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Diverse Responses of Neurons and Monocytes to Titanium Dioxide Nanoparticle Exposure

Titanyum Dioksit Nanopartikül Maruziyetinde Nöron ve Monositlerin Farklı Yanıtları

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Abstract

Introduction: The toxicity of titanium dioxide nanoparticles (TiO_2NPs) in neurons occurs by glutamate signaling via N-methyl-d-aspartate (NMDA) receptors. Although cellular uptake of TiO_2NPs may lead to oxidative stress in macrophages, it is not known whether TiO_2NPs have toxic effects on U937 monocytic cell line.

Material and Methods: Human neuroblastoma (SH-SY5Y) and U937 human monocytic cell lines were exposed to 25nm and 10nm TiO_2NPs, in medium with or without fetal bovine serum (FBS). Mitochondrial metabolic activity was assessed using the MTT-assay before and after treatment with 15 mM N-acetylcysteine (NAC) and 0.1μM or 10μM neopterin.

Results: TiO_2NPs displayed no toxicity on SH-SY5Y and U937 cells in FBS-free medium. The addition of FBS resulted in a significant reduction in cell viability with both sizes of TiO_2NPs on SH-SY5Y and U937 cells. In FBS-containing medium, NAC pretreatment significantly increased cell viability of SH-SY5Y cells in comparison to U937 cells. Both neopterin doses enhanced cell viability of TiO_2NPs-exposed SH-SY5Y cells for all concentrations. Only a limited increase in the cell viability was achieved in 10nm TiO_2NPs-exposed neurons by pretreatment with neopterin. Whereas, neopterin could not provide a constant amelioration for both 25nm and 10nm sized TiO_2NPs-exposed U937 monocytic cells. TiO_2NPs displayed size-dependent neuronal toxicity. In FBS-containing medium, both sizes of TiO_2NPs caused reduction in cell viability of both cell lines.

Conclusion: While toxicity of TiO_2NPs emerged via NMDA and AMPA receptors in SH SY5Y cells, U937 cells were most probably activated by AMPA receptors only. Unlike SHSY5Y cells, NADPH oxidase complex inhibition was not effective in TiO_2NPs exposed U937 cells.

Keywords: Titanium dioxide nanoparticle, N-acetyl cysteine, neopterin, U937 monocytic cells, SH-SY5Y neuroblastoma cells, N-methyl-D-aspartate receptors

Öz

Giriş: Titanyum dioksit nanopartiküllerinin (TiO_2NP) toksisitesi nöronlarda N-metil-D-aspartat (NMDA) reseptörleri aracılığıyla glutamat sinyal iletimi ile meydana gelir. TiO_2NP makrofajlar tarafından hücre içine alınması oksidatif strese neden olmakla birlikte, U937 monositik hücreleri üzerine TiO_2NP toksik etki gösterip göstermediği bilinmemektedir.

Gereç ve Yöntemler: İnsan nöroblastoma (SH-SY5Y) ve U937 insan monositik hücre hatları 25nm ve 10nm TiO_2NP ile fetal sığır serumu (FBS) varlığında ya da yokluğunda inkübe edildi. Mitokondriyal metabolik aktivite 15 mM N-asetil sistein (NAC) ve 0.1μM veya 10μM neopterin uygulanmadan önce ve sonra MTT ile tayin edildi.

Bulgular: TiO_2NP, FBS içermeyen besi yerinde SH-SY5Y ve U937 hücrelerinde canlılıkları değiştirmedi. Her iki boyuttaki TiO_2NP için de ortama FBS eklenmesi SH-SY5Y ve U937 hücrelerinin canlılıklarının anlamlı olarak azalmasına neden oldu. FBS içeren besi yerinde NAC ile önceden muamele, SH-SY5Y hücre canlılıklarını U937'lere göre anlamlı olarak artırdı. Her iki neopterin dozu da bütün TiO_2NP konsantrasyonları için SH-SY5Y hücrelerinin canlılıklarında artışa neden oldu. 10nm TiO_2NP'e maruz kalan SH-SY5Y hücrelerinin canlılığı neopterin ile kısıtlı miktarda düzeltilebildi. Ancak, neopterin ilavesi, 10nm ve 25nm TiO_2NP maruz kalan U937 monositik hücrelerinin canlılıkları üzerine etkili olamadı. TiO_2NP nörotoksisitesi partikül büyüklüğü bağımlıdır. FBS içeren besi yerlerinde her iki hücre hattının da canlılığı TiO_2NP ile azalmıştır.

Sonuç: TiO_2NP toksisitesine SH-SY5Y hücrelerinde hem NMDA hem de AMPA reseptörleri aracılık ederken, U937 hücrelerinde büyük olasılıkla sadece AMPA reseptörleri görev almaktadır. SH-SY5Y hücrelerinin aksine U937 hücrelerinde NADPH oksidaz kompleksinin inhibisyonu TiO_2NP toksisitesi üzerine etkili olmamıştır.

Anahtar Kelimeler: Titanyum dioksit nanopartikülü, N-asetil sistein, neopterin, U937 monositik hücreleri, SH-SY5Y nöroblastoma hücreleri, N-metil-D-aspartat reseptörü

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Introduction

Titanium dioxide (TiO_2) nanoparticles (NPs) are manufactured in large quantities for uses including in plastics industry, electronics, cosmetic products and nanomedicine.[1]

Degradation products of TiO_2NPs include free ions, organo-metallic complexes particles that are ranged from nano to macro sizes.[2,3] Several studies have reported that degradation products of TiO_2NPs can be absorbed into the human body by inhalation, ingestion and dermal penetration.[4-8] Following the entry to the systemic circulation, NPs can be distributed to vital organs and readily cross the cell membranes and blood-brain barrier, and are acummulated.[4-8] In this context, toxicity risk of TiO_2 arises with the accumulation of NPs in the neurons and other cells.[4–8] Furthermore, many factors including particle size, shape, chemical composition and surface charges affect the magnitude of TiO_2NPs toxicity.[9–11] The aggregation of TiO_2NPs is important for their toxicity.[12] Thus, the colloidal stability of TiO_2NPs is dependent on the presence and concentration of human serum albumin. Indeed, albumin prevents high aggregation rate of NPs.[13,14]

Reactive oxygen species (ROS) generation mediated oxidative stress and impaired antioxidant capacity are the major causes of cytotoxicity of TiO_2NPs.[15,16] Recently, Coccini et al. showed that TiO_2NPs show neurotoxic effects on human glial and neuronal cell lines.[17] Thus, TiO_2NPs pass through the cell membranes into the cytoplasm or nucleus, and significantly suppress hippocampal neurons in a concentration-dependent manner.[8,18] Furthermore, they induce a marked release of glutamate to the extracellular region and significantly inhibit the expression of N-methyl-D-aspartate (NMDA) receptor subunits including NR1, NR2A, and NR2B.[8,18] Although TiO_2NPs have attracted extensive interest due to their use in wide range of applications, their toxicity mechanisms on different human cells are still quite uncertain.[17] Cellular internalization of TiO_2NPs activates macrophages and neutrophils that contributes to superoxide anion production by the hyper-activation of NADPH oxidase (NOX).[19] It has been shown that TiO_2NPs cause production of ROS, nitric oxide, and activation of NF-κB pathway in endothelial cells through stimulating the expression of adhesion molecules in monocytic cells.[20,21] The human leukemic monocyte lymphoma (U937) cell line treated with nanoparticles exhibit a distinct signaling pathway response to inhibit or stimulate cytokine production.[22] Considering the widespread use of titanium in various prostheses in clinical practice, this is the first study to demonstrate the effect of neopterin, a chronic immune activation mediator, on cell death induced by TiO_2NPs in neurons and monocytic cells.

However, it is well-known that the toxicity mechanism of TiO_2NPs is related to the glutamatergic signaling in neurons.[8,18] But it is not clear whether similar mechanism is valid in monocytic cells. In addition, there is very limited evidence about TiO_2 toxicity in many pathologies where serum neopterin level is increased or N-acetylcysteine (NAC) therapy is used in clinical practice.[23,24] In this study, responses of TiO_2NPs exposed SH-SY5Y human neuroblastoma cells were compared with the U937 monocytic cell line in the medium with or without fetal bovine serum (FBS) before and after treatment with NAC and neopterin.

Materials and methods

In this study, SH-SY5Y, human neuroblastoma and U937 human monocytic cell lines were exposed to ten different concentrations of 25 nm and 10 nm TiO_2NPs in medium with or without fetal bovine serum (FBS), for three different time periods. Afterwards, the cells were treated with 15 mM NAC and 0.1 μM or 10 μM neopterin in addition to the NPs, in the determined incubation periods and medium composition. Toxicity patterns of TiO_2NPs were compared by determining mitochondrial metabolic activity (MTT) of these cell lines in conditioned mediums.

Nanoparticle preparation

TiO_2 NPs were a gift from Dr. Marie Carrière (Université Grenoble Alpes, Grenoble, France). The TiO_2 bulk material was purchased from Sigma-Aldrich (Paris, France) and P25 nanomaterial was from Degussa-Evonik (Germany). OCTi60 were laboratory samples from the Service des Photons (Saclay, Gif-sur Yvette, France). The samples were synthesized by gas phase methods, combustion (P25; 25 nm) or laser pyrolysis (OCTi60; 10 nm) (Pignon et al., 2008) and were composed of a major phase of anatase and a minor phase of rutile (Table 1). The preparation of suspensions was done using a several steps method detailed elsewhere.[25,26] Stock suspensions of 10 nm and 25 nm sized TiO_2 NPs (10 mg/mL) were prepared in water.

Cell culture

The human monocytic U937 cell line was obtained from Ankara University Biotechnology Institute and cultured

Table 1. Characteristics of TiO2 P25 and OCTi60 NPs[19,20]

TiO_2 NPs	Anatase/ rutile	Diameter (nm) (TEM)	Diameter (um) (BET)	Diameter in water Z average	Z-potential in water (mV)	Diameter in medium Z average	Z-potential in medium (mV)
P25 (25nm)	85/15	23	25 (60 m^2/g)	140	+1	220	-8 to -12
OCTi60 (10nm)	90/10	10	16 (95 m^2/g)	70	-6	170	-9 to -12

in RPMI 1640 (Sigma-Aldrich Co., USA) supplemented with 10% FBS and 1% antibiotic and antimycotic solution (Penicillin-streptomycin, 100x, Biological Industries, Israel) at 37°C under a humidified atmosphere of 5% CO2/95% air. SH-SY5Y cells were incubated in Ham F12: EMEM (1:1) medium (SigmaAldrich Co.) supplemented with 15% FBS. The cells were incubated with NPs in three time points as 6, 24 and 48 hours. For 6 hours of incubation the cells were seeded in medium without FBS (incomplete medium) and the medium was supplemented with FBS (complete medium) for the incubation periods of 6, 24 and 48 hours. NP solutions were diluted with medium according to the cell line that is used.

Mitochondrial activity

Mitochondrial activity was assessed spectrophotometrically, by using MTT-assay according to a modified method of Mosmann.[27] Cells (10^4 cells/well) were seeded in 96-well plates. Twenty-four hours after seeding (one cell cycle), the cells were exposed to 10 different concentrations of TiO_2NPs, ranging between 0.2 and 100 µg/mL, for three time periods, 6 (with and without FBS), 24 and 48 hours. All the assays performed in triplicates in three sets of experiments. For each experiment, the particle suspension was freshly prepared and diluted to appropriate concentrations. Culture medium without TiO_2NPs served as the control in each experiment. Cells were counted by using trypan blue for each time point and for each concentration in every assay condition.

By the evaluation of the first experiment series, the NP doses and exposure periods were chosen for NAC and neopterin assays. NAC concentration was relevant to the treatment doses[23,28,29], while neopterin doses were chosen according to our previous studies and human serum levels.[30] For further experiments, the cells were pre-incubated for 30 minutes with 15 mM NAC or 0.1 or 10 µM neopterin. After this period, the cells were incubated with 0.8 µg/mL, 50 µg/mL and 100 µg/mL, 25 nm or 10 nm TiO_2NPs for 24 hours in FBS containing or FBS-free medium. For all treatments MTT assay was performed.

Statistical Analysis

The significance of the difference between control and compound treated cell groups were analyzed by Mann-Whitney U test and $p<0.05$ was considered statistically significant. The calculations were performed by using the statistical package SPSS, version 13.0 (SPSS Inc., Chicago, Illinois, USA).

Results

The concentration-dependent effects of 25 or 10 nm TiO_2NP on the viability of SH-SY5Y and U937 cells cultured in FBS-containing or FBS-free medium, were compared. The results show that both sizes of TiO_2NPs-exposed SH-SY5Y and U937 cells display viability more than 80% in FBS-free medium at the end of 6-hour incubation period. (Fig. 1 and 2). The addition of FBS to the cell culture medium resulted in a statistically significant TiO_2NPs size-dependent reduction in cell viability of U937 cells (Fig. 1) ($p<0.05$). On the other hand, the viability of SH-SY5Y cells exposed to 10 nm TiO_2NPs significantly decreased at 24 hours while 25 nm was more effective at 6 hour-incubation period (Fig. 2) ($p<0.05$). The metabolic activity percentile per cell and mean value of cell viabilities of ten different concentrations (from 0.39 ug/mL to 100 ug/mL for 10 or 25 nm) of TiO_2NPs-exposed SH-SY5Y neurons were significantly lower than U937 monocytic cells at the end of 24-hour incubation period in FBS containing medium (Fig. 1 and 2). These results might indicate that the different effects of TiO_2NPs on mitochondrial dysfunction/cell viability of SH-SY5Y cells in comparison to the U937 cells, were related to the different regulatory mechanisms of redox signaling of SH-SY5Y cells. In FBS-containing medium, decreases in cell viabilities of NAC pretreated human U937 monocytic cells were negatively correlated with the size of TiO_2NPs for 0.8, 50 and 100µg/mL. Whereas, NAC pretreated SH-SY5Y cells displayed highly significant increase in viability (mitochondrial metabolic activity) of cells when compared to U937 cells (Fig. 4) ($p<0.05$). The lack of the

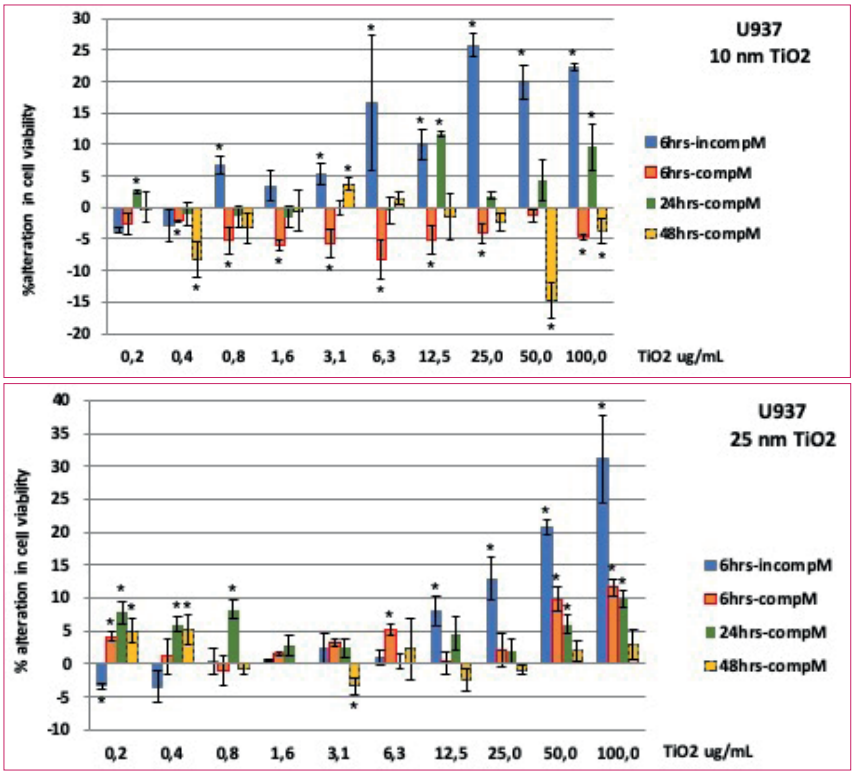

Figure 1. Alteration in cell viabilities of TiO_2NPs-exposed U937 monocytic cells. 10 nm TiO_2NPs (a); 25 nm TiO_2NPs (b) (*p<0.05; TiO2NPs free group vs TiO2NPs treated groups).

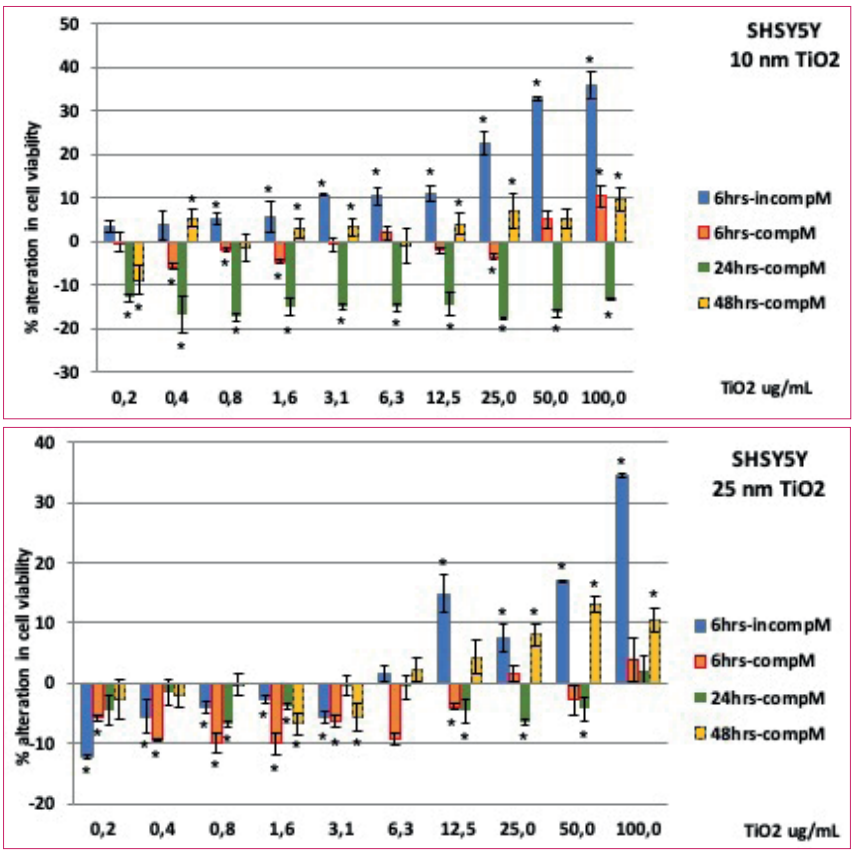

Figure 2. Alteration in cell viabilities of TiO_2NPs-exposed SH-SY5Y neurons. 10 nm TiO_2NPs (a); 25 nm TiO_2NPs (b) (*p<0.05; TiO2NPs free group vs TiO2NPs treated groups).

protective effects of NAC pretreatment on the cell viabilities (mitochondrial metabolic activities) of U937 monocytic cells in comparison to only-TiO_2NP treated U937 cells indicated that the glutamatergic mechanism was negligible in the harmful effects of TiO_2NP upon U937 cells (Fig. 3) ($p>0.05$). However, NAC pretreatment provided a significant increase in viability of U937 cells against 25 nm of TiO_2NPs at 50μg/mL concentration (2.4 fold increase, $p=0.033$). This unusual increase in cell viability was attributed to glutathione formation from NAC by U937 cells.

In FBS containing medium, although both 25 and 10 nm TiO_2NPs showed significant decreases in viability of SH-SY5Y cells ($p<0.05$), 10 nm TiO_2NPs caused 4.4 folds decrease in cell viability in comparison to 25nm NPs ($p<0.05$). This ratio was 1.8 for U937 cells. The addition of 0.1 μM or 10 μM neopterin to the cell culture medium could not provide an efficient increase in monocytic cell viability for both sizes of TiO_2NPs, for all concentrations when compared to only-TiO_2NP treated U937 cells ($p>0.05$, for all concentrations). 10 μM neopterin provided a remarkable increase in viability of 25 nm TiO_2NPs-exposed SH-SY5Y cells in comparison to 0.1 μM neopterin supplemented medium, for all NP concentrations ($p=0.006$-0.028). Both neopterin concentrations did not protect the SH-SY5Y neurons against the harmful effect of 10 nm sized TiO_2NPs. (Fig. 4)

Discussion

FBS is one of the most popular complements for cell culture and a significant source of glutamate that can be at concentrations sufficient to kill primary cultured hippocampal neurons. The glutamate concentration in several batches of FBS is close to 1 mM, thus 10% serum supplement to culture media

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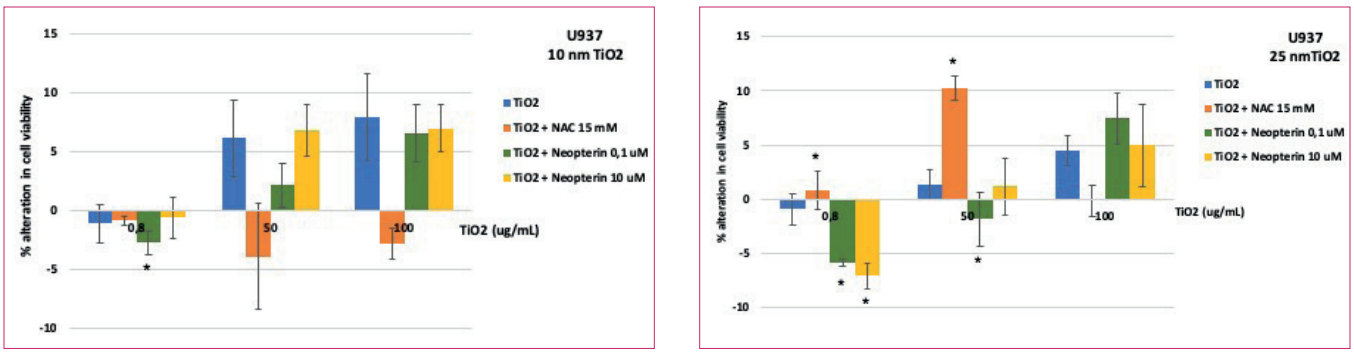

Figure 3. Alteration in cell viabilities of TiO_2NPs + NAC or Neopterin exposed U937 monocytic cells. 10 nm TiO_2NPs (a); 25 nm TiO_2NPs (b) (*p<0.05; TiO2NPs treated group vs TiO2NPs+ NAC or TiO2 + neopterin treated groups).

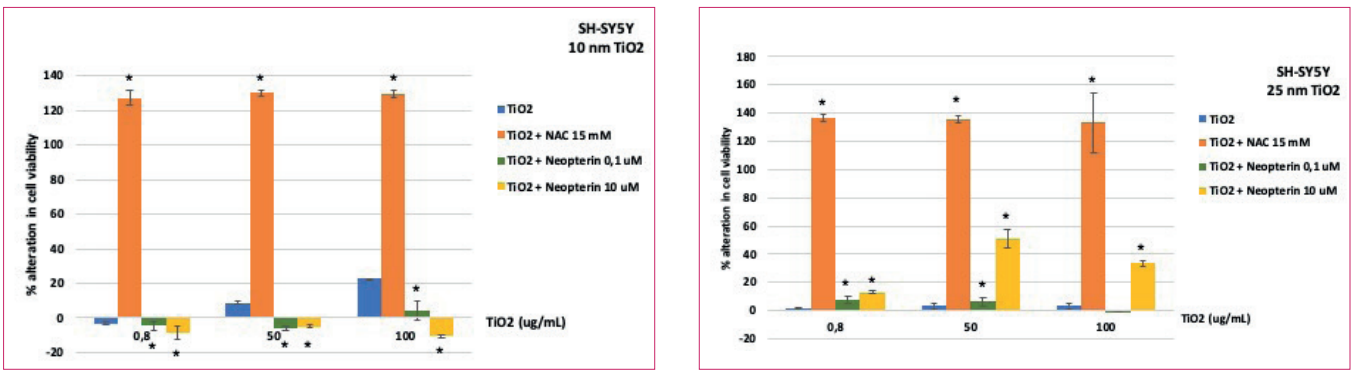

Figure 4. Alteration in cell viabilities of TiO_2NPs + NAC or Neopterin exposed SH-SY5Y neurons. 10 nm TiO_2NPs (a); 25 nm TiO_2NPs (b). (*p<0.05; TiO2NPs treated group vs TiO2NPs+ NAC or TiO2 + neopterin treated groups).

results in glutamate concentration of 30–100 μM due to serum itself. The vulnerability of neurons to medium changes can be solely explained by excitotoxicity resulting from serum-borne glutamate.[31] Furthermore, the addition of FBS prevents high agglomeration, leading to a stable dispersion of TiO_2NPs for at least 24 h, possibly due to steric stabilization of the particles.[32] NP-protein complexes indicate that cellular response is associated with both NP and specific features of the NP environment.[14] Hence proteins compete for the NP surface, leading to a protein corona that largely defines the biological identity of the particle and further interaction pattern with biological systems.[33,34] Although cellular uptake is clearly NPs size-dependent, a higher mass concentration in the case of larger NPs, but a higher particle number for the smaller NPs is required.[35] Small groups of nano sized particles are engulfed by the monocytes and sequestered as intracytoplasmic aggregates after 24-h exposure to TiO_2NPs. The accumulation of TiO_2NPs favors mitochondrial dysfunctions and oxidative stress.[36] The TiO_2NPs-induced cytotoxicity on monocytes is associated with intracellular ROS generation, collapse of the mitochondrial membrane potential (MMP) and depletion of glutathione.[24,37] The release of TNF-α is enhanced by TiO_2NPs-exposure in U937 cells.[38–40] TNF-α secretion, increases ROS production, and lowers cAMP levels in U937 cells.[41] U937 cells cannot express NMDA receptor.[42] U937 monocytes most probably responded to glutamatergic stimulation through α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor only (Fig. 5).[43] Elevation of intracellular Ca^{2+} levels causes the activation of p38 MAPK upon apoptotic stimulus in U937 cells.[44] TiO_2NPs induce both apoptotic and necrotic cell death via TNF-α expression in U937 cells.[38,45] In this study, although TNF-α was not measured, our results clearly supported by the evidences described above.

The uptake of TiO_2NPs by SH-SY5Y cells in cultures is time-and concentration-dependent.[46] While NAC increases glutamate levels, it is converted to cysteine, which causes the reverse transport of glutamate into the extracellular space from neurons.[47,48] Therefore, it is presumed that NAC administration directly regulates the amount of glutamate present in the extracellular space.[49,50] The amount of cysteine in the system as well as the feedback via reduced glutathione (GSH) production by neurons may directly regulate the amount of glutamate present in the extracellular space.[51,52] Furthermore, GSH

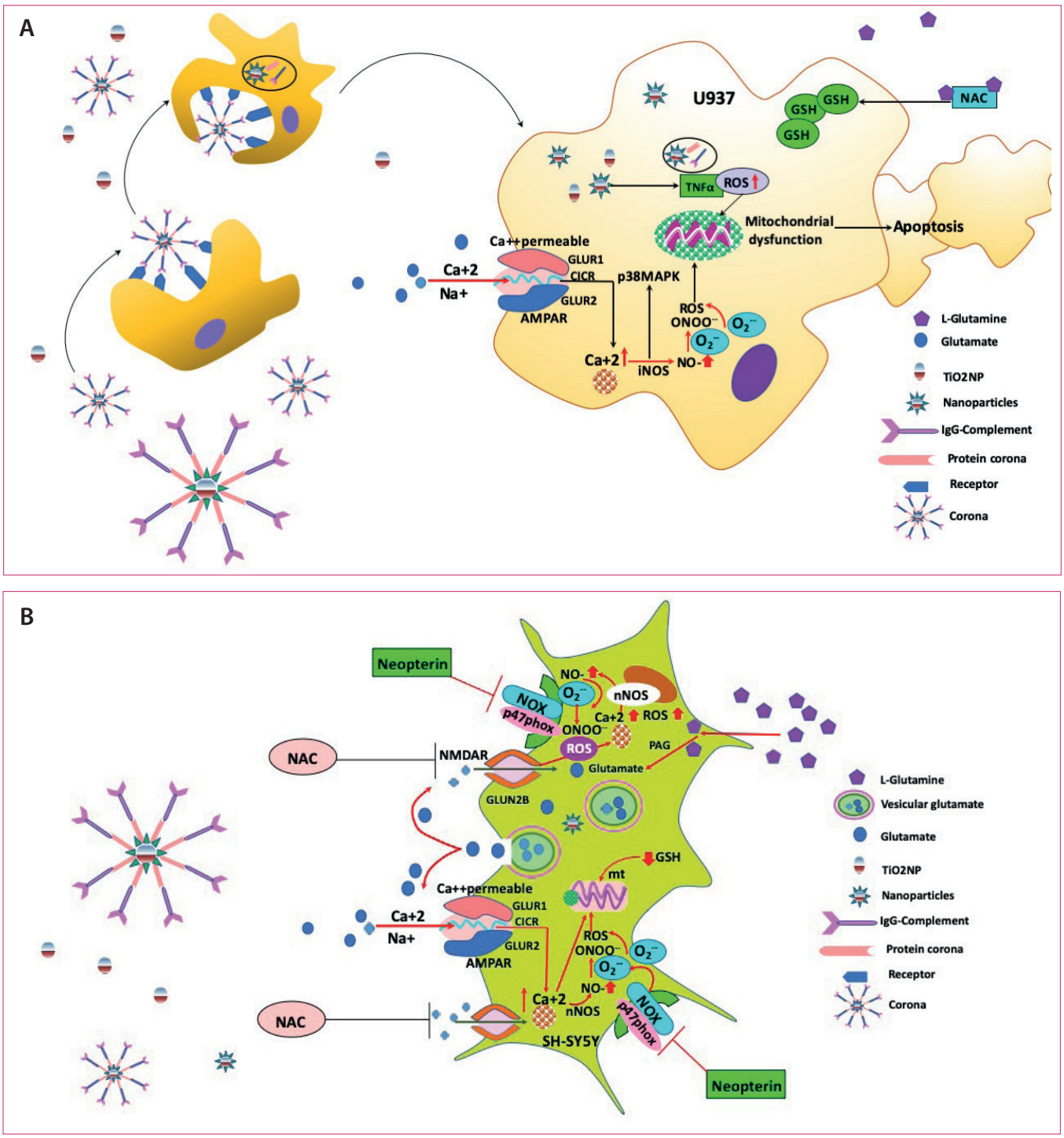

Figure 5. Effects of TiO_2NPs on U937 human monocytic cells (a) and on SH-SY5Y human dopaminergic cells (b). **a:** The TiO_2NPs-induced cytotoxicity on human U937 monocytic cells occurs through AMPA receptor stimulation, which is associated with intracellular ROS generation, mitochondrial dysfunction, glutathione depletion. NAC and neopterin could not increase the viability of U937 cells. **b:** ROS generation mediated oxidative stress and impaired antioxidant capacity are the major causes of cytotoxicity of TiO_2NPs on SH-SY5Y human dopaminergic cells. NAC pretreated SH-SY5Y cells, which were exposed to TiO_2NPs displayed highly significant increase in viability for both sizes of NPs at all concentrations. However, only 10 μM neopterin provided a significant protection against 25 nm TiO_2NPs toxicity at all concentrations. (TiO2NPs, titanium dioxide nanoparticles; NAC, N-acetylcysteine; NMDAR, N-methyl-D-aspartate receptor; GLUN2B, NMDAR subunit; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (iontotropic glutamate receptor); GLUR1 and GLUR2, AMPAR subunits; NOX, NADPH oxidase; mt, mitochondria, ROS, reactive oxygen species; nNOS, neuronal nitric oxide synthase; PAG, Phosphate activated glutaminase; GSH, reduced glutathione; CICR, calcium-induced calcium release; iNOS, inducible nitric oxide synthase; TNFα, tumor necrosis factor alpha.)

itself has been shown to potentiate neuronal NMDA receptor response to glutamate.[51,52] NAC-derived GSH is the major endogenous antioxidant and is critical for the maintenance of the redox potential in neuron. Therefore, changes in the levels of neuronal GSH may alter available glutamate levels.[53–55] In our study, the addition of FBS to the cell culture medium resulted in a statistically significant reduction in cell viability of SH-SY5Y and U937 cells, with both sizes of TiO_2NPs.

NAC has been well established as a cysteine donor.[56] Thereby, it also acts as an antioxidant itself and may modulate the redox properties of NMDA receptors.[57] The function of NMDA receptors can be modulated through the reduction of extracellular cysteine residues[55], arguing for a role for an extracellular action of GSH (Fig. 5). In addition, GSH has been shown to target the glutamatergic system through the activation of the cystine-glutamate exchanger.[58] In FBS-containing medium, NAC pretreatment significantly increased viability of SH-SY5Y cells in comparison to both TiO_2NP-treated SH-SY5Y cells alone and TiO_2NP plus NAC-pretreated U937 monocytic cells. It is fact that, FBS supplementation in culture medium, did not only enhance the toxicity of TiO_2NP, but also strongly supported antioxidant effect of NAC, on SH-SY5Y dopaminergic neurons. The lack of significant difference between the mitochondrial metabolic activities/cell viabilities of NAC-pretreated U937 monocytic cells and only TiO_2NP treated U937 cells indicates that the glutamatergic mechanism was negligible in the TiO_2NP effects upon U937 cells. However, a significant increase in viability of U937 cells against 25 nm of TiO_2NPs at 50μg/mL concentration suggested that NAC stimulated GSH formation together with glutamine. This resulted in a concomitant decrease in the level of ROS.[59]

These findings indicated that the harmful effects of TiO_2NPs on SH-SY5Y cells were related to glutamate signaling through NMDA receptors, but this seem not to be applicable for U937 human monocytic cells.

Glutamate exposure may also increase Ca^{2+} influx into neurons and may stimulate the generation of oxidative/nitrosative species that damage the cell.[60] The plasma membrane-bound NADPH oxidase complex (NOX) may play an essential role in the glutamate-induced apoptotic cell death through increased production of ROS. Stimulation of glutamate induced apoptotic cell death via increase in the level of ROS, is significantly suppressed by the inhibitor of NOX, the neopterin.[61]

Ten μM neopterin provided a remarkable restoration and increase in cell viability despite the increased concentration of 25 nm TiO_2NPs. Whereas, both doses of neopterin could not be protective against the toxicity of 10 nm TiO_2NPs. On the other hand, 0.1 μM neopterin supplementation to the cell culture medium in addition to 100 μg/mL 25 nm TiO_2NP was not sufficient to increase the viability of SH-SY5Y cells in comparison to TiO_2NP exposed cells only. However, 0.1 μM neopterin enhanced cell viability of neurons that were exposed to 25 nm TiO_2NP at lower concentrations. Thus, our study demonstrated that the particles' size can influence the cytotoxicity of nanoparticles. In this respect, smaller particles tend to have higher cytotoxicity.

NOX, which produces superoxide is a major metabolic pathway of oxidative stress in the presence of TiO_2NPs.[19] The functional NOX is characteristically expressed in mature monocytes.[62,63] Induction of a functional NOX in U937 cells that lacks the capacity to generate ROS, requires the combined actions of either retinoic acid or IFN-γ.[62,63] Although it is claimed that the treatment with neopterin significantly blunts the generation of ROS, and induction of apoptosis in different cell lines[48,49], in our study, neopterin pretreatment could not provide a constant amelioration in human U937 cells, which were exposed to TiO_2NPs. Inefficiency of neopterin supplementation confirmed that AMPA receptor or TNF-α-mediated apoptosis due to TiO_2NP-induced toxicity could not be prevented in U937 monocytic cells.

Clinical perspectives

Adverse effects of TiO_2 NPs on living cells have raised serious concerns for their use in health care and consumer sectors and implanted biomaterials[64]. In this context, TiO_2 NPs are released mostly around the implants and affect epithelial cells, connective tissue, macrophages, and bone. Long-term local and systemic effects of titanium particles and ions on cells and tissues remain unknown. They may contribute to the disruption of epithelial barriers, induce oxidative stress and may have cytotoxic and genotoxic potential for tissues.[65–68] In fact, NAC, as a precursor of GSH, is used as an adjunct to standard therapy for the treatment of chronic obstructive pulmonary disease via improving the neurogenic inflammatory response, a deleterious condition that may support the vicious circle between oxidative stress and inflammation.[69,70] In this study, NAC has been shown to provide a significant protection against titanium toxicity.

Although not routinely used in daily clinical practice, activation of cell-mediated immunity is demonstrated by high neopterin levels in neurological, cardio-vascular, malignant and autoimmune diseases.[71, 72, 73] In addition, measurements of neopterin concentrations are helpful in monitoring the immune modulating therapy of patients.[73] However, no toxicity has been reported due to endogenous overexpression of neopterin. In our study, it was tested whether neopterin, which is synthesized by the activated macrophages, had an antioxidant effect in the environment without macrophages. Unfortunately, there is no practical experience outlining how much of NAC or neopterin should be used to facilitate the increase in the antioxidant capacity of the patient against TiO_2 NPs toxicity. Further clinical studies are necessary to determine health-hazardous amount of TiO_2 NPs and corresponding doses of NAC or neopterin.

Conclusions

The addition of FBS to the cell culture medium resulted in a statistically significant reduction in cell viability of SH-SY5Y and U937 cells, with both sizes of TiO_2NPs. The effect of TiO_2NPs on mitochondrial dysfunction/cell viability of SH-SY5Y cells is related to the regulation of redox signaling mechanisms of SH-SY5Y cells via glutamate uptake, whereas U937 human monocytes do not respond to glutamatergic stimulation. Unlike SH-SY5Y cells, NOX inhibition is not effective on viability of TiO_2NPs-exposed U937 cells. In this context, calcium influx and the function of AMPA receptor antagonists on U937 cells were not detected in this study, thereby, further investigation of different activated mechanisms of ROS generation, which are induced by TiO_2NPs, is necessary.

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Ethics Committee Approval: In this study, commercially available cell lines were used. Therefore, ethical committee approval was not required.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The Authors declare no conflict of interest.

Author Contributions: Concept: ABE, EDE; Design: ABE; Supervision: EDE; Resources: ABE, EDE; Materials: ABE, EDE; Data collection and/or processing: ABE, EDE; Analysis and/or interpretation: ABE, EDE; Literature search: ABE, EDE; Writing manuscript: ABE, EDE; Critical review: EDE.

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