05). It was found that there is a significant relationship between telomerase activity and the total contents of rare earth elements (p<0.01) (Table 2). It was found that there was a high correlation

Table 2
Relationship Analysis Between Telomerase and Some Factors

	Exposure group $\bar{X}\pm s$ (n = 30)	Control group $\bar{X}\pm s$ (n = 30)	P	Ralationship with telomerase	P
Age (years old)	38.69±8.02	40.45±9.02	>0.05	0.062	>0.05
WBC ($\times 10^9$/L)	6.157±1.8921	6.843±1.452	>0.05	0.12	>0.05
T ($\times 10^9$/L)	1.900±0.6151	1.968±4.04	>0.05	0.171	>0.05
N ($\times 10^9$/L)	4.030±1.5421	4.207±1.109	>0.05	0.241	>0.05
Total rare earth content (ng/ml)	3.470±2.267	0.966±0.536	<0.01	0.558	<0.01

Abbreviation: WBC: white blood cell; T: T-lymphocyte; N: neutrophil.

Table 3
Apoptosis and Cell Cycle of the Exposure Group and Control Group

Groups	G_0/G_1 (%)	G_2/M (%)	S (%)	Apoptotic rates (%)
Exposure group (n=30)	99.27±0.09	0.331±0.069*	0.364±0.033*	0.272±0.127
Control group (n=30)	99.91±0.02	0.058±0.016	0.128±0.013	0.203±0.179

* $p < 0.05$, compared with the control.

between telomerase activity and the total content of rare earth elements (r=0.558, p<0.001). Hiyama et al. *(7).* found that the telomerase activity signal was generally strong in PHA stimulating T-cells (80.6%). Thus, we think that rare earth exposure could improve the proliferation of T-lymphocytes. Some investigators reported that the telomerase activated was in the G_0- to G_1-phase, and we also found that the cells in the S-phase was increased by flow cytometry analysis.

The percentage of apoptotic cells was not significant different between the two groups (p>0.05), but the percentage of cells in S-phase and G_2/M-phase increased (p<0.05) (Table 3). Experimental results by Wang et al. *(8)* showed that Ce and Gd at a narrow concentration field promoted DNA synthesis and increased the cellular total protein content and S-phrase or G_2/M-phase cell proportion The role of cell apoptosis is maintaining the balance of the proliferation and dead of cells. Our investigation displays that the proportion of apoptosis was not different between the rare earth exposure group and the control group, but the S-phrase and G_2/M-phrase cells increased.

Thus, rare earth exposure could improve the activity of telomerase and increase the percentage of cells in the S-phrase and G_2/M-phrase, and has no influence on the apoptosis of PBMNCs.

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