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Cytotoxicity and mutagenicity of retinol with ultraviolet A irradiation in mouse lymphoma cells☆

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Abstract

Vitamin A (all-*trans*-retinol; retinol) is an essential human nutrient and plays an important role in several biological functions. However, under certain circumstances, retinol treatment can cause free radical generation and induce oxidative stress. In this study, we investigated photocytotoxicity and photomutagenicity of retinol using L5178Y/*Tk*^{+/-} mouse lymphoma cells concomitantly exposed to retinol and ultraviolet A (UVA) light. While the cells treated with retinol alone at the doses of 5 or 10 µg/ml in the absence of light did not increase the mutant frequency (MF) in the *Tk* gene, the treatment of the cells with 1–4 µg/ml retinol under UVA light (1.38 mW/cm² for 30 min) increased the MF in the *Tk* gene in a dose-responsive manner. To elucidate the underlying mechanism of action, we also examined the mutational types of the *Tk* mutants by determining their loss of heterozygosity (LOH) at four microsatellite loci spanning the entire chromosome 11 on which the *Tk* gene is located. The mutational spectrum for the retinol + UVA treatment was significantly different from those of the control and UVA alone. More than 93% of the mutants from retinol + UVA treatment lost heterozygosity at the *Tk1* locus and the major type (58%) of mutations was LOHs extending to *D11Mit42*, an alternation involving approximately 6 cM of the chromosome, whereas the main type of mutations in the control was non-LOH mutations. These results suggest that retinol is mutagenic when exposed to UVA in mouse lymphoma cells through a clastogenic mode-of-action.

Keywords

Retinol; UVA; Mouse lymphoma assay; Photomutagenicity; Loss of heterozygosity

☆The views presented in this article do not necessarily reflect those of the US Food and Drug Administration.

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1. Introduction

Vitamin A (all-*trans*-retinol; retinol) is an essential human nutrient because it cannot be synthesized de novo within the body (Bendich and Langseth, 1989), and belongs to a family of molecules known as retinoids. One of the essential roles of vitamin A is in the maintenance of normal skin function, including the regulation of epidermal cell growth and differentiation (IARC, 1998; Ries and Hess, 1999; Tee, 1992). Although vitamin A in skin is predominantly derived from the diet, skin care products containing vitamin A are increasingly used as a source of cutaneous vitamin A in some populations, because topical application of vitamin A is believed to have beneficial effects on human skin. People using these products are unavoidably exposed to sunlight. However, the possible toxic effects of vitamin A on skin exposed to sunlight have not been investigated fully.

Vitamin A is commonly viewed as an antioxidant agent. However, under certain conditions, vitamin A can have pro-oxidant activity (Murata and Kawanishi, 2000; Polyakov et al., 2001), with the rate of radical generation depending on several factors including the concentration of the antioxidant, the partial pressure of oxygen, the presence of metal ions, the availability of ancillary antioxidant systems to remove reactive oxygen species (ROS), and UV exposures (Fu et al., 2007). It has been reported that retinol induces oxidative stress on different cellular constituents, leading to increased lipid peroxidation, protein carbonylation, and DNA oxidative damage (Dal-Pizzol et al., 2001a,b; Pasquali et al., 2008). Up to 5 μ M of retinol in cells is considered to be within the normal physiological range, and the deleterious effects are observed with the administration of higher concentrations (Gelain and Moreira, 2008). In addition, pre-treatment with antioxidants (e.g., *N*-acetyl-cysteine, α -tocopherol, and Trolox) is able to inhibit the pro-oxidant effect of retinol. Previous studies have demonstrated that retinol supplementation has a genotoxic effect in various types of bioassays. Retinol caused an increased recombinogenic activity in the SMART assay with *Drosophila melanogaster*, induced DNA fragmentation in Chinese hamster lung fibroblasts in the comet assay, induced single- or double-strand breaks in primary rat Sertoli cells, increased chromosomal aberrations in human lymphocytes, and caused DNA damage in HL-60 cells (Badr et al., 1998; Klamt et al., 2003b; Murata and Kawanishi, 2000).

In our previous studies, we observed that photoirradiation of retinyl palmitate (RP), the major storage form of retinoids, by UVA light resulted in 14 photodecomposition products in vitro, including 5,6-epoxyretinyl palmitate (5,6-epoxy-RP) and anhydroretinol (AR), which were formed through a light-initiated free radical chain reaction, or an ionic photodissociation mechanism (Cherng et al., 2005). Application of RP to skin can increase the levels of retinol and RP, and have a significant impact on vitamin A homeostasis in the skin. When male and female SKH-1 mice received repeated topical application of creams containing 1–13% of RP 5 days a week for 13 weeks with exposure to solar light, the levels of both retinol and RP in the skin were higher than the control mice (Yan et al., 2007). In addition, the levels of retinol and RP were increased in the skin of mice immediately following topical treatment with RP and decreased with the time after termination of the treatments (Yan et al., 2006). In vitro study showed that photoirradiation of RP, 5,6-epoxy-RP, and AR by UVA light generated ROS, such as singlet oxygen and superoxide, which initiated lipid peroxidation (Xia et al., 2006). Results from in vitro studies using the comet

assay and the mouse lymphoma assay suggest that RP, AR and 5,6-epoxy-RP when exposed to UVA can result in cytotoxicity, DNA damage, and mutations (Mei et al., 2005, 2006; Yan et al., 2005).

Since RP, the principal storage form of retinol in humans and animals, can be enzymatically hydrolyzed back to retinol in vivo (Boehnlein et al., 1994), the retinol could be subject to photodecomposition like RP to generate phototoxicity. The mouse lymphoma assay (MLA) using the *Thymidine kinase (Tk1)* gene is widely used to detect the potential genotoxicity of a wide variety of chemical agents. The assay is recommended as a part of the core battery of genetic toxicology tests (Dearfield et al., 1991; DHHS, 1997; ICH, 1995). In this study, we assessed the mutagenic potential of retinol when exposed to UVA using MLA. By using loss of heterozygosity (LOH) analysis of the *Tk* mutants produced in the experiment, we also determined the types of mutations induced by the combination treatment of retinol and UVA irradiation to explore the underlying mechanism of the mutation induction.

2. Materials and methods

2.1. Materials

Retinol (CAS 68–26–8, minimum 95% in purity), dimethyl sulfoxide (DMSO, 99.9% in purity) and trifluorothymidine (TFT) were purchased from the Sigma Chemical Company (St. Louis, MO). Fischer's medium was obtained from Quality Biological Inc. (Gaithersburg, MD), and all other cell culture supplies were acquired from Invitrogen Life Technologies (Carlsbad, CA). PCR Master Mix was from Promega Company (Madison, WI). The primers used for detection of LOH at the *Tk* locus and the *D11Mit42*, *D11Mit29* and *D11Mit74* loci were synthesized by Invitrogen Life Technologies.

2.2. UVA light source

The UVA light box was custom-made using 4 UVA lamps (National Biologics, Twinsburg, OH) (Mei et al., 2005). The irradiance of the light box was determined using an Optronics OL754 Spectroradiometer (Optronics Laboratories, Orlando, FL), and the light dose was routinely measured using a Solar Light PMA-2110 UVA detector (Solar Light Inc., Philadelphia, PA). The maximum emission of the UVA light box was 350–352 nm with the following spectral distribution: UVA (315–400 nm), 98.93%; UVB (280–315 nm), 1.07%; and UVC (250–280 nm), <0.0001%.

2.3. Cells and culture conditions

The L5178Y/*Tk*^{+/–}–3.7.2C mouse lymphoma cell line was utilized for the mutation assay. Cells were grown according to the methods described in our previous study (Mei et al., 2005). Briefly, the basic medium was Fischer's medium for leukemic cells of mice with L-glutamine supplemented with pluronic F68 (0.1%), sodium pyruvate (1 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml). The treatment medium (F_{5p}), growth medium (F_{10p}), and cloning medium (F_{20p}) were the basic medium supplemented with 5%, 10%, and 20% heat-inactivated horse serum, respectively. The cultures were maintained in a humidified incubator with 5% CO₂ in air at 37 °C.

2.4. Cell treatment with retinol in the absence of UVA irradiation

The retinol working solution (100×) was prepared just prior to use by dissolving in anhydrous DMSO. The cells were suspended in 100-mm diameter tissue culture dishes at a concentration of 6×10^6 cells in 10 ml of treatment medium. One hundred microliters of the retinol working solutions were added to give the final concentrations of 5 or 10 µg/ml, and the cells were incubated for 4 h at 37 °C. In all cases, including the solvent controls (DMSO only) and positive controls (0.1 µg/ml 4-nitroquinoline-1-oxide), the final concentration of DMSO in the medium was 1%.

2.5. Cell treatment with retinol and UVA light

Cells were treated with different concentrations of retinol (0.25–4 µg/ml) and exposed to 2.48 J/cm² of UVA light during a period of 30 min (1.38 mW/cm²). The treated cultures were then incubated at 37 °C (without UVA irradiation) for an additional 3.5 h. After treatment, the cells were centrifuged and washed twice with fresh medium, and then resuspended in growth medium at a density of 3×10^5 cells/ml in 25 cm² cell culture flasks to begin the 2-day phenotypic expression.

2.6. The Tk microwell mutation assay

Mutant selection was performed as described previously (Mei et al., 2005). Briefly, the cells were counted and the densities were adjusted using fresh medium at approximately 1 and 2 days following exposure. For mutant enumeration, 3 µg/ml of TFT was added to the cells in the cloning medium, and cells were seeded into four 96-well flat-bottom microtitre plates using 200 µl per well with a final density of 2000 cells/well. For the determination of plating efficiency, cultures were adjusted to 8 cells/ml medium and aliquoted in 200 µl per well into two 96-well flat-bottom microtitre plates. All plates were incubated at 37 °C in a humidified incubator with 5% CO₂ in air. After 11 days of incubation, colonies were counted and mutant colonies were categorized as small or large. Small colonies are defined as those smaller than 25% of diameter of the well. Mutant frequencies (MFs) were calculated using the Poisson distribution. Cytotoxicity was measured using the relative suspension growth (RSG) and the relative total growth (RTG). RSG is calculated using the suspension growth (SG) for the treatment cultures during the 2-day phenotypic expression ($SG1 \times SG2$) divided by the SG for the control culture. The RTG as another measure of cytotoxicity includes a measure of growth during treatment, expression (RSG), and cloning (relative cloning efficiency, RCE). The equations for the calculation of RSG, RCE, and RTG are described by Chen and Moore (2004).

2.7. LOH analysis of the Tk mutants

Mutant clones were directly taken from TFT-selection plates. Forty-five large and 45 small mutant colonies resulting from the treatment with 4 µg/ml retinol and UVA were analyzed. The mutant cells were washed once with PBS by centrifugation, and cell pellets were quickly frozen and stored at –20 °C. Genomic DNA was extracted by digesting the cells in lysis buffer [10 mM Tris–HCl (pH 7.5), 5 mM MgCl₂, 1% (v/v) Triton X-100, 1% (v/v) Tween 20] with 200 µg/ml of proteinase K at 60 °C for 90 min, followed by inactivation of proteinase K at 95 °C for 10 min. For PCR analysis of LOH at the *Tk* and other loci

(*D11Mit42*, *D11Mit29* and *D11Mit74* loci), the amplification reactions were carried out in a total volume of 20 μ l using 2 $\times$ PCR Master Mix and pairs of primers described previously (Singh et al., 2005). The thermal cycling conditions were as follows: initial incubation at 94 °C for 3 min, 40 cycles of 94 °C denaturation for 30 s, 55 °C annealing for 30 s, and 72 °C extension for 30 s, and a final extension at 72 °C for 7 min. The amplification products were scored for the presence of one band (indicating LOH) or two bands (retention of heterozygosity at the given locus) after 2% agarose gel electrophoresis.

2.8. Statistical analysis

The data evaluation criteria developed by the Mouse Lymphoma Assay (MLA) Expert Workgroup of the International Workshop for Genotoxicity Tests were used to determine whether a specific treatment condition was positive, and positive responses are defined as those where the induced MF in one or more treated cultures exceeds the global evaluation factor (GEF) of 126 mutants per 10^6 cells and there is also a dose related increase with MF (Moore et al., 2002, 2006). For comparison of the MFs from the cells treated with different agents, one-way ANOVA followed by the Tukey test was used to evaluate the differences between the treatment groups and $p < 0.05$ has been chosen as the criteria for statistical significance. LOH patterns of mutants were compared using the computer program written by Cariello et al. (1994) for the Monte Carlo analysis developed by Adams and Skopek (1987).

3. Results

Previous studies showed that combined exposure to RP and its photodecomposition products, AR and 5,6-epoxy-RP, are mutagenic in mouse lymphoma cells in the presence of UVA (Mei et al., 2005, 2006). In this study, we used similar experimental conditions to evaluate the cytotoxicity and mutagenicity of retinol when exposed to UVA light. The cells were treated with retinol at concentrations of 5 or 10 μ g/ml for 4 h without UVA exposure, or treated with retinol (0.25–4 μ g/ml) for 4 h and exposed to UVA at a total light dose of 2.48 J/cm² during the first 30 min of 4 h incubation. The data of relative suspension growth (RSG), relative total growth (RTG), and *Tk* MF from one representative MLA experiment are presented in Table 1. These results were replicated in two additional experiments with retinol range of 1–4 μ g/ml and the data are displayed as mean $\pm$ 1 standard deviation in Fig. 1. The treatments with retinol alone (5 and 10 μ g/ml) for 4 h caused minimal cytotoxicity and no mutagenicity. In contrast, the treatments of cells with various concentrations (1–4 μ g/ml) of retinol and concomitantly exposed to UVA light resulted in an increase of both the cytotoxicity and mutagenicity (Fig. 1). When all concomitant treatment groups (retinol and UVA) were compared with UVA alone, the treatments with 3 or 4 μ g/ml of retinol under UVA exposure resulted in significantly greater cytotoxicity and mutagenicity ($p < 0.001$). Although the combined treatment with 2 μ g/ml retinol and UVA irradiation significantly produced a cytotoxic effect ($p < 0.05$), it did not significantly increase the *Tk* MF. The MFs in the *Tk* gene for the UVA alone, the concomitant treatment of 3 μ g/ml retinol, and the concomitant treatment of 4 μ g/ml retinol were $175 \pm 9 \times 10^6$, $249 \pm 19 \times 10^6$, and $312 \pm 9 \times 10^6$, respectively. The MFs for the doses greater than 4 μ g/ml retinol in the combinational exposure were not determined due to significant cytotoxicity and low plating efficiency.

To determine whether the pre-irradiation of cells with UVA light before adding retinol treatment had an effect on mutagenicity of retinol, we irradiated the cells with 2.48 J/cm² UVA in the absence of retinol and then immediately added 3 µg/ml of retinol for 4 h treatment. The MF for the retinol treatment after the irradiation of cells with UVA was not different from that for UVA alone, and significantly less ($p < 0.001$) than that for the concomitant exposure of retinol and UVA (Fig. 2). The MF for the treatment of 3 µg/ml retinol and UVA were similar to the observation in Fig. 1.

LOH analysis of the *Tk* mutants from retinol + UVA treatment was conducted using allele-specific PCR for four microsatellite loci (the *Tk1* locus, *D11Mit42*, *D11Mit29*, and *D11Mit74*) spanning the entire chromosome 11 (Fig. 3B). DNA samples for 45 large and 45 small mutant colonies collected from the treatment with 4 µg/ml retinol + UVA were analyzed for LOH. More than 93% of the mutants from retinol + UVA treatment lost heterozygosity at the *Tk1* locus (Table 2). The MFs of different types of mutations for all (large and small) colonies are shown in Fig. 3A. The major type of mutation for the retinol + UVA treatment was LOH extending to *D11Mit42* (58%), an alternation involving approximately 6 cM of the chromosome. For comparison, we have included the microsatellite mutant spectra previously obtained for negative control, UVA alone, and RP + UVA in Fig. 3A (Mei et al., 2005). Statistical analysis of the spectra revealed that the mutational spectra induced by retinol + UVA were significantly different from the vehicle control and UVA alone groups ($p < 0.001$), but not different from RP + UVA group ($p = 0.124$).

4. Discussion

Ultraviolet A (UVA) is a major component of UV-irradiation in natural sunlight, and contributes to induction of skin cancer in humans (IARC, 1992). Chronic exposure to UVA from sunlight is a common scenario for humans due to working under the sun or during outdoor recreational activities. In the present study, the UV-irradiation dose was chosen to be environmentally relevant. In this regard, 2.48 J/cm² of UVA, equates to about 30 min at the noontime of sunny days during the summer around world. This dose is based upon observations of UVA intensity of 2.1 mW/cm² in Okayama, Japan in September (Arimoto-Kobayashi et al., 2000), 3.6 mW/cm² in Jackson, MS, USA in August (Yu et al., 2001), 5.4 mW/cm² in Paris, France in July (Jeanmougin and Civatte, 1987), 6.6 mW/cm² in Coimbatore, India in July (Balasaraswathy et al., 2002). In our laboratory, we used approximately 30 min at the dose rate of 1.38 mW/cm² to obtain 2.48 J/cm² of UVA irradiation. Therefore, the dose of irradiation used in the present study approximates environmentally relevant UVA doses at which humans could be exposed (Mei et al., 2009).

Vitamin A and its metabolites play an important role in many critical biological processes including vision, reproduction, and the integrity of membrane structures. However, the potential adverse health effects resulting from high vitamin A intakes from both diet and vitamin supplements have been addressed (Hathcock et al., 1990). It has been suggested that the toxic effects of retinol and other retinoids are related to pro-oxidant properties (Pasquali et al., 2008; Polyakov et al., 2001). In cultured Sertoli cells (one of the important physiological targets of retinol), 7 and 14 µM (about 2 and 4 µg/ml) of retinol enhance

intracellular reactive species production and increase catalase activity (Pasquali et al., 2008). Retinol also has a clear effect of increasing ROS production in a time- and concentration-dependent manner in rat PC12 cells (Gelain and Moreira, 2008).

In the present study, retinol treatment in combination with UVA exposure of 2.48 J/cm² induced cytotoxicity and mutagenicity in mouse lymphoma cells, which is consistent with our previous studies on the photomutagenicity of RP using the same test system (Mei et al., 2005). Retinol alone in the concentrations of 5 and 10 µg/ml did not increase the MF over the vehicle control. However, combined treatment with lower concentrations (1–4 µg/ml) of retinol and UVA light resulted in increases of MFs, in a dose-dependent manner (Fig. 1). There were significant differences between the MFs induced by UVA alone and 3 or 4 µg/ml retinol + UVA. In the MLA, clastogens tend to result in a relative higher proportion of small colony of *Tk* mutants and predominantly LOH types of mutations, whereas chemical compounds that induce point mutations result in a relative higher proportion of large colony mutants and less LOH (Applegate et al., 1990). In this study, LOH at the *Tk* locus occurred in 93% of large colony mutants and 100% of small colony mutants from 4 µg/ml retinol + UVA treatment (Table 2), and the major type of mutation for the retinol + UVA treatment was the LOH involving approximately 6 cM of the chromosome 11 (58%, Fig. 3A). These results indicate that the mutagenicity of retinol in combination with UVA irradiation results from a clastogenic mode-of-action. In addition, the mutational spectra induced by retinol + UVA and RP + UVA was not significantly different ($p = 0.124$, Fig. 3A). This similarity suggests that retinol + UVA may induce mutations through a similar mechanism as RP + UVA. The clastogenic effect of retinol is also evident from elevation of different types of chromosomal aberrations in cultured human lymphocytes in vitro (Badr et al., 1998) and in rat bone marrow cells in vivo (Gulkac et al., 2004).

Retinol-related toxicity is associated with cellular redox modifications, often leading to oxidative stress (Gelain and Moreira, 2008). It has been reported that retinol treatment (7 µM) resulted in a 3-fold increase in UV-mediated free radical generation, a 40% increase in lipid peroxidation, and increased DNA fragmentation and mitochondrial oxidative damage (Klamt et al., 2003a). Irradiation of RP with UVA light can also generate ROS and lipid peroxides (Cherng et al., 2005). Retinol and its esters have an absorption maximum in the UV spectral region at approximately 325 nm, and they can be efficiently photoexcited by sunlight in both the UVA and UVB spectral regions to produce a singlet excited state (Fu et al., 2007). Photodynamic action results in the production of free radicals, which can attack cellular constituents including proteins, nucleic acids, and lipids. In addition, Hydroxyl radicals can initiate a chain reaction that produces multiple lipid hydroperoxide molecules from a single initial event (Gutteridge and Halliwell, 1990). The chain reaction amplifies the initial oxidative insult and the resulting ROS can damage DNA and produce chromosome mutations.

To determine whether retinol under UVA irradiation produces mutations by increasing the sensitivity of mammalian cells as suggested by Dufour et al. (2006), we pre-irradiated the cells with UVA for 30 min in the absence of retinol and then treated the cells with 3 µg/ml retinol for 4 h. The MF for pre-irradiation with UVA and subsequent retinol treatment was significantly less than that for the concomitant exposure to retinol and UVA, and was not

different from that for UVA alone treatment (Fig. 2). Previously we observed that the MF for pre-irradiated RP treatment (i.e., irradiating RP solution with UVA for 30 min immediately before the cell treatment) was less than that for the concomitant exposure to RP and UVA, and greater than RP alone (Mei et al., 2005). These results demonstrate that the increased mutagenicity resulted from UVA irradiation of retinol and that pre-irradiating the cells did not increase the sensitivity of the cells to retinol treatment.

It is noteworthy that retinol is shown to be more cytotoxic and mutagenic than RP in combination with UVA light by the fact that the highest dose resulting in a RTG between 20% and 10% for retinol and RP was 4 and 25 $\mu\text{g/ml}$ (about 14 and 47 μM), respectively. Since retinol has shorter length in its chemical structure than RP, it may more easily diffuse across the plasma membrane. Retinol and its esters have a rich photochemistry involving the formation of free radicals, ROS and multiple photoproducts (Fu et al., 2007), and could be photocytotoxic and photosensitize damage to cellular components such as lipids and DNA. Photooxidative damage may play a significant role in the observed in vitro effects elicited by photoexcited vitamin A.

In conclusion, our study demonstrated that retinol treatment when combined with UVA irradiation produced a clearly mutagenic effect at the heterozygous *Tk* locus in mouse lymphoma cells. The *Tk* mutants induced by the concomitant treatment mainly resulted from LOH, indicating a clastogenic mode-of-action for the photomutagenicity of retinol. The similarity of the mutational spectra between retinol and RP in combination with UVA exposure suggests that their mutation induction results from a common mechanism, possibly oxidative DNA damage.

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Fig. 1.

Comparison of cytotoxicity (the top panel) and mutagenicity (the bottom panel) of retinol (ROH) and ROH + UVA in mouse lymphoma cells. The cells were treated with retinol alone (5 and 10 μg/ml) or with different concentrations of retinol (0–4 μg/ml) for 4 h in combination with 1.38 mW/cm² UVA irradiation for 30 min. The data points represent the mean ±1 standard deviation from 3 independent experiments. The dashed line represents the baseline for vehicle control (DMSO, dimethyl sulfoxide), and the solid line indicates the baseline for UVA alone. The concomitant treatment groups (ROH + UVA) with 2–4 μg/ml of retinol were significantly different from the UVA alone (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Fig. 2.

Comparison of the mutant frequencies in the *Tk* gene of mouse lymphoma cells treated with UVA alone, pre-irradiation + ROH, and ROH + UVA. The retinol (ROH) concentration used for cell treatment was 3 $\mu\text{g}/\text{ml}$. The UVA irradiation was at a dose rate of 1.38 mW/cm^2 for 30 min. For pre-UVA + ROH, the cells were irradiated for 30 min and then immediately treated with retinol for 4 h. For ROH + UVA treatments, the cell suspensions were irradiated with UVA for 30 min during the first 30 min of 4-h incubation with retinol. The data points represent the mean ± 1 standard deviation from 4–6 independent experiments. The asterisks indicate that the mutant frequency in this treatment is significantly different from those in UVA alone and pre-ROH + UVA groups ($p < 0.001$).

Fig. 3.

Comparison of mutation frequencies of mutational types of all (large and small) colonies (A) produced in mouse lymphoma cells treated with vehicle, UVA alone, RP + UVA, and ROH + UVA. The data for control (dimethyl sulfoxide), UVA alone, and RP + UVA are from our previous study (Mei et al., 2005). The concentration for retinol and RP was 4 and 25 $\mu\text{g}/\text{ml}$ (about 14 and 47 μM), respectively. Different types of mutations showed in the histograms indicate the range of LOH at the same scale as used in the ideogram of mouse chromosome 11 (B). The loci that were analyzed for LOH (*Tk1*, *D11Mit42*, *D11Mit29*, and *D11Mit74*) are marked. The ruler in cM indicates the distance from the top of the chromosome. Type 1 mutation, Non-LOH; 2, LOH at *Tk1* locus only; 3, LOH extending to *D11Mit42* (about 6 cM); 4, LOH extending to *D11Mit29* (about 38 cM); and 5, LOH extending to the top of chromosome 11.

Table 1

Photocytotoxicity and photomutagenicity of retinol in L5178Y/Tk^{+/−} mouse lymphoma cells from one representative experiment.

Treatment	Dose	Relative suspension growth (%)	Relative total growth (%)	Mutant frequency (×10 ^{−6})
Control ^a	1% DMSO	100	100	78
	1% DMSO	100	100	74
Retinol	5 µg/ml	91	86	83
	10 µg/ml	82	84	74
UVA ^b	2.48 J/cm ²	82	78	181
Retinol + UVA ^c	0.25 µg/ml	77	79	164
	0.5 µg/ml	61	60	191
	1 µg/ml	51	57	201
	2 µg/ml	45	53	216 ^d
	4 µg/ml	21	19	312 ^d

^aEach experiment had two vehicle controls (DMSO, dimethyl sulfoxide).

^bCells received 1.38 mW/cm² UVA irradiation for 30 min.

^cCells were concomitantly exposed to different concentrations of retinol for 4 h and 1.38 mW/cm² UVA irradiation for 30 min.

^dIndicates a positive response.

Table 2

Loss of heterozygosity (LOH) at four loci along chromosome 11 in the Tk mutant from the cells exposed to retinol (4 µg/ml) with UVA.

Locus	Position (cM) ^a	Retinol + UVA	
		No. of large colonies (%)	No. of small colonies (%)
<i>D11Mit74</i>	0.0	6 (14)	4 (9)
<i>D11Mit29</i>	40.0	13 (31)	7 (16)
<i>D11Mit42</i>	72.0	28 (67)	38 (86)
<i>Tk</i>	78.0	42 (93)	45 (100)
Mutants screened		45	45

^aLocus position in centimorgans (cM) is the distance to the top of chromosome 11.