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Protective effect of TiO_2 particles on UV light induced pyrimidine dimer formation

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Abstract

UV light irradiation is known to induce DNA damage, e.g. pyrimidine dimers, which is expected to introduce genetic damage in tissues and cells. Here, we have studied the effects of TiO_2 particles on UV-induced pyrimidine dimer formation.

Pyrimidine photoproducts (6-4 type) and cyclobutane-type pyrimidine dimers were detected by using monoclonal antibodies for these dimers in both normal human cells (TIG-1) and human cancer cells (T24) after UVC light irradiation. Application of TiO_2 particles to cell surfaces reduced the extent of UVC-induced dimer formation. On mouse skin, UVC introduced both types of pyrimidine dimers, and the presence of TiO_2 particles on the surface of mouse skin also reduced the UVC-induced pyrimidine dimer formation.

UVA light irradiation, on the other hand, did not introduce dimer formation in either cultured cells or in mouse skin tissues, and TiO_2 did not have a significant effect on UVA irradiation. The presence of TiO_2 did not influence the repair of the UVC-induced pyrimidine dimers. These effects confirm the usefulness of TiO_2 as a protective agent against UV-induced DNA damage in cells and tissues.

Keywords: TiO_2 particles; Pyrimidine dimer; UV light

1. Introduction

Several types of cosmetics contain particulate TiO_2 as a UV absorber or scatterer. Usually, in skin cells exposed to UV light (especially UVC), adjacent pyrimidines in DNA are known to form dimers. Normally, almost all of these dimers are excised and repaired, except in repair-deficient cells, such as xerodermia pigmentosum cells [1–3]. However, when a large number of dimers are formed, unexcised dimers can persist as damaged DNA in cells. If the damaged DNA forms in important genes, e.g. tumor suppresser genes, such damage might possibly introduce skin cancer [4,5]. Also, the increase of pyrimidine dimer formation has been suggested to be associated with the cell killing effect of UV light [1].

On the other hand, TiO_2 excited by UV light with wavelengths less than 380 nm is known to photocatalyze $OH^{\bullet}$ and/or $O_2^{-\bullet}$ anion formation in the presence of water and oxygen, respectively [6,7]. These radicals introduced by UV light have been applied to anti-bacterial materials [8]. No data, however, existed concerning the effect of TiO_2 on the UV-induced pyrimidine dimer formation in DNA.

Here, we have investigated the relationship between the formation of pyrimidine dimers by UV light and the exposure of TiO_2 to cultured cells and skin tissues and have shown that the presence of TiO_2 reduced the dimer formation introduced by UVC light in human cells in vitro as well as mouse skin in vivo, and UVA, which is usually applied for TiO_2 excitation for photocatalysis, did not influence this dimer formation.

2. Materials and methods

2.1. Cells and cell culture

The T24 cells derived from human bladder cancer tissues were cultured in Ham's F-12 medium supplemented with 10% fetal calf serum at 37°C in a 3.5% CO_2 incubator. The TIG-1 normal fibroblast cells derived from human

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embryonal lung tissue were cultured in MEM medium supplemented with 10% fetal calf serum at 37°C. The T24 cells (4×10^5 to 8×10^5) were inoculated in dishes and cultured for 2–3 days before the experiments. TIG-1 cells (1×10^6) were inoculated in dishes and cultured for 3 days before the experiments.

2.2. Skin tissue

Nude mice (BALB-C/NU, male, CLEA Japan) were used for in vivo experiments. Skin tissue was obtained from 5- to 6-week old nude mice at constant volume of skin using a 3 mm diameter skin cutter (Kai Medical Co.) immediately after light exposure.

2.3. UV-irradiation

UV irradiation at a wavelength of 254 nm for UVC and 360 nm for UVA was performed with a UV lamp (UVGL-25, UVP Inc., USA). The dose rates were 0.02 (UVC) and 0.04 mW/cm^2 (UVA), and were monitored with a Topcon UV Radiometer (UVR-2, Topcon). In the case of in vivo experiments, the dose rate was 0.2 mW/cm^2.

2.4. Preparation of TiO_2 particles

The P25 TiO_2 particles (anatase, Nippon–Aerosil, Tokyo) were used. At first, the TiO_2 particles were autoclaved and then were ultrasonically dispersed in PBS solution. Cell-cultured media were discarded and washed with PBS. Cells were then treated with PBS solution containing TiO_2 particles (1 mg/ml). For mouse skin experiments, glycerol (final 0.08%) was added to the TiO_2 particle suspension.

2.5. DNA extraction from cells

The genomic DNA from cultured cells was purified by use of the phenol/chloroform procedure. At first, the cells were added to TE (10 mM Tris, 1 mM EDTA, pH 8.0) solution. They were then lysed with 10% SDS and proteinase K (100 μg/ml Boehringer Mannheim) at 37°C for 16 h. The DNA was extracted into phenol/chloroform after treatment with RNase A (100 μg/ml, Boehringer Mannheim) at 37°C for 1 h. The DNA obtained was then dissolved in PBS (pH 7.4) and washed with 70% ETOH.

2.6. DNA extraction from skin tissues

Each skin tissue was homogenized in TE (10 mM Tris–1 mM EDTA pH 8.0) solution. Then, 10% SDS and RNase A (100 μg/ml, Boehringer Mannheim) were added and allowed to react at 37°C for 1 h. Proteinase K (50 μg/mg, Boehringer Mannheim) was added after the reaction and was then maintained at 50°C overnight (16 h). The sample was then centrifuged, and the DNA was extracted into phenol/chloroform, as described for the cells.

2.7. Quantification of pyrimidine dimer formation

For the extracted DNA, mouse monoclonal antibodies that recognize either of the pyrimidine dimers (provided by Dr. Toshio Mori, Nara Medical University, Nara, Japan) [9] were applied for the quantification of the relative number of 6-4 photoproducts and cyclobutane-type dimers in the total genomic DNA.

The assay method to measure the 6-4 photoproducts, and the cyclobutane-type dimers (CPDs) was the direct binding of monoclonal antibodies to antigens measured by the ELISA method. Details of the method have been described previously [10]. In brief, polyvinylchloride flat-bottom microtiter plates, pre-coated with 1% protamine sulfate (50 μl/well, Wako), were incubated with UV-irradiated DNA in PBS at 37°C for 24 h. After drying, the plates were washed five times with PBS containing 0.05% Tween 20 (Sigma). The plates were incubated with 1% newborn calf serum in PBS (150 μl/well) at 37°C for 30 min and then washed again. One hundred microliter of the monoclonal antibody was added to the wells, with incubation at 37°C for 30 min. The plates were washed five times with PBS containing 0.05% Tween 20 (Sigma) and were then incubated again with 100 μl per well of affinity-purified goat anti-mouse immunogloblin G (IgG) conjugated with peroxidase (in PBS, Zymed) at 37°C for 30 min. Finally, five times washings were carried out with PBS containing 0.05% Tween 20 (Sigma), and two subsequent washings were carried out with citrate–phosphate buffer, and 100 μl of substrate solution, consisting of 0.04% *o*-phenylene diamine, and then 0.007% H_2O_2 in citrate–phosphate buffer was added to each well. After 30 min incubation at 37°C, 50 μl of 2 M H_2SO_4 was added to stop the reaction, and the absorbance at 490 nm was measured by use of a Microplate Reader (Bio-Rad). Experiments were performed at least twice. Data shown are typical results except as mentioned.

3. Results

Pyrimidine dimer formation was analyzed after UV irradiation to cultured human cells with the use of monoclonal antibodies for each type of pyrimidine dimer by the ELISA method [9]. In cancer-derived cells (T24), both 6-4 photoproducts and cyclobutane-type pyrimidine dimers were formed with 254 nm UV (UVC) irradiation. It was confirmed that the number of these dimers formed increased in proportion to the total amounts of incident ultraviolet light. However, UVA light introduced none of these pyrimidine dimers in cells, even with very high doses of light (Fig. 1).

Next, we added TiO_2 particles to the cell layers and irradiated with UVC light. It was found that the formation of

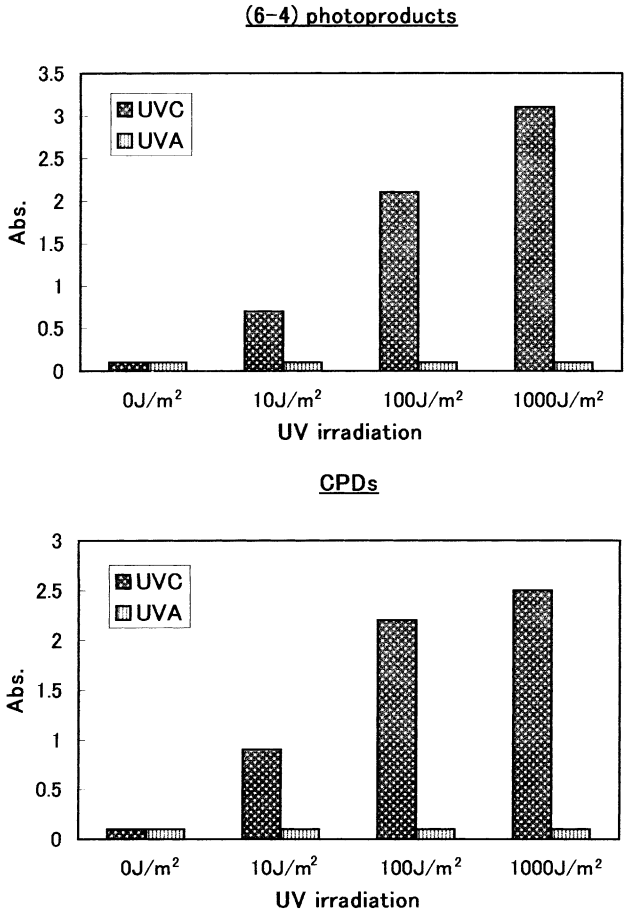

Fig. 1. Pyrimidine dimer formation in T24 cells irradiated with 254 nm UV (UVC) light (▩) or 360 nm UV (UVA) light (□). Data are shown as an average of duplicate experiments.

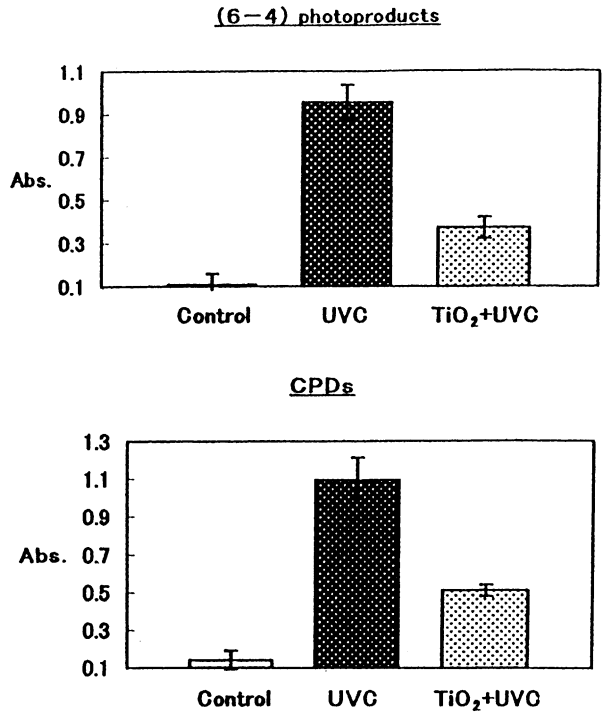

Fig. 2. Pyrimidine dimer formation resulting from irradiation with 254 nm UV light (10 J/m^2) in T24 cells with (▩) or without (□) TiO_2 particles(1 mg/ml).

the 6-4 photoproducts and the cyclobutane-type pyrimidine dimer in T24 cells was suppressed in the presence of the TiO_2 particles, as seen in Fig. 2. The inhibitory effect of TiO_2 on the formation of these pyrimidine dimers by UVC in T24 cells was directly dependent on the amount of TiO_2 particles added (Fig. 3).

On TIG-1 cells, which are considered to be normal human fibroblast cells, the addition of TiO_2 particles was shown to suppress the formation of both types of dimers by UVC irradiation, as seen in Fig. 4. To this normal cell line, we irradiated with high doses of UV light. The addition of TiO_2 to the cultured dishes also decreased the formation of both pyrimidine dimers by irradiation with 100 J/m^2 UVC light. UVA light (360 nm) did not induce dimer formation in the cells (Fig. 5).

Pyrimidine dimer formation in mouse skin by the irradiation of UV light and the effects of TiO_2 were then analyzed. We applied TiO_2 particles in suspension (see Section 2) directly on the skin of nude mice. Then, 254 nm UV light (100 J/m^2) was irradiated. Immediately after the UV irradiation, a piece of skin was resected and frozen in liquid

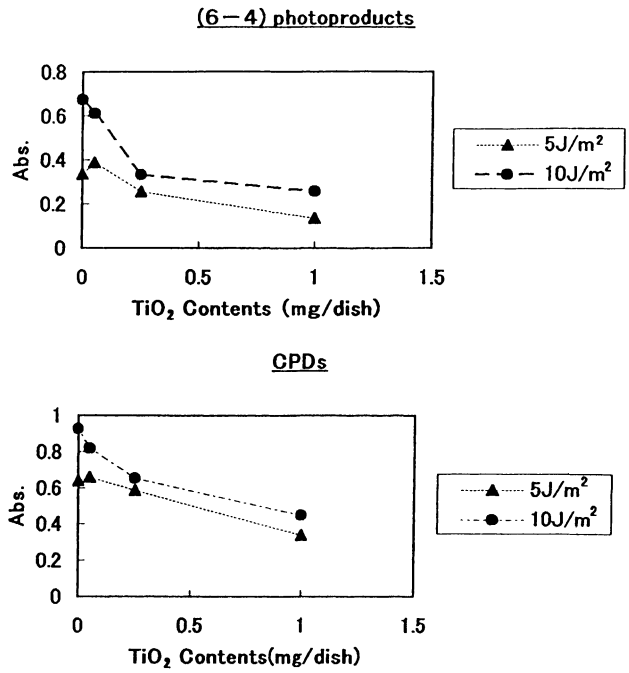

Fig. 3. Effect of different concentrations of TiO_2 on the formation of both types of pyrimidine dimers in T24 cells resulting from 254 nm UV irradiation with an intensity of 5 J/m^2 (▲) or 10 J/m^2 (●).

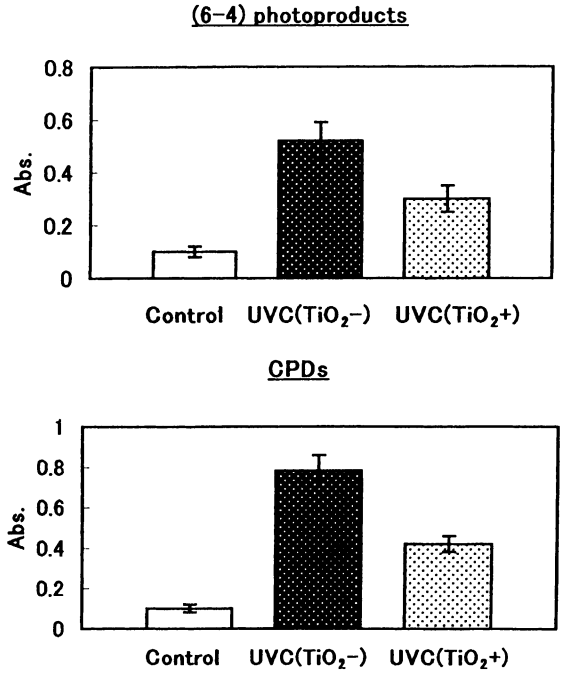

Fig. 4. Pyrimidine dimer formation in TIG-1 cells irradiated with 254 nm UV light (10 J/m^2) with TiO_2 (⊡) or without TiO_2 (▩) particles(1 mg/ml).

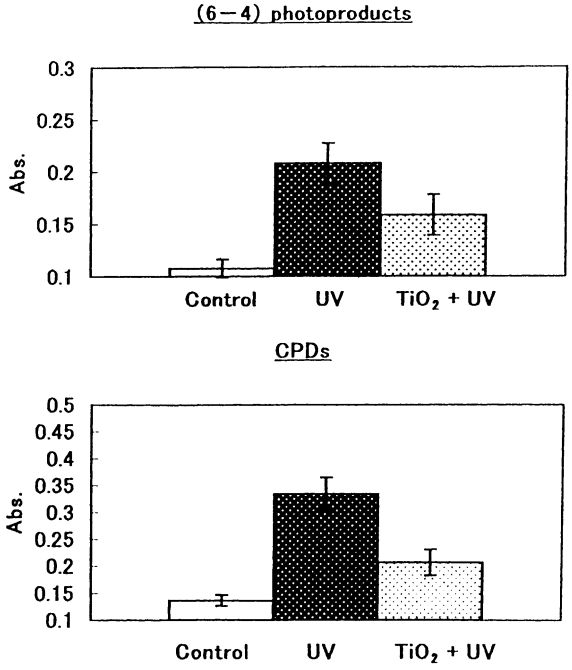

Fig. 6. Pyrimidine dimer formation in mouse skin resulting from irradiation with 254 nm UV light (100 J/m^2) with (⊡) or without (▩) TiO_2 particles (1 mg/ml) on the skin ($n = 5$).

nitrogen. DNA extracted from the frozen skin was then analyzed for the formation of pyrimidine dimers using monoclonal antibodies. UVC was found to induce both types of pyrimidine dimer formation on mouse skin. The application of TiO_2 on mouse skin was found to inhibit the formation of both types of pyrimidine dimers ($P < 0.01$), as seen in Fig. 6. UVA, however, did not induce the formation of pyrimidine dimers (data not shown).

Pyrimidine dimer are usually repaired during 24–48 h after exposure to UV light. We then sought to observe this repair phenomenon in T24 cells, and the effect of TiO_2 on this repair phenomenon was studied.

As in Fig. 7, the 6-4 pyrimidine photoproduct was reduced to control levels during a 24 h repair period in T24 cells. For cyclobutane-type pyrimidine dimers, on the other hand, half of the dimers were still observed 24 h after UV irradiation.

In the presence of TiO_2 particles, less of both types of pyrimidine dimers were formed by UVC irradiation than for UVC alone, but the addition of TiO_2 to the cells did not influence the amount of pyrimidine dimers after a 24 h repair period (Fig. 7).

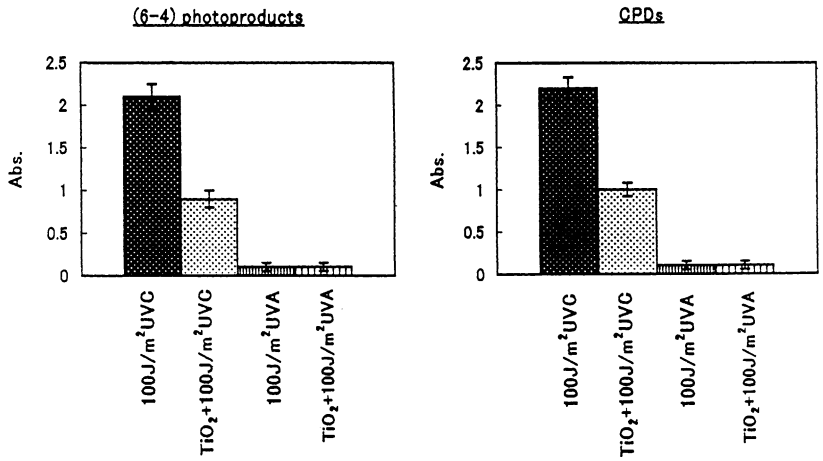

Fig. 5. Pyrimidine dimer formation in TiG-1 cells irradiated with high doses (100 J/m^2) of 254 nm UVC ((⊡), (▩)) or 360 nm UVA ((▥), (▨)) irradiation with or without TiO_2 particles(1 mg/ml).

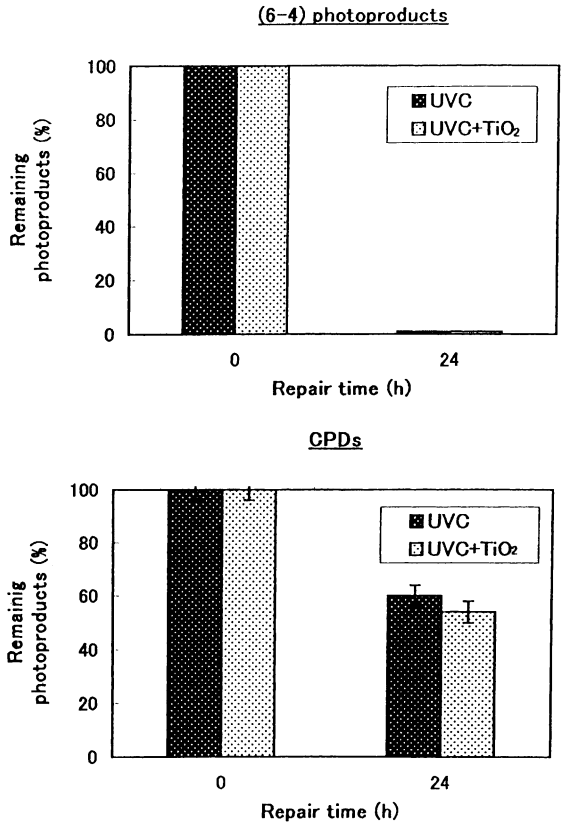

Fig. 7. Comparison of the formation of pyrimidine dimers in T24 cells with (⊡) or without (▩) TiO_2 immediately after 254 nm UV irradiation and 24 h after UV irradiation.

4. Discussion

In both normal human cells (TIG-1) and cancer cells (T24), pyrimidine dimers (6-4 photoproducts and cyclobutane-type) were formed by the irradiation of UVC in a dose-dependent manner as expected, and TiO_2 was shown to reduce the dimer formation. On mouse skin tissue, an inhibitory effect of TiO_2 on the UVC-induced pyrimidine dimer formation was also observed.

TiO_2 is well known to absorb UV light. This might be thought to be the reason for the reduction of the UVC-induced pyrimidine dimer formation. For example, in mouse skin experiments, we also examined the effect of a TiO_2 suspension that was indirectly placed on the skin on dimer formation by UVC irradiation. The result was exactly the same as that for the experiments in which TiO_2 was placed on the skin directly (data not shown). In several preliminary experiments, we also found that the inhibitory effect of TiO_2 on pyrimidine dimer formation by UVC was reduced when the TiO_2 particles were agglomerated (data not shown). Agglomerated TiO_2 might decrease the effectiveness of the absorbance of UV light and thus, might reduce the inhibitory effect on pyrimidine dimer formation.

There was no basic difference between cultured cells and skin tissues for UVC-induced pyrimidine dimer formation and for the inhibitory effect of TiO_2 on dimer formation. In the mouse skin experiments, however, we used relatively high doses of UVC irradiation (100 J/m^2) and two times more concentrated doses of TiO_2. Increasing the dose of UVC was also shown to increase the formation of pyrimidine dimers. Considering the difference between in vitro and in vivo experiments, we then chose to use 100 J/m^2 doses of UVC for the estimation of the effect of TiO_2 on the formation of pyrimidine dimers on skin tissues. Cells in skin tissue are overlapped, not single layers, as in a cell culture system with respect to the incident light, and the surface of the skin is microscopically irregular enough to scatter light.

UV light with wavelengths shorter than 310 nm is known to be responsible for dimer formation [10]. As expected, UVA irradiation (360 nm) did not induce either type of pyrimidine dimer formation in cells nor did it introduce dimer formation in mouse skin in our experiments. Even with a 1000-fold higher dose compared to that of UVC, we confirmed that these pyrimidine dimers were not formed after the UVA irradiation. The presence of TiO_2 particles did not have any effect on this.

The compound TiO_2 is known to excited by UV light with wavelength shorter than 380 nm. UVA (360 nm) used in this experiment possibly introduced photocatalytic reactions on the surface of TiO_2 particles on cultured cells or skin tissues.

In our experiment, even with the possible photocatalytic reactions of TiO_2 particles, which might produce several types of radicals or radical anions, e.g. $OH^{\bullet}$, the formation of pyrimidine dimers in the cells or skin tissues were not observed, as expected. The production of radicals on the TiO_2 particles by UVA light was evidently taking place outside the cells. The radicals usually do not penetrate inside the cells. Thus, pyrimidine dimer formation, which is an event talking place inside the cell nucleus, would not be affected by the radicals formed on the TiO_2 particles. Our experiments might also confirm this point.

Pyrimidine dimers formed by UVC in mammalian cells are known to be repaired during the 24–48 h after exposure to UV light. Pyrimidine dimers introduced by UVC in the cells are usually repaired by nucleotide-excision repair, except in repair-deficient cells, such as xerodermia pigmentosum cells [11–13]. In our experiments with T24 cells, almost all pyrimidine dimers formed by UVC were repaired during the 24–48 h after irradiation. In particular, the 6-4 photoproduct which is considered to be more responsible for the cell killing effect and to have greater mutagenic and carcinogenic potential than the cyclo-butane type pyrimidine dimer, was repaired more quickly than the cyclobutane type pyrimidine dimer, as in other reports [14,15]. The presence of TiO_2 particles did not influence the repair in our experiment as in Fig. 7. Since the repair process proceeds mainly inside the cell (cell nucleus) [11–13] the presence of TiO_2 or chemical reactions occurring on the TiO_2 particles which are outside the cells [7,8] seems not to influence the repair process either.

Considering these results, TiO_2 particles are effective agents for protecting against UV-induced DNA damage in cells and tissues.

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