JrnlID 12011_ArtID 8407_Proof# 1 - 18/05/2009

Biol Trace Elem Res
DOI 10.1007/s12011-009-8407-9

The Organic Selenium Compound Selenomethionine Modulates Bleomycin-Induced DNA Damage and Repair in Human Leukocytes

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Received: 29 April 2009 / Accepted: 8 May 2009 /

Abstract The objective of this work was to evaluate the effects of selenomethionine (SeMet) on the induction, repair, and persistence of DNA damage in human leukocytes challenged with bleomycin (BLM). Comet assay was used to determine DNA strand breaks and hOGG1 for the specific recognition of oxidative damage. Leukocytes were (A) stimulated with phytohemagglutinin, (B) damaged with BLM, and (C) incubated to allow DNA repair. Comet assay was performed after each phase. SeMet (50 μM) was supplemented either during phase A, B, or C, or AB, or ABC. Treatment with SeMet decreased BLM-induced stand breaks when added during phase AB. Results obtained after the repair period indicate that SeMet favors repair of DNA damage especially when applied during phase AB. The comparison between DNA damage before and after repair showed that BLM-induced damage was repaired better in the presence of SeMet. Our results showed antigenotoxic effect of SeMet on BLM-induced DNA and also on repair and persistence of this damage when applied before and simultaneously with BLM.

Keywords Selenomethionine · Bleomycin · Comet assay · Oxidative damage · Antigenotoxicity

Introduction

Bleomycin (BLM) is a mixture of glycopeptide antibiotics with antitumor activity against several cancers; its clinical effectiveness is based on its cytotoxicity backed up by its interaction with DNA, chelation of metal ions, and generation of oxygen-free radicals in the presence of molecular oxygen [1]. BLM behaves as a radiomimetic agent capable of

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inducing a wide spectrum of mutagenic lesions in mammalian cells including DNA base damage, abasic sites, and alkali-labile sites [1–3], which result in DNA single- and double-strand breaks. BLM is classified as a direct clastogenic agent (it does not need metabolic activation) and acts in S-independent fashion [4]. Challenging doses of BLM have been frequently used in DNA repair competence studies [5, 6].

The aim of this work was to evaluate the effects of the organic selenium compound selenomethionine (SeMet) on the induction, repair, and persistence of DNA damage (strand breaks and oxidative) in human leukocytes challenged with BLM. Comet assay was used to determine DNA damage and the human DNA-glycosylase hOGG1 for the specific recognition of 8-hydroxy-2′-deoxyguanosine (8-OHdG), main result of oxidative DNA damage [7], and suitable biomarker of oxidative stress [8].

Materials and Methods

Chemicals

L-Selenomethionine (CAS No. 1464-42-2) and bleomycin (CAS No. 9041-93-4) were purchased from Sigma–Aldrich Co. (Madrid, Spain). Both compounds were dissolved in sterile distilled water prior to use.

Preparation of Human Leukocytes

Human peripheral blood was collected using heparinized vials from three healthy nonsmoker male donors aged 23–30. The University of A Coruña Research Ethics Committee approved the protocol of the experiments (process No. PI 1/2007). Written consent was obtained from each donor prior to joining the study. Mononuclear cells (lymphocytes and monocytes) were isolated on a Ficoll–Hypaque density gradient as described earlier [9]. The cells were suspended in freezing medium (50% fetal calf serum, 40% RPMI 1640, 10% DMSO) to obtain 10^7 cells/ml, and frozen at −80°C in Nalgene® Cryo 1°C Freezing Container (Nalgene Nunc International, Hereford, UK). At the time of experiment performing, cells were quickly thawed at 37°C.

Treatment of Leukocytes and Comet Assay

A preliminary experiment was performed to evaluate the possible genotoxic effects of SeMet and to establish the dose of SeMet to be subsequently used. Leukocytes (10^6 cells/ml) were cultured at 37°C for 20 h in supplemented RPMI 1640 medium containing 15% heat inactivated foetal calf serum, 1% phytohemagglutinin (PHA), 1% L-glutamine (200 mM), and 1% penicillin (5,000 U/ml)/streptomycin (5,000 μg/ml), all from Invitrogen (Barcelona, Spain), in presence of SeMet at 1% of final volume (1, 5, 10, 50, and 100 μM). Viability was then assessed by trypan blue exclusion technique, being higher than 85% in all cases. Alkaline comet assay was performed following the general protocol proposed by Singh et al. [10] with minor changes [11]. Alternatively, modified comet assay incorporating incubation with hOGG1 was carried out as described by Smith et al. [12]. One hundred cells were examined for each experimental point and donor (50 from each replica). Image capture and analysis were done using the QWIN Comet software (Leica Imaging Systems, Cambridge, UK). Percentage of DNA in the comet tail (%TDNA), measured from the estimated centre of the cell, was evaluated as DNA damage parameter.

The next experimental protocol was designed to evaluate the different effects of SeMet on induction, repair, and persistence of DNA damage (stand breaks and oxidative damage) and comprised three consecutive phases. Phase A (PHA stimulation): cells were incubated for 20 h with or without SeMet at 37°C in supplemented RPMI 1640 medium. Phase B (damage induction): leukocytes were centrifuged at 1,500 rpm for 10 min and resuspended in RPMI 1640, and DNA damage was induced by treatment with BLM (20 μg/ml) for 30 min at 37°C in presence or absence of SeMet. BLM concentration was chosen on the basis of previously published papers by other authors [6, 13]. Phase C (repair): cells were washed, resuspended in fresh RPMI 1640 medium, and incubated with or without SeMet for 15 min at 37°C to allow DNA repair. Comet assay (standard protocol and modified with hOGG1 incubation) was performed after each phase as described above. SeMet (50 μM) was supplemented either during phase A, B, or C, or during phases A and B (AB), or during the whole process (phase ABC).

Statistical Analysis

Three independent experiments were performed for each experimental condition tested, and statistical analysis was done using the SPSS for Windows statistical package (version 15.0). Distribution of the response variable departed significantly from normality (Kolmogorov–Smirnov goodness-of-fit test); therefore, nonparametric tests were considered adequate for the statistical analysis of these data. The differences between groups were tested with Mann–Whitney *U* test. Experimental data are expressed as median ± interquartile range. Dose-response relationship for SeMet was determined by Pearson's correlation. A *p* value of less than 0.05 was considered significant.

Results

The incubation of human leukocytes with SeMet (1–100 μM), carried out to analyze the possible genotoxicity of this selenium organic compound, resulted in significant decreases in DNA damage in the two highest concentrations tested (50 and 100 μM; Fig. 1a), and a significant inverse dose-response relationship was obtained ($r = -0.870$, $P<0.05$). In the

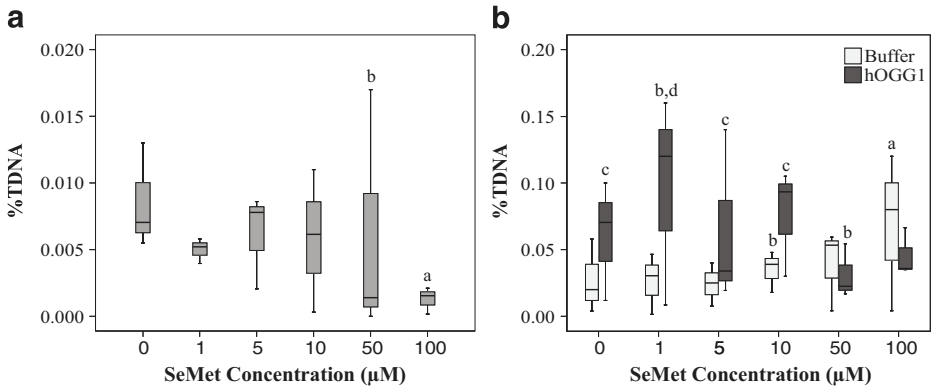

Fig. 1 Results of standard comet assay (**a**) and modified comet assay with hOGG1 incubation (**b**) in human leukocytes treated with SeMet. [a]$P<0.01$, [b]$P<0.05$, significant difference with regard to the control. [c]$P<0.01$, [d]$P<0.05$, significant difference with regard to the buffer

evaluation of the SeMet-induced oxidative damage (Fig. 1b), cells exposed to 1 μM SeMet showed a significantly increased damage regarding to control cells, while a significant decrease in 50 μM SeMet dose was detected as compared with the control. Moreover, the lowest SeMet doses (1–10 μM) showed significant increases in %TDNA after hOGG1 incubation with regard to buffer incubation as control did, but 50 and 100 μM SeMet did not show any difference. On the basis of the results obtained in this preliminary experiment, 50 μM was selected as the most appropriate SeMet dose for the evaluation of its possible antigenotoxic effects on BLM-induced DNA damage, since it not only does not increase DNA strand breaks or oxidative damage but also seems to show a certain antioxidant effect. The experiment was conducted in three consecutive phases: A (PHA-stimulation), B (BLM treatment), and C (repair), and comet assay (standard protocol and modified with hOGG1 incubation) was performed after each phase.

With the objective of determining the influence of SeMet on BLM-induced DNA damage, human leukocytes were treated with SeMet during phases A, B, and AB, and comet assay was performed immediately after BLM incubation. SeMet treatment during phase AB induced a significant decrease in BLM-induced DNA damage; however, treatment during phase A resulted in a significant damage increase (Fig. 2a). After hOGG1 incubation (Fig. 2b), consecutive treatment with SeMet during both phases (AB) induced a significant increase in %TDNA with regard to BLM treatment. In the comparison with buffer incubation, significant increases in DNA damage were observed in treatments with BLM alone and with SeMet during phases B and AB.

The possible modulation of SeMet on repair of BLM-induced damage was analyzed by incubating the leukocytes with SeMet during phases A, B, C, AB, and ABC, and DNA damage was evaluated after a 15-min repair period in BLM-free medium. SeMet treatment during phases AB and ABC decreased %TDNA, significantly in the case of phase AB (Fig. 3a). All treatments showed increased DNA damage after incubation with hOGG1, except for a significant decrease in SeMet incubation during phase ABC. Furthermore,

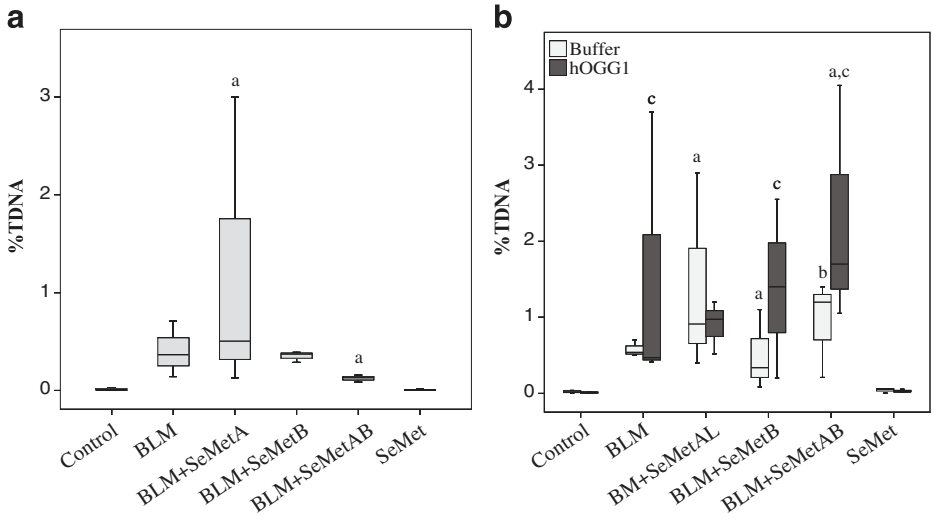

Fig. 2 Effect of SeMet treatment during phases A, B and AB in human leukocytes challenged with BLM. (**a**) Standard comet assay, (**b**) modified comet assay with hOGG1 incubation. $^{a}P<0.01$, $^{b}P<0.05$, significant difference with regard to the BLM treatment. $^{c}P<0.01$, significant difference with regard to the buffer

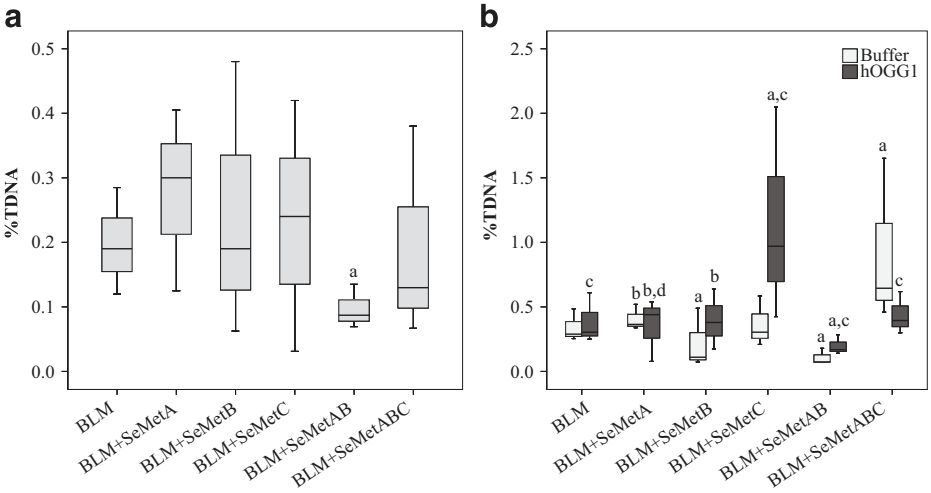

Fig. 3 Effect of SeMet treatment during phases A, B, C, AB, and ABC in human leukocytes challenged with BLM and incubated 15 min for DNA repair. (**a**) Standard comet assay, (**b**) modified comet assay with hOGG1 incubation. $^{a}P<0.01$, $^{b}P<0.05$, significant difference with regard to the BLM treatment. $^{c}P<0.01$, $^{d}P<0.05$, significant difference with regard to the buffer

oxidative damage was lower in SeMet treatment during phase AB than the one detected in BLM treatment (Fig. 3b).

Comparison between results obtained in the different treatments before and after the repair period was made to determine the effect of SeMet on persistence of BLM-induced DNA damage. Strand breaks decreased significantly after the repair period in all treatments, significantly in BLM alone and with SeMet during phase A (Fig. 4a). Moreover, all tested treatments reduced significantly their oxidative damage during the 15-min incubation for repair (Fig. 4b).

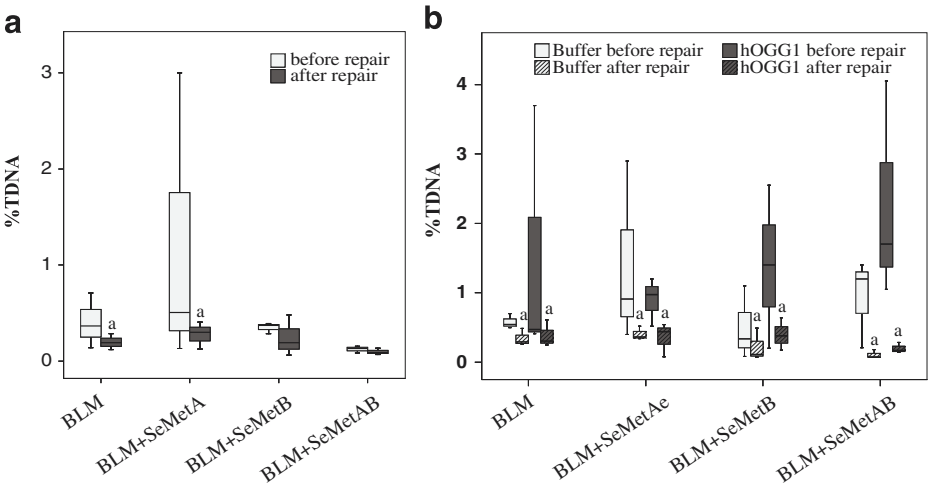

Fig. 4 Effect of SeMet treatment during phases A, B, and AB in human leukocytes challenged with BLM before and after a 15-min incubation for DNA repair. (**a**) Standard comet assay, (**b**) modified comet assay with hOGG1 incubation. $^{a}P<0.01$, significant difference with regard to the same treatment before repair

Discussion

SeMet is the predominant selenium compound in common food and in Se-enriched yeast, so it is the most suitable organic form to introduce a selenium supplement in the diet [14]. In this study, the modulation of SeMet on DNA damage induction by the chemotherapeutic drug BLM, and also on its repair and persistence, was analyzed in human leukocytes. For this purpose, a preliminary experiment was carried out to discard genotoxicity of SeMet (1–100 μM) and to determine the most appropriate dose to be used together with BLM treatment. The highest doses tested (50 and 100 μM) showed a significant decrease in the DNA damage, and significant inverse dose-response relationship was obtained. We also evaluated strand breaks plus hOGG1 sensitive sites, which additionally reveal oxidized DNA guanines (8-OHdG, main representative of oxidative damage) more specifically than formamidopyrimidine DNA-glycosylase that also recognizes alkylation damage [12]. Again, the two highest doses analyzed did not induce oxidative damage, and even at 50 μM SeMet, a significant decrease in oxidative damage was observed. Literature data reviewed by Alaejos et al. [15] show that selenium levels in body fluids (serum, plasma, and whole blood) are lower in cancer patients than in control subjects, and the difference between populations is not usually higher than selenium concentrations corresponding to 50 and 100 μM SeMet (4 and 8 mg/l, respectively). Although low selenium levels in body fluids can be due to the malnutrition observed in cancer patients, there are also evidences that high selenium status are associated with lower cancer mortality [16]. On this regard, literature supporting the cancer-protecting effects of selenium has been reviewed [17], emphasizing that in the physiological dosage range, this trace element appears to prevent the malignant transformation of normal cells and the activation of oncogenes.

Besides, cells treated with low concentrations of SeMet showed increases in %TDNA after hOGG1 incubation with regard to control cells. These data confirm the importance of selenium dose level regarding its activity, agreeing with the results of previous studies where adverse effects of low concentrations of SeMet were reported [18, 19].

The experimental design to achieve the raised objective was conducted in three consecutive phases: A (PHA-stimulation), B (BLM treatment), and C (repair). Addition of SeMet was carried out either to each separate phase or consecutively to phases A and B (AB) or to the whole process (ABC). Treatment with SeMet showed to decrease BLM-induced DNA damage when added during phase AB. This apparent protection offered by SeMet is in line with the protective effect of this selenium compound against DNA damage caused by several chemical or physical agents [20, 21] and with the recently reported antigenotoxic properties of this selenium compound on DNA damage induced by the anticancer drug doxorubicin, both in rats [22] and in human lymphocytes [23]. As for other chemotherapeutic drugs, Rao et al. [24] reported that SeMet protected against cisplatin-induced nephrotoxicity without interfering with antitumor activity, and a study by Cao et al. [25] showed that SeMet offered selective protection against toxicity induced by several chemotherapeutic agents and simultaneously augmented the antitumor activity and the cure rate. Nevertheless, no protective effect of SeMet was detected in our results on the induction of oxidative damage by BLM. On the contrary, Kaur et al. [26] observed that preincubation of glioma and neuronal cells with SeMet prevents against increased methylmercury-induced reactive oxygen species generation.

Cells were allowed to repair BLM-damaged DNA for 15 min, as recommended by Schmezer et al. [27], since longer repair incubations did not result in an increase in repaired DNA (data not shown). Results obtained after the repair period indicate that SeMet favors repair of BLM-induced DNA damage (strand breaks and oxidative) when applied during

phase AB or ABC but not when applied during phases A, B, or C. Therefore, a net protective effect is only achieved when cells are in presence of SeMet during the whole process or at least during PHA stimulation and BLM treatment. A role for selenium in DNA repair was first noticed when selenium treatment was shown to enhance host cell reactivation of a UV-damaged reporter plasmid template by enhancing DNA repair protein complexes [28]. Moreover, a recent study showed that SeMet pretreatment causes a p53-dependent DNA repair response, elevating the expression of proteins responsible for recognition of DNA damage and increasing the rate of DNA repair and overall DNA repair synthesis, which protects from subsequent challenge with DNA-damaging agents [29]. Enhancement of DNA repair could be a mechanism of chemoprevention and only very few compounds have been yet shown to act by this mechanism [30]. Furthermore, Zhang et al. [31] inferred that selenium only enhances DNA repair of normal tissues as a consequence of the selective modulation of selenium on Nrf2 in tumor and normal tissues [32], supporting the SeMet supplementation of cancer patients treated with chemotherapeutic drugs.

The comparison between DNA damage before and after the repair period showed that BLM-induced damage, both strand breaks and oxidative, was repaired better in presence of SeMet. Despite the BLM-induced damage was higher in presence of SeMet, except for phase AB, the differences between before and after repair were always greater when SeMet was used, showing an increase in DNA repair ability of the cells. This increase is especially noteworthy in the case of treatment with SeMet during phase AB with regard to the oxidative damage.

In conclusion, our results showed protective effect of SeMet on BLM-induced DNA damage in human leukocytes, and also on repair and persistence of this damage, when applied before and simultaneously with BLM. These data support the previously proposed mechanisms of chemoprotection of SeMet as related to its ability to interfere with DNA repair pathways [23].

Acknowledgements This work was funded by a grant from the Xunta de Galicia (INCITE08P-XIB106155PR). V. Valdiglesias was supported by a fellowship from the University of A Coruña.

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