Can rare metal elements show co-genotoxic effects by inhibiting DNA repair?

ABSTRACT

Along with increasing relevance of rare earth (RE) elements in industrial technology, the risk of their environmental release and occupational exposure on human health is of concern. Although many toxicological studies were reported for REs, it is not known how they affect DNA repair. In this study, the effects on DNA repair of all RE ions except radioactive Pm were studied. Human lymphiblastoid WTK1cells were irradiated to UV followed by 2h exposure to each RE with and without DNA repair inhibitor cytosine-1β-D- arabinofuranoside (araC), and then single strand breaks (SSBs) were detected by the comet assay. SSBs are generated in the incision step of nucleotide excision repair (NER) of pyrimidine dimers and disappear in the re-synthesis step of NER. Seven REs, Yb, Lu, Dy, Er, Sc, Pr, and Ce, enhanced comet positive response without araC but not with araC, suggesting that araC is antagonistic to the 7 REs. Since araC inhibits re-synthesis step of NER, they would inhibit the re-synthesis step of NER. Six REs, Tm, Sm, Tb, Gd, Eu, and Y suppressed comet positive responses with and without araC, suggesting that they decreased comet assay detectable SSBs. Therefore, these 6 REs are considered to inhibit the incision step of NER. Only La decreased tail length without araC but increased with araC, suggesting that La increased comet assay detectable SSBs. Therefore, only La would enhance the incision step of NER. Neither Nd nor Ho affected tail length with or without araC.

Keywords: Co-genotoxic potential; incision step;re-synthesis step; inhibition of nucleotide excision repair; rare earth element.

1. INTRODUCTION

Rare earth (RE) elements involve scandium, yttrium and 15 lanthanides and are indispensable materials for improving the performance of electronic products such as storage batteries, light emitting diodes, and magnets. RE elements, especially lanthanoids, exhibit physically unique properties because their electron configurations are different from those of ordinary elements. It is used as a material for hydrogen storage alloys, secondary battery raw materials, optical glass, strong rare earth magnets, phosphors, and abrasives. Mechanical properties are improved by adding a small amount to the magnesium alloy [1]. Along with their increasing relevance in industrial technology, the risk of environmental release and occupational exposure of REs on human health is of concern [2–4]. Considering that we are always exposed to various kinds of environmental mutagens, interactions between them might present a serious problem to our health. β-Carbolines and heterocyclic amines, such as harman, Trp-P-1, and Trp-P-2, show co-genotoxic activity in human cells by inhibiting DNA repair [5]. Not only those organic compounds but an inorganic metal ion, Cd^{2+} also shows co-genotoxic activity by inhibiting nucleotide excision repair (NER) [6]. Although many toxicological studies using animal models and cultured cell lines were reported for REs [7], it is not known whether they can show co-genotoxic activity by inhibiting NER. In this study, we examined how all RE ions, except radioactive promethium, affect NER.

2. MATERIAL AND METHODS

2.1 Reagents

The rare earth metal salts used were $ScCl_3$, Y $(NO_3)_3$, $La(NO_3)_3$, $CeCl_3$, $PrCl_3$, $Nd(NO_3)_3$, , $SmCl_3$, $Eu(NO_3)_3$, $GdCl_3$, $TbCl_3$, $DyCl_3$, $HoCl_3$, $Er(CH_3COO)_3$, $ErCl_3$, $TmCl_3$, $YbCl_3$, and $LuCl_3$. All reagents used were of the highest grade commercially available.

Cells: *TK*$^{+/-}$ heterozygotes of WTK1 human lymphoblastoid cells exhibiting mutant-type *p53* (kindly provided by Dr. Honma, National Institute of Health Sciences, Tokyo) were used. WTK1 cells were maintained using RPMI 1640 medium (Nissui Pharmaceutical Co., Ltd.) supplemented with 10% horse serum (SAFC Biosciences), 200 μg/mL sodium pyruvate and 200 μg/mL streptomycin at 37^{o}C under a 5% CO_2 atmosphere.

2.2 Cell treatment with mutagens

This study was conducted as the experimental design described in our previously study [8,9]. One mL of cell suspension in saline (5 x 10^5 cells/mL) in a 6-cm dish was irradiated with a germicide lamp (National GL15, 15 W, Matsushita Electric Industrial Co., Japan) at 25 ·W/cm^2 the UVC.

2.3 Comet assay

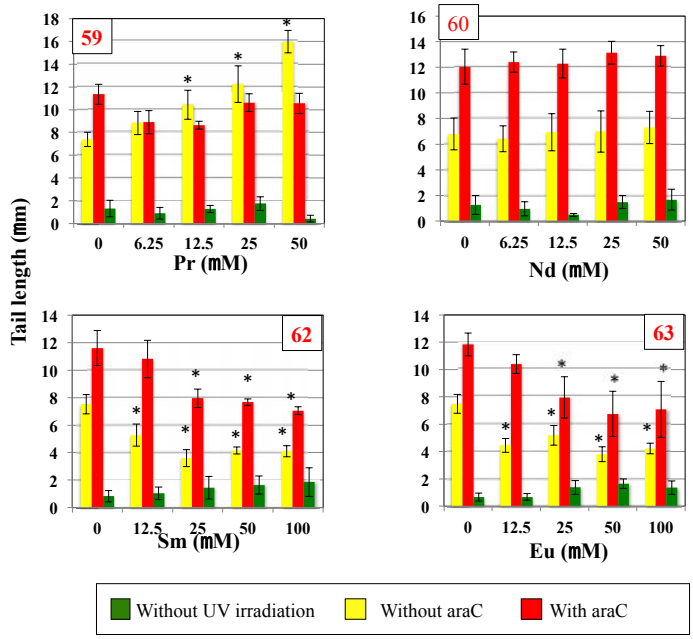
Cells exposed to UVC were post-treated with each RE for 2 h in the presence or absence of the DNA repair inhibitors cytosine-1-β-D-arabinofuranoside (araC, 1.8 mM). AraC (Wako Pure Chemical Industries, Ltd.) was dissolved in physiological saline. Exposed cells were sampled immediately after chemical treatment and the percentage of viable cells was measured by the trypan blue exclusion test. Relative survivals (survivals of treated cells compared with that of an untreated control cells) were obtained.

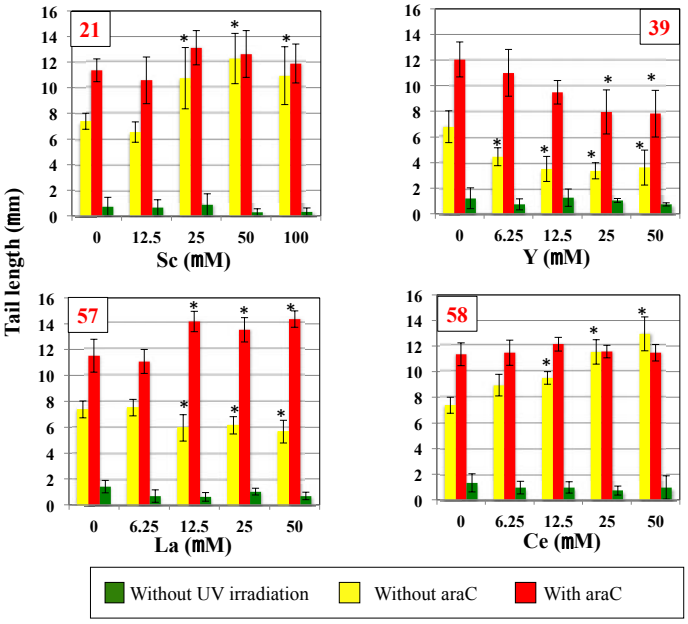
Sampled cells were suspended in 1% agarose-LGT at 5×105 cells/75 μL, and 75 μL of cell suspension was immediately deposited on a fully frosted slide (Matsunami Glass Ind., Ltd., Osaka, Japan), which was coated with 1% agarose GP-42 and then covered with another slide glass. The slides were placed so as to allow the agarose to gel. The samples on the slides were then immediately exposed to a lysing solution (pH 10) of 2.5 M NaCl, 100 mM EDTA disodium (Na_2EDTA), 10 mM Trizma, 1% sarkosyl, 10% DMSO and 1% Triton X-100, and left at 4 ^{o}C for 1 h. The slides were then placed on a horizontal gel electrophoresis platform and covered with pH >13 alkaline solution composed of 300 mM NaOH and 1 mM Na_2EDTA. The slides were left in solution at 0 ^{o}C for 20 min to allow unwinding of the DNA and expression of alkali-labile sites to occur. The power supply was set at 25 V and 250 mA. The DNA was subjected to electrophoresis at 0 ^{o}C for 20 min and the slides were rinsed with 400 mM Trizma (pH 7.5) to neutralize the excess alkalinity. Each slide was stained with 50 μL of 20 μg/mL ethidium bromide (Wako Pure Chemical Industries, Ltd.) and covered with a cover slip. Fifty cells on one slide per dose (one slide was prepared for each dose) were examined and photographed (black and white ASA 400 Fuji film) at 200× magnification using a fluorescence microscope (Olympus) equipped with a G filter. The tail length

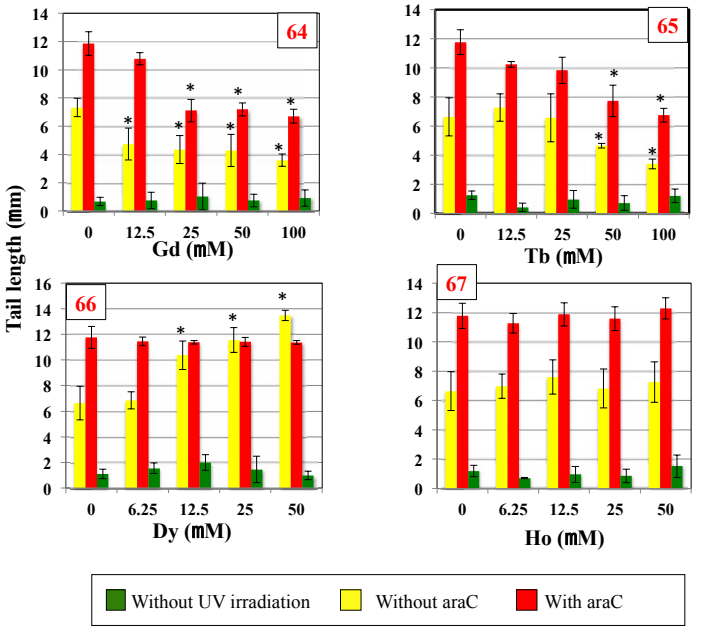
of the comet image was measured. The effect of chemical treatment on tail length was analyzed using ANOVA and the Dunnett test.

3. RESULTS

The effects of REs on the tail length in UV exposed WTK1 cells are shown in Fig 1 and summarized in Table 1. Relative survivals in WTK1 cells irradiated to UV followed by RE exposure are shown in Table 2. Sc, Ce, Pr, Dy, Er, Yb, and Lu increased tail length statistical significantly in the absence of araC but did not affect tail length in the presence of araC. Y, La, Sm, Eu, Gd, Tb, and Tm decreased tail length statistical significantly in the absence of araC. In the presence of araC, they decreased tail length statistical significantly except for La, but La on the contrary, increased tail length. Neither Nd nor Ho affected tail length with or without araC. Relative survival was ≥70% with and without DNA repair inhibitors, showing that observed decrease and increase in tail length were not due to cytotoxic effect of REs. REs did not affect tail length in UV-unexposed WTK1cells, suggesting that they do not have genotoxic potential in WTK1 cells.







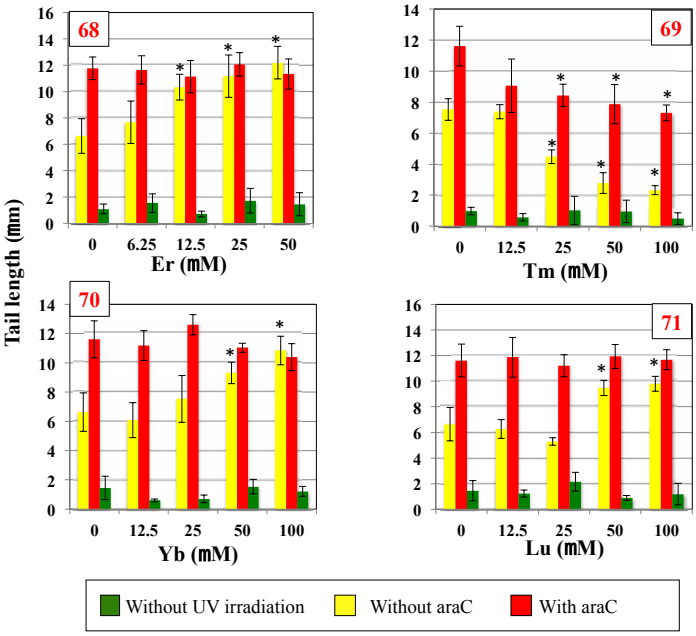


Fig. 1. Effects of post-treatment with REs on the tail length in WTK1 cells.

Cells were exposed to UV were post-treated with REs for 2 h. The error bars indicate standard errors of the mean. Numbers in red are atomic numbers of REs.

**Significant difference from RE-untreated control: $p<0.05$*

Table 1. Summary of effects of REs on comet positive response by UV.

Atomic No.	REs	-araC*	+araC*	Category**
21	Sc	E	—	1
39	Y	S	S	2
57	La	S	E	3
58	Ce	E	—	1
59	Pr	E	—	1
60	Nd	—	—	4
62	Sm	S	S	2
63	Eu	S	S	2
64	Gd	S	S	2
65	Tb	S	S	2
66	Dy	E	—	1
67	Ho	—	—	4
68	Er	E	—	1
69	Tm	S	S	2
70	Yb	E	—	1
71	Lu	E	—	1

*E, enhance; S, suppress; —, no effect **see discussion for the category

Table 2. Cytotoxic effects of post-treatment with REs in WTK1 cells.

REs	araC	Relative survival (%, mean ± SD)					
		Dose of Res (μM)					
		0	6.25	12.5	25	50	100
Sc	-	100		100.5 ±8.82	96.0 ± 6.22	98.9 ± 3.82	96.7 ± 4.81
	+	100		95.7 ± 8.85	94.0 ± 7.83	93.4 ± 9.71	86.9 ± 4.50
Y	-	100		99.1 ± 3.53	99.0 ± 5.37	95.3 ± 10.9	103.8 ± 3.41
	+	100		92.9 ± 1.52	93.7 ± 2.25	92.6 ± 5.69	89.7 ±2.50
La	-	100	99.5 ± 6.49	90.3 ± 14.8	77.1 ± 9.46	84.2 ± 7.02	
	+	100	106 ± 5.94	95.6 ± 10.4	95.7 ± 0.519	97,1 ± 5.70	
Ce	-	100	94.4 ± 2.32	98.6 ± 5.05	92.3 ± 4.53	92.8 ± 2.82	
	+	100	90.5 ± 2.21	93.6 ± 2.34	95.3 ± 3.84	97.5 ± 2.08	
Pr	-	100	97.3 ± 4.23	98.5 ± 4.21	96.9 ± 6.16	78.7 ± 2.22	
	+	100	100.2 ± 4.21	97.5 ± 7.73	90.7 ± 14.9	79.9 ± 12.0	
Nd	-	100	97.2 ± 6.36	93.2 ± 7.11	98.6 ± 10.3	93.7 ± 9.81	
	+	100	98.6 ± 8.42	99.1 ± 7.68	90.0 ± 7.88	90.2 ±7.96	
Sm	-	100		92.4 ± 2.79	95.2 ± 8.24	100.8 ± 8.95	87.7 ± 3.28
	+	100		94.4 ± 8.19	93.7 ± 4.63	83.0 ± 5.10	89.6 ± 5.12
Eu	-	100		94.3 ± 4.08	99.2 ± 7.16	84.9 ± 12.3	89.8 ± 4.50.
	+	100		93.9 ± 10.00	95.3 ± 13.2	90.9 ± 3.89	90.4 ± 11.4
Gd	-	100		99.0 ± 3.12	97.0 ± 6.18	92.2 ± 5.68	85.6 ± 6.67
	+	100		97.3 ± 5.72	95.3 ± 10.6	100.9 ± 5.13	100.4 ± 8.29
Tb	-	100		102.2 ± 6.04	84.9 ± 10.7	70.4 ± 1.93	58.1 ± 2.72
	+	100		101.8 ± 2.83	90.6 ± 6.04	81.0 ± 4.78	75.2 ± 3.93
Dy	-	100	105.1 ± 4.16	87.4 ± 7.13	79.1 ± 2.08	74.0 ± 3.31	
	+	100	105.6 ± 5.08	102.2 ± 3.24	101 ± 4.21	96.1 ± 4.24	

Ho	-	100	101.9 ± 6.32	87.67 ± 7.15	92.1 ± 9,88	96.6 ± 6.77	
	+	100	98.9 ± 6.68	103.8 ± 1.91	95.1 ± 3.57	90.2 ± 0.05	
Er	-	100	99.1 ± 3.31	95.3 ± 4.12	91.7 ± 3.66	73.3 ± 6.53	
	+	100	100.2 ± 3.45	101.9 ± 1.34	96.9 ± 6.16	79.5 ± 8.35	
Tm	-	100		101.9 ± 2.82	97.0 ± 6.27	90.1± 2.73	89.8 ± 8.84
	+	100		91.1 ± 2.91	82.9 ± 3.80	88.5 ± 6.26	95.7 ± 11.1
Yb	-	100		101.8 ± 3.25	95.7 ± 5.97	76.0 ± 6.55	79,8 ± 6.83
	+	100		84.5 ±4.25	96.1 ± 6.47	95.1 ± 3.66	87.6 ± 2.08
Lu	-	100		99.2 ± 5.00	88.7 ± 4.45	95.6 ± 6.67	95.8 ± 2.37
	+	100		90.5 ± 2.71	91.0 ± 5.59	93. 3 ± 12.64	96.9 ± 2.11

4. DISCUSSION

Based on the obtained results, studied REs are classified into four categories (Table 1). Seven REs, Yb, Lu, Dy, Er, Sc, Pr, and Ce, are classified into category 1. They enhanced comet positive response without araC but not with araC. Six REs, Tm, Sm, Tb, Gd, Eu, and Y, are classified into category 2. They suppressed comet positive responses with and without araC. The comet assay detects single strand breaks (SSBs) produced as initial lesions and also those that are generated during the repair of initial lesions such as alkylated bases, bulky base adducts and pyrimidine dimers [10]. It is known that pyrimidine dimers are repaired by NER, which consists of the following four steps: recognition of the DNA lesion, excision of a 24–32 nucleotide stretch containing the lesion by dual incision of the damaged DNA strand on both sides, filling in of the resulting gap by DNA polymerase and ligation of the nick. During the process, SSBs are produced as intermediates, which can be visualized as a comet tail in the comet assay. Therefore, the inhibition of SSB formation in the incision step and promotion of SSB disappearance in re-synthesis step would result in the suppression of comet positive responses. In the case of REs in category 2, their effects with araC are the same to those without araC, suggesting that their effects are independent to the re-synthesis step. Therefore, the suppression of comet responses by them would reflect the decrease in comet-detectable SSB by the inhibition of initiation step. The DNA re-synthesis inhibition by araC is due to either direct inhibition of DNA polymerase when araCTP is bound to the dCTP binding site of the enzyme, or indirect inhibition through araCMP incorporation into a repaired region of DNA rendering it unsuitable for further polymerase action [11]. In the case of REs in category 1, their effects on comet responses were canceled by araC, suggesting that araC is antagonistic to them. Therefore, they are considered to inhibit re-synthesis step to increase tail length. Only La decreased tail length without araC but increased with araC. La in category 3 enhanced comet responses when re-synthesis step is cancelled by araC, suggesting that it promotes the production of SSBs in the incision step. Produced SSBs disappear in the re-synthesis step following to the incision step. Like as La, aqueous extracts of *Sophora japonica* L. and *Connarus ruber* have been shown to increase and decrease tail length with or without araC, respectively [8, 9]. Both aqueous extracts were discussed to enhance the incision of GGR sub-pathways in NER (where SSBs are formed), followed by SSB rejoining. Like as both aqueous extracts La could be considered to enhance the incision of GGR, from which only La has an anti-genotoxic but not co-genotoxic effect. RE ions also have nematicidal activities as they strongly perturb the embryonic development of the nematode, *Caenorhabditis elegans* [12]. Although it increased with increasing atomic number of lanthanide ions, the correlation between their effects on NER and atomic number was not observed. Inorganic metal ions, such as Fe^{2+}, Cu^{+} and Zn^{2+}, act as co-factors of various proteins including respiratory enzymes, transcription factors, etc. that are essential for cellular metabolism. Metal complexes with biological activity are of increasing importance in medicine as potential alternatives for biologically active organic compounds, which often show severe side effects [13]. It has been reported that xanthine derivatives, such as caffeine, an inhibitor of post-replication repair, enhance the antitumor actions of cisplatin, UV, and X-rays [14, 15]. Although there are still many unclear points about this mechanism, the inhibition of DNA repair is considered as one possible mechanism [16]. Therefore, RE ions are presently shown to inhibit DNA repair, which might suggest the possibility of their medicinal applications as antitumor drug enhancers.

5. CONCLUSION

Except for Nd and Ho, REs affect NER by the different mode of mechanisms. Seven REs inhibit the re-synthesis step of NER and 6 REs inhibit the incision step of NER. Although those 13 REs inhibit NER, only La enhances he incision

step of NER. To our knowledge, this is the first report that shows REs have inhibitory effects on NER by different modes of mechanisms.

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