PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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• BRIEF REPORTS •

Photocatalytic killing effect of TiO_2 nanoparticles on Ls-174-t human colon carcinoma cells

Ai-Ping Zhang, Yan-Ping Sun

Ai-Ping Zhang, Yan-Ping Sun, Chemical Engineering Department, Taiyuan University of Technology, Taiyuan 030024, Shanxi Province, China
Supported by the National Natural Science Foundation of China, No. 29776032 and the Natural Science Foundation of Shanxi Province, No.971013
Correspondence to: Yan-Ping Sun, Chemical Engineering Department, Taiyuan University of Technology, Taiyuan 030024, Shanxi Province, China. ypsun@tyut.edu.cn
Telephone: +86-351-6010070
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Abstract

AIM: To investigate the photocatalytic killing effect of photoexcited TiO_2 nanoparticles on human colon carcinoma cell line (Ls-174-t) and to study the mechanism underlying the action of photoexcited TiO_2 nanoparticles on malignant cells.

METHODS: Ls-174-t human colon carcinoma cells were cultured in RPMI 1640 medium supplemented with 199 mL/L calf serum in a humidified incubator with an atmosphere of 50 mL/L CO_2 at 37 ℃. Viable cells in the samples were measured by using the MTT method. A GGZ-300 W high pressure Hg lamp with a maximum ultraviolet-A (UVA, 320-400 nm) irradiation peak at 365 nm was used as light source in the photocatalytic killing test.

RESULTS: The photocatalytic killing of Ls-174-t cells was carried out *in vitro* with TiO_2 nanoparticles. The killing effect was weak by using UVA irradiation without TiO_2 nanoparticles. In our studies, the photocatalytic killing effect was correlated with the concentration of TiO_2 and illumination time. Once TiO_2 was added, Ls-174-t cells were killed at a much higher rate. In the presence of 1 000 μg/mL TiO_2, 44% of cells were killed after 10 min of UVA irradiation, and 88% of cells were killed after 30 min of UVA irradiation.

CONCLUSION: When the concentration of TiO_2 is below 200 μg/mL, the photocatalytic killing effect on human colon carcinoma cells is almost the same as that of UVA irradiation alone. When the concentration of TiO_2 is above 200 μg/mL, the remarkable killing effect of photoexcited TiO_2 nanoparticles can be found.

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INTRODUCTION

The application of TiO_2 photocatalysis has received increasing attention since the first report of microbiocidal effects by Matsunaga *et al*. in 1985[1]. In recent years, in contrast to many studies using TiO_2 powder for photodecomposition of organic pollutants[2-9], few studies have investigated the application of TiO_2 in life science, especially in the field of cancer treatment[10-12]. The incidence of colon cancer is rising in China. Despite that surgical operation is used currently, people have recognized its limitation. The way to treat cancer usually includes radiation therapy and chemical therapy, which may generate severe side effects in human body. Therefore, this study tried to investigate a new therapy for cancer. Ls-174-t cells were used as experiment objects in this study. The photocatalytic killing effect of TiO_2 nanoparticles on malignant cells and its killing mechanism were investigated.

MATERIALS AND METHODS

Reagent preparation

TiO_2 colloid solutions were prepared[13,14] by hydrolysis of titanium isopropoxide, $Ti[OCH(CH_3)_2]_4$ (97%, Aldrich Chemical Co). In brief, 12.5 mL of $Ti[OCH(CH_3)_2]_4$ was added to 2 mL isopropanol, then the mixture was added to 150 mL of distilled deionized water containing 2 mL of 700 mL/L nitric acid and vigorously stirred for 6 h at 75 ℃. Approximately 150 mL of TiO_2 colloid solution being stable for several months at 4 ℃ was obtained after the organic layer was removed. The average diameter determined by Zetasizer $3000HS_A$ (USA) was 21.2 nm.

The pH value of TiO_2 colloid solutions used in the subsequent experiment had to be adjusted from 1.8 to 5.5-6.5 in order not to damage the normal growth of cells. Therefore, 1 mol/L NaOH aqueous solution and 1.5 mL/L polyvinyl alcohol were added to the colloid solutions before the pH adjustment to prevent the TiO_2 from precipitation. The final TiO_2 colloid solutions were sterilized by autoclaving and then diluted to the required concentration. Other chemical reagents used were all of analytical purity from commercial sources.

Cell culture and treatment

Human colon carcinoma cell lines Ls-174-t were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai, China. The cell lines were cultured in RPMI 1640 (Gibco) medium supplemented with calf serum 100 ml/L, penicillin (100×10^3 μ/L) and streptomycin (100 mg/L). pH was maintained at 7.2-7.4 by equilibration with 50 mL/L CO_2. Temperature was maintained at 37 ℃. Cells were sub-cultured with a mixture of ethylenedinitrile tetraacetic acid (EDTA) and trypsin. All experiments were performed using cells during the exponential growth phase. Cell concentration was determined by using a hemocytometer and the cell density was adjusted to the required final concentration.

Ls-174-t cells were treated with TiO_2 diluted in RPMI 1640 medium for 2 h at 37 ℃. Then the solutions were irradiated with a GGZ-300W high pressure Hg lamp (E_{max} = 365 nm) at room temperature. A UV pass filter was used to obtain a light wavelength between 300-400 nm. The light intensity at the liquid surface was measured by a VLX-3W radiometer-photometer (USA). The incident light intensity was 3.7 mW/cm^2. In our study, three groups of tests were carried out. One group was treated in the absence of TiO_2. Another group was treated in the absence of UVA. The third group was treated with different TiO_2 concentrations and irradiated by UVA.

Measurement of the viability of Ls-174-t cells

Viable cells in the samples were measured by using the MTT staining method[15]. MTT [3- (4, 5-dimethylthiazol-2-yl) -2, 5-diphenyl tetrazolium bromide] was dissolved in phosphate-buffered saline (PBS, pH 7.4) at 5 mg/mL and filtered to be sterilized. Twenty microliters of stock MTT solution were added to all wells for an assay, and plates were incubated at 37 ℃ for 4 h. One hundred and fifty microliters of DMSO were added to all wells and mixed thoroughly to dissolve the blue-violet crystals. After a few minutes at room temperature to ensure that all crystals were dissolved, the plates were read on a Bio-Rad Novapath^{tm} microplate reader (Japan), using a test wavelength of 595 nm, taking the solution without MTT as control. Then optical absorptions [A] were obtained. Plates were normally read within 1 h after DMSO was added. The survival rate could be calculated according to $[A]_t/[A]_i$, where $[A]_i$ is the optical absorption of untreated cells.

RESULTS

Determination of the average diameter of TiO_2 particles

Zetasizer 3000HSA (USA) was used to determine the average diameter of TiO_2 nanoparticles. The result is shown in Figure 1. The average volume size of TiO_2 nanoparticles was 21.2 nm.

Cytotoxicity of TiO_2 nanoparticles

The cytotoxicity of TiO_2 nanoparticles (without UVA irradiation) was determined by exposing cells to various concentrations of TiO_2 in RPMI 1640 (Gibco) medium for 24 h. The surviving fraction of the cells was greater than 90% when the concentration of TiO_2 was in the range of 1 000 μg/mL, as shown in Figure 2. The result confirmed that nonirradiated TiO_2 nanoparticles were not toxic to the Ls-174-t cells. It was consistent with those in literatures[16-17].

Effect of photoexcited TiO_2 nanoparticles on Ls-174-t cells

The fact that the surviving fraction was greater than 90% after 30 min as shown in Figure 3 (A) indicated that TiO_2 nanoparticles without UVA irradiation showed little toxicity to living cells. The killing effect of UVA without TiO_2 is shown in (B) with the surviving fraction of Ls-174-t cells given as a function of the UVA light irradiation time. About 20% cells were killed after 30 min exposure, whereas after 20 min exposure, more than 90% of the cells survived. Once TiO_2 was added, the Ls-174-t cells were killed at a much higher rate as shown in (C). For example, in the presence of 1 000 μg/mL of TiO_2, 44% of the cells were killed after 10 min of UVA irradiation, and after 30 min irradiation, 80 % of the cells were killed. Therefore it was concluded that photoexcited TiO_2 nanoparticles had an active killing effect on Ls-174-cells.

Effect of TiO_2 concentration on Ls-174-t cells activity

The effect of the TiO_2 concentration ranging from 200 to 1 000 μg/mL on the rate of cell killing by UVA light is shown in Figure 4. The light intensity was 3.7 mW/cm^2 and kept constant. The experimental results demonstrated that cell viability decreased monotonically as TiO_2 concentration increased and cell viability decreased with time.

Although a higher concentration of TiO_2 could achieve a higher reaction rate, the difficulties in separation and measurement had to be considered. So the effect of a higher TiO_2 concentration (C>1 029 μg/mL) on Ls-174-t cell activity was not investigated. The concentration of TiO_2 was set at <1 029 μg/mL with which the dark cytotoxicity was considered to be negligible. The range of TiO_2 concentrations was close to that used previously[18].

Morphological changes of Ls-174-t cells

Untreated and TiO_2-treated cells were collected by centrifugation and resuspended in RPMI 1640 medium. The samples were pipetted into a 24-well plate, which was directly observed with an inverted phase-contrast microscope. When treated by photoexcited TiO_2, the cellular shape was condensed and nuclei were dispersed in fragments.

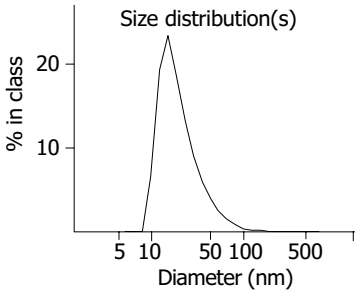

Figure 1 Volume size distribution of TiO_2.

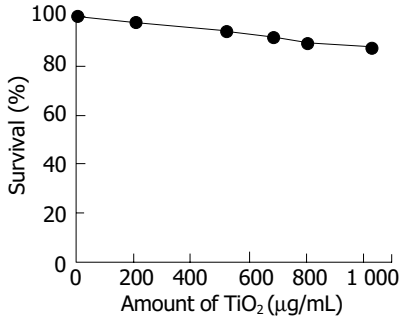

Figure 2 After Ls-174-t cells were incubated in RPMI 1640 medium for 24 h without irradiation, the survival of Ls-174-t cells was shown.

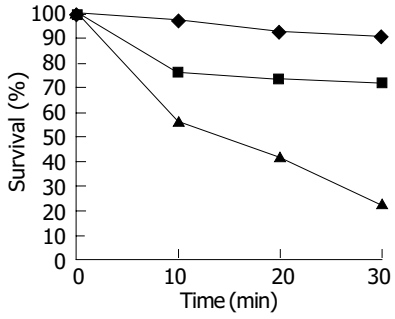

Figure 3 Effect of light and TiO_2 on viability of Ls-174-t cells. (A) TiO_2 (1 000 μg / mL) in the dark; (B) no TiO_2 in the light; (C) TiO_2 (1 000 μg / mL) in the light. Initial cell concentration: 5×10^5 cell / mL, light intensity: 3.7 mW / cm^2.

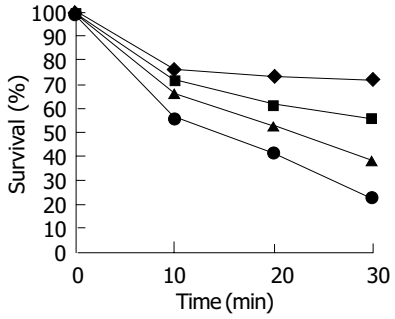

Figure 4 Influence of TiO_2 concentration on Ls-174-t cells activity. (A) 204 μg / mL, (B) 524 μg / mL, (C) 804 μg / mL, (D) 1 029 μg / mL.

DISCUSSION

In this experiment, the TiO_2 nanoparticle system was used, displaying its superiority. TiO_2 nanoparticles were easy to attach

to the cellular membranes and accumulate. They were also easy to enter into the cytoplasm via phagocytosis[19]. It could lead to accumulation of ROS on the surface of cell membranes and in the cytoplasm. Hence under light irradiation, TiO_2 nanoparticles had more significant cell killing effect *in vitro*.

Human colon carcinoma cells treated with photoexcited TiO_2 nanoparticles (C>200 μg/mL) were effectively damaged, with cells contracted and a lot of cell fragments simultaneously observed by an inverted phase-contrast microscope[18].According to these characteristics, we assumed that the mechanism of photoexcited TiO_2 in killing human colon carcinoma cells might be through a series of oxidized chain reactions and in inducing cell death by reactive oxygen species[20-25]. Human colon carcinoma cell damages occurred in two stages. The initial oxidative damage took place on the cell membranes, where the TiO_2 photocatalytic surface had its first contact with intact cells, the membranes became somewhat permeable. At this stage the cells did not lose their viability. Photocatalytic action made the cell membranes permeable, intracellular components began to leak from the cells and free TiO_2 nanoparticles might also diffuse into the damaged cells and directly attack intracellular components, eventually leading to cell death. It is different from the bactericidal effect of TiO_2 photocatalytic reaction. Bacteria are simple prokaryotic cells that do not contain the nucleus characteristics of eukaryotic cells. Whereas human colon carcinoma cells are eukaryotic cells and their structure is complex. Based on their structural differences, we assumed that killing cancer cells might be more difficult than killing bacteria by the photocatalytic reaction of TiO_2 nanoparticles.

In the present study, cultured human colon carcinoma cells were effectively killed by photoexcited TiO_2 nanoparticles *in virto*. The concentration of TiO_2 affected the photocatalytic killing effect. When the concentration of TiO_2 was below 200 μg/mL, there was only a slight decrease in survival ratio after UVA irradiation for more than 30 min. It was almost the same as that of UVA irradiation alone. It indicated that minor cell membrane leakage might occur and the cell viability was not lost. When the concentration of TiO_2 was above 200 μg/mL, the survival ratio decreased rapidly with increasing TiO_2 concentration. It indicated that major rupture of cell membranes and decomposition of essential intracellular components might take place, thus accelerating cell death. It verified the mechanism of TiO_2 nanoparticles in killing human colon carcinoma cells.

The photocatalytic killing effect of TiO_2 nanoparticles on human colon carcinoma cells suggested the idea of cancer treatment using TiO_2 nanoparticles and light irradiation. Under these conditions, it could be adapted to an anticancer modality by the local or regional treatment of the tumor with TiO_2 nanoparticles, followed by light irradiation focusing on the tumor. Although UVA light (320-400 nm) cannot penetrate the human body deeply, it may be possible that the modality will be applied to several human tumors in the future.

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