Chemosphere 135 (2015) 217–224

Contents lists available at ScienceDirect

Chemosphere

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

Silicon as neuroprotector or neurotoxic in the human neuroblastoma SH-SY5Y cell line

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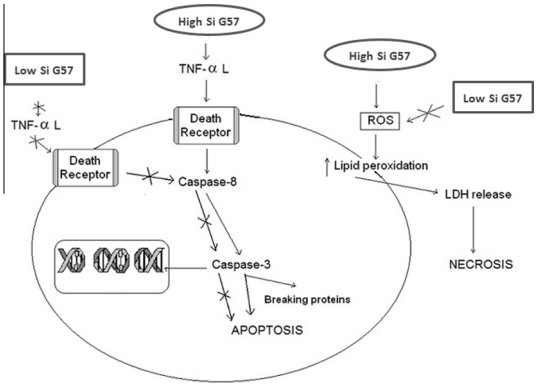
HIGHLIGHTS

- Silicon show a double-edged-sword effect depends on the concentrations used.
- Silicon at high doses may act as neurotoxic by necrosis and apoptosis mechanisms.
- Silicon at low doses may act as neuroprotector by inducing anti-apoptotic effects.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 November 2014
Received in revised form 14 April 2015
Accepted 22 April 2015
Available online 15 May 2015

Keywords:
Silicon
Cell death
Proliferation
Neuroprotection
Neurotoxic
SH-SY5Y

ABSTRACT

Silicon (Si) is a trace element that has been considered to be an environmental contaminant for many years, although different studies have recently reported it is an essential element for living cells. The present study tested the ability of different concentrations of Si G57™ to induce neuroprotection or neurotoxicity over 24 h in the SH-SY5Y human neuroblastoma cell line. Cell viability, cellular proliferation, LDH release, ROS, antioxidant capacity, TBARS, caspase-3, -8 and -9, DNA fragmentation, and TNF-α levels were evaluated. Low Si doses (50–250 ng mL^{-1}) increased the cell viability and reduced caspase-3 and -8 activities and TNF-α level. The increase in cell viability was independent of any proliferative effect as there was no variation in cyclin E and PCNA levels. At higher concentrations, Si increased caspase-3, as well as TBARS, LDH, DNA fragmentation, and TNF-α releases. Altogether, these results suggest that Si could act either as a neuroprotector or a neurotoxic agent depending on the concentration tested. This study emphasizes the importance of developing new neuroprotective therapies based on low Si doses.

Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; AD, Alzheimer's disease; AFC, 7-amino-4-trifluoromethyl-coumarin; Al, aluminium; AMC, fluorochrome 7-amino-4-methyl-coumarin; BHT, butylated hydroxytoluene; H_2DCF–DA, 2,7-dichlorodihydrofluorescein diacetate; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; FBS, foetal bovine serum; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide; Si, silicon; PCNA, proliferating cell nuclear antigen; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances.

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http://dx.doi.org/10.1016/j.chemosphere.2015.04.060
0045-6535/

1. Introduction

Silicon (Si) is the second most prevalent element in the Earth's crust (27.2) (Greenwood and Earnshaw, 1997). Si participates in the life processes of animals (Carlisle, 1972, 1974) with important role in connective tissue (Carlisle, 1981). Si has been related to collagen synthesis (Carlisle, 1972), bone mineralization (Eisinger and Clairet, 1993), atherosclerosis (Schwarz, 1977), hypertension (Maehira et al., 2011), Alzheimer's disease (AD) (Candy et al., 1985; Gonzalez-Muñoz et al., 2008), as well as other biological disorders (Jurkić et al., 2013). The protective effect of Si against neurodegenerative disease is mainly based on the ability of silicic acid to act as a natural antagonist of aluminium (Al). Si prevents Al toxicity (Birchall et al., 1989) by reducing its absorption and facilitating its excretion (Popplewell et al., 1998). Besides, our group has recently suggested (Garcimartín et al., 2014) the role of Si as a protective agent against hydrogen peroxide effects on neuroblastoma human SH-SY5Y cell line. In fact, Si partially blocks cellular damage related to oxidative stress, a known cause of cell death in neurodegenerative diseases.

Despite all previous cited properties, the Si essentiality has not been broadly accepted. Moreover, their adequate intake/recommended daily allowances and tolerable upper intake level have not been set (Institute of Medicine, Food and Nutrition Board, 2001). The reduced Si levels detected in the aged population might be associated to some degenerative diseases, including atherosclerosis (Pérez-Granados and Vaquero, 2002). Thus, the supplementation with bioavailable forms of Si might have a potential therapeutic interest.

On the other hand, Si as silica nanoparticles shows deleterious effects (Gazzano et al., 2012). Rong et al. (2013) found that crystalline silica can induce cell injury and inflammation in endothelial cells. Besides, epidemiological studies reported that silica dust exposure is associated with airway diseases (Erdogdu and Hasirci, 1998).

Neuronal models, as SH-SY5Y human neuroblastoma cell line, appear as a useful preliminary tool to evaluate the neuroprotective/neurotoxic effects of different substances (Xie et al., 2010). However, according to Jantas et al. (2014) such effects could be different depending on the cell model used (e.g.: SH-SY5Y *vs.* primary cortical neurons). Nevertheless, to the best of our knowledge the effect of Si in a neuronal cell model has not yet been studied. Considering our previous results (Garcimartín et al., 2014) as well as the paradoxical beneficial/deleterious effects of Si described in bibliography, we hypothesised that organic Si, a bioavailable form of Si, exerts these dual effects depending on its concentration. The objective of the present study was to evaluate the protective or deleterious effects of different concentrations of organic Si (from 50 to 20,000 ng mL^{-1}) on SH-SY5Y human neuroblastoma cell line.

2. Material and methods

2.1. Chemicals

Silicium organique G57™ as Si source was purchase from Glycan Group (Geneva Switzerland). Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS), 0.25% trypsin–EDTA, and penicillin/streptomycin mixture were obtained from GIBCO–BRL (Grand Island, NA, USA). 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), aprotinin, leupeptin, phenyl-methane-sulfonyl fluoride (PMSF), Ac-DEVD-AMC-[N-acetyl-Asp-Glu-Val-Asp-(7-amino-4-methyl-coumarin)], Ac-LETD-AFC-[Leu-Glu-Thr-Asp-(7-amino-4-trifluoromethyl-coumarin)], Ac-LEHD-AFC-[Leu-Glu-His-Asp-(7-amino-4-trifluoromethyl-coumarin)], barbituric acid, butylated hydroxytoluene (BHT), 2,7-dichlorodihydrofluorescein diacetate (H_2DCF–DA), fluorescein, dimethyl sulfoxide (DMSO), 2,2′-azobis-(2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethil-chromal-2-carboxylic acid (Trolox), dithiothreitol (DTT) and 1,1,3,3-tetramethoxypropane were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cyclin E antibody (M-20) (sc-481), Proliferating cell nuclear antigen (PCNA) antibody (FL-261) (sc-7907) and goat anti-rabbit IgG-HRP (sc-2004) were supplied by Santa Cruz Biotechnology, Quimigen (Madrid, Spain). PVDF membrane and ECL select were purchased from GE Healthcare (Madrid, Spain). Gel red (41003) was purchased from Biotium Inc. (California, USA). Other chemicals were reactive grade products from Merck (Darmstadt, Germany).

2.2. Cell culture and treatment

SH-SY5Y cells were grown in DMEM supplemented with 10% FBS and 100 IU mL^{-1} penicillin/streptomycin, maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. The culture medium was removed every three days and a sub-cultured was used once they reached 80–90% confluence. SH-SY5Y cells were tested between 3 and 15 passages. Experiments were performed seeding cells in the 24- or 96-well culture plates and maintaining them in DMEM with 10% FBS for 24 h. Later, cells were treated with Si (50–20,000 ng mL^{-1}) in DMEM containing 1% FBS for 24 h, except in the experiment of reactive oxygen species (ROS) measurement in which cells were treated for only 2 h due to the known DMSO toxicity in this cell line. For treatments, Si G57 (100,000 ng/mL Si element) was diluted with culture medium to obtain a stock solution of the highest concentration needed.

2.3. Protein measurement

The protein concentrations of cell extracts were determined by the Bradford (1976) methods using bovine serum albumin as standard.

2.4. Cell viability (MTT assay)

This assay is based on the ability of living metabolic active cells to reduce the MTT given a compound with purple colour. It is used to evaluate cell viability. SH-SY5Y were seeded into 96-well culture plates (3×10^4 cells *per* well) and treated as described above. MTT solution (2 mg mL^{-1}) was added to each well and incubated for 1 h at 37 °C. The blue formazan crystals inside the cells were dissolved in DMSO. Finally, the absorbability was read at 595 nm using a microplate reader (LT-4000, Labtech International Ltd., United Kingdom). The results expressed as the percentage of MTT reduction relative to control cells.

2.5. Cyclin E and PCNA levels

Cyclin E and PCNA levels were measured by Western Blot following Figueroa et al. (2005) protocol. SH-SY5Y cells were plated on 100 mm dishes and treated with Si (50–750 ng mL^{-1}). After treatment, cells were washed twice with PBS, harvested, and lysed with buffer enriched with aprotinin, leupeptin and PMSF as protease inhibitors. Cell lysates were centrifuged at 14,000*g* for 10 min at 4 °C. Equal amounts of protein (30 μg) were separated in 10% polyacrylamide gel (SDS–PAGE), and then transferred to PVDF membrane (GE Healthcare, Madrid, Spain) at 400 mA for 1 h, at 4 °C. For immunodetection, membranes were incubated overnight at 4 °C with the appropriate primary antibodies: rabbit polyclonal anti-cyclin E (1/1000) and rabbit polyclonal anti-PCNA (1/1000) followed by incubation with peroxide-conjugated secondary antibodies for 1 h at room temperature (Santa Cruz Biotechnology, Quimigen, Madrid, Spain). Blots were developed

by enhanced chemoluminescence (ECL select; GE Healthcare, Madrid, Spain) according to the manufacturer's instructions. Anti β-actin was used as loading control.

2.6. LDH measurement

Intracellular enzyme lactate dehydrogenase (LDH) is released to the cell culture medium when cell membrane is damaged; thus, it is considered a good marker of necrosis detection. The enzyme activity was determined by spectrophotometric assay according to López et al. (2003). The absorbance decrease, resulting from NAD(H) oxidation, was measured at 340 nm.

SH-SY5Y cells (3×10^5 cells *per* well in 24-well plates) were treated and incubated 24 h at 37 °C. Culture medium from all samples was collected. In addition, the cells were homogenized with 0.1 M KH_2PO_4/K_2HPO_4 (pH 7.4), containing 0.1% Triton X-100. Cell homogenates were centrifuged at 13,000*g* for 10 min and LDH activity measured in both cell supernatants and culture medium. LDH release was expressed as the percentage of LDH activity present in the culture medium according to the following formula (1):

$$\text{LDH release } (\%) = \frac{(\text{LDH activity release in the culture medium})}{\text{Total LDH activity } (\text{LDH cell supernatant} + \text{LDH release})} \times 100 \quad (1)$$

2.7. ROS measurement

ROS formation was assayed using H_2DCF–DA which enters the cells and is transformed into 2,7-dichlorodihydrofluorescein (H_2DCF). H_2DCF is later oxidized into fluorescent DCF by H_2O_2.

SH-SY5Y cells were seeded into 96-well culture plates (3×10^4 cells *per* well). H_2DCF–DA (5 μM) was added to the cell culture. After 30 min the cells were treated with Si dissolved in glucose–PBS for only 15 min. The fluorescence was measured every 15 min during 2 h in a FL800-BioTek spectrofluorometer (Bio-Tek Instruments INC, Germany) fitted with 485/20 nm excitation and 528/20 nm emission filters. Results were expressed as arbitrary fluorescence units (AFU).

2.8. Antioxidant capacity assessment

The ability of Si to capture ROS, out of the cells, was evaluated by a modification of the ORAC method (Ganske and Dell, 2006). Fluorescein, under its reduced form, is a fluorescent molecule; however, when it is oxidized by a ROS source as AAPH, the fluorescent properties falls. The presence of an antioxidant substance in the reaction medium partially avoids the loss of fluorescence capturing ROS produced by AAPH. Fluorescein (79 ng mL^{-1}), AAPH (10.8 mg mL^{-1}), and Si samples (at different concentrations) were mixed. The fluorescence was read at 37 °C, every minute for 2 h in a fluorescence plate-reader (FL800, Bio-Tek Instruments INC, Germany) at 485/20 nm excitation and 528/20 nm emission wavelengths. The results were expressed as fluorescence fall (final fluorescence–initial fluorescence). The fluorescence decrease in absence of sample was used as positive control.

2.9. Lipid peroxidation assay

Lipid peroxidation was estimated by measuring TBARS after addition of BHT as antioxidant (Mihara and Uchiyama, 1978). SH-SY5Y cells were treated during 24 h and the cell extracts prepared with the lyses buffer. Briefly, 500 μL cell extract, BHT (0.01% w/v), phosphoric acid (1% v/v), and barbituric acid (0.6% w/v) mixture was incubated at 100 °C for 45 min. Samples were read at 485/20 nm excitation and 528/20 nm emission wavelengths in a fluorescence plate-reader (FL800, Bio-Tek Instruments INC, Germany). Quantification was performed against a standard curve of malondialdehyde generated by acid hydrolysis of 1,1,3,3-tetramethoxypropane.

2.10. Caspase-3, -8, and -9 activity measurements

SH-SY5Y cells were seeded into 24-well culture plates (3×10^5 cells *per* well). Cells were lyses 24 h after treatment using lyses buffer (10 mM Tris–HCl, 10 mM NaH_2PO_4/Na_2HPO_4, pH 7.5, 130 mM NaCl, 0.5% Triton X-100, 10 mM $Na_4P_2O_7$ and 2 mM DTT) and centrifuged at 13,000*g* for 5 min. Supernatants, with at least 20 μg of protein, were incubated at 37 °C for 1 to 6 h in assay buffer (20 mM, HEPES, pH 7.5, with 2 mM DTT) and 20 mM Ac-DEVD-AMC [Nacetyl-Asp-Glu-Val-Asp-(7-amino-4-methyl-coumarin)]. The fluorochrome 7-amino-4-methyl-coumarin (AMC) is released from the substrate Ac-DEVD-AMC after reacting with caspase-3. Fluorescence signal produced by free AMC is proportional to the caspase-3 activity present in the sample. This assay was monitored using a fluorescence plate-reader (FL800, Bio-Tek Instruments INC, Germany) at 360/40 nm excitation and 460/20 nm emission wavelengths. Finally, enzymatic activity was expressed as arbitrary fluorescence unit after 1 h *per* mg protein.

Caspase-8 and -9 activities were measured under similar experimental conditions to caspase-3, but using Ac-LETD-AFC-[Leu-Glu-Thr-Asp-(7-amino-4-trifluoromethyl-coumarin)] and Ac-LEHD-AFC-[Leu-Glu-His-Asp-(7-amino-4-trifluoromethyl-coumarin)] for caspase-8 and caspase-9 determinations, respectively. The fluorochrome 7-amino-4-trifluoromethylcoumarin (AFC) released was measured at 360/40 nm excitation and 530/25 nm emission wavelengths.

2.11. Determination of apoptosis by DNA fragmentation

Cells were seeded onto 100 mm dishes and treated with Si. After 24 h, adherent and floating SH-SY5Y cells were pooled and washed twice on ice-cold PBS. DNA was isolated using the DNAasy Blood and Tissue kit (Qiagen, Hilden, Germany). The precipitated DNA was resuspended in TE buffer and loaded onto a 0.7% agarose gel containing gel red to visualize DNA fragmentation by UV illumination.

2.12. Evaluation of the tumour necrosis factor

Tumour necrosis factor alpha (TNF-α) was measured in culture medium after 24 h of treatment using enzyme-linked immunoabsorbent assay (ELISA) (Human TNF alpha ELISA Kit, 950.090.096, Diaclone, France) according to the manufacturer's manual. The colour intensity increase was evaluated at 450 nm using microplate reader (LT-4000, Labtech International Ltd., United Kingdom).

2.13. Statistical analysis

Data were expressed as mean ± SEM from two independent experiments using different cultures. Each experiment was performed at least in triplicate with different cell batches (total 6–12 measurement/condition). Statistical analyses were performed using SigmaPlot 11.0 software. Data were tested by One Way ANOVA on Ranks followed by the Dunn's test or by the Kruskal–Wallis test when the variance normality test failed. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Effects of Si on cell viability

Fig. 1 shows that Si increased cell viability at low doses while at higher concentrations it decreased the basal viability up to 50% as compared to control cells.

3.2. Effect of Si on cell proliferation

The increase in cell viability at low Si doses (200–400 ng mL^{-1}) could provide protection through an anti-apoptotic effect or cell division. In order to confirm both possibilities, levels of cyclin E, which is responsible for the transition from stage G1 to S, and PCNA, which is associated with DNA replication were measured. Results from Fig. 2A and B indicate that at low Si concentrations these protein levels were not modified while at the highest one PCNA significantly decreased.

Cell viability losses may mean the existence of cell death or breathe capacity decrement. To distinguish these events, some experiments were performed in this cell line employing different Si concentrations.

3.3. Si effect on LDH release, ROS formation and lipid peroxidation levels

LDH release is a cell necrosis biomarker. Fig. 3A shows that Si was able to release LDH only at the highest doses (750–1000 ng mL^{-1}) while did not exert any effects at low Si concentrations. Cell necrosis could be due to ROS formation and lipid peroxidation induction. Thus, both possibilities were tested.

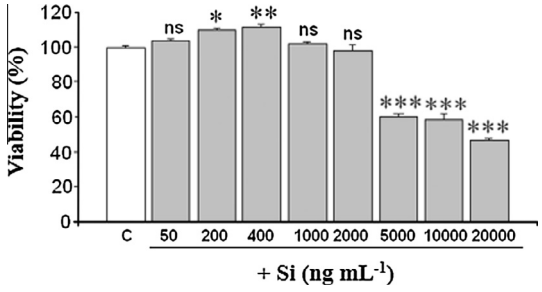

Fig. 1. Effect of Silicon (Si) G57 on cell viability. Results are expressed as means ± SEM of two experiments from different cultures, each one performed in quadruplicate with different batches of cells (total 8 measurements/condition). (*)/ns = Statistical significances are referred to the control. ns = Non-significant; (*) = $p < 0.05$; (**) = $p < 0.01$; (***) = $p < 0.001$.

Results from Fig. 3B show that Si in the 50–750 ng mL^{-1} range did not modify ROS production. Higher Si doses (750–20,000 ng mL^{-1}) decreased ROS generation ($p < 0.001$).

Lipid peroxidation is one of the mechanisms through cells die by necrosis. It is mediated by the ·OH radical and O_2. Lipid peroxidation significantly rose at the 250–750 ng mL^{-1} Si range. The increase was very significant at 750 ng mL^{-1} (Fig. 3C).

Present results suggest that Si-induced necrosis was not the result of ROS formation because Si diminished this mechanism. In order to confirm it, the own Si antioxidant capacity was tested against AAPH in absence of cells.

3.4. Antioxidant capacity of Si against AAPH

Fig. 4 illustrates the fluorescence fall induced by AAPH at different Si concentrations. Si at any concentration tested partially blocked the fluorescence fall in comparison to control. In the range 25–1500 ng mL^{-1}, as higher the concentration of Si tested was as lower the decrease of fluoresce found. A nearly steady-state was observed for Si concentrations higher than 1500 ng mL^{-1}. The EC50 of Si was 532 ng mL^{-1}.

3.5. Effect of Si on caspase-3 activity and DNA fragmentation

Once demonstrated that high Si doses induced necrosis, the possibility of apoptotic death was checked by the quantification of caspase-3 activity. Fig. 5A suggests a U-shape Si dose–response curve with a significant anti-apoptotic effect at 100 and 250 ng mL^{-1}. The caspase-3 activity increased significantly at 500–750 ng mL^{-1} with respect to control.

Normally, an increase in caspase-3 activity is usually followed by DNA fragmentation. In order to check this event, the effect of Si on DNA fragmentation was evaluated. Fig. 5B confirms that Si at concentrations 500 and 750 ng mL^{-1} induced DNA fragmentation.

3.6. Effect of Si on caspase-8 and caspase-9 activities

In the caspase family, caspase-3 is one of the effector enzymes responsible for apoptosis, as it degrades DNA and proteins. This enzyme may be activated through an extrinsic or an intrinsic pathway with the intervention of other caspases. Thus, caspase-9 is activated when the intrinsic pathway is involved; while caspase-8 activity is underlining in the case of the extrinsic pathway. In order to check these possibilities, the activity of both

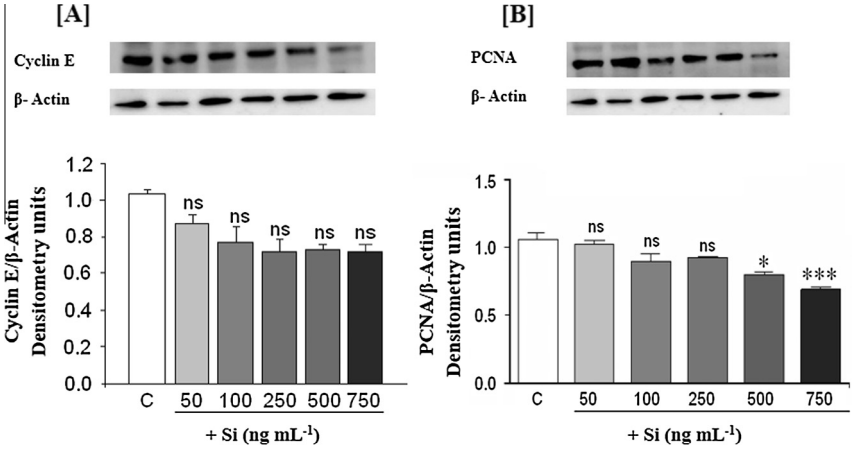

Fig. 2. Effect of Silicon (Si) on cell proliferation. (A) Cyclin E and (B) PCNA levels. Results are the means of two experiments from different cultures each one performed in triplicated. ns/(*) = Statistical significances are referred with respect to the control. (ns) = Non-significant, (*) = $p < 0.05$ and (***) = $p < 0.001$.

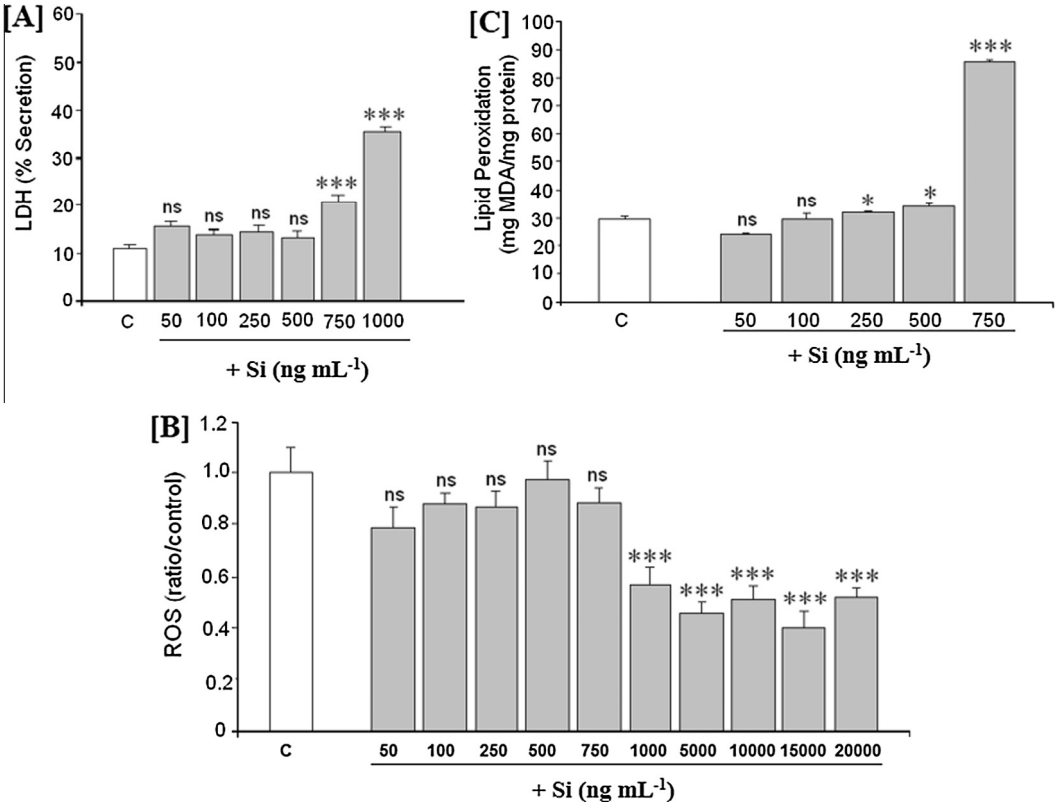

Fig. 3. Effect of Si on (A) LDH release, (B) ROS formation and (C) lipid peroxidation. Results are expressed as means ± SEM of two experiments from different cultures, each one performed in quadruplicate with different batches of cells (total 8 measurements/condition). (*)/ns = Statistical significances are referred to the control. ns = Non-significant; (*) = $p < 0.05$ and (***) = $p < 0.001$.

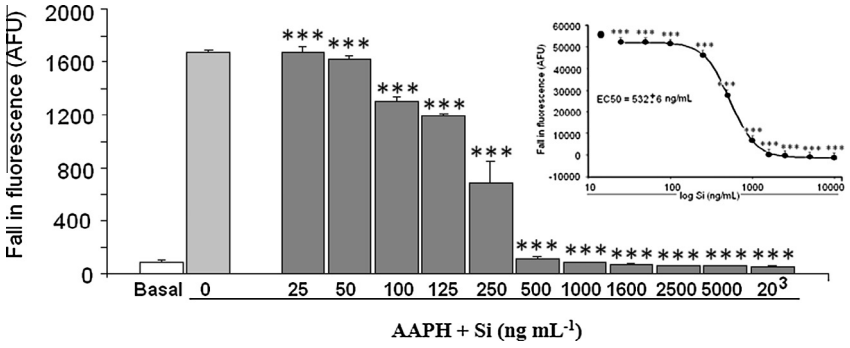

Fig. 4. Effect of Si on the lost in fluorescence of the fluorescein due to the pro-oxidant action of AAPH. Results are expressed as means ± SEM of three experiments. (*) = Statistical significances referred to the control (***) = $p < 0.001$.

enzymes was assayed. Si did not have any effect on caspase-9 (data not shown); however, it decreased the basal caspase-8 activity at the 100–500 ng mL^{-1} range (Fig. 6A).

3.7. Effect of Si on TNF-α

As caspase-8 activation is mediated through death receptor, TNF-α, one ligand of these receptors, was measured. Results from Fig. 6B indicate that Si, at 250 ng mL^{-1}, decreased basal TNF-α levels but significantly increased TNF-α levels at 750 ng mL^{-1}.

4. Discussion

Results highlight the dual function of Si, either as a protector or as a neurotoxic agent depending on the concentration. At the lower doses used (50–400 ng mL^{-1}), organic Si increased basal cell viability and reduced caspase-3 and -8 activation without affecting LDH release.

At lower doses, the significant increase in cell viability found was not caused by a proliferative effect given that, here was no change in either cyclin E or PCNA levels with respect to control. Thus, these results suggest that low Si concentrations have a protective effect on the SH-SY5Y cell line, probably mediated by an anti-apoptotic mechanism. However, at higher concentrations Si acted as a neurotoxic agent, as evidenced by increased LDH release and caspase-3 activation.

The mechanisms whereby high Si concentrations caused cell death were necrosis and apoptosis. When necrosis occurs, some hazardous molecules are released, which are damaging to surrounding cells and ultimately produce inflammation. Thus, Si toxicity at 750 ng mL^{-1} seems to involve inflammation, which was corroborated by the increase found in TNFα, a pro-inflammatory

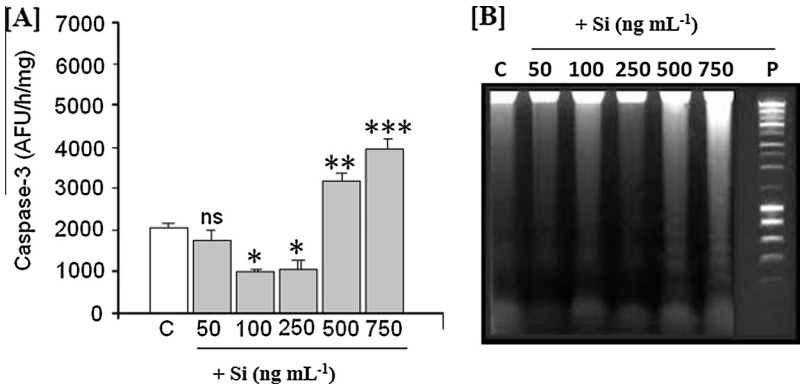

Fig. 5. (A) Action of Si on caspase-3. Results are expressed as means ± SEM of two experiments from different cultures, each one performed in quadruplicate with different batches of cells (total 8 measurements/condition). (*)/ns = Statistical significances referred to the control. ns = Non-significant; (*) = $p < 0.05$; (**) = $p < 0.01$; (***) = $p < 0.001$. (B) Effect of Si on DNA fragmentation. Results are expressed as means ± SEM of two experiments from different cultures, each one performed in duplicated with different batches of cells.

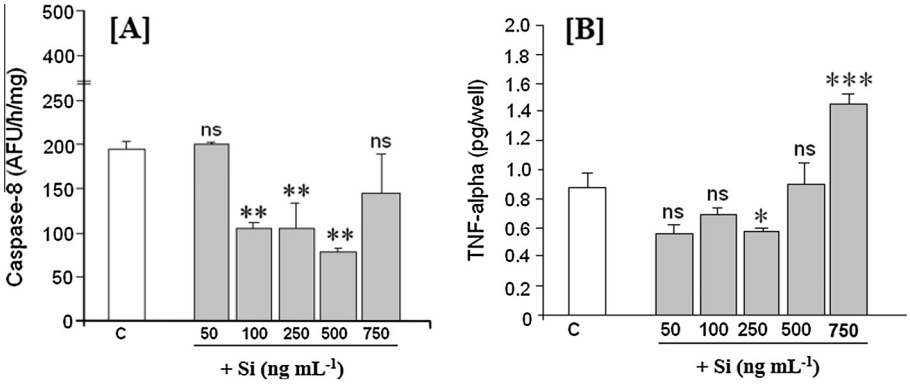

Fig. 6. (A) Action of Si on caspase-8 activation. Results are expressed as means ± SEM of two experiments from different cultures, each one performed in quadruplicate with different batches of cells (total 8 measurements/condition). (*)/ns = Statistical significances referred to the control. ns = Non-significant; (**) = $p < 0.01$. (B) Effect of Silicon (Si) on TNF-α. Results are expressed as means ± SEM of two experiments from different cultures, each one performed in quadruplicate with different batches of cells (total 8 measurements/condition). (*)/ns = Statistical significances referred to the control. ns = Non-significant; (*) = $p < 0.05$; (***) = $p < 0.001$.

cytokine. Several other researchers (Fujimura, 2000; Zhou et al., 2012) have demonstrated that TNF-α plays a pivotal role in mediating inflammatory reactions in silicosis.

Si did not increase ROS production with respect to control cells. Moreover, at the highest doses Si did significantly reduce the ROS concentration, despite a considerable increase of lipid peroxidation at a dose of 750 ng mL^{-1}. These results are consistent with evaluations of the capacity of Si to capture ROS released by AAPH. In light of the TBARS result, the ROS decrease would seem contradictory, or at least paradoxical, since ROS should cause lipid peroxidation. However, there are possible explanations for these results: (a) ROS production was measured after the first two hours of treatment due to limitations affecting this cellular model while the rest of the experiments were conducted after 24 h treatment following standard protocols; (b) Henderson and Chapell (1993) reported that the 2,7-DCF-DA method detects ROS as H_2O_2, while lipid peroxidation is mainly induced by the OH· radical; (c) Fubini and Hubbard (2003) reported that Si may generate OH· through the subsequent reaction (2): $SiO_2 + H_2O_2 = SiOH_{(s)} + OH^{\cdot} + O_2$, and this OH· would be responsible for the lipid peroxidation found in the present paper. The necrotic cell death induced by Si was caused by lipid peroxidation-OH· formation would not have been detected since the 2,7-DCF-DA method determines H_2O_2.

Regarding cell death by apoptosis it seems possible that a high concentration of Si activated caspase-3 in SH-SY5Y cells and also promoted DNA fragmentation. No significant caspase-8 activation was found at the higher Si concentrations, despite the increase in caspase-3. However, the increase of TNFα suggests that apoptosis was mediated through an extrinsic pathway. The lack of caspase-8 activity could be related to the length of experiment chosen, in a situation where 50% cells are already dead. All this suggests that Si led to apoptosis through the mediation of death receptors as summarized in Fig. 7. Conversely, low Si levels increased cell viability as well as reducing basal caspase-3 activity and TNF-α levels. Furthermore, there was no change in the proliferation markers, suggesting that organic Si exerted a protective effect through an anti-apoptotic mechanism rather than through proliferation. This mechanism could involve the inhibition of TNF-α synthesis, since a decrease in the latter leads to a lack of death receptor stimulation and hence no caspase-8 and/or -3 activation. The reason for the significant increase in cell viability with respect to control values (above the 100% of the control) could be that under normal conditions some cells may die as a consequence of environmental aggression from different sources. Langley et al. (2010) reported that chronic exposure to silica raises expression of anti-apoptotic markers including caspase-3 in rat lungs. On the contrary, acute exposure to a large dose of silica produces severe inflammation. Our results agree with those of Langley et al. (2010). However, the latter attributed the dual effect of silica to differences (chronic or acute) in exposure, while in the present study we demonstrate that the ranges of Si concentration are critical for predicting and understanding the effect of this mineral.

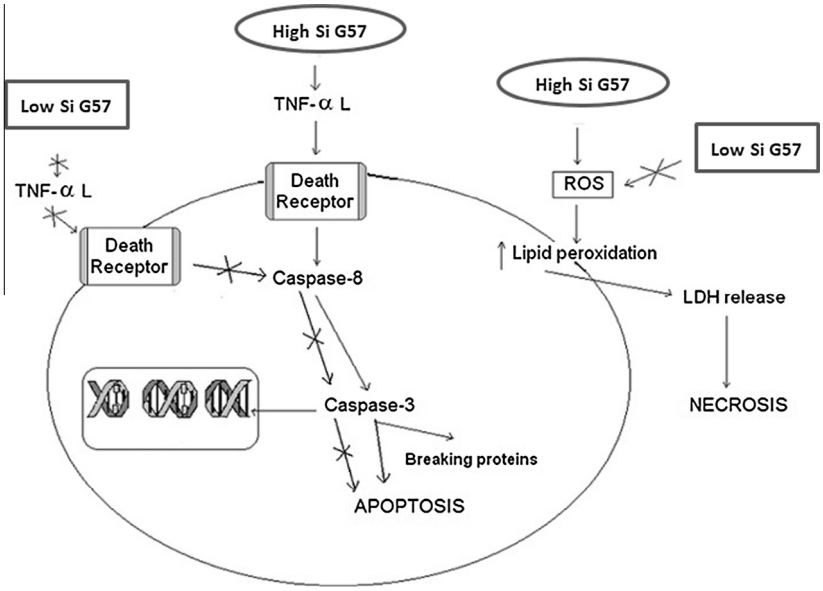

Fig. 7. Diagram of the mechanism by which Silicon (Si) induces cellular damage and neuroprotection. Si, at high concentrations, increases TNF-α and promotes activation of death receptors. This activation is responsible for increasing caspase-3 activity (effectors enzyme) which damages DNA and produces apoptosis. Besides, Si increases lipid peroxidation, LDH release and hence, necrosis. Si, at low concentrations, does not increase TNF-α; thus, death receptors, caspase-8 and caspase-3 are not activated and apoptosis is not produced.

Gonzalez-Muñoz et al. (2008) found that Si normalizes gene expression of TNFα and several antioxidant enzymes depleted by Al administration. The present study suggests that the neuroprotective effect of Si was the result of anti-apoptotic mechanisms.

The complex effects of Si in the human SH-SY5Y cell line are summarized in Fig. 7. Si raises TNF-α levels, which can activate cell death receptors, thereby leading to activation of caspase-3. This caspase-3 damaged DNA, producing apoptosis. On the other hand, high Si concentrations induced lipid peroxidation, probably through $OH^{\cdot}$ formation, leading to LDH release and necrotic cell death. Otherwise, Si protection would occur through inhibition of caspase-3 and -8 activities and thus prevent apoptosis. Present results also confirm the mechanism proposed in a previous paper (Garcimartín el al., 2014), which posited that Si exerted protective effects against the pro-oxidant insult mediated by hydrogen peroxide.

5. Conclusions

The results of this study demonstrate that the effect of Si depends on the concentrations tested. At high concentrations, Si acts as a neurotoxic agent through two mechanisms: necrosis, inducing LDH release; and apoptosis, increasing caspase-3 activity and producing DNA fragmentation. At low concentrations, Si acts as a neuroprotector by inducing anti-apoptotic effects and reducing inflammation by lowering TNF-α levels. Further research in primary brain cell cultures from different animal models needs to be done to confirm the mode of action of Si and to ascertain the most suitable therapeutic doses.

Conflict of interest

The authors declare that they are not conflicts of interest.

Acknowledgements

The authors gratefully acknowledge the financial support by the Spanish project AGL2011-29644-C02-02 and the pre-doctoral fellowship granted to Alba Garcimartín by BES-2012-054752 (FPI).

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