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Hypoxanthine phosphoribosyl transferase assay of lead mutagenicity on keratinocytes

YE Shun-Hua
(School of Public Health, Shanghai Medical University, Shanghai 200032, China)

ABSTRACT An improved hypoxanthine phosphoribosyl transferase (HPRT) assay system was used to investigate the genotoxicity in human and rat keratinocytes exposed to Pb^{2+} 0.1-100 $\mu mol \cdot ml^{-1}$ *in vitro*. Autoradiography was applied to determine the number of labeled cells / cm^2 of culture with [^{3}H]TdR and liquid scintillation spectrometry was used to determine the incorporation of [^{3}H]TdR into DNA counting of 6-thioguanine (TG)-resistant cells. The ratio between the number of labeled cells in the Pb^{2+} treated group (T) and in the control group (C) was calculated. When the cells exposed to Pb^{2+} 6 $\mu mol \cdot L^{-1}$ for 4 h, the T / C ratios reached 1.75 (scintillation, S), and 2.07 (autoradiography, A) in human and 1.37 (S), and 1.77 (A) in rat cells. A positive relation existed between the concentration of Pb^{2+} and mutagenicity. Lead should be considered as a weak mutagen in human and rat keratinocytes.

KEY WORDS lead; keratinocytes; hypoxanthine phosphoribosyl transferase; mutagenicity tests; thioguanine; cultured cells

Both inorganic and organic lead compounds are carcinogenic in experimental animals[1]. There is as yet no proof that lead is carcinogenic in humans. The association of sister chromatid exchange (SCE) in peripheral lymphocytes and exposure to lead in industrial workers is still in doubt[2-5]. No change was seen in the rates of micronuclei in lead exposed V79 cells *in vitro*, but there was slight mutagenic activity *in vivo*[6]. The above results were concordant with those of the experiments from rat keratinocytes *in vitro*[7]. In this paper, a modified hypoxanthine phospboribosyl transferase (HPRT) assay was adopted for evaluation of the mutagenic intensity of human and rat keratinocytes exposed to lead *in vitro*, since keratinocyte is suitable to indentify the environmental carcinogens.

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MATERIALS AND METHODS

Culture of epidermal keratinocytes The culture technique for the human and rat keratinocytes followed Brown *et al*[8] with some modifications. Keratinocytes were isolated from skin of 10 normal human adults (33± 0.8 a, 5 M, 5 F) and 98 new bornrats (CFN strain, 1-2 d). After purified by centrifugation at 18 000× *g* for 15 min at 4℃, the basal cells were collected from the band at a density of 1.062 $g \cdot ml^{-1}$ and were suspended in normal minimal essential medium (MEM) at a concentration of 2×10^6 $cells \cdot ml^{-1}$ 0.3 ml of basal cells suspension was placed upon a cover glass 484 mm^2 in a culture dish (35 mm) and incubated in 5% CO_2 at 35℃. The medium was not changed until the cells adhered onto cover glass after about 24 h. When the culture was about 80% confluent, the medium was changed to low calcium MEM (LCMEM), which rendered the cells growing in monolayers without stratification[9].

Lead exposure The cultures were exposed lead acetate to 0.1, 1, 6, 10, and 100 $\mu mol \cdot ml^{-1}$ for 2, 4, 8, and 24 h. They were washed twice with Eagle's Buffered Saline Solution (EBSS) and incubated in LCMEM with 2% Byclone Bovine Calf Serum (BCS) at 35℃ for 3, 5, 7, and 9 d to allow expression of the mutated gene, when cultures were treated with TG 1 $\mu mol \cdot ml^{-1}$ for 2 d.

Autoradiographic counting of TG-resistant (TG^r) cells After an exposure to TG for 48 h, all cultured cells were labeled with [^{3}H]thymidine 74 $kBq \cdot ml^{-1}$ in the presence of TG for 2 h at 35 ℃. The number of labeled cells / cm^2 (area randomly selected) was counted. T / C (autoradiography, A) represented the ratio of the number of labeled cells in lead treated group (T) to that in the control group (C).

Liquid scintillation spectrometry The cultured cells, labeled with [^{3}H]TdR 74 $kBq \cdot ml^{-1}$ for 2 h at

35℃ were rinsed twice with ice-cold PBS. Then, 1 ml of 7% trichloroacetic acid was added to each dish and kept overnight at 4℃ [11]. Radioactivity was determined by liquid scintillation spectrometry (Packard Instruments). T / C (scintillation, S) represented the ratio of counts per min in the DNA of treated cells (T) to those of the control group(C).

RESULTS

T / C increased with the rising concentration of lead. The maximal value was seen at 6 μmol · L^{-1}. Owing to cytotoxicity, the higher concentrations resulted in a lower T / C (Tab 1).

Human keratinocyte cultures were exposed to Pb^{2+} 6 μmol · L^{-1} for 2, 4, 8, and 24 h on d 5 after the cells were plated. The [^{3}H]TdR incorporation into DNA and the number of labeled cells / cm^2 were the highest after exposure for 4 h.

After human keratinocytes were exposed to Pb^{2+} 6 μmol · L^{-1} for 4 h and treated with TG 1 μmol · L^{-1} for 2 d, 5 d was found to be the optimal time for expression of the mutated gene. With a longer cultivation time, more cells died while labeled cells decreased in both control and treated cultures (Tab 2).

DISCUSSION

The HPRT assay has been widely applied as a component of genotoxicity test batteries. A modified HPRT assay was adopted in this project. TG^r cells are considered to be mutant at the HPRT locus. In the modified method radioactivity was used to measure the amount of labeled cells instead of counting their number under microscope. This analytical approach reduced the labor intensity of the test. It has 3 advantages: (1) the time needed for carrying out the determination is greatly reduced. (2) the method is easy to control, simpler than that of autoradiography, thus making the assay more attractive as a

Tab 1. Incorporation of [^{3}H]TdR into DNA of keratinocytes after exposed to Pb^{2+} for 4 h. n = 12 samples from one man and 10 samples pooled from 98 rats. $\bar{x} \pm s$. $^*P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* control.

Cells	Pb^{2+}, μmol · L^{-1}	Incorporation of [^{3}H]TdR into DNA dpm / ml extract	T/C	Labeled cells / cm^2	T/C
Man	0	5 270± 230	1.00	9.9± 5.2	1.00
	0.1	5 770± 2 510*	1.10	11.0± 5.6*	1.11
	1	7 080± 1 150*	1.34	10.4± 5.2*	1.05
	6	9 230± 1 920***	1.75	20.4± 6.0**	2.07
	10	8 310± 1 840**	1.58	12.8± 3.0*	1.30
	100	6 770± 380*	1.27	6.0± 3.0*	0.60
Rat	0	4 350± 1 000	1.00	9.7± 2.1	1.00
	0.1	4 920± 1 580*	1.09	10.6± 5.9*	1.22
	1	4 880± 1 350*	1.12	11.8± 3.9*	1.36
	6	5 920± 1 150***	1.37	15.4± 2.3***	1.77
	10	3 150± 460*	0.73	5.7± 1.7*	0.66

Tab 2. Incorporation of [^{3}H]TdR into DNA of human keratinocytes after exposed to Pb^{2+} 6 μmol · L^{-1} and treated with 6-TG. n = 16 and 8 samples pooled from 5 women and 4 men, respectively. $\bar{x} \pm s$. $^*P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* control.

Time		Samples	Incorporation of [^{3}H]TdR into DNA dpm / ml extract			Labeled cells / cm^2		
			Treated	Control	T / C	Treated	Control	T / C
Exposure / h	2	16	8 080± 1 000*	7 770± 1 150	1.03	42± 10*	41± 11	1.02
	4	16	9 690± 1 150***	7 380± 770	1.30	50± 12***	39± 10	1.28
	8	16	9 040± 1 190*	7 630± 730	1.18	44± 11*	38± 10	1.15
	24	16	9 120± 1 720*	7 690± 1 420	1.18	45± 12*	42± 10	1.07
Expression / d	3	8	11 810± 3 850*	13 150± 4 380	0.90	43± 11*	38± 10	1.02
	5	8	8 730± 1 230**	7 920± 1 150	1.10	38± 8***	27± 6	1.4
	7	8	6 730± 1 770*	6 650± 1 920	1.00	21± 4*	18± 3	1.20
	9	8	5 230± 1 540*	5 080± 1 340	1.00	6± 1*	7± 1	0.85

screening tool for mammalian cell mutagens and the approach is more objective than the autoradiographic one. When cultures of human and rat keratinocytes were exposed to Pb^{2+} 6 $\mu mol \cdot L^{-1}$ for 4 h *in vitro* and then, were incubated in the presence of TG 1 $\mu mol \cdot L^{-1}$ for 2 d, the incorporation of [^{3}H]TdR and the number of labeled cells / cm^2 increased 1.37 (rat, S), 1.75, and 1.77 (rat, A), 2.07 folds over the control values, respectively. The maximal responses both in human and rat keratinocytes *in vitro* were observed at Pb^{2+} 6 $\mu mol \cdot L^{-1}$. When the concentration of Pb^{2+} was raised to 10 $\mu mol \cdot L^{-1}$, the number of labeled cells and the level of incorporation of tracer into DNA decreased rapidly due to cytotoxicity, resulting in a lower T / C value. The results showed that human keratinocytes are concordant with rat cells, and human keratinocytes are more sensitive than the rat cells ($P < 0.05$).

Bradley, *et al*[11] established a T / C of 3 as the minimum value for a positive response in the HPRT assay. Although the highest value of T / C obtained in this study was less than 3, it appeared that lead should be considered as a weak mutagen since at Pb^{2+} 6 $\mu mol \cdot L^{-1}$ the treated group showed values for percentage of labeled cells, and the incorporation of tracer into DNA that were signficantly higher than those of the controls ($P < 0.05$).

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用次黄嘌呤磷酸核糖转移酶试验来检测铅对角朊细胞的诱变性

R 979.5

叶舜华 (上海医科大学公共卫生学院，上海 200032，中国)

摘要 以改良的次黄嘌呤磷酸核糖转移酶检测系统研究人及大鼠角朊细胞体外接触铅的遗传毒性. 放射自显影法观察[^{3}H]TdR 掺入抗 6-TG 标记细胞数和液体闪烁仪测定 DNA 的放射量，计算实验组标记细胞(T)和对照组细胞(C)的比值，当 Pb^{2+} 6 $\mu mol \cdot L^{-1}$ 与细胞接触时，T / C 值分别为 1.75，2.07 (人)和 1.37，1.77 (鼠). Pb^{2+}浓度与突变性具有正相关. 铅呈弱诱变性.

关键词 铅; 角质细胞; 次黄嘌呤磷酸核糖转移酶; 诱变性试验; 硫鸟嘌呤; 培养的细胞