Catalysis Today 151 (2010) 58–63

Contents lists available at ScienceDirect

Catalysis Today

journal homepage: www.elsevier.com/locate/cattod

Effects of UV-irradiated titania nanoparticles on cell proliferation, cancer metastasis and promotion

Ekaterini Stefanou[a], Angelos Evangelou[b], Polycarpos Falaras[a,*]

[a] Institute of Physical Chemistry, NCSR Demokritos, 15310 Aghia Paraskevi, Attiki, Greece
[b] Laboratory of Physiology, Faculty of Medicine, University of Ioannina, Ioannina 45110 Greece

ARTICLE INFO

Article history:
Available online 12 March 2010

Keywords:
Titanium dioxide
Anticancer activity
Sarcoma cells killing
Lipooxygenase enzymic action inhibition
Human platelets aggregation

ABSTRACT

The antitumor activity of irradiated Degussa P25 TiO_2 nanoparticles (anatase/rutile ratio of about 3:1; average diameters of the anatase and rutile particles estimated at 25 and 85 nm, respectively) was investigated in vitro: sarcoma cells killing, lipooxygenase enzymic action inhibition; and ex vivo: human platelets aggregation, in order to get insight into the possible effects of photoexcited titanium dioxide in cancer treatment. Irradiated and non-irradiated TiO_2 concentrations (varying from 0.8 μM to 1000 μM) did not show any toxicity when cultured with fibroblasts MRC-5. In vitro cultured sarcoma cells, derived and isolated from sarcoma tumors produced on experimental Wistar rats after their treatment with benzo-[a]-pyrene, were completely killed in the presence of TiO_2 (40 μg/ml). The antimetastatic capability of titanium oxide in haematogenous spread of cancer cells was also confirmed in tests involving fresh human platelets with a number of different stimulators, including sarcoma cells, platelet activated factor (PAF), diphosphoric adenosine (ADP) and arachidonic acid. Sarcoma cells-human platelets mixtures irradiated by UV-C in the presence of TiO_2 showed a significant decrease in aggregation, effect that becomes more pronounced by increasing the titania concentration. Platelet aggregation triggered by PAF and arachidonate addition was totally inhibited by photoexcited TiO_2, while partial inhibition of aggregation triggered by ADP was observed. Addition of TiO_2 followed by irradiation with UV-C also resulted in an important decrease of lipooxygenase enzyme's activity.

1. Introduction

Titanium is widely used in biomedical applications. Its mechanical properties and biocompatibility, conferred by a layer of oxide present on its surface, make titanium the material of choice for various implants (artificial hip and knee joints, dental prosthetics, vascular stents, heart valves). Furthermore, the high refractive index of titanium oxide is advantageous in biosensor applications based on optical detection methods. In both of the above fields of application, novel surface modification strategies leading to biointeractive interfaces (that trigger specific responses in biological systems) are continuously sought [1]. Titanium implants have been used widely and successfully for various types of bone-anchored reconstructions. It is believed that properties of oxide films covering titanium implant surfaces are of crucial importance for a successful osseointegration, in particular at compromized bone sites [2].

It the literature it is well established that malignant cells are inactivated by photoexcited titania. In fact, TiO_2 particles are under intensive investigation for the photodecomposition of tumors, as they can drive various chemical reactions due to their strong oxidizing and reducing ability. In one study, photoexcited TiO_2 particles completely killed HeLa cells cultured in vitro and significantly suppressed the growth of HeLa cells implanted in nude mice. In contrast, very little effects were observed from TiO_2 treatment without UV irradiation [3]. The TiO_2 antitumor photoinduced activity was also investigated with success in vivo, under the skin of mice [4]. However, no relevant work was reported on sarcoma cells, one of the most aggressive and easily become resistant to medicines and treatments kinds of cancer.

It is also well established that platelets are involved in the haematogenous process of cancer metastasis. Thus, it has been observed that inhibition of platelets aggregation results in discontinuing of haematogenous metastasis, which it is proved that highly depends on the platelets environment [5–6]. Up to now however, practically very little is known about the behavior and quantification of the different factors expressing the platelets aggregation [7] in the presence of UV-treated titania nanoparticles and these effects need further clarification.

According to several research studies, lipoxygenase enzyme is involved in expression of some types of cancer. In fact, it has been reported that lipoxygenase inhibitors significantly sup-

* Corresponding author. Tel.: +30 2106503644; fax: +30 2106511766.
E-mail address: papi@chem.demokritos.gr (P. Falaras).

0920-5861/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.cattod.2010.02.016

pressed cell growth [8,9], inhibit proliferation and induce apoptosis in breast cancer cells in vitro [10]. Additionally, they inhibited proliferation of human bronchiolar lung cancer cell lines [11,12] whereas they hampered cell division and induced apoptotic cellular death on murine adenocarcinomas and lympholeukemia cells 388 [13,14]. Furthermore, lipoxygenase enzyme also augments tumor cell metastatic potential and it may serve as key target for the development of anti-invasive agents useful for combating the spread of prostate cancer [15–17]. However, besides a very rich literature on the above pure biological processes, no data exist on the lipoxygenase enzyme expression modifications induced by photoexcited titania nanoparticles, fact that could be very important in the case of sarcoma cells treatment.

Although the discovery of the photoinduced effects of titanium dioxide created new hopes for photocatalytic cancer treatment and therapy, in the literature there is a lack of systematic and global approach on the anticancer activity of titanium dioxide, especially in terms of process specification (in vitro and ex vivo), selectivity on neoplasm and inflammatory cells, and parallel processes such as cancer metastasis and promotion. To account for these effects, in the present study the antitumor activity of UV-C irradiated TiO_2 nanoparticles (versus non-illuminated TiO_2) was studied towards leiomyosarcoma cells (compared to normal fibroblast cells), platelet's aggregation and 2,3-lipoxygenase enzymic activity.

2. Experimental

2.1. General

In the biological experiments (sarcoma cells, fibroblasts, aggregation, lipoxygenase) Degussa P25 TiO_2 nanopowder, containing anatase and rutile phases in a ratio of about 3:1 (where the average diameters of the anatase and rutile particles are estimated to be about 25 and 85 nm, respectively), was used [18]. UV-C irradiation at 290 nm was assured using a 30 W lamp (Philips, 9 mm in diameter) for 10 min. During treatment with TiO_2 and or UV-C special care was taken in order to keep stable sterilization conditions, pH and incubation temperature. Statistical analysis was performed in all the experimental data, that were analysed using one-tailed *t*-test, considering $p < 0.005$ as significant level (Microsoft office excel 2003).

2.2. Cells toxicity

2.2.1. Toxicity of sarcoma cells

Sarcoma cells (mesenchymal tissue) cultures were treated with photoexcited TiO_2 nanoparticles at different concentrations: 0.8 μM, 1 μM, 5 μM, 10 μM, 30 μM, 50 μM, 100 μM, 200 μM, 400 μM, 800 μM, and 1000 μM. The cell line was established using chemically induced carcinogenesis in experimental Wistar rats [19]. This model uses a polycyclic aromatic hydrocarbon, benzo-[a]-pyrene (B[a]P) to produce tumor cells of mesenchymal origin that have particularly aggressive growth patterns and demonstrate rampant metastasis upon infusion. Cancer cells were seeded into multiwell dishes. The cells were exposed to multiplex concentrations of a test compound for 24-h and 48-h time periods. Log dilutions from the highest concentration were diluted using Dulbecco's modified Eagle's medium (DMEM, GIBCO BRL). At the end of the exposure period, the cultures were trypsinized and counted on a hemocytometer equipped with a microscope (Nikon Labophot, Japan, line: 220/240 V, 50–60 Hz lamp: 6 V, 20 W, fuse: 0.5 A).

Measurements of in vitro cell toxicity have been carried out in preliminary repetitions. Cell proliferation/survival was measured (%) in DMEM with 10% fetal calf serum (FCS, Sigma Chem. Co., St. Louis, MO, USA), incubated at 37 °C, 5% CO_2. The test compounds were each weighed and dissolved to equal a 1 mM solution. From this solution a dilution was made into the test range concentration of 0.8 mM down to 0.008 mM. Exponentially growing cancer cells were seeded in 3.5 cm, six-well. Culture plates at 50,000 cells/ml and allowed a 24-h doubling time then treated with 100 μl of various concentrations of tested compounds. After 24 and 48 h the cells were washed with buffer solution detached from support with trypsin-EDTA (Sigma Chem. Co., St. Louis, MO, USA), and counted on a hemocytometer (Bauer slide, Germany) [3].

2.2.2. Toxicity of fibroblast cells (MRC-5)

The same concentrations of photoexcited TiO_2 nanoparticles (0.8–1000 μM) were also tested for toxic activity against fibroblast cells (MRC-5), used as a biocompatibility reference [20]. The cell line used was established following the literature [21]. Fibroblast cells were seeded into multiwell dishes. The cells were exposed to multiplex concentrations of a test compound for 24-h time period. Log dilutions from the highest concentration were diluted using Dulbecco's modified Eagle's medium (DMEM). The procedure that was carried out and the concentrations of the tested compound were the same as described above for sarcoma cells. Exponentially growing fibroblast cells were seeded in 3.5 cm, 24-well. Culture plates at 250,000 cells/1.5 ml and allowed a 24-h doubling time then treated with 100 μl of various concentrations of tested compounds and were treated as sarcoma cells.

2.3. Platelet aggregation

The platelet antiaggregation function of TiO_2 was evaluated using an aggregometer from Chronolog Corporation with Aggro/Link software for Windows was tested. Fresh blood samples were obtained and centrifuged immediately at low speed ($1000 \times g \times 10$ min) to obtain platelet rich plasma (prp) used as test samples. The fresh blood was centrifuged again at high speed ($3000 \times g \times 10$ min) to obtain a reference sample of plasma with no platelets (platelet pure plasma or ppp). The platelet activators ADP, Arachidonic acid, and PAF were provided by Chronolog and prepared according to reagent directions.

The leiomyosarcoma cell line used was isolated from Wistar rats. These cells are of mesenchyme origin and metastatic to the lungs. Cells are maintained in DMEM with 10% FCS at 37 °C with 5% CO_2. Following trypsinization the cells are counted on a hemocytometer and resuspended at 500,000/50 μl. It has been determined in unpublished work that this is the dose giving peak reaction (500,000 cells in a 500 μl platelet sample) in aggregation studies. For test sample the titanium oxide dilutions (from 0.015 mM to 1 mM) were incubated in the platelet samples prior to adding the activator [22].

2.4. Enzymatic kinetics of 2,3-lipoxygenase

Lipoxygenase activity was assayed by UV analysis of the kinetic reactions of the enzyme at 254 nm at 37 °C, using a UV-VIS 1601 spectrophotometer form Shimadzu Corporation. The linear parts of the curves were used to calculate reaction rates using a molar extinction coefficient of 23 mM cm^{-1} for the conjugated dien formation. The biosynthesis of 13-HPODE was accomplished by 2000 μl linoleic acid (0.2 mM), by 950 μl buffers solution (boric acid 0.2 M, NaOH 50% pH 9) and by 50 μl enzyme solution (50 μl buffers or 500 units enzyme) in a total of the above 3 ml. The absorbance of the reaction mixture at 234 nm was recorded every second for 16.5 min [23–26]. All the reagents we used (including linoleic acid, boric acid and 2,3-lipooxygenase) were purchased from the Sigma Chem. Co. (St. Louis, MO).

3. Results and discussion

3.1. Photocytotoxicity of TiO_2 towards sarcoma cells and fibroblast cells (MRC-5)

Photoexcited TiO_2 nanoparticles were tested in leiomyosarcoma cells to evaluate their cytotoxicity. Sarcoma cells were harvested by leiomyosarcoma tumor that was produced by the means of B[a]P chemical carcinogenesis on experimental Wistar rats. These cells were cultured and incubated with titanium oxide. The cultures were performed in duplicates for each concentration of titanium oxide tested. One set of these duplicates was irradiated with UV-C for 10 min and the other set was not. Fig. 1 shows that at 24 h the population of sarcoma cells treated with TiO_2 only (without UV) is high and it is about the same amount as the population of control cells without any TiO_2 treatment. On the contrary, the photoexcited TiO_2-treated cells seem to be significantly lowered in population. At 5 μM concentration of the photoexcited compound only half of the cell population survives. From 10 μM up to 15 μM concentration a significant inhibition of the population is observed with a survival ratio approximately 35% at 10 μM and 4% at 15 μM. At concentrations of 50 μM and up cells are completely killed.

The above effects are more pronounced were the irradiation time was increased. Fig. 2 shows the cell population as it was recorded at 48 h of incubation. The cell number starts to decrease

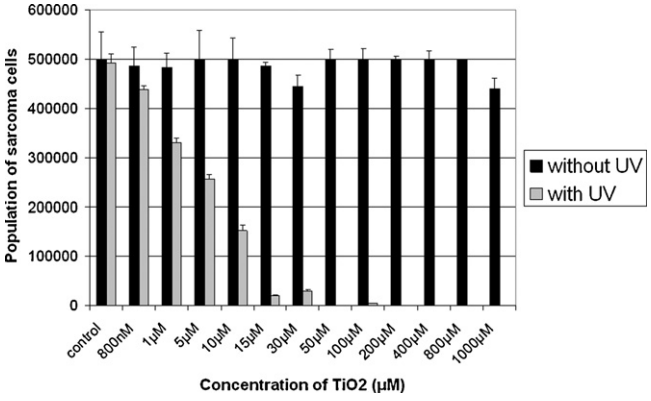

Fig. 1. Sarcoma cell population survived (average values ± SD), after 24 h of incubation in the presence of titanium oxide without (black) and with (grey) UV-C synergy (10 min), versus TiO_2 concentration.

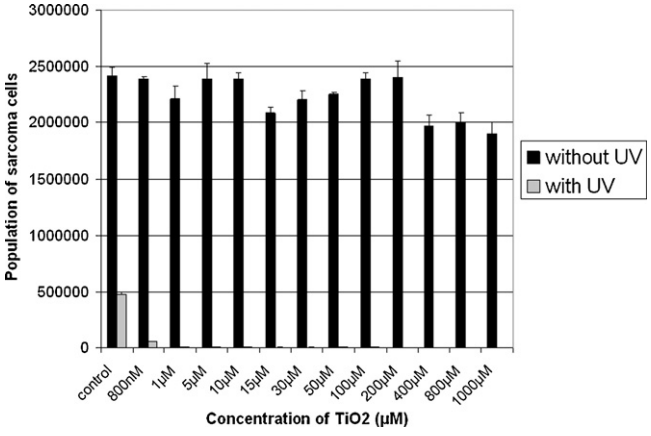

Fig. 2. Sarcoma cell population survived (average values ± SD), after 48 h of incubation in the presence of titanium oxide without (black) and with (grey) UV-C synergy (10 min), versus TiO_2 concentration.

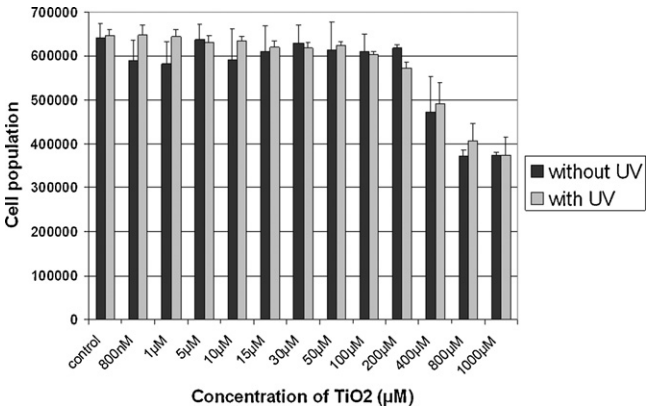

Fig. 3. Fibroblast (MRC-5) cell population survived (average values ± SD) after 24 h of incubation in the presence of titanium oxide without (black) and with (grey) UV-C synergy (10 min), versus TiO_2 concentration.

significantly even at 0.08 μM concentration of photoexcited TiO_2, and there is a complete cell death at 15 μM. Only 11% of the population of sarcoma cells survived after 48 h from treatment with 800 nM photoexcited TiO_2, while all the cell population was killed after 24 h when treated with the concentration of 50 μM. On the other hand, the population remained also practically unaffected, when the cells were incubated for 48 h in the presence of TiO_2, without UV irradiation.

Normal fibroblast cells were used to test the biocompatibility, specificity and selectivity of photoexcited TiO_2. The toxicity of both irradiated and non-irradiated TiO_2 was evaluated using the fibroblast cells MRC-5a normal cell line and TiO_2 concentrations varying from 0.8 μM to 1000 μM. In fact, fibroblast cells (MRC-5) were cultured and incubated with titanium oxide following the same procedure that was carried out for sarcoma cells. Cell counts were taken only at 24-h after incubation with the tested compound. The results presented in Fig. 3, confirm that cell population remained unchanged using titanium oxide with or without UV-C synergy and the same as the control ones.

Although misclassification of exposure and low exposure prevalence might have resulted in false negative results, there are studies suggesting that occupational exposure to titanium dioxide does not increase the risk of lung cancer [27]. As far as fibroblasts are concerned, biocompatibility of TiO_2 nanoparticle films has been demonstrated by the successful cell culture of human dermal fibroblast [20]. Concerning the mechanism of photoinduced cancer cell death, the exact mechanism of particle-induced cytotoxicity remains to be established. However, in vivo work on HeLa cells implanted in nude mice suggested that the cells were killed by the $OH^{\bullet}$ and H_2O_2 produced from photoexcited TiO_2 particles [3]. Under this option, the resistance of fibroblasts (normal cells) may be attributed to the presence of species that can act as quenchers of hydroxyl radicals and scavengers of hydrogen peroxide, respectively. On the other hand, direct attack on the cell membrane can not be excluded. In fact, the cell death process of the human bladder cell line T24 in the presence of UV-irradiated TiO_2 particles was associated with rapid increase in intracellular calcium concentration attributed to the fact that cell membrane permeability to Ca^{2+} cations was promoted prior to cell death [28]. Some other studies also refer to the role of reactive oxygen species (ROS) involving TiO_2 [29]. However, the wide range of proposed applications of oxide nanoparticles has raised questions about the potential impact of these nanoparticles and their risks on the environment and human health [30,31] and for this reason special care has to be taken to use them properly, especially for their application in cancer treatment.

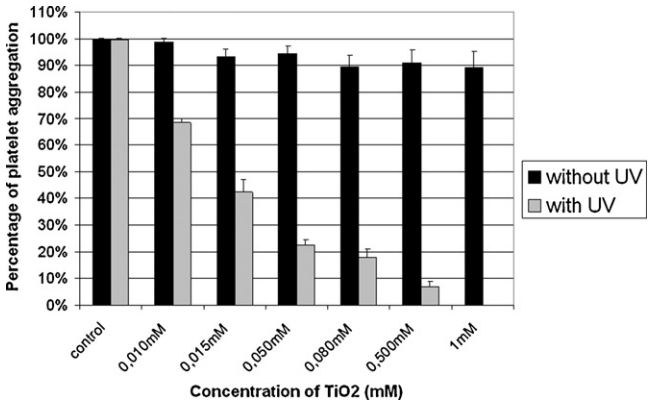

Fig. 4. Percentage of platelet's aggregation triggered by arachidonic acid (average values ± SD), after incubation in the presence of titanium oxide without (black) and with (grey) UV-C synergy (10 min), versus TiO_2 concentration.

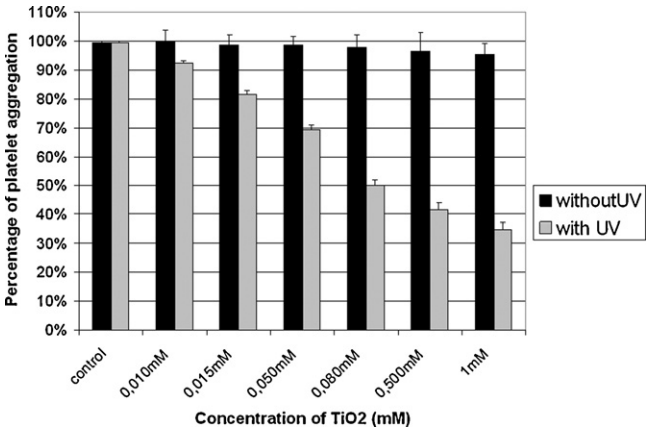

Fig. 6. Percentage of platelet's aggregation triggered by platelet activating factor (average values ± SD), after incubation in the presence of titanium oxide without (black) and with (grey) UV-C synergy (10 min), versus TiO_2 concentration.

3.2. Desegregation of human platelets

It is well known that platelets are involved in the haematogenous process of cancer metastasis. To put more insight in the intimetastatic behavior induced by the UV-irradiated TiO_2, the titania nanoparticles were also tested on fresh human platelet's aggregation, by considering and evaluating a number of factors stimulating the aggregation effect, including: platelet activating factor (PAF), arachidonic acid, diphosphate adenocine (ADP) and sarcoma cells.

Photoexcited TiO_2 (concentrations tested were 0.015 mM, 0.010 mM, 0.050 mM, 0.080 mM, 0.5 mM and 1 mM) were applied to human's platelets aggregation. Thus, for low concentrations, we confirmed no effect of TiO_2 (without UV-C synergy) on PAF platelet's aggregation, although statistically it may be a small effect, when concentration is equal or above 0.015 mM; Fig. 4). On the contrary, important PAF triggered desegregation was observed, when human platelets were treated with photoexcited titania. The results noted on platelet's aggregation were: 63% aggregation with addition 0.015 mM of the photoexcited compound, 7% aggregation with 0.5 mM and complete desegregation (0%) with 1 mM. Similar effects were observed in the case of arachidonic acid (Fig. 5). In fact, when 0.010 mM of photoexcited TiO_2 is added to arachidonate-triggered platelet aggregation it is recorded a 60% of aggregation, 0.015 mM of photoexcited TiO_2 gives 30% aggregation, 10% aggregation with 0.080 mM, and at 0.500 mM there is no aggregation at all. When it comes to ADP-triggered aggregations, the corresponding concentrations of the photoexcited compound result in higher percentages of platelet aggregation than the ones that were triggered by arachidonate. In that case (ADP-triggered platelet aggregation) the results (Fig. 6) confirmed: for 0.010 mM of the photoexcited compound there was a 93% aggregation, for 0.015 mM there was 82%, for 0.08 mM there was 50%, for 0.500 mM we recorded 42% and 35% for 1 mM. It was of a great interest to verify analogous effects using the sarcoma cell stimulators to trigger platelet aggregation. Indeed, when the platelets was incubated with the photoexcited TiO_2 and then were activated by the means of sarcoma cells, significant desegregation was initiated (Fig. 7): about 43% aggregation was recorded at 0.010 mM while no aggregation was observed at concentrations of the photoexcited compound higher than 0.080 mM. In contrast, when non-photoexcited TiO_2 was tested on human platelets, the platelets aggregation was unaltered (no change in aggregation was observed), when one considers the corresponding controls tests for each type of aggregation (Figs. 4–7). The above results are in excellent agreement with the literature. In fact, the blood compatibility of the titanium oxide film was established by measurements of blood clotting time and platelet adhesion [32]. Moreover, surfaces blasted with TiO_2 do not inhibit fibroblast attachment and smooth or finely grooved surfaces could be conducive to cellular attachment [33]. Concerning the photoinduced mechanism, the antiaggregation activity of the photoexcited TiO_2 particles may be attributed to the increase the system hydrophilic-

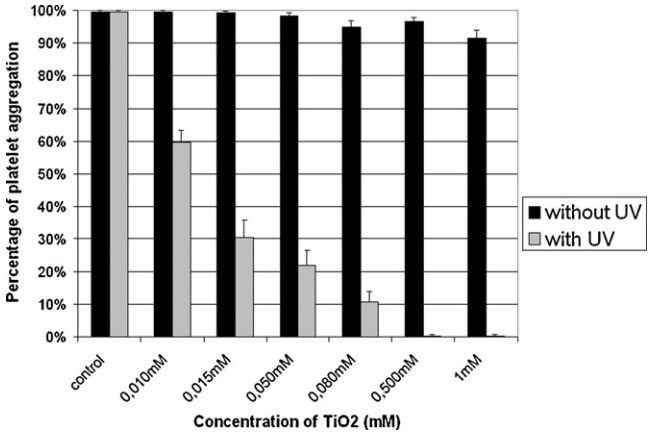

Fig. 5. Percentage of platelet's aggregation triggered by ADP (average values ± SD), after incubation in the presence of titanium oxide without (black) and with (grey) UV-C synergy (10 min), versus TiO_2 concentration.

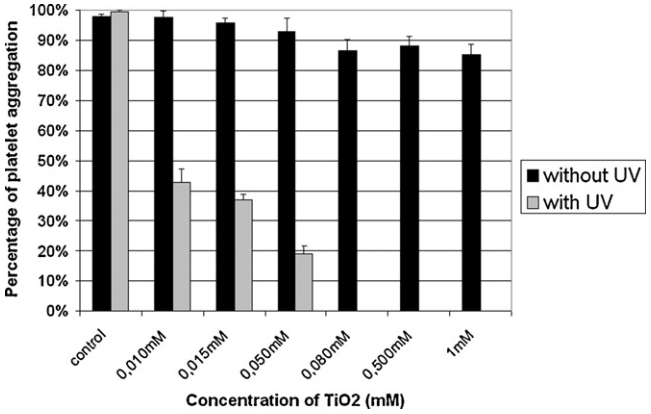

Fig. 7. Percentage of platelet's aggregation triggered by sarcoma cells (average values ± SD), after incubation in the presence of titanium oxide without (black) and with (grey) UV-C synergy (10 min), versus TiO_2 concentration.

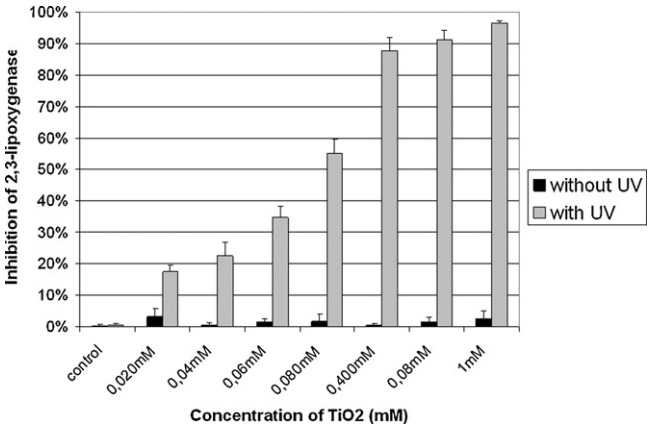

Fig. 8. Percentage of inhibition of 2,3-lipoxygenase (average values ± SD), after incubation in the presence of titanium oxide without (black) and with (grey) UV-C synergy (10 min), versus TiO_2 concentration.

ity, thus decreasing possible aggregation triggered by different stimulators [34–35].

3.3. Enzymatic kinetics of 2,3-lipoxygenase

To account for the activity photoexcited TiO_2 as a non-promoter of cancer, its inhibitory effect on the lipoxygenase enzyme action was also investigated. The kinetic method of lipooxygenase enzyme actually detects the production of 13-hyperoxolinoleic acid which the precursor molecule of leukotrienes. In this study, titanium oxide was incubated with the substrate and was irradiated for 10 min with UV-C. Then the kinetic reaction and its energetic (activity of the enzyme) was performed and recorded. The irradiated samples were compared to control irradiated samples containing only the enzyme, the substrate and the buffer with absence of the TiO_2 compound. The irradiation of all samples had the same duration time and no significant changes in the kinetic reaction of the enzyme were recorded.

Fig. 8 shows that titanium oxide is non-reactive without UV-C synergy as there is no change in the enzymic kinetic reaction. In contrast, significant alterations in the enzymatic activity were recorded when UV-C synergy was applied, even for concentrations as low as 0.020 mM. For the kinetic experiments with photoexcited TiO_2 on lipooxygenase enzyme activity the following results were recorded: 18% inhibition of the enzymic activity of lipooxygenase with addition of 0.02 mM TiO_2 irradiated with UV-C, 23% inhibition with 0.04 mM, 35% inhibition with 0.06 mM, 55% inhibition with 0.08 mM, 88% inhibition with 0.40 mM, 93% inhibition with 0.8 mM. Complete inhibition (100%) of the enzymatic activity which was achieved at 1 mM TiO_2 concentration, although no alteration in lipooxygenase's activity was observed, when only UV-C irradiation was applied.

The above results are well supported by the literature, postulating that TiO_2 alone did not affect enzymatic release [36], whereas it strongly influences the expression of adenosine triphosphate (ATP), a nucleotide that is produced following the action of lipoxygenase. Although a direct interaction between the lipoxygenase enzyme and the semiconductor cannot be excluded, our results however are consistent with electrophilic enzyme properties and especially their proved ability to capture electrons from the photoexcited TiO_2 [37].

Thus, the findings of our experiments indicate that TiO_2 when irradiated with UV-C is effective in all three biological systems (waster rat's sarcoma cell cultures, human's platelet aggregation and lipooxygenase enzymatic activity), while TiO_2 without synergy of UV-C is not active. Additionally, the final concentration of the photoexcited TiO_2 that is noted to have the best effect when all three biological systems (cells, platelets and enzyme) are combined is 0.08 μM.

4. Conclusions

The antitumor activity of photoexcited TiO_2 particles, studied in vitro and in vivo as well as ex vivo, gave very promising results for the possible use of this effect for cancer treatment. The performed work indicates that titanium dioxide is effective in all three experimental anticancer aspects: sarcoma cell cultures, platelet aggregation and lipooxygenase enzyme's activity. The results confirm that TiO_2 nanoparticles irradiated with UV-C are able to inhibit proliferation of sarcoma cells while it was found that they had no effect when applied to a normal fibroblast cell line. Cell counts at 24 h after the addition of 5 μM photoexcited TiO_2 showed that only 50% of the cell population survived while at 48 h no cell survived. In contrast, when the cells were treated with the corresponding concentrations of non-photoexcited TiO_2 showed no alteration in numbers compared to the control ones.

The irradiated titania nanoparticles also inhibit platelet's aggregation and hinder the expression of 2,3-lipoxygenase reaction. This does not take place when TiO_2 alone is applied. The optimum concentration where the photoexcited TiO_2 has the best effect when all three biological systems (cells, platelets and enzyme) are combined is 0.08 μM. Thus, TiO_2 in conjunction with UV-C synergy are excellent candidates for anticancer, antimetastatic and anti-inflammatory applications. Although work in progress shows that the mechanism of photocatalytic cancer cells killing involves apoptosis, however, further insight is required into intracellular signaling and on the role of oxygen radicals. In addition, optimization of the processes in terms of the nano-photocatalyst properties (structure, particle size, surface area, band-dap) and corresponding light parameters (intensity, wavelength) is also underway.

Acknowledgements

The authors gratefully acknowledge financial support from the GSRT/Ministry of Development, Greece (PEP ATTIKIS project) and "Clean Water" Collaborative Project (Grant Agreement Number 227017), co-funded by the Research DG of the European Commission within the joint RTD activities of the Environment and NMP Thematic Priorities/FP7. The authors also thank T. Kartsiouni, S. Karkabounas (Laboratory of Physiology, Faculty of Medicine, University of Ioannina), as well as D. Rizos, C. Gogos (Intensive Care Unit) and C. Pliakos (Microbiology Laboratory) from Xatzikosta Hospital of Ioannina for valuable assistance.

References

[1] F.F. Rossetti, M. Bally, R. Michel, M. Textor, I. Reviakine, Langmuir 21 (2005) 443–450.
[2] Y.T. Sul, C.B. Johansson, S. Petronis, A. Krozer, Y. Jeong, A. Wennerberg, T. Albrektsson, Biomaterials 23 (2002) 491–501.
[3] R.I. Cai, Y. Kubota, T. Shuin, H. Sakai, K. Hashimoto, A. Fujishima, Cancer Res. 52 (1992) 2346–2348.
[4] A. Fujishima, K. Hashimoto, T. Watanabe, TiO_2 Photocatalysis, Fundamentals and Applications, BKC, Inc., Tokyo, 1999.
[5] G. Cox, R.A. Walker, A. Andi, W.P. Steward, K.J. O'Byrne, Lung Cancer 29 (2000) 169–177.
[6] A. Östman, Cytokine Growth Factor Rev. 15 (2004) 275–286.
[7] R.E. Shepherd, S.K. Hadjikakou, C.D. Antoniadis, N. Hadjiliadis, M. Kubicki, J. Binolis, S. Karkabounas, K. Charalabopoulos, Inorg. Chim. Acta 358 (2005) 2861–2866.
[8] E. Mitsuharu, N. Masakuni, K. Kazuo, T. Motohiro, Oncology 52 (1995) 150–155.
[9] N. Rama, Front. Biosci. 3 (1998) 81–88.
[10] W.G. Tong, X.Z. Ding, T.E. Adrian, Biochem. Biophys. Res. Commun. 296 (2002) 942–948.
[11] D. Ou, P. Bonomi, W. Jao, S. Jadko, J.E. Harris, K.M. Anderson, Cancer Lett. 166 (2001) 223–231.

[12] I.M. Avis, M. Jett, T. Boyle, M.D. Vos, T. Moody, A.M. Treston, A. Martinez, J.L. Mulshine, J. Clin. Invest. 9 (1996) 806–813.
[13] V.V. Shaposhnikova, L.N. Kublik, A.A. Narimanov, M.Kh. Levitman, O.E. Orlova, A.A.I. Kudriavtsev, V.V. Leshchenko, Iu.N. Korystov, Zv. Akad, Ser. Nauk. Biol. 2 (2001) 249–252.
[14] H.J. Hussey, M.J. Tisdale, Br. J. Cancer 74 (1996) 683–687.
[15] B. Liu, R.J. Maher, Y.A. Hannun, A.T. Porter, K.V. Honn, J. Natl. Cancer Inst. 86 (1994) 15–18.
[16] F.A. Attiga, P.M. Fernandez, A.T. Weeraratna, M.J. Manyak, S.R. Patierno, Cancer Res. 60 (2000) 4629–4637.
[17] J. Hosh, C.E. Mayers, Biochem. Biophys. Res. Commun. 235 (1997) 418–423.
[18] T. Ohno, K. Sarukawa, K. Tokieda, M. Matsumura, J. Catal. 203 (2001) 82–86.
[19] S. Karkabounas, D. Assimakopoulos, M. Malamas, A.L. Skaltsounis, S. Leonce, J. Zelovitis, D. Stefanou, A. Evangelou, Anticancer Res. 20 (2000) 4391–4395.
[20] D.S. Kommireddy, A.A. Patel, T.G. Shutava, D.K. Mills, Y.M. Lvov, J. Nanosci. Nanotechnol. 5 (2005) 1081–1087.
[21] E. Kolettas, A. Evangelou, E.S. Gonos, Anticancer Res. 21 (2001) 435–444.
[22] S. Karkabounas, G. Sofis, A. Evangelou, Epitheorese Klinikes Pharmakologias kai Farmakokinetikes. International Edition, vol. 10, 1996, pp. 84–91.
[23] W. Chamulitrat, R.P. Mason, J. Biol. Chem. 264 (1989) 20968–20973.
[24] J.M. Nelson, D.G. Batt, J.S. Thompson, S.W. Wright, J. Biol. Chem. 266 (1991) 8225–8229.
[25] J.M. Nelson, S.P. Seitz, R.A. Cowling, Biochemistry 29 (1990) 6897–6903.
[26] C. Kemal, P.L. Flamberg, R. Krupinski-Olsen, A.L. Shorter, Biochemistry 26 (1987) 7064–7072.
[27] P. Boffetta, V. Gaborieau, L. Nadon, M.F. Parent, E. Weiderpass, J. Siemiatycki, J. Work Environ. Health 27 (2001) 227–232.
[28] H. Sakai, E. Ito, R.X. Cai, T. Yoshioka, Y. Kubota, K. Hashimoto, A. Fujishima, Biochim. Biophys. Acta 1201 (1994) 259–265.
[29] T.C. Long, N. Saleh, R.D. Tilton, G.V. Lowry, B. Veronesi, Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): Implications for nanoparticle neurotoxicity, Environ. Sci. Technol. 40 (2006) 4336–4345.
[30] M. Auffan, L. Decome, J. Rose, T. Orsiere, M. De Meo, V. Briois, C. Chaneac, L. Olivi, J.L. Berge-Lefranc, A. Botta, M.R. Wiesner, J.Y. Bottero, Environ. Sci. Technol. 40 (2006) 4367–4373.
[31] M.R. Wiesner, G.V. Lowry, P. Alvarez, D. Dionysiou, P. Biswas, Environ. Sci. Technol. 40 (2006) 4336–4345.
[32] L. Xianghuai, Z. Zhihong, Z. Zhuyao, H. Nan, Y. Ping, C. Guanjun, J. Yuanru, Biomater. Appl. 10 (1996) 330–337.
[33] K. Mustafa, B. Silva Lopez, K. Hultenby, A. Wennerberg, K. Arvidson, Clin. Oral Implants Res. 9 (1998) 195–207.
[34] A.I. Kontos, A.G. Kontos, D.S. Tsoukleris, G.D. Vlachos, Polycarpos Falaras, Thin Solid Films 515 (2007) 7370–7375.
[35] A.G. Kontos, A.I. Kontos, D.S. Tsoukleris, V. Likodimos, J. Kunze, P. Schmuki, P. Falaras, Nanotechnology 20 (2009) 045603, 9 pp.
[36] J.K. Kim, W.K. Lee, E.J. Lee, Y.J. Cho, K.H. Lee, H.S. Kim, Y. Chung, K.A. Kim, Y. Lim, J. Toxicol. Environ. Health A 58 (1999) 437–450.
[37] M.L. Ganadu, L. Andreotti, I. Vitali, A. Maldotti, A. Molinari, G.M. Mura, Photochem. Photobiol. Sci. 1 (2002) 951–954.