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Selenium Modulates Oxidative Stress-Induced Cell Apoptosis in Human Myeloid HL-60 Cells Through Regulation of Calcium Release and Caspase-3 and -9 Activities

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Abstract Selenium is an essential chemopreventive antioxidant element to oxidative stress, although high concentrations of selenium induce toxic and oxidative effects on the human body. However, the mechanisms behind these effects remain elusive. We investigated toxic effects of different selenium concentrations in human promyelocytic leukemia HL-60 cells by evaluating Ca^{2+} mobilization, cell viability and caspase-3 and -9 activities at different sample times. We found the toxic concentration and toxic time of H_2O_2 as 100 μM and 10 h on cell viability in the cells using four different concentrations of H_2O_2 (1 μM–1 mM) and six different incubation times (30 min, 1, 2, 5, 10, 24 h). Then, we found the therapeutic concentration of selenium to be 200 nM by cells incubated in eight different concentrations of selenium (10 nM–1 mM) for 1 h. We measured Ca^{2+} release, cell viability and caspase-3 and -9 activities in cells incubated with high and low selenium concentrations at 30 min and 1, 2, 5, 10 and 24 h. Selenium (200 nM) elicited mild endoplasmic reticulum stress and mediated cell survival by modulating Ca^{2+} release, the caspases and cell apoptosis, whereas selenium concentrations as high as 1 mM induced severe endoplasmic reticulum stress and caused cell death by activating modulating Ca^{2+} release, the caspases and cell apoptosis. In conclusion, these results explained the molecular mechanisms of the chemoprotective effect of different concentrations of selenium on oxidative stress-induced apoptosis.

Keywords Selenium · Endoplasmic reticulum stress · Apoptosis · Oxidative stress

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Introduction

Apoptosis in cancer cells is a gene-regulated form of cell death that is critical for normal development and tissue homeostasis. Up to now, three predominant apoptotic pathways—the death receptor–mediated extrinsic pathway, the mitochondria-mediated intrinsic pathway and the endoplasmic reticulum stress–mediated apoptotic oxidative stress pathway—have been elucidated (Chen and Wong 2009). Apoptosis can be initiated by extracellular and intracellular signals that trigger a complex machinery of proapoptotic proteases and mitochondrial changes, leading to activation of specific endoneucleases and DNA fragmentation (Shi 2002; Brookes et al. 2004). If mitochondria are exposed to proapoptotic signals, mitochondrial cytochrome *c* is released into cytosol. The released cytochrome *c* binds to Apaf-1 and then participates in caspase-9 activation. The activated capase-9 consequently is able to activate caspase-3, which in turn activates a caspase-activated DNase from an inhibitor of caspase-activated DNase by cleaving the caspase-activated DNase protein (Yoon et al. 2002). This process leads to DNA degredation, a hallmark event in apoptosis. A variety of stress-related stimuli activate apoptotic factors, including reactive oxygen species (ROS). Recent studies have indicated that ROS such as H_2O_2, which are formed in association with a variety of oxidative stress–induced disorders, may be

related to cell death and, hence, play an important role in apoptosis (Halliwell 2006; Nazıroğlu 2007a).

Ca^{2+} is a key regulator of cell survival since cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$) is a major regulatory factor for a large number of cellular processes such as muscle contraction, metabolism, secretion or even cell differentiation and apoptosis. However, the sustained elevation of intracellular Ca^{2+} plays a role in cell death (Demaurex and Distelhorst 2003). The proapoptotic effects of Ca^{2+} are mediated by a diverse range of Ca^{2+}-sensitive factors that are compartmentalized in various intracellular organelles, including endoplasmic reticulum and mitochondria (Hajnóczky et al. 2006). If the free intracellular Ca^{2+} concentration increases due to degeneration of cation channels, physiologic cell functions will be lost (Halliwell 2006; Nazıroğlu 2009). Excessive Ca^{2+} load to the mitochondria may induce apoptosis by both stimulating the release of apoptosis-promoting factors from the mitochondrial intermembrane space to the cytoplasm and impairing mitochondrial function (Wang 2001).

Selenium is well established as an essential trace mineral, which plays critical roles in many biological processes; and adequate amounts of this element are therefore required for optimal human health (Rayman 2000). The requirement of selenium for life and its beneficial role in human health have been known for several decades. In fact, selenium is known primarily for its antioxidant activity as a component of glutathione peroxidase (GSH-Px) and, in therapeutic aspects, for its chemopreventive, antiinflammatory and antiviral properties (Rayman 2000; Zeng and Combs 2008). The known functions of selenium as an essential element in animals are attributed to ~12 known mammalian selenoproteins, GSH-Px, thioredoxin reductase, selenoprotein P and W and phospholipid hydroperoxide, which contain selenocysteine, specifically incorporated through a unique cotranslational mechanism (Rayman 2000; Zeng and Combs 2008). However, there are few reports on the relationships between selenium and signal molecules.

The effects of selenium on the organism are concentration-dependent, ranging from essential to antioxidant in the nanomolar to micromolar range to potentially prooxidant at concentrations above (Vinceti et al. 2001). At even higher concentrations, selenium compounds may accumulate, leading to oxidative stress and damage to cellular components, thus having toxic effects (Papp et al. 2007). Interestingly, it has been reported that selenium in HL-60 and U937 cells at low concentrations may increase cell proliferation and suppress apoptosis caused by some stimuli while, at higher concentrations, it may decrease cell proliferation and cause apoptosis (Gopee et al. 2004; Guan et al. 2009). In this sense, it has been recently postulated that low concentrations of selenium in NB4 cells may preferentially activate the survival responses that depend on the unfolded protein response pathway, whereas high concentrations of selenium may lead to assembly of the apoptotic molecules and ROS, which induce mitochondrial membrane permeabilization and caspase activation (Guan et al. 2009). Different concentrations of selenium could have different effects on the cells. However, the mechanisms of these effects are not fully understood.

Since the modulatory effects of distinct concentrations of selenium on cellular survival and death have not been clarified, we focused on the dual effect of selenium in human promyelocytic leukemia HL-60 cells by checking its role in cell viability and Ca^{2+} release from intracellular stores evoked by hydrogen peroxide as well as by analyzing the activation of caspase-3 and -9 induced through oxidative stress.

Materials and Methods

Cells and Chemicals

The HL-60 15-12 cell line (ECACC 88120805) is a variant of HL-60 which is differentiates toward either neutrophils or monocytes and was purchased from the European Collection of Cell Cultures (ECACC, Dorset, UK). Fetal bovine serum (FBS) and penicillin/streptomycin were obtained from HyClone (Aalst, Belgium). L-Glutamine and RPMI 1640 medium were acquired from Cambrex (Verviers, Belgium). Dimethylsulfoxide (DMSO) and *N*-acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin (AC-DEVD-AMC) were obtained from Sigma (Madrid, Spain). *N*-Acetyl-Leu-Glu-His-Asp-7-amido-4-trifluoromethylcoumarin (AC-LEHD-AMC) was purchased from Bachem (Bubendorf, Switzerland). Fura-2 acetoxymethylester (fura-2/AM) was from Molecular Probes (Leiden, The Netherlands). All other reagents were of analytical grade.

Cell Culture

HL-60 cells (passages 6–12) were grown in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% heat-inactivated FBS, 100 U/ml penicillin, 100 μg/ml streptomycin and 1.25% DMSO at 37°C under humidified conditions of 95% air and 5% CO_2. Cells were routinely plated at a density of 3×10^5/ml in fresh flasks and resuspended in fresh medium before the experiments.

Cell Viability (MTT) Assay

Cell viability was evaluated by the MTT assay based on the ability of viable cells to convert a water-soluble, yellow tetrazolium salt into a water-insoluble, purple formazan

product. The enzymatic reduction of the tetrazolium salt happens only in living, metabolically active cells but not in dead cells. Cells were seeded in 96-well plates at a density of 2×10^5/well and subsequently exposed to several concentrations of sodium selenite (10 nM–1 mM) and H_2O_2 (1 μM–1 mM) at different incubation times (1–72 h for sodium selenite and 0.5–24 h for H_2O_2) at 37°C. After the treatments, the medium was removed and MTT was added to each well and then incubated for 90 min at 37°C in a shaking water bath. The supernatant was discarded and DMSO was added to dissolve the formazan crystals. Treatments were carried out in duplicate. Optical density was measured in an automatic plate reader at 490 nm and 650 nm and presented as the fold increase over the pretreatment level (experimental/control).

Assay for Caspase Activities

To determine caspase-3 and -9 activities, stimulated or resting cells were sonicated and cell lysates were incubated with 2 ml of substrate solution (20 mm HEPES [pH 7.4], 2 mm EDTA, 0.1% CHAPS, 5 mm DTT and 8.25 μM of caspase substrate) for 1 h at 37°C. The activities of caspase-3 and -9 were calculated from the cleavage of the respective specific fluorogenic substrate (AC-DEVD-AMC for caspase-3 and AC-LEHD-AMC for caspase-9). Substrate cleavage was measured with a fluorescence spectrophotometer, with excitation wavelength of 360 nm and emission wavelength at 460 nm. Preliminary experiments reported that caspase-3 or -9 substrate cleaving was not detected in the presence of the inhibitors of caspase-3 or -9, DEVD-CMK or z-LEHD-FMK, respectively. The data were calculated as fluorescence units/mg protein and presented as the fold increase over the pretreatment level (experimental/control) (González et al. 2009).

Measurement of $[Ca^{2+}]_c$

Cells were loaded with fura-2 by incubation with 4 μM fura-2/AM for 30 min at room temperature according to a procedure published elsewhere (González et al. 2009). Once loaded, the cells were washed and gently resuspended in Na-HEPES solution containing (in mM) NaCl 140, KCl 4.7, $CaCl_2$ 1.2, $MgCl_2$ 1.1, glucose 10 and HEPES 10 (pH 7.4). Fluorescence was recorded from 2-ml aliquots of magnetically stirred cellular suspension (2×10^6 cells/ml) at 37°C using a spectrofluorometer (RF-5301-PC; Shimadzu, Tokyo, Japan) with excitation wavelengths of 340 and 380 nm and emission at 505 nm. Changes in $[Ca^{2+}]_c$ were monitored using the fura-2 340/380 nm fluorescence ratio and calibrated according to the method of Grynkiewicz et al. (1985). In the experiments where calcium-free medium is indicated, Ca^{2+} was omitted and 2 mM ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA) was added.

Ca^{2+} release was estimated using the integral of the rise in $[Ca^{2+}]_c$ for 2.5 min after addition of H_2O_2 (Espino et al. 2009). Ca^{2+} release is expressed as nanomoles, taking a sample every second (nM · s), as previously described (Heemskerk et al. 1997).

Statistical Analysis

Data are expressed as means ± SEM of the number of determinations. Statistical significance was analyzed using Student's *t*-test. To compare the different treatments, statistical significance was calculated by one-way analysis of variance followed by Tukey's multiple comparison tests. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Determination of Toxic Concentration of H_2O_2 and Therapeutic Concentration of Selenium on HL-60 Cell Viability (MTT)

The effects of H_2O_2 and selenium on the MTT in HL-60 cells are shown in Figs. 1 and 2, respectively. Cells were incubated with increasing concentrations of H_2O_2 (1 μM–1 mM) for six different time periods (0.5–24 h). The toxic effect of moderate H_2O_2 started at the 100 μM concentration and 10 h after H_2O_2 incubation ($P < 0.05$). For 5 h of incubation, the toxic effect started at a higher concentration of H_2O_2 (1 mM) exposure. Hence, we found the toxic concentration of H_2O_2 to be 100 μM in the cell culture system. Then, we investigated the toxic concentration and duration of selenium exposure in HL-60 cell culture. Cells were incubated at eight different concentrations of selenium (10 nM, 100 nM, 200 nM, 500 nM, 1 μM, 10 μM, 100 μM, 1 mM) for 1 h. Cell samples were taken at six different times (1, 5, 10, 24, 48 and 72 h), and the MTT test was done in the samples. We observed a 50% decrease in MTT by 10 μM and 10 h of selenium exposure ($P < 0.01$). Hence, we found that the toxic effects of selenium started at 10 μM at 10 h. MTT levels at the highest concentration of selenium, 1 mM, decreased significantly ($P < 0.001$) at 1 h.

Effects of Moderate and High Concentrations of Selenium and H_2O_2 on the MTT Test

The effect of moderate and high concentrations of selenium and H_2O_2 on the MTT levels in HL-60 cells is shown in Fig. 3. Cells were preincubated with 200 nM selenium

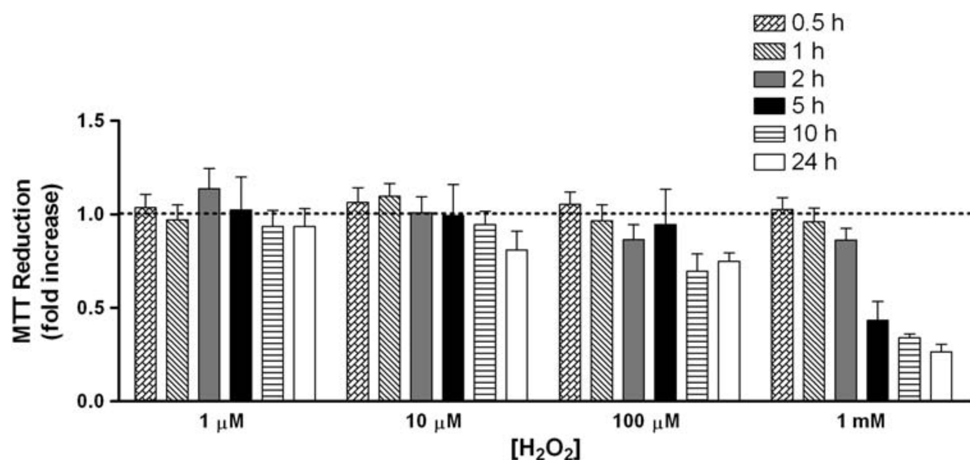

Fig. 1 Effect of H_2O_2 on HL-60 cell viability (MTT). Cells were incubated with increasing concentrations of H_2O_2 (1 μM–1 mM) for various periods of time (0.5–24 h). The toxic effect of H_2O_2 started at 100 μM concentration and 10 h after incubation ($P < 0.05$). Concentrations of H_2O_2 as high as 1 mM started the toxic effect at 5 h after incubation. MTT at 1 mM concentration of selenium increased significantly ($P < 0.001$) at 5, 10 and 24 h. Hence, we found the toxic concentration of H_2O_2 to be 100 μM in our cell culture system

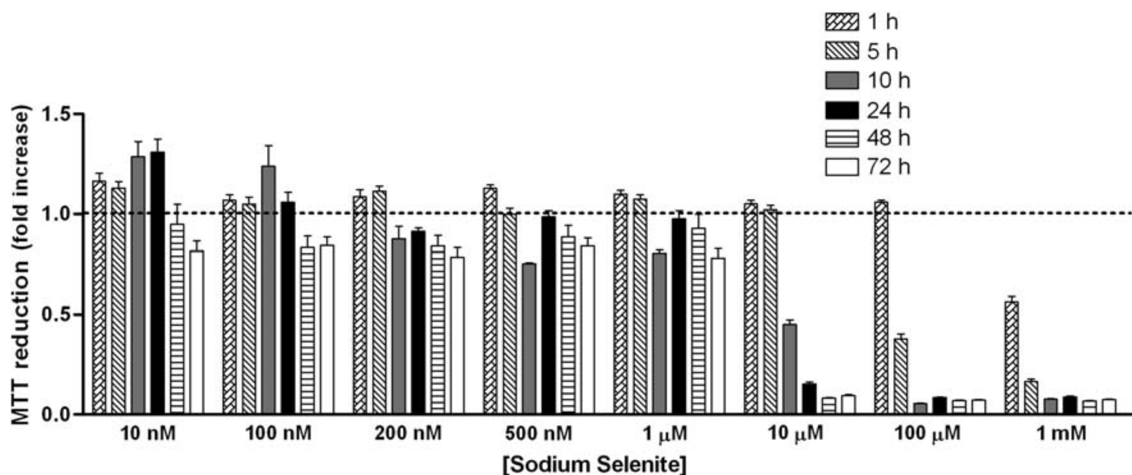

Fig. 2 Effects of selenium concentrations on HL-60 cell viability (MTT). There was statistical significance at five concentrations of selenium between 10 nm and 1 μM. The toxic effect of selenium on MTT started at 10 μM at 5 h incubation. MTT at 10 μM, 100 μM and 1 mM was significantly lower than control ($P < 0.001$)

(Fig. 3a, b) for 1 h and then stimulated with 1 mM (Fig. 3a) or 100 μM (Fig. 3b) H_2O_2 for 10 h. MTT levels decreased significantly ($P < 0.05$ and 70%) in 1 mM H_2O_2 (group A), and the cells did not tolerate 1 mM H_2O_2. The cell viability did not recover in the selenium and H_2O_2 group. However, cell viability decreased at the 100 μM concentration ($P < 0.05$). Cell viability was recovered in the 100-μM group by selenium supplementation, although selenium did not affect cell viability in the 1-mM group.

The effects of moderate and high concentrations of selenium on cell viability induced by H_2O_2 in HL-60 cells are shown in Fig. 4. Cells were preincubated with 1 mM H_2O_2 (Fig. 4a) and 100 μM H_2O_2 (Fig. 4b) for 10 h. Cell viability decreased by 70% ($P < 0.001$) and 25% ($P < 0.05$) at 1 mM and 100 μM H_2O_2, respectively. However, cell viability was significantly (70% and $P < 0.05$) lower in the H_2O_2 group than in the selenium group. Cell viability was recovered ($P < 0.05$) in the 100-μM group by selenium supplementation, although selenium did not affect cell viability in the 1-mM group. High selenium and high H_2O_2 induced the greatest decrease (75%) in cell viability (Fig. 4a).

Effects of Moderate and High Concentrations of Selenium and H_2O_2 on Caspase-3 and -9 Activities

The effects of moderate concentrations of selenium and H_2O_2 on caspase-3 and -9 in HL-60 cells are shown in Fig. 5. Cells were preincubated with 200 nM selenium (Fig. 5a, b) for 1 h and then stimulated with 1 mM (Fig. 5a)

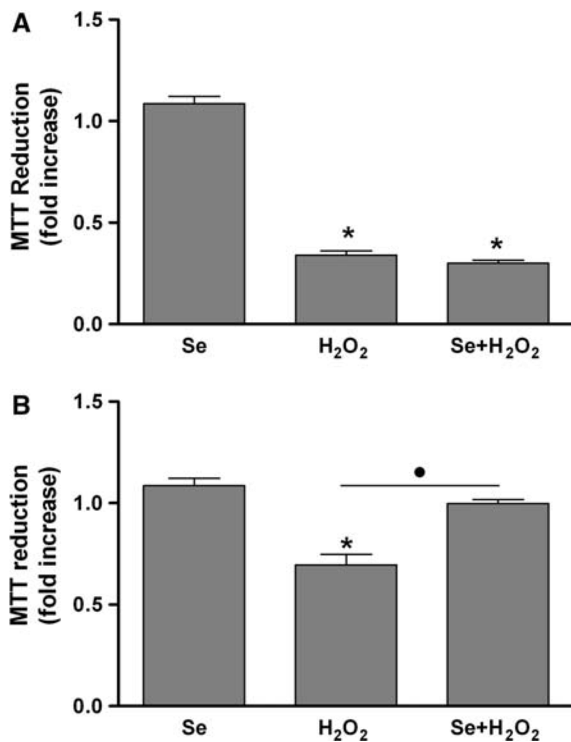

Fig. 3 Effect of low concentration of selenium (Se) on HL-60 cell viability (MTT) induced by H_2O_2. Cells were preincubated with 200 nM selenium (**a**, **b**) for 1 h and then stimulated with 1 mM (**a**) or 100 µM (**b**) H_2O_2 for 10 h. MTT was significantly ($P < 0.05$) decreased in the H_2O_2 group versus the selenium group. MTT was recovered by selenium supplementation in the 100-µM group, although selenium did not affect MTT in the 1-mM group

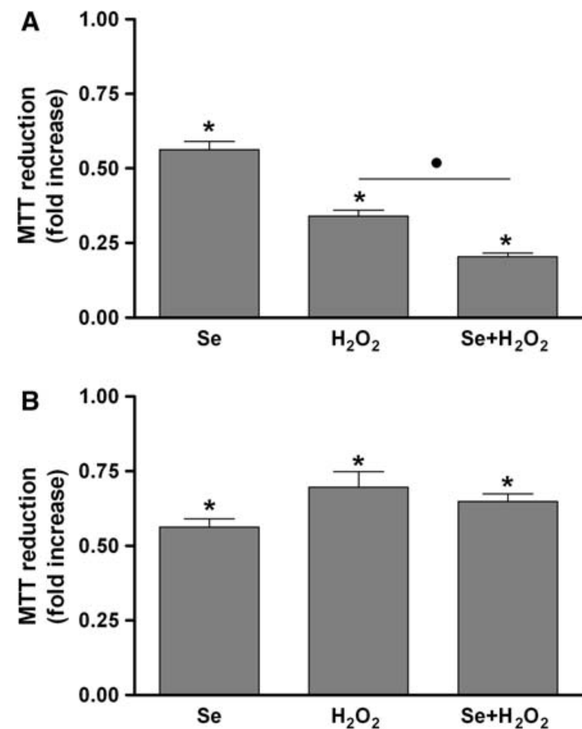

Fig. 4 Effect of high concentration of selenium (Se) and H_2O_2 on HL-60 cell viability (MTT). Cells were preincubated with 1 mM (**a**) or 100 µM (**b**) H_2O_2 for 10 h and then stimulated with 1 mM selenium (**a**, **b**) for 1 h. The effects of 1 mM (**a**) or 100 µM (**b**) H_2O_2 and 1 mM selenium (**a**, **b**) alone are shown for comparison. MTT reduction was determined as described in "Materials and Methods." Values are represented as means ± SD of six separate experiments in duplicate. Values are expressed as fold increase over the pretreatment level (experimental/control). * $P < 0.05$ compared to control, ● $P < 0.05$ compared to H_2O_2 alone

or 100 µM (Fig. 5b) H_2O_2 for 10 h. Caspase-3 and -9 activities were significantly ($P < 0.001$) higher in the H_2O_2 group than in the selenium group. Caspase-3 and -9 activities were significantly ($P < 0.001$) lower in the selenium (200 nM) plus H_2O_2 (100 µM) group than in the H_2O_2-only group (100 µM).

The effects of high concentrations of selenium with H_2O_2 on caspase-3 and 9 in HL-60 cells are shown in Fig. 6. Cells were incubated with 1 mM H_2O_2 (Fig. 6a, b) for 10 h and then treated with 1 mM selenium. Caspase-3 and -9 activities were significantly ($P < 0.001$) higher in the H_2O_2 group than in the selenium-only group.

Effects of Moderate and High Concentrations of Selenium and H_2O_2 on Calcium Release

Effects of moderate concentrations of selenium and H_2O_2 on Ca^{2+} release in HL-60 cells are shown in Fig. 7. Cells were preincubated with 200 and 500 nM selenium (Fig. 7a, b) for 1 h and then stimulated by 1 mM (Fig. 7a) or 100 µM H_2O_2. Ca^{2+} release into cells was increased by 1 mM and 100 µM H_2O_2. Ca^{2+} release into cells was significantly ($P < 0.001$) lower in the 200 and 500 nM selenium plus H_2O_2 group (100 µM) than in the H_2O_2-only group (100 µM or 1 mM).

Discussion

Selenium could cause thiol/disulfide redox modification of numerous proteins that may result in protein unfolding or misunfolding in the endoplasmic reticulum, triggering endoplasmic reticulum stress (Guan et al. 2009). Thus, it is highly plausible that selenium could induce endoplasmic reticulum stress and oxidative stress. We speculated that oxidative stress–induced pathways in endoplasmic reticulum probably mediate the chemoprotective effects of selenium. Treatment with selenium in oxidative stress-induced apoptosis induces a number of signaling markers in a concentration-dependent manner. These markers can be apoptotic molecules such as Ca^{2+} release and caspases. Low concentrations of selenium as sodium selenite preferentially activated the survival responses, whereas high concentrations of selenium led to assembly of the apoptotic

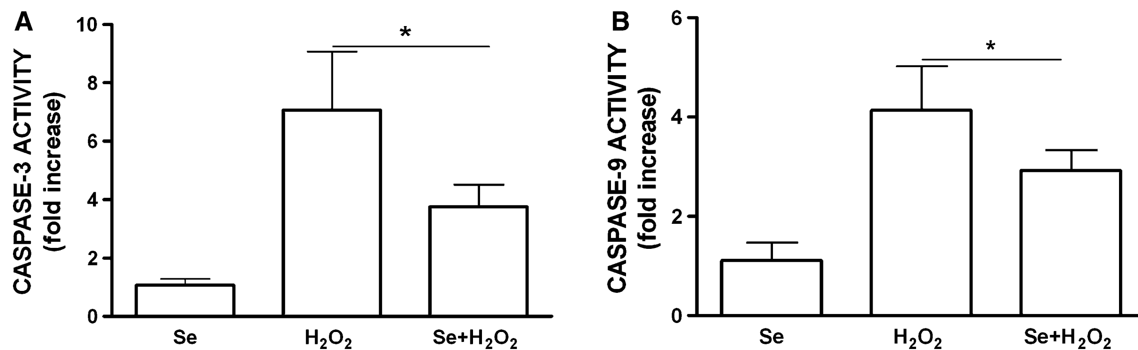

Fig. 5 Effect of low concentration of selenium (Se) on caspase-3 and -9 activities induced by H_2O_2 in HL-60 cells. Cells were preincubated with 200 nM Se for 1 h and then stimulated with 100 µM H_2O_2 for 10 h. The effects of 200 nM sodium selenite and 100 µM H_2O_2 alone are shown for comparison. Caspase-3 and -9 activities were determined as described in "Materials and Methods." Values are represented as means ± SD of 10 separate experiments. Values are expressed as fold increase over the pretreatment level (experimental/control). * $P < 0.05$

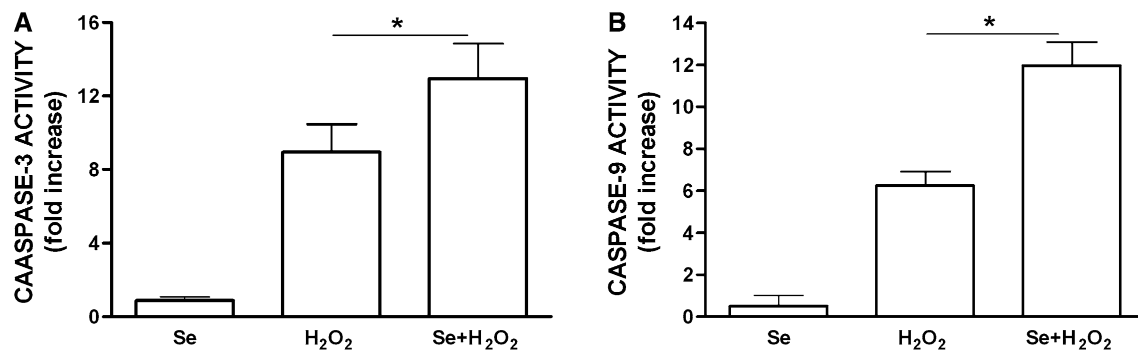

Fig. 6 Effect of high concentration of selenium (Se) on caspase-3 and -9 activities induced by H_2O_2 in HL-60 cells. Cells were preincubated with 1 mM H_2O_2 for 10 h and then stimulated with 1 mM sodium selenite for 1 h. The effects of 1 mM H_2O_2 and 1 mM sodium selenite alone are shown for comparison. Caspase-3 and -9 activities were determined as described in "Materials and Methods." Values are represented as means ± SD of five separate experiments. Values are expressed as fold increase over the pretreatment level (experimental/control). * $P < 0.05$

molecules and ROS, which induced mitochondrial membrane permeabilization and caspase activation. The net balance between these signaling cascades probably governs cell survival or apoptosis (Guan et al. 2009).

We observed that incubation with 100 µM H_2O_2 for 10 h induced a toxic effect on cell viability, Ca^{2+} release and caspase-3 and -9 activities, whereas 200 nM selenium had a protective effect on 100 µM H_2O_2-induced toxicity, Ca^{2+} release and caspase activities in HL-60 cells. Hence, the concentration of selenium induced a protective effect on oxidative stress–induced cell apoptosis. Apoptosis by oxidative stress has been implicated in several biological and pathological processes—aging, inflammation and carcinogenesis—and in diseases such as AIDS, Parkinson, Huntington and cataract formation (Chandra et al. 2000). However, the mechanisms of cell death by oxidative stress are not yet clarified, whereas the effects of oxidative damage are well known on mitochondria failure and Ca^{2+} homeostasis alterations (Hyslop et al. 1988). H_2O_2 acts on mitochondria, causing a disruption of mitochondrial membrane potential and the release of cytochrome *c* (Stridh et al. 1998). H_2O_2 can be produced by stresses as well as by normal function of mitochondria. One possible mechanism of H_2O_2-induced apoptosis is activation of the ASK1-JNK–mitochondrial dysfunction–caspase activation pathway. Because ROS induce ASK1 activation in cells (Stridh et al. 1998; Shi 2002), ASK1 and downstream JNK activation leads to an increase in mitochondrial dysfunction and caspase activation. Furthermore, ASK1(–/–) cells are resistant to TNF-α- and H_2O_2-induced apoptosis (Yoon et al. 2002). Thus, ASK1 is selectively required for H_2O_2-induced prolonged activation of JNK/p38 and apoptosis (Yoon et al. 2002).

Cytosolic Ca^{2+} has been presented as a key regulator of cell survival, but this ion can also induce apoptosis in response to a number of pathological conditions (Nazıroğlu

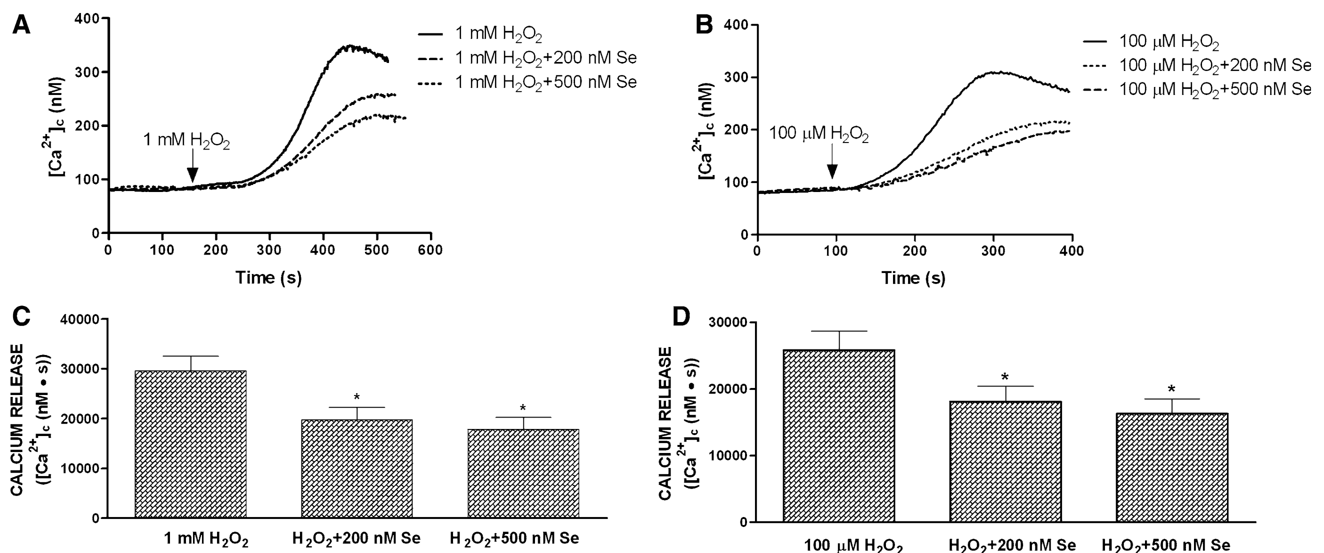

Fig. 7 Effect of selenium (Se) on calcium mobilization evoked by H_2O_2 in HL-60 cells. Fura-2-loaded cells were preincubated with 200 or 500 nM sodium selenite for 1 h and then stimulated with 1 mM (**a**) or 100 μM (**b**) H_2O_2 in a calcium-normal solution (1.2 mM $[Ca^{2+}]_o$). Traces are representative of four to six independent experiments. **c**, **d** Histograms represent the integral for 5 min of Ca^{2+} release induced by 1 mM (**c**) or 100 μM (**d**) H_2O_2, calculated as described in "Materials and Methods." Values are means ± SD of four to six independent experiments. * $P < 0.05$ compared to H_2O_2 alone

2009). In addition, the mitochondria act as Ca^{2+} buffers by sequestering excess Ca^{2+} from the cytosol (Hajnóczky et al. 2006). Ca^{2+} mobilizing agonists can effectively produce a rapid, simultaneous and reversible cessation of the movements of both endoplasmic reticulum and mitochondria, which is strictly dependent on a rise in $[Ca^{2+}]_c$. This inhibition in mitochondrial motility reflects an increased mitochondrial Ca^{2+} uptake and, thus, enhances the local Ca^{2+} buffering capacities of mitochondria, with important consequences for signal transduction (Brough et al. 2005). Ca^{2+} overloading in mitochondria can induce an apoptotic program by stimulating the release of apoptosis-promoting factor like cytochrome *c* and by generating ROS due to respiratory chain damage (Brookes et al. 2004; Hajnóczky et al. 2006). Furthermore, mitochondria have been found to play a pivotal role in Ca^{2+} signaling (Hajnóczky et al. 2006). In fact, the release of Ca^{2+} from endoplasmic reticulum stores by IP3 receptors has been implicated in multiple models of apoptosis as being directly responsible for mitochondrial Ca^{2+} overload (Giorgi et al. 2009). Stored Ca^{2+} is crucial for a number of cellular functions, including signal-transduction cascades that respond to stress conditions (Meldolesi and Pozzan 1998). The filling state of the intracellular Ca^{2+} stores has been described as a candidate to trigger the initiation of apoptotic events (Rayman 2000). Our findings indicate that the caspase activation caused by depletion of intracellular Ca^{2+} stores is probably due to a mitochondrial membrane depolarization and subsequent release of intramitochondrial apoptosis-promoting factors. We provide compelling evidence that mitochondrial Ca^{2+} uptake evoked by rises in $[Ca^{2+}]_c$ induces mitochondrial membrane depolarization and caspase-9 and -3 activation. Our results indicate that the blockade of both Ca^{2+} uptake into mitochondria with low selenium (200 nM) and increases in $[Ca^{2+}]_c$ was able to decrease caspase activation mediated by selenium and to release Ca^{2+} from intracellular stores.

ROS act as subcellular messengers in such complex processes as mitogenic signal transduction, gene expression and regulation of cell proliferation when they are generated excessively or when enzymatic and nonenzymatic defense systems are impaired (Nazıroğlu 2007a, b). The major intracellular antioxidant enzyme GSH-Px detoxifies H_2O_2 to water and removes organic hydroperoxides (Halliwell 2006). Selenium has a major antioxidant function as a cofactor for GSH-Px. It is therefore essential in removing free oxygen radicals from the body and preventing oxidative stress (Papp et al. 2007). However, reports in the last decade have revealed that low or moderate concentrations of selenium provide protection against ROS-induced apoptosis, whereas high concentrations induce ROS production and apoptosis (Gopee et al. 2004; Li et al. 2007). In the current study, Ca^{2+} release, cell apoptosis and caspase activities were higher in high selenium (1 mM) plus H_2O_2 groups than in the H_2O_2 group. Hence, higher concentrations of selenium induce activation of endoplasmic reticulum–induced oxidative stress instead of playing an antioxidant role.

In most of the selected cancer cell types, caspases acts as key executors of selenium-induced apoptotic cell death. It was also found that selenium induced caspase-independent apoptosis in the absence of caspase-3 and without typical nuclear fragmentation with the involvement of p53 and p38 pathways (Jiang et al. 2004), which was mainly mediated by nuclear translocation of apoptosis-inducing factors from mitochondria (Susin et al. 1999). Studies have demonstrated that selenite and selenomethionine induced apoptosis in human prostate cancer cells with involvement of p53 and caspase activation as mediated by superoxide overproduction (Jiang et al. 2004; Zhao et al. 2006). The induction of caspase-dependent or -independent apoptotic pathways depends on the chemical forms of selenium and the cell types involved. To our knowledge, there is no report about the effects of selenium concentrations on oxidative stress–induced caspase activities and apoptosis. In the current study we observed that high concentrations of selenium induced caspase-3- and -9-dependent apoptosis in HL-60 cells. Hence, these observations demonstrated that selenium maintained cell survival by activating an antiapoptotic signal. Similarly, Uezono et al. (2006) reported that 100 μM selenium as sodium selenite modulated Ca^{2+} release and catecholamine secretion in bovine adrenal chromaffin cells. A physiological concentration of selenium (<3 μM) in HT1080 increased cell proliferation and cell survival, blocking the apoptotic signal (Yoon et al. 2002).

Low concentrations of selenium elicit mild endoplasmic reticulum stress and active survival pathways, which help cells to cope with and recover from endoplasmic reticulum oxidative stress. Apoptosis markers such as caspase-3 and -9 activities and Ca^{2+} release are activated before selenium treatment at 200 nM. Taken together, low concentrations of selenium activate multiple survival mechanisms that counteract oxidative stress–induced cell apoptosis by inhibiting ROS production and supporting the antioxidant redox system, while high concentrations of selenium block these survival pathways and at the same time activate multiple mechanisms that directly trigger cell apoptosis. These different molecular mechanisms control oxidative stress–induced apoptosis.

Acknowledgements J. A. P. and M. N. formulated the present hypothesis and were responsible for writing the report. A. C. U., J. E., I. B., D. G. and A. B. R. were responsible analyses the data. J. A. P. and M. N. made critical revisions to the manuscript.

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