Biol Trace Elem Res (2013) 154:440–447
DOI 10.1007/s12011-013-9749-x

Selenium Compounds Induced ROS-Dependent Apoptosis in Myelodysplasia Cells

Ana Cristina Gonçalves · André Barbosa-Ribeiro · Vera Alves · Teresa Silva · Ana Bela Sarmento-Ribeiro

Received: 14 May 2013 / Accepted: 1 July 2013 / Published online: 31 July 2013

Abstract Several authors have demonstrated the chemoprotective and anti-carcinogenic role of selenium. However, the therapeutic potential of selenium in myelodysplastic syndrome (MDS) as single agent and as co-adjuvant of the current therapies has not been previously studied. Sodium selenite and selenomethionine, alone and in combination with cytarabine, induce a decrease in cell viability in a time-, dose- and administration-dependent manner inducing cell death by apoptosis in F36P cells (MDS cell line). These compounds increased superoxide production and induced mitochondrial membrane depolarization. The increase in BAX/BCL-2 ratio and in the activated caspase 3 expression levels, the decrease in mitochondria membrane potential, as well as the increase in superoxide production, supports the mitochondria contribution on selenium-induced apoptosis. These findings suggest that selenium may offer a new therapeutic approach in myelodysplastic syndrome in monotherapy and/or as co-adjuvant therapy to conventional anti-carcinogenic.

Keywords Apoptosis · Myelodysplastic syndrome · Oxidative stress · Selenomethionine · Sodium selenite · Mitochondrial dysfunction

A. C. Gonçalves · A. Barbosa-Ribeiro · A. B. Sarmento-Ribeiro
Applied Molecular Biology, University Clinic of Haematology and Center of Investigation in Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal

A. C. Gonçalves · A. B. Sarmento-Ribeiro
Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal

V. Alves
Immunology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

T. Silva
Hematopathology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

A. B. Sarmento-Ribeiro (✉)
Biologia Molecular Aplicada e Clínica Universitária de Hematologia, Faculdade de Medicina da Universidade de Coimbra, Azinhaga de Santa Comba, Celas, 3000-548 Coimbra, Portugal
e-mail: absarmento@fmed.uc.pt

Introduction

Many observational epidemiological studies and also some randomised clinical trials have demonstrated the potential preventive application of natural compounds in cancer, in particular vitamins and minerals, and some of them may decrease the risk of developing cancer. The main group of carcinogenesis inhibiting agents is dietetic antioxidants; however, natural compounds may have several other properties such as inhibiting apoptosis, cytochrome P450 enzymes and angiogenesis but also antagonising growth factors, hormones or DNA-repairing enzymes [1, 2]. Despite this, their molecular activity is not yet clear and identifying their molecular targets may bring some enlightenment on their potential therapeutic role in several diseases such as cancer. These compounds may activate or inhibit several cell signalling pathways such as those involved in proliferation, survival, differentiation and cell death. Apoptosis may be induced by stimulating several intra or extracellular targets regarding oxidative stress, cell cycle changes, hypoxia induction and loss of cell adherence [3, 4]. On the other hand, the simultaneous use of antioxidant compounds with the anti-carcinogenic therapy has proven to be of benefit in cancer patients being associated with a lesser secondary toxicity, decreasing the premature interruption rate of the antineoplastic therapy [5–8]. The chemoprotective and anti-carcinogenic effects of selenomethionine (SeMt) and sodium selenite (NaSe), being considered an active product in the chemoprevention of cancer, has been proposed by several authors. However, these effects are limited when intracellular

methionine levels are low. In the absence of adequate concentrations of methionine, selenomethionine is incorporated into proteins, replacing methionine [9–14]. Besides, it has been demonstrated in previous studies that the interaction of Se with other trace elements, namely lead (Pb) and cadmium, may result in an increased cancer susceptibility and in the abolishment of its anti-carcinogenic effect [15, 16]. Moreover, the anti-carcinogenic effect exhorted by selenium is dependent on the available form for cell use, organic or inorganic form, i.e. selenomethionine or sodium selenite, respectively. While selenomethionine induces apoptosis and cell cycle block in G1 phase [9, 10], sodium selenite induces a cytotoxic and anti-proliferative effect by cytoplasmatic vacuolization, cell membrane damage, cell cycle arrest in S/G2 phase and breakdown of the DNA double helix-originating cell death by necrosis. In fact, some studies demonstrate that the presence of selenium in cell cultures prevents cell mutation or transformation into neoplastic cells. These observations may be related with the increased in antioxidant capacity, namely in glutathione peroxidase activity, with the inhibition of the enzymes responsible for converting carcinogenics, with the increase in immunological response and the decreased in the proliferation rate [11–14]. Myelodysplastic syndrome (MDS) is a group of clonal diseases of the hematopoietic stem cell characterised by dysplasia, ineffective haematopoiesis and a high risk of progression to acute myeloblastic leukaemia. Several mechanisms are involved in MDS pathophysiology, namely those related to genetic and/or epigenetic modifications, apoptosis deregulation, oxidative stress and mitochondrial dysfunction [17, 18]. The therapeutic approach to MDS is far from satisfactory and only stem cell bone marrow transplantation offers a potentially curative approach but it is associated with a high morbidity and mortality. As far as chemotherapy is concerned, MDS is considered to be particularly refractory to cytotoxic chemotherapy regimens; when remissions are accomplished, MDS are usually brief and associated with a high incidence of drug toxicity and thus a high morbidity and mortality, especially in the elderly [19]. Therefore, new therapeutic approaches and new molecular targets should be pursued and so, in this study, we intend to evaluate the therapeutic potential of selenium in MDS, both in monotherapy and/or as an adjuvant to conventional anti-carcinogenic therapies.

Materials and Methods

Materials

RPMI 1640 medium, foetal bovine serum (FBS), penicillin/streptomycin and recombinant interleucin-3 (rh-Il-3) were from GIBCO, Invitrogen (Barcelona, Spain). Phosphate-buffered saline (PBS), cytarabine (Ara-C), hydroetidine (HE), mercury orange (MO), selenomethionine and sodium selenite were from Sigma (Sintra, Portugal). Annexin V (AV)-fluorescein isothiocyanate (FITC) and propidium iodide (PI) were from Immunostep kit (Salamanca, Spain). 2′,7′-Dichlorodihydrofluorescin diacetate (H2DCF-DA) and JC-1 were from Molecular Probes, Invitrogen (Barcelona, Spain). Culture flasks and multi-well plates were from Sarstedt (Rio Tinto, Portugal). Phycoerythrin (PE) anti-BAX and fluorescein isothiocyanate (FITC)-labeled anti-BCL-2 antibodies were from Santa Cruz, CA, USA. PE anti-activated caspase 3 was from Pharmingen, Becton Dickinson (San Jose, CA, USA). Monoclonal mouse antibody isotype immunoglobulin G (IgG)1/FITC and antibody isotype IgG2b/PE was from Dako (Glostrup, Denmark).

Cell Culture

The F36P cells, a myelodysplastic syndrome cell line established from a patient with refractory anaemia with excess of blast in transformation (RAEB-t), was purchased from the European Collection of Cell Cultures (UK). Cell line was routinely grown in RPMI-1640 medium (L-glutamine 2 mM, HEPES-Na 25 mM, penicillin 100 U/mL and streptomycin 100 μg/mL) supplemented with 10 ng/mL rh-Il-3 and 10 % heat-inactivated FBS at 37 °C in a humidified atmosphere containing 5 % CO_2. All experiments were performed between 10 and 25 passage numbers and F36P cells were seeded at a density of 5×10^5 cells/ml. For the preparation of NaSe and SeMt stock solutions, these compounds were dissolved in water, shacked and stored at 4 and −20 °C, respectively. Cells were incubated in the absence and in the presence of increasing NaSe and SeMt concentrations ranging from 0.1 to 250 and 10 to 1,000 μM, respectively, in monotherapy or in association with 50 nM Ara-C.

Cell Viability Assay

Cell viability was assessed by resazurin metabolic assay. This assay is based on the addition of a redox fluorogenic indicator to the cell culture. When resazurin is added to the cell culture, viable cells reduce resazurin into resofurin changing colour from non-fluorescent indigo blue to fluorescent pink. This colour change can be measured by spectrophotometry. Therefore, resazurin, in a final concentration of 10 μg/ml, was added to the plates 2 h prior to the reading in a spectrophotometer (Synergy™ HT Multi-Mode Microplate Reader, BioTek Instruments) at 580 and 600 nm and compared to the control.

Cell Death Evaluation

F36P cell death was evaluated under the conditions describe above by optical microscopy through morphological assessment of May–Grünwald–Giemsa-stained slides and by flow cytometry using annexin V and propidium iodide double

staining. For morphological assessment, cells were transferred to slides fixed, stained and evaluated under light microscopy, using a Nikon Eclipse 80i equipped with a Nikon Digital Camera DXm 1200F. For flow cytometry analysis, F36P cells were stained simultaneously with AV, labelled with FITC and PI. After drug treatments in the above conditions, cells were co-stained with AV-FITC and PI according to the manufacturer's recommended protocol. Briefly, cells were washed with ice-cold PBS (centrifuged at 500×*g* for 5 min), resuspended in 100 μl of binding buffer and incubated with 5 μl of AV-FITC solution and 5 μl of PI solution for 15 min in the dark. After incubation time, cells were diluted in 400 μl of binding buffer, and analysed by flow cytometry. Flow cytometry analyses were performed using a six-parameter, four-colour FACSCalibur^{TM} flow cytometer (Becton Dickinson). For each assay, 1×10^6 cells were used and at least 10,000 events were collected by acquisition using CellQuest software (Becton Dickinson) and analysed using Paint-a-gate software (Becton Dickinson).

Evaluation of Reactive Oxygen Species Production

The reactive oxygen species (ROS) production in F36P cells was determined by oxidation of H2DCF-DA and HE. Cells cultured in the absence and presence of NaSe and SeMt were incubated with 5 μM H2DCF-DA for 30 min and with 2 μM of HE during 15 min at 37 °C in the dark. Cells were then washed twice with PBS, resuspended in the same solution and fluorescence was detected by flow cytometry [20].

Mitochondrial Membrane Potential Measurement

Mitochondrial membrane potential ($\Delta\psi_{mit}$) in F36P cells treated with and without selenium compounds were measured using JC1 (Molecular Probes) as described by others [20]. Briefly, after incubation in the absence or in the presence of NaSe and SeMt, cells were washed with PBS (centrifugation at 300×*g* during 5 min) and incubated with JC-1 at final concentration 5 μg/ml for 15 min at 37 °C in the dark. At the end of the incubation period, the cells were washed twice in PBS, resuspended in a total volume of 500 μl and analysed by flow cytometry.

Evaluation of Apoptotic Proteins Expression by Flow Cytometry

The modulation of BAX, BCL-2 and activated caspase 3 expression levels were analysed in cells cultured in the absence and in the presence of NaSe and SeMt. F36P cells were centrifuged and incubated with monoclonal antibodies anti-BAX, anti-BCL-2 and anti-cytochrome c antibodies, according to manufacturer's protocols. For all the assays, negative controls were established with isotype IgG, IgG1 and IgG2b, and submitted to the same procedures.

Statistical Analysis

Data were expressed as mean±SD of the number of independent experiments indicated in the figure legends, each one performed in triplicate. Student's *t* test and/or analysis of variance were used to determine the statistical significance, considering a p value of <0.05.

Results

NaSe and SeMt Have an Anti-Proliferative Effect on the F36P Cell Line

NaSe and SeMt induce a decrease in F36P cell viability in a time- and dose-dependent manner, with a medial lethal concentration (LC_{50}) of 5 and 250 μM, respectively, at 48 h of exposure to the compounds (Fig. 1a, b). In order to assess whether the frequency of administration had influence in the anti-proliferative effect of these compounds, a low dose of NaSe and SeMt ($\pm LC_{20}$) were added every 24 h. As we can observe in Fig. 1c, cells treated with this daily dose administration of NaSe (1 μM) and SeMt (50 μM), achieved a greater anti-proliferative effect than with 5 μM and 250 μM (LC_{50}) in single administration. We also investigated if the association of these compounds with the conventional anti-carcinogenic therapy, Ara-C, has any influence on cell growth. The F36P cells were incubated with a concentration of 50 nM of Ara-C, with slight effect on cell growth (LC_{20} after 96 h of exposure). When this concentration of Ara-C was administrated simultaneously in association with the same concentrations for NaSe and SeMt used in daily administration, we observed a potentiation synergistic anti-proliferative effect with the association of NaSe and Ara-C (Fig. 1d), whilst when SeMt and Ara-C were combined, this effect was not observed.

Cytotoxicity Induced by NaSe and SeMt is Mediated by Late Apoptosis/Necrosis

In order to assess if the anti-proliferative effect was accomplished by cytotoxicity, we analysed cell viability and death by flow cytometry using an annexin V/propidium iodide double staining incorporation, by flow cytometry. As we can observe in Fig. 2, both NaSe and SeMt induce cell death mainly by late apoptosis/necrosis in a concentration and selenium form dependent manner. In fact, a fivefold increase in concentration of SeMt was needed to obtain an equivalent cytotoxic effect as those observed with NaSe. Sodium selenite and SeMt in combination with Ara-C also presents a synergistic cytotoxic effect. Morphological features of F36P cells confirm the results obtain by flow cytometry, considering the smears of the F36P cell line—using a May–Grünwald–Giemsa staining—which showed morphological features typical of apoptosis (data not shown).

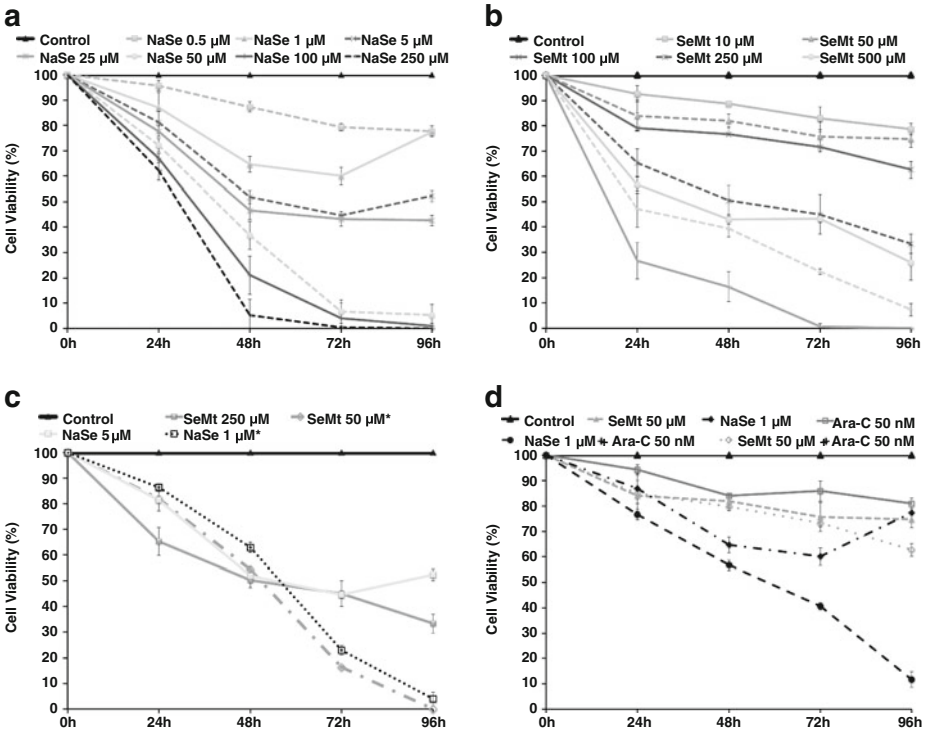

Fig. 1 Dose–response curves. F36P cells were incubated in a density of 0.75×10^6 cells/ml, during 96 h in the absence or in the presence of increasing concentrations of sodium selenite, selenomethionine, alone or in combination with cytarabine, as indicated in the figure. Dose–response curves were established by resazurin reduction for 24 h each, as described in "Materials and Methods" section. Results are expressed in percentage normalised to control. Data are expressed as mean±SD obtained from five independent experiments. *Asterisk* daily administration, *NaSe* sodium selenite, *SeMt* selenomethionine, *Ara-C* cytarabine

NaSe and SeMt Cytotoxicity is Mediated by Oxidative Stress and Mitochondrial Membrane Dysfunction

To elucidate the cytotoxic mechanisms involved in cell death mediated by the selenium compounds, intracellular expression of hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$) and reduced glutathione (GSH) were analysed (Figs. 3 and 4a). As far as H_2O_2 is concerned, we detected a decrease in these levels both when the F36P cells were incubated with NaSe and with SeMt. Moreover, when we measured $O_2^{\cdot-}$ levels in cells incubated with low concentrations of NaSe and SeMt, we detected a decrease and a maintenance of $O_2^{\cdot-}$ levels,

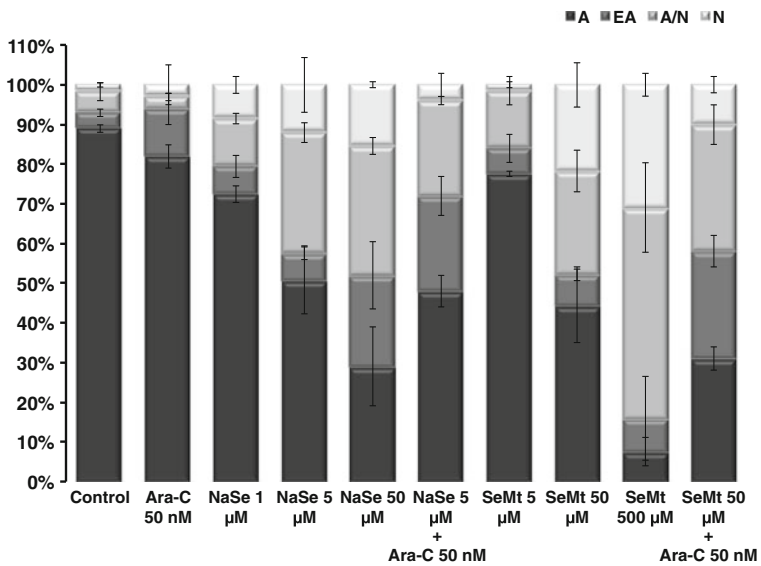

Fig. 2 Analysis of cell death induced by sodium selenite and selenomethionine in F36P cells by flow cytometry. F36P cells were incubated in a density of 0.75×10^6 cells/ml, during 48 h, in the absence or in the presence of increasing concentrations of sodium selenite, selenomethionine, alone or in combination with cytarabine, as indicated in the figure. Cell death was detected by annexin V-FITC and propidium iodide staining and analysed by flow cytometry. Data are expressed as percentage of alive cells (*A*), late apoptotic/necrotic cells (*A*/*N*), necrosis (*N*) and early apoptotic cells (*EA*) as mean±SD obtained from three independent determinations

respectively, for each form of selenium. When cells were incubated with high concentrations of these compounds, alone or in combination with Ara-C, we detected an increase in $O_2^{\cdot-}$ levels. Next, we analysed the changes in GSH levels in cells treated in the same conditions. Treatment with NaSe increases GSH levels in spite of the concentration. However, when cells were incubated with SeMt, low concentrations induce a decrease in GSH levels, but high concentrations induce a statistically significant increase in such levels. Since apoptosis is closely related to the collapse of $\Delta\psi_{mit}$, we assessed the effect of SeMt and NaSe on $\Delta\psi_{mit}$ (Fig. 4b), by flow cytometry using the JC1 dye. We detected an increase in monomeres/aggregates ratio (M/A ratio), which is correlated with a decrease in mitochondrial transmembrane potential, except for the low-dose 50 μM SeMt, in which there was a decrease in M/A ratio, but was not statistically significant. We also observed a decrease in $\Delta\psi m$ in cells treated with the combination of selenium with Ara-C.

NaSe and SeMt Induce Apoptosis by Modulation of Apoptotic Proteins

BCL-2 and BAX are members of the BCL-2 family involved in apoptosis regulation, playing an anti-apoptotic and pro-apoptotic role, respectively. As observed in Fig. 5a, a significant increase of intracellular concentration of BAX/BCL-2 ratio is observed when cells are treated with both compounds alone or in combination. Moreover, the changes in BAX and BCL-2 expression levels are accompanied by an increase in activated caspase 3 expression level (Fig. 5b).

Discussion

Ever since the first civilizations, plants have been used as chemoprotective approaches on several diseases and its

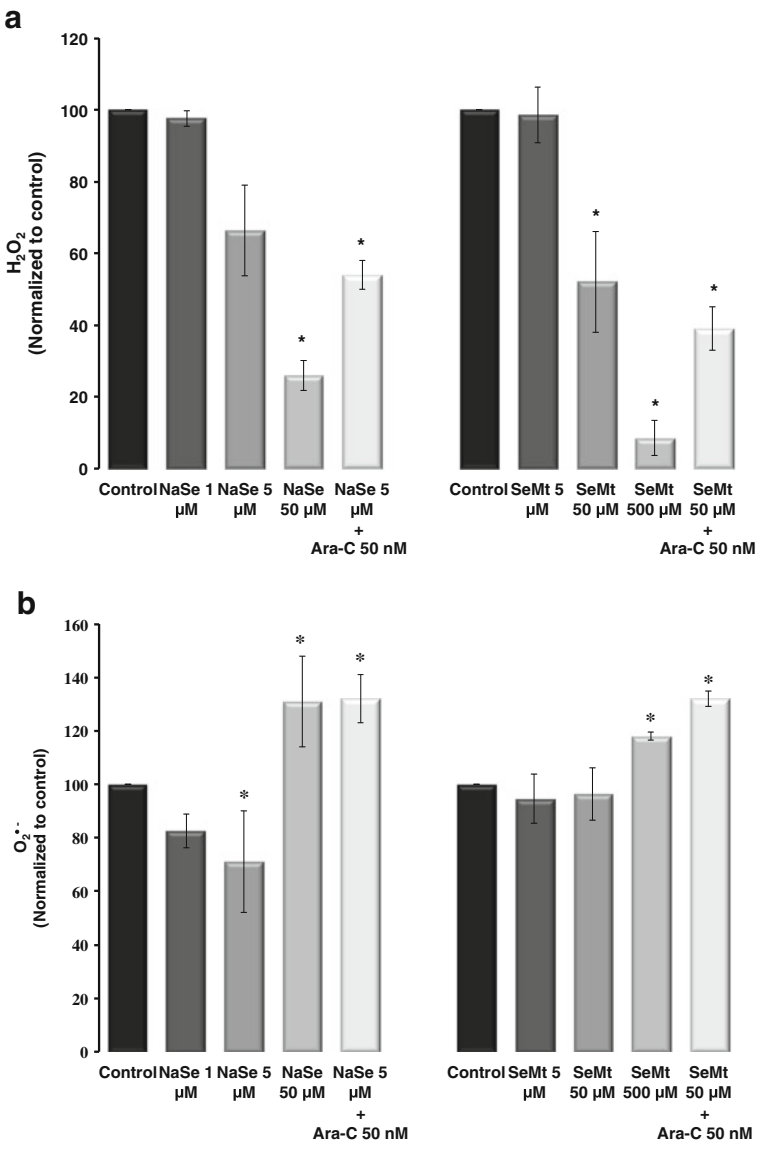

Fig. 3 Evaluation of ROS expression levels in F36P cells treated with selenium by flow cytometry. In **a** hydrogen peroxide (H_2O_2) and in **b** superoxide anion ($O_2^{\cdot-}$), levels were analysed by flow cytometry using DCFH2-DA and DHE fluorescent probes, respectively. F36P cells were incubated in a density of 0.75×10^6 cells/ml, during 48 h, in the absence or in the presence of sodium selenite (NaSe), selenomethionine (SeMt) and cytarabine (Ara-C). Results are expressed as medium fluorescence intensity (MFI) normalised to control and represents mean±SD of fluorescence intensity detected in three independent experiments. *$p<0.05$

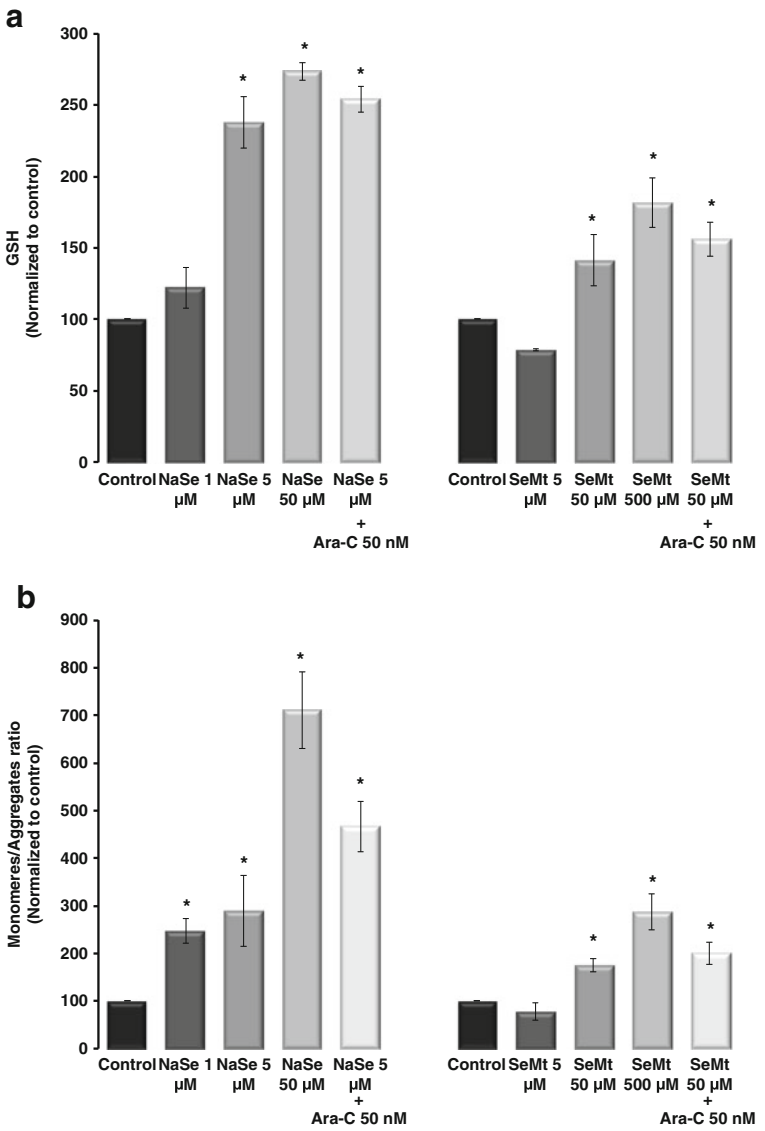

Fig. 4 Analysis of reduced glutathione (**a**) and mitochondrial membrane potential (**b**), by flow cytometry, in F36P cells treated with selenium. The reduced glutathione (GSH) and mitochondrial membrane potential ($\Delta\psi_{mit}$) were analysed by flow cytometry using mercury orange and JC1 fluorescent probes, respectively, as describe in material and methods. F36P cells were incubated in a density of 0.75×10^6 cells/ml, during 48 h, in the absence or in the presence of sodium selenite (NaSe), selenomethionine (SeMt) and cytarabine (Ara-C). JC-1 probe coexist in monomeric or aggregate form depending on the mitochondrial membrane potential and an increase in the monomer/aggregate ratio (M/A ratio) indicates a decreased in the mitochondrial membrane potential. Results are expressed in mean±SD of monomer/aggregate ratio of JC-1 and this ratio was calculated as the fraction of MFI observed for each molecule. *$p<0.05$

compounds used as useful therapeutic agents. In recent decades, this approach has resurfaced and many researchers have been trying to isolate potentially bioactive compounds and analysed its potential use as therapeutic agents in cancer therapy [5, 6, 8]. The precise molecular mechanisms of how these compounds work is not yet fully understood but it is thought that it may interfere in cell signalling pathways either by activation or inhibition of proliferation, survival, differentiation and cell death pathways. As an attempt to answer this question, we evaluated the potential use of NaSe and SeMt as anti-cancer agents. Several studies have shown that selenium has cytotoxic effect in multiple cell lines, such as breast cancer, prostate cancer, melanoma and some haematological tumours cell lines [11–14, 21]. However, the use of this compound in MDS is not yet studied.

Our results show that both selenium forms have anti-proliferative and cytotoxic effect dependent of the concentration, the time of exposure and the selenium form used. This cytotoxic effect was induced mainly by apoptosis. The inorganic selenium form —NaSe— demonstrated an earlier cytotoxic effect and at lower concentrations compared with the inorganic form. The daily dose administration appears to be the best regimen of administration considering that it achieved the same cytotoxic effect but at a lower accumulated dose. When a low concentration of Ara-C was tested simultaneously with lower concentrations than the LC_{50} of NaSe and SeMt, we observed a potentiation synergistic cytotoxic effect with the association of NaSe and Ara-C. When SeMt and Ara-C were combined, this effect was not observed suggesting that inorganic form of selenium is more effective than organic form.

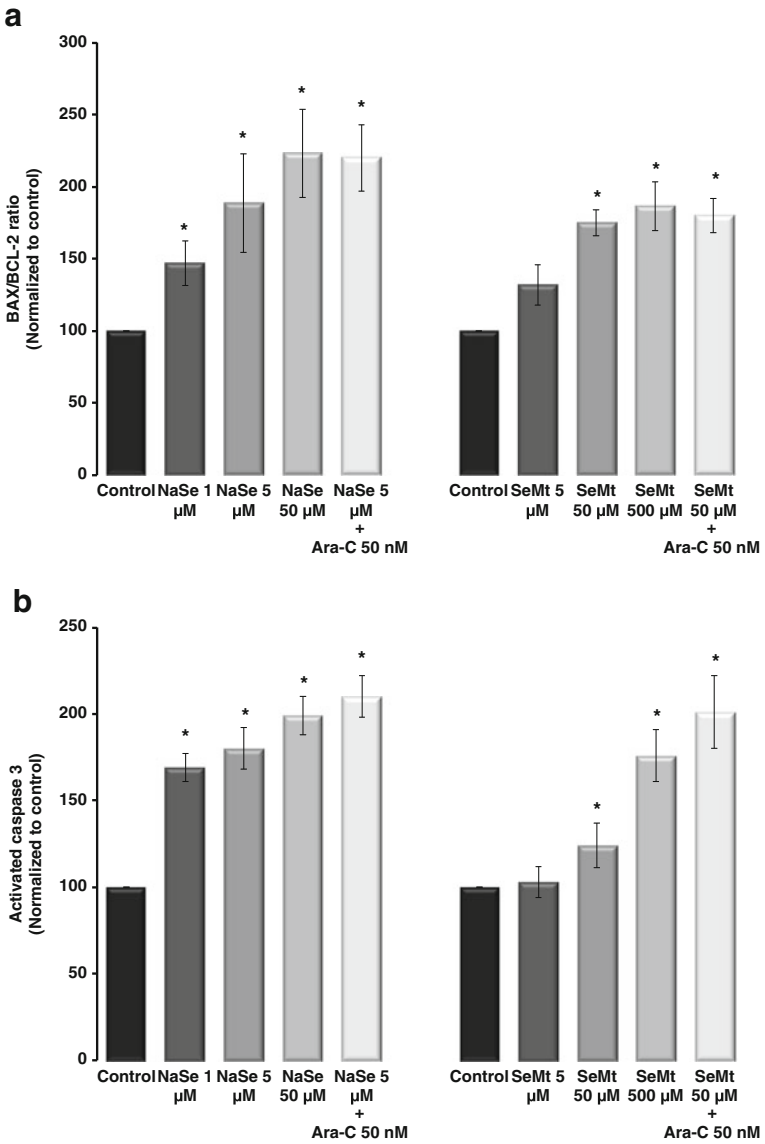

Fig. 5 Evaluation of apoptotic protein expression levels in F36P cells treated with selenium by flow cytometry. BAX/BCL-2 ratio (**a**) and activated caspase 3 (**b**) levels were analysed by flow cytometry using monoclonal antibodies labelled with fluorescent probes. F36P cells were incubated in a density of 0.75×10^6 cells/ml, during 48 h, in the absence or in the presence of sodium selenite (NaSe), selenomethionine (SeMt) and cytarabine (Ara-C) in the concentrations indicated in the figure. BAX/BCL-2 ratio was calculated as the ratio of MFI observed for each molecule. Results are expressed as medium fluorescence intensity (MFI) normalised to control and represents mean±SD of fluorescence intensity detected in three independent experiments. *$p<0.05$

Since selenium is one of the key antioxidants that maintain cell redox balance in account of its role as a co-factor of the antioxidant enzyme glutathione peroxidase, we analysed cell oxidative stress status, in particular the ROS production and GSH levels. We observed a significant increase in $O_2^{\cdot-}$ levels in cells treated with high SeMt concentrations and a significant decrease in H_2O_2 in this cell line. These results may be explained through the reaction of NaSe with GSH, forming selenodiglutathione, which is then reduced to selenopersulfide anion producing $O_2^{\cdot-}$ [13]. However, when these cells were incubated with high concentrations of NaSe and SeMt, we also observed a significant increase in GSH production, which may be related to an increase in glutathione reductase. Furthermore, selenium have a role in the synthesis of seleno-proteins such as the antioxidant defences glutathione peroxidase and thioredoxin reductase, and higher concentrations are related with increased oxidative stress by decreasing reduced glutathione/oxidised glutathione (GSH/GSSG) ratio and also by altering manganese superoxide dismutase (MnSOD) subcellular distribution [22]. Besides that, the increase in GSH may not be enough to neutralise the amount of ROS generated and thus, unable to prevent oxidative damage.

Cell death and the correspondent increase in $O_2^{\cdot-}$ is also associated with the mitochondrial dysfunction induced by selenium since a significant decrease in the mitochondrial membrane potential was observed, as previously reported by others [11–14]. There is yet evidence that selenium leads to the opening of pores in the mitochondrial membrane through oxidation of thiol groups in mitochondrial proteins and $O_2^{\cdot-}$ synthesis in reaction with GSH. Kim et al. observed the induction of apoptosis in hepatocellular carcinoma cell line (HepG2) when cultivated with NaSe and the subsequent decrease in mitochondrial membrane potential and cytochrome c release. According to these authors, the opening

of the pores in the mitochondrial cell membrane was induced by proteins thiol groups oxidation since they observed thiol groups oxidation in isolated mitochondria in HepG2 cell lysate. However, most studies show that the induction of apoptosis depends mainly on GSH depletion and ROS formation [11, 14]. Moreover, selenium alters subcellular distribution of MnSOD, another important antioxidant defence, inducing its depletion in mitochondria and increase in cytosol [23]. Within mitochondria, MnSOD provides a major defence against oxidative damage induced by reactive oxygen species, converting the superoxide radical into hydrogen peroxide. Depletion of MnSOD in the mitochondrial matrix may result in inefficient dismutation of superoxide anion into hydrogen peroxide contributing to the observed increase in superoxide anion and decrease in hydrogen peroxide levels. The increase in BAX/BCL-2 ratio and the decrease in mitochondria membrane potential, as well as the increase in superoxide production reinforces the contribution of mitochondria on selenium-induced apoptosis.

These findings suggest that sodium selenite and selenomethionine alone and in combination with cytarabine may be an effective MDS treatment and should be evaluated as a new therapeutic approach in these patients, in monotherapy or as an adjuvant to conventional anti-carcinogenic therapy. Moreover, this association and/or daily administration, by lowering drug concentration, may be also useful in reducing drug side effects related with systemic toxicity.

Acknowledgments Centro de Investigação em Meio Ambiente, Genética e Oncobiologia (CIMAGO) and Clinical Haematology Service, Centro Hospital e Universitário de Coimbra (HUC/CHUC) funded this study.

Conflict of Interest The authors declare that they have no conflict of interests.

References

1. Halliwell B (1999) Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. Nutr Rev 57:104–113
2. Kelloff GJ, Crowell JA, Steele VE, Lubet RA, Boone CW, Malone WA, Hawk ET, Lieberman R, Lawrence JA, Kopelovich L, Ali I, Viner JL, Sigman CC (1999) Progress in cancer chemoprevention. Ann N Y Acad Sci 889:1–13
3. Aggarwal BB, Shishodia S (2006) Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharm 71:1397–1421
4. Martin KR (2006) Targeting apoptosis with dietary bioactive agents. Exp Biol Med 231:117–129
5. Ripoll EA, Rama BN, Webber MM (1986) Vitamin E enhances the chemotherapeutic effects of adriamycin on human prostatic carcinoma cells in vitro. J Urol 136(2):529–531
6. Kurbacher CM, Wagner U, Kolster B, Andreotti PE, Krebs D, Bruckner HW (1996) Ascorbic acid (vitamin C) improves the antineoplastic activity of doxorubicin, cisplatin and paclitaxel in human breast carcinoma cells in vitro. Cancer Lett 103(2):183–189
7. Woźniak G, Anuszewska EL (2002) Influence of vitamins C and E on cytotoxic activity of adriamycin in chosen cell cultures. Acta Pol Pharm 59(1):31–35
8. Prasad KN, Kumar B, Yan XD, Hanson AJ, Cole WC (2003) α-Tocopheryl succinate, the most effective form of vitamin e for adjuvant cancer treatment: a review. J Am Coll Nutr 22(2):108–117
9. Ip C (1998) Lessons from basic research in selenium and cancer prevention. J Nutr 128(11):1845–1854
10. Novotny L, Rauko P, Kombian SB, Edafiogho IO (2010) Selenium as a chemoprotective anti-cancer agent: reality or wishful thinking? Neoplasma 57(5):383–391
11. Zhong W, Oberley TD (2001) Redox-mediated effects of selenium on apoptosis and cell cycle in the LNCaP human prostate cancer cell line. Cancer Res 61(19):7071–7078
12. Zeng H (2002) Selenite and selenomethionine promote HL-60 cell cycle progression. J Nutr 132(4):674–679
13. Kim TS, Yun BY, Kim IY (2003) Induction of the mitochondrial permeability transition by selenium compounds mediated by oxidation of the protein thiol groups and generation of the superoxide. Biochem Pharmacol 66(12):2301–2311
14. Schrauzer GN (2008) Effects of selenium and low levels of lead on mammary tumor development and growth in MMTV-infected female mice. Biol Trace Elem Res 125(3):268–275
15. Drasch G, Schöpfer J, Schrauzer GN (2005) Selenium/cadmium ratios in human prostates: indicators of prostate cancer risk of smokers and nonsmokers, and relevance to the cancer protective effects of selenium. Biol Trace Elem Res 103(2):103–107
16. Hesketh J (2008) Nutrigenomics and selenium: gene expression patterns, physiological targets, and genetics. Annu Rev Nutr 28:157–177
17. Nimer SD (2008) Myelodysplastic syndromes. Blood 111(10):4841–4851
18. Farquhar MJ, Bowen DT (2003) Oxidative stress and the myelodysplastic syndromes. Int J Hematol 77(4):342–350
19. Young NS (2007) Aplastic anemia, myelodysplasia and related marrow failure syndromes. In: Fauci AS (ed) Harrison's principles of internal medicine, 17th edn. McGraw-Hill, London, pp 663–671
20. Almeida S, Sarmento-Ribeiro AB, Januário C, Rego AC, Oliveira CR (2008) Evidence of apoptosis and mitochondrial abnormalities in peripheral blood cells of Huntington's disease patients. Biochem Biophys Res Commun 374:599–603
21. Redman C, Scott JA, Baines AT, Basye JL, Clark LC, Calley C, Roe D, Payne CM, Nelson MA (1998) Inhibitory effect of selenomethionine on the growth of three selected human tumor cell lines. Cancer Lett 125(1–2):103–110
22. Freitas M, Alves V, Sarmento-Ribeiro AB, Mota-Pinto A (2011) Combined effect of sodium selenite and docetaxel on PC3 metastatic prostate cancer cell line. Biochem Biophys Res Commun 408:713–719
23. Guan L, Jiang Q, Li Z, Huang F, Ren Y, Yang Y, Xu C (2009) The subcellular distribution of MnSOD alters during sodium selenite-induced apoptosis. BMB Rep 42(6):361–366