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2-Hydroxy-(4-methylseleno)butanoic Acid Is Used by Intestinal Caco-2 Cells as a Source of Selenium and Protects against Oxidative Stress

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ABSTRACT

Background: Selenium (Se) participates in different functions in humans and other animals through its incorporation into selenoproteins as selenocysteine. Inadequate dietary Se is considered a risk factor for several chronic diseases associated with oxidative stress.

Objective: The role of 2-hydroxy-(4-methylseleno)butanoic acid (HMSeBA), an organic form of Se used in animal nutrition, in supporting selenoprotein synthesis and protecting against oxidative stress was investigated in an in vitro model of intestinal Caco-2 cells.

Methods: Glutathione peroxidase (GPX) and thioredoxin reductase (TXNRD) activities, selenoprotein P1 protein (SELENOP) and gene (*SELENOP*) expression, and *GPX1* and *GPX2* gene expression were studied in Se-deprived (FBS removal) and further HMSeBA-supplemented (0.1–625 μM, 72 h) cultures. The effect of HMSeBA supplementation (12.5 and 625 μM, 24 h) on oxidative stress induced by H_2O_2 (1 mM) was evaluated by the production of reactive oxygen species (ROS), 4-hydroxy-2-nonenal (4-HNE) adducts, and protein carbonyl residues compared with a sodium selenite control (SS, 5 μM).

Results: Se deprivation induced a reduction ($P < 0.05$) in GPX activity (62%), *GPX1* expression, and both SELENOP (33%) and *SELENOP* expression. In contrast, an increase ($P < 0.05$) in *GPX2* expression and no effect in TXNRD activity ($P = 0.09$) were observed. HMSeBA supplementation increased ($P < 0.05$) GPX activity (12.5–625 μM, 1.68–1.82-fold) and SELENOP protein expression (250 and 625 μM, 1.87- and 2.04-fold). Moreover, HMSeBA supplementation increased ($P < 0.05$) *GPX1* (12.5 and 625 μM), *GPX2* (625 μM), and *SELENOP* (12.5 and 625 μM) expression. HMSeBA (625 μM) was capable of decreasing ($P < 0.05$) ROS (32%), 4-HNE adduct (49%), and protein carbonyl residue (75%) production after H_2O_2 treatment.

Conclusion: Caco-2 cells can use HMSeBA as an Se source for selenoprotein synthesis, resulting in protection against oxidative stress. *J Nutr* 2019;149:2191–2198.

Keywords: poultry, organic selenium, selenoproteins, oxidative stress, intestine, selenium deprivation, hydroxy-selenomethionine

Introduction

Selenium (Se) is an essential trace element that plays a role in many important biological functions in animals and humans (1, 2). Se mainly performs biological functions through its incorporation into selenoproteins in the form of selenocysteine (SeCys). Although in general selenoproteins are classified as antioxidants, they exhibit a wide range of functions [see (3) for a review]. The most abundant selenoproteins expressed in the intestine are glutathione peroxidases (GPXs), thioredoxin reductases (TXNRDs), and selenoprotein P1 (SELENOP) (4).

In humans, the GPX family contains 8 isoforms from which 4 members are selenoproteins expressed in the intestine (5, 6): the ubiquitous cytosolic GPX (GPX1), gastrointestinal GPX (GPX2), plasma GPX (GPX3), and phospholipid hydroperoxide GPX (GPX4). These enzymes catalyze the reduction of H_2O_2 and organic hydroperoxides using glutathione as a reducing cofactor.

TXNRDs are NAD(P)H-dependent flavoenzymes that regulate intracellular redox by reducing thioredoxin, a small active redox protein distributed ubiquitously in various mammalian tissues (7). In mammals, 3 TXNRD isoforms have been

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reported: TXNRD1, found in the cytoplasm; TXNRD2, in mitochondria; and TXNRD3, expressed only in specialized tissues such as testis (3).

SELENOP is unique because it contains 10 SeCys residues in humans and 15 in dogs (8). The main function of SELENOP is to deliver Se to the whole body and it has been reported that the C-terminal domain, with ≤9 SeCys residues, is required for this transport function. In contrast, the N-terminal domain contains just 1 SeCys residue, with putative enzymatic antioxidant activity in the form of GPX4 and peroxynitrite reductase (9, 10). This selenoprotein is mainly produced in the liver and then secreted into the plasma, where SELENOP and GPX3 represent most of the Se. Nevertheless, it is also expressed, at the mRNA and protein levels, in the intestine, where it is secreted through the basolateral membrane (11). The function of SELENOP secreted in the intestine might be to protect epithelial membranes and to participate in the transport of the absorbed Se from the intestinal epithelium to the liver via the portal circulation or to support other cells with Se, in particular cells of the mucosa-associated lymphoid tissue in the gut (11).

In the intestine, inadequate dietary Se is considered a risk factor for several chronic diseases associated with oxidative stress and inflammation (12). The Se sources commonly used in dietary supplements are selenomethionine (SeMet) as well as sodium selenite (SS) and selenate. Most studies of the effects of Se are performed using Se in its inorganic form, usually SS. In contrast, here, we studied the role of 2-hydroxy-(4-methylseleno)butanoic acid (HMSeBA) as a source of Se in intestinal Caco-2 cells. Based on the homology between SeMet and methionine (Met), HMSeBA has been developed similarly to 2-hydroxy-(4-methylthio)butanoic acid, a precursor of Met and widely used in animal nutrition as a source of Met (13). The nutritional efficacy of HMSeBA in broilers, layers, swine, and dairy cows has also been demonstrated (14–18). In fact, Fisinin et al. (19) consider that the enrichment of animal-derived foods (mainly meat, milk, and eggs) with Se via supplementation of animal feeds can be an effective way of increasing human Se status. To this end, we established a model of Se deprivation by removing FBS, because it naturally contains organic Se forms. Se-deprived cells were further supplemented with HMSeBA to study its capacity to support selenoprotein synthesis and to protect against oxidative stress, in comparison with SS.

Methods

Materials

DMEM, trypsin, TRI-reagent, penicillin, and streptomycin were supplied by Life Technologies. Nonessential amino acids, sterile PBS, 2′,7′-bis(2-carboxyethyl)-5(6-carboxyfluorescein) (BCECF), and SS were supplied by Sigma. FBS was purchased from GE Healthcare Life Sciences. HMSeBA (Selisseo) was provided by Adisseo France SAS. Tissue culture supplies, including Transwells, were obtained from Costar. L-[U-^{14}C]-lactic acid (specific activity 165 mCi/mmol) was purchased from PerkinElmer. Filtron-X was supplied by National Diagnostics.

Caco-2 cell culture

Caco-2 cells were purchased from European Collection of Authenticated Cell Cultures. The cells were routinely grown in plastic flasks and cultured at a density of 10^3 cells/cm^2 as previously described (20). Cells were subcultured in Transwells for uptake experiments and intracellular pH (pH_i) measurements; in 24-well clusters to determine intracellular reactive oxygen species (ROS), protein carbonyl, and 4-hydroxy-2-nonenal (4-HNE) production; or in 75-cm^2 flasks to determine GPX and TXNRD activity, SELENOP protein expression, as well as *SELENOP*, *GPX1*, and *GPX2* gene expression.

Experiment 1: HMSeBA uptake

Transport experiments were performed as previously described (13). Only cell monolayers with transepithelial electrical resistance values >1000 Ω · cm^2 were used. Briefly, for cis-inhibition experiments, monolayers grown on filters were incubated at 37°C for 5 min in Krebs buffer containing 5 μCi/mL L-[U-^{14}C]-lactic acid in the absence (control) or presence of 500 μM unlabeled HMSeBA in the apical compartment. Moreover, total Se in the basolateral medium after 2, 20, and 100 μM HMSeBA incubation was determined by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500ce) using Rh as the internal standard.

pH_i was measured, as an indicator of H^+-coupled HMSeBA transport, in Caco-2 cells grown on filters using the pH-sensitive fluorescent dye BCECF, as previously described (13). The preloaded monolayers with 7.5 μM BCECF were then incubated at an apical pH of 5.5, in the absence (control) or presence of 10 μM HMSeBA.

Experiment 2: Se deprivation

The model of Se deprivation was established taking into account that the only Se source in the culture medium is FBS. We analyzed the 2 batches of FBS used in this study by ICP-MS and the mean ± SEM concentration of Se was found to be 153 ± 3.6 nM (n = 6). The final mean ± SEM Se concentration in the culture medium was 13.9 ± 0.3 nM in the presence of FBS. Moreover, the main source of Met is DMEM, where the concentration of this amino acid is 0.2 mmol/L.

To establish the Se-deprivation model, Caco-2 cells were maintained in the presence or absence of FBS [referred to in these experiments as control and (-)FBS, respectively]. The control condition refers to cultures maintained with FBS for 2 wk. In contrast, (-)FBS refers to cultures maintained with FBS for 8 d (to allow the epithelium to reach confluence) and without FBS for 6 additional days (2 wk in total). In these conditions, GPX and TXNRD activity, SELENOP and *SELENOP* expression, as well as *GPX1* and *GPX2* gene expression were determined. Moreover, ROS production was determined as described previously (21).

For GPX and TXNRD activity and SELENOP protein expression, cells were collected by scrapping with 600 μL PBS and subjected to ultrasonic oscillation for 15 s with a 1.27-cm-tip probe, while maintaining the samples <4°C. The supernatant obtained was collected and centrifuged at 9600 × g for 5 min at 4°C (Accuspin Micro 17R, Fisher Scientific) and stored at −80°C until the day of the experiment.

GPX activity was determined with a commercial GPX assay kit (Cayman Chemical) following the manufacturer's instructions. The decrease in absorbance per minute from the supernatant was determined at an emission wavelength of 340 nm (Benchmark Plus Reader; BioRad). TXNRD activity was determined with a commercial TXNRD assay kit (Sigma). The increase in absorbance per minute from the supernatant was determined at an emission wavelength of 412 nm (TECAN). SELENOP protein expression was determined using a sandwich enzyme

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Abbreviations used: BCECF, 2′,7′-bis(2-carboxyethyl)-5(6-carboxyfluorescein); GPX, glutathione peroxidase; GPX4, phospholipid hydroperoxide glutathione peroxidase; HMSeBA, 2-hydroxy-(4-methylseleno)butanoic acid; ICP-MS, inductively coupled plasma mass spectrometry; LDH, lactate dehydrogenase; MCT1, monocarboxylate transporter 1; NC, negative control; pH_i, intracellular pH; ROS, reactive oxygen species; Se, selenium; SeCys, selenocysteine; SeMet, selenomethionine; SELENOP, selenoprotein P1; SS, sodium selenite; TXNRD, thioredoxin reductase; 4-HNE, 4-hydroxy-2-nonenal.

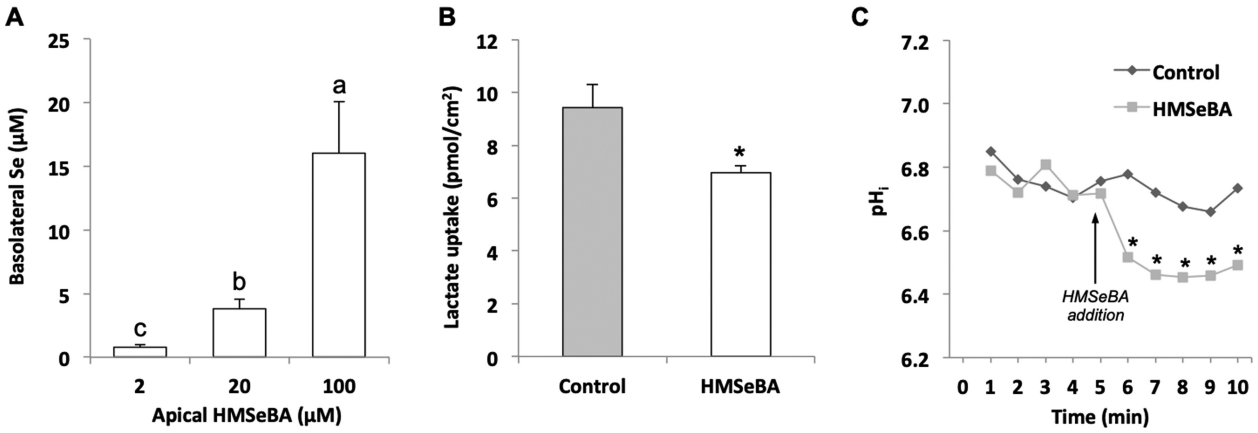

FIGURE 1 HMSeBA uptake (experiment 1). (A) Total basolateral Se after apical 2, 20, and 100 μM HMSeBA incubation; (B) L-lactate transport in the presence of 500 μM HMSeBA; and (C) pH_i in the presence of 10 μM HMSeBA in Caco-2 cells. The results were expressed as mean ± SEM of $n = 3$–4 cultures. Different letters denote significant differences ($P < 0.05$). *Significant differences with respect to control ($P < 0.05$). HMSeBA, 2-hydroxy-(4-methylseleno)butanoic acid; pHi, intracellular pH.

immunoassay technique performed with a commercial ELISA kit (Cusabio). Absorbance was measured at an emission wavelength of 450 nm (TECAN).

For *SELENOP* (SELENOP_selenoprotein P1, Life Technologies), *GPX1* (GPX1_glutathione peroxidase 1, Life Technologies), and *GPX2* (GPX2_glutathione peroxidase 2, Life Technologies) gene expression, cells were collected as aforementioned but using the pellet obtained after ultrasonic oscillation and processed as previously described (22). After testing different reference genes (ACTB_β-actine; GAPDH; and RPLP0_ribosomal protein lateral stalk subunit P0; Life Technologies), RPLP0 was used for normalization purposes. mRNA expression was calculated as fold changes using the $\Delta\Delta$Ct method. RT-PCR analysis was performed at the Centres Científics i Tecnològics of the University of Barcelona.

Experiment 3: HMSeBA supplementation

For the Se-supplementation experiments, Se-deprived cells [referred to in these experiments as negative control (NC)] were supplemented after 14 d in culture with HMSeBA or SS during an additional period of 72 h. First, to evaluate the possible cytotoxic effect of these sources, lactate dehydrogenase (LDH) release to the medium after the incubation with HMSeBA (12.5 μM, 125 μM, 625 μM, and 1.25 mM) or SS (5 μM, 12.5 μM, and 50 μM) was analyzed as previously described (21). Then, GPX activity and SELENOP protein expression were evaluated in cultures supplemented with 0.1–625 μM HMSeBA, using SS (5 μM) for comparative purposes. Moreover, TXNRD activity was tested in cultures supplemented with 12.5 μM HMSeBA. Finally, *SELENOP*, *GPX1*, and *GPX2* gene expression was evaluated in cultures supplemented with 12.5 and 625 μM HMSeBA or 5 μM SS.

Experiment 4: HMSeBA and oxidative stress

Oxidative stress after H_2O_2 treatment was evaluated by determination of ROS, protein carbonyl, and 4-HNE production. Se-deprived cells (NC) were preincubated with 12.5 and 625 μM HMSeBA or SS (5 μM) for 24 h and then stimulated with H_2O_2 (1 mM). After 3 h, ROS production was determined as aforementioned. 4-HNE and protein carbonyl production were determined using a commercial lipid peroxidation kit (Cell Biolabs Inc.) and with a commercial protein-damage kit (Cell Biolabs Inc.), respectively. The absorbance of the supernatant was determined at an emission wavelength of 450 nm (TECAN) for both determinations.

Statistical analysis

Results are given as means ± SEMs. Significant differences were detected by 1-factor ANOVA (for results in **Figures 1**B and **2**) followed by Bonferroni's post hoc test (for results in Figure 1A and **Figures 3–6**) or Student's *t* test (for results in Figure 1C for each incubation time after HMSeBA addition with respect to control) using the SPSS software (SPSS Inc.). $P < 0.05$ was considered to denote significance.

Results

In experiment 1, Caco-2 cell incubation with increasing HMSeBA concentrations produced a dose-dependent increase of Se concentration in the basolateral compartment ($P < 0.05$ between HMSeBA concentrations) (Figure 1A). HMSeBA (used in cis-experiments as a competitive inhibitor) inhibited lactate uptake ($P < 0.05$) by 26 % (Figure 1B) and produced a decrease in pH_i ($P < 0.05$ for each incubation time) (Figure 1C).

In experiment 2 (Figure 2), Se deprivation (cultures maintained with FBS for 8 d and without FBS for 6 additional days) induced a reduction ($P < 0.05$) in GPX activity (62%), *GPX1* gene expression, and both SELENOP (33%) and *SELENOP* expression. In contrast, an increase ($P < 0.05$) in *GPX2* gene expression and ROS production (2.87-fold) was observed. TXNRD activity revealed no changes ($P = 0.09$) with FBS removal (control: 3.98 ± 0.49 nmol · min^{-1} · mg $protein^{-1}$; (-)FBS: 6.00 ± 1.11 nmol · min^{-1} · mg $protein^{-1}$, mean ± SEM of $n = 4$ cultures).

In experiment 3, HMSeBA did not compromise cell viability relative to control ($P \geq 0.05$), whereas SS produced an increase ($P < 0.05$) in LDH release starting at 12.5 μM (1.49- and 1.46-fold) (Figure 3). Therefore, for comparative purposes, SS was used in further experiments at the highest nontoxic concentration available (5 μM). As shown in Figure 4A, GPX activity increased ($P < 0.05$) with HMSeBA supplementation at ≥12.5 μM relative to NC (1.68–1.82-fold), reaching similar values to those obtained with SS ($P \geq 0.05$) except for 0.1 μM HMSeBA ($P < 0.05$). The values obtained with HMSeBA and SS supplementation did not reach FBS-containing results ($P < 0.05$, Figure 2A compared with Figure 4A). Further gene expression experiments were performed with 2 HMSeBA concentrations: the lowest that increased GPX activity (12.5 μM) and the highest one tested (625 μM). HMSeBA supplementation increased ($P < 0.05$) *GPX1* (12.5 and 625 μM) and *GPX2* (625 μM) gene expression relative to NC (Figure 4B, C). In both cases, no differences were detected between SS and NC ($P = 1.00$ for *GPX1* and $P = 0.76$ for

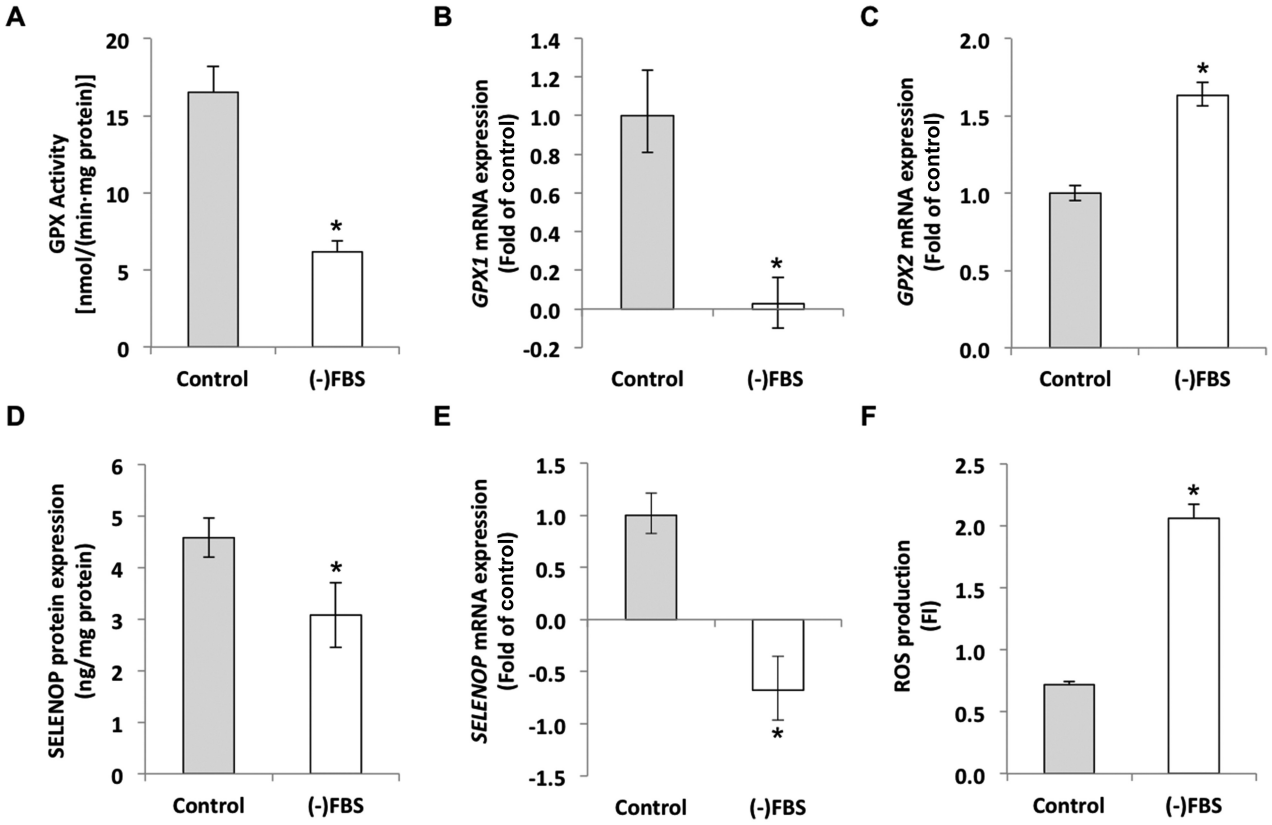

FIGURE 2 Se deprivation (experiment 2). (A) GPX activity ($n = 6$), (B) *GPX1* relative gene expression ($n = 8$), (C) *GPX2* relative gene expression ($n = 8$), (D) SELENOP protein expression ($n = 8$), (E) *SELENOP* relative gene expression ($n = 8$), and (F) ROS production ($n = 9$) were determined in Caco-2 cells cultured in the presence and absence of FBS [control and (-)FBS, respectively]. In the case of gene expression, the results were expressed relative to control values. The results were expressed as mean ± SEM of *n* cultures. *Significant differences with respect to control ($P < 0.05$). FI, fluorescence intensity; GPX, glutathione peroxidase; ROS, reactive oxygen species; SELENOP, selenoprotein P1.

GPX2). In the case of SELENOP protein expression (Figure 5A), an increase ($P < 0.05$) was also observed at high concentrations of HMSeBA (250 and 625 μM) relative to NC (1.87- and 2.04-fold, respectively), reaching similar values to those obtained with SS ($P \geq 0.05$) and with FBS ($P \geq 0.05$, Figure 2D compared with Figure 5A). For *SELENOP* gene expression (Figure 5B), an increase relative to NC ($P < 0.05$) for both HMSeBA concentrations tested was observed. Here again, no differences ($P = 0.11$) were detected between SS and NC. In the case of TXNRD activity, an increase by 1.94-fold with 12.5

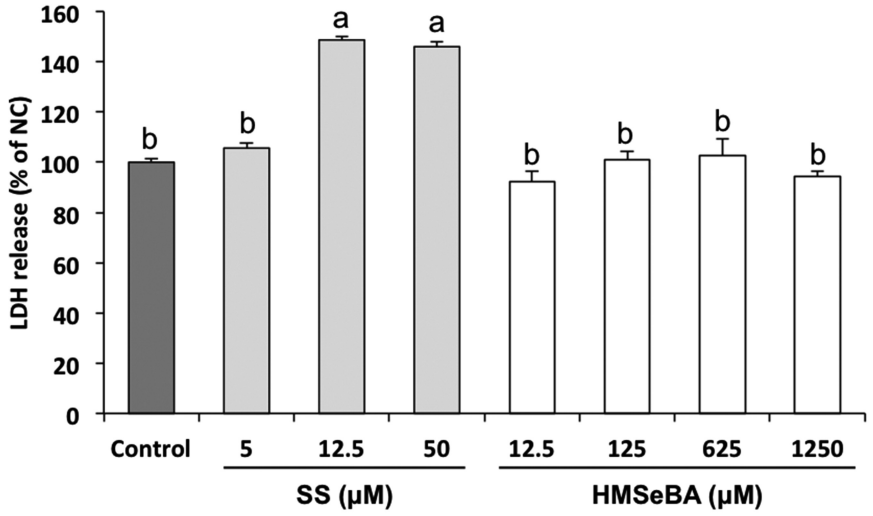

FIGURE 3 HMSeBA supplementation: LDH release (experiment 3). LDH release was determined in Caco-2 cells cultured in the presence of FBS (control, $n = 12$); or supplemented with 5–50 μM SS ($n = 6$) or 12.5 μM–1.25 mM HMSeBA ($n = 6$). The results were expressed as mean ± SEM of *n* cultures. Different letters denote significant differences ($P < 0.05$). HMSeBA, 2-hydroxy-(4-methylseleno)butanoic acid; LDH, lactate dehydrogenase; NC, negative control; SS, sodium selenite.

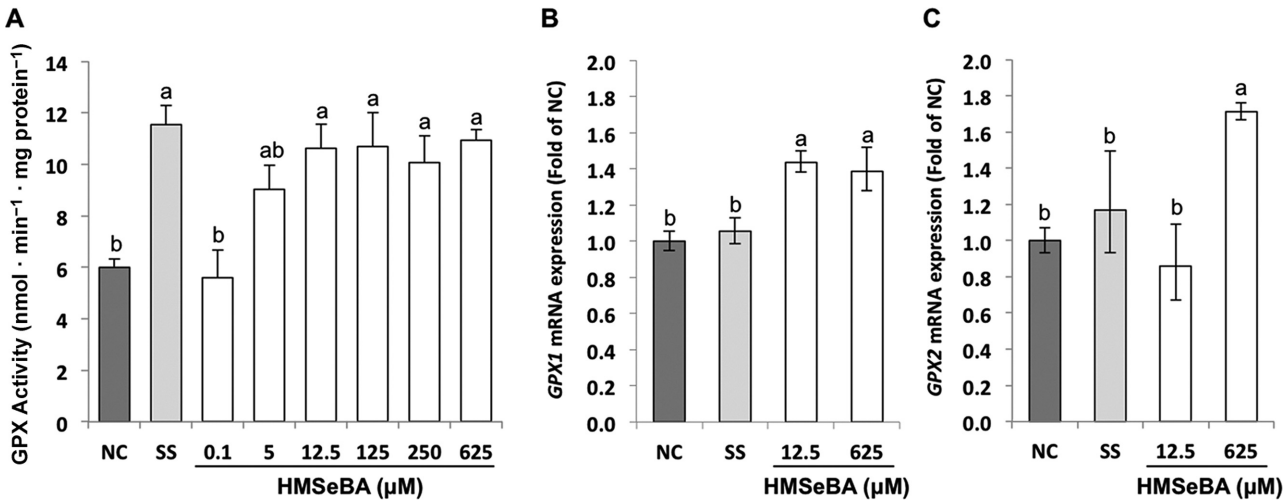

FIGURE 4 HMSeBA supplementation: GPX activity, and *GPX1* and *GPX2* relative gene expression (experiment 3). (A) GPX activity ($n = 4$), (B) *GPX1* relative gene expression ($n = 6$), and (C) *GPX2* relative gene expression ($n = 6$) were determined in Caco-2 cells maintained in the absence of FBS (NC); and supplemented with 5 μM SS or 0.1–625 μM HMSeBA. Different letters denote significant differences ($P < 0.05$). In the case of gene expression, the results were expressed relative to NC values. The results were expressed as mean ± SEM of *n* cultures. GPX, glutathione peroxidase; HMSeBA, 2-hydroxy-(4-methylseleno)butanoic acid; NC, negative control; SS, sodium selenite.

μM HMSeBA was observed (NC: 6.42 ± 0.96 nmol · min^{-1} · mg protein^{-1}; 12.5 μM HMSeBA: 12.3 ± 2.37 nmol · min^{-1} · mg protein^{-1}, mean ± SEM of $n = 4$ and 5 cultures, respectively, $P < 0.05$).

In experiment 4 (Figure 6), the stimulation of NC cells with H_2O_2 increased ($P < 0.05$) ROS (2.24-fold), 4-HNE adduct (2.22-fold), and protein carbonyl (5.19-fold) production. In these conditions, only the supplementation with HMSeBA at a concentration of 625 μM was capable of counteracting the effect of H_2O_2, reaching values of nonstimulated cells ($P \geq 0.05$) in the case of 4-HNE adduct and protein carbonyl production.

Discussion

Se deficiency is associated with several diseases in animals and humans (1, 23). Relatively low Se intakes determine the expression of selenoproteins, of which it is an essential constituent. In addition, higher intakes have been shown to have anti-inflammatory and antitumorigenic potential; but very high Se intakes can produce adverse effects (4). Because it is well reported that Se supplementation is required for optimal growth (15), its incorporation in animal diets is of great importance.

To study the capacity of Caco-2 cells to use HMSeBA as a Se source, the uptake of this molecule as well as its

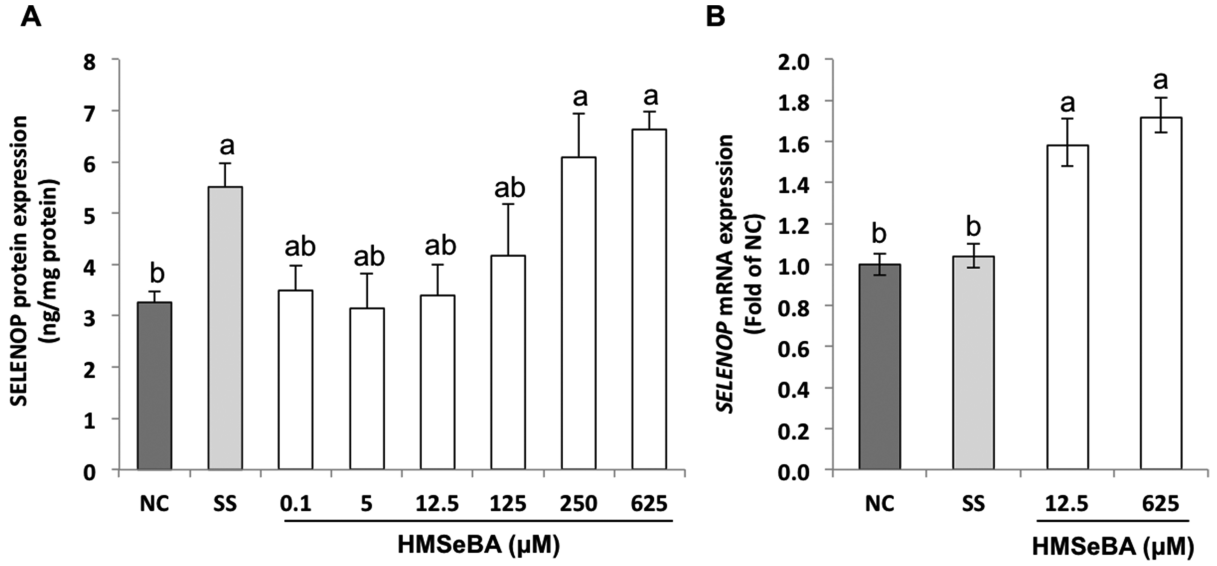

FIGURE 5 HMSeBA supplementation: SELENOP protein and *SELENOP* relative gene expression (experiment 3). (A) SELENOP protein expression and (B) *SELENOP* relative gene expression were determined in Caco-2 cells maintained in the absence of FBS (NC) and supplemented with 5 μM SS or 0.1–625 μM HMSeBA. Different letters denote significant differences ($P < 0.05$). In the case of gene expression, the results were expressed relative to NC values. The results were expressed as mean ± SEM of $n = 5$ cultures. HMSeBA, 2-hydroxy-(4-methylseleno)butanoic acid; NC, negative control; SELENOP, selenoprotein P1; SS, sodium selenite.

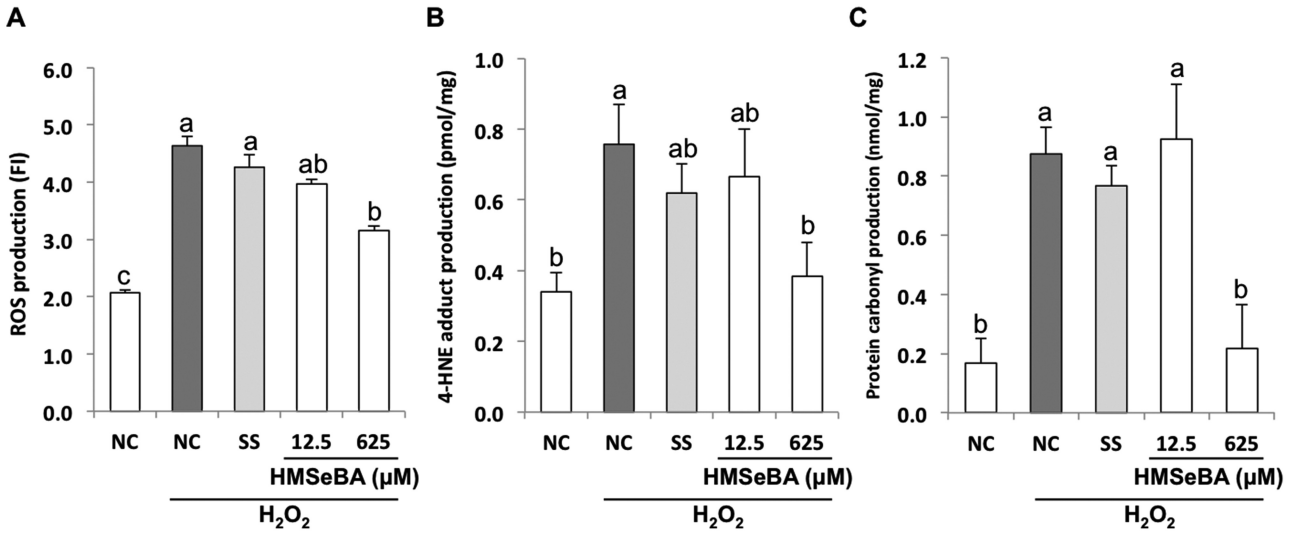

FIGURE 6 HMSeBA and oxidative stress (experiment 4). (A) ROS ($n = 9$), (B) 4-HNE ($n = 5$), and (C) protein carbonyl ($n = 5$) production were determined in Caco-2 cells maintained in the absence of FBS (NC) and treated with H_2O_2 (1 mM) in the absence of any Se source, or in cultures supplemented with 5 μM SS, or 12.5 and 625 μM HMSeBA. Different letters denote significant differences ($P < 0.05$). The results were expressed as mean ± SEM of n cultures. FI, fluorescence intensity; HMSeBA, 2-hydroxy-(4-methylseleno)butanoic acid; NC, negative control; ROS, reactive oxygen species; SS, sodium selenite; 4-HNE, 4-hydroxy-2-nonenal.

potential toxicity needed to be investigated. Taking into account the results of Nickel et al. (24) indicating that SeMet shares 2 transport systems with Met, our hypothesis was that HMSeBA could share monocarboxylate transporter 1 (MCT1) with the hydroxyanalog of Met (13). Because MCT1 is a H^+-dependent transporter with lactate as a specific substrate, the results obtained in cis-inhibition experiments as well as the reduction in pH_i confirm this hypothesis. Moreover, HMSeBA did not show cytotoxicity at any of the concentrations tested. In contrast, according to Barrera et al. (25) and Takahashi et al. (26), SS showed a remarkable cytotoxicity; in our case, already at 12.5 μM. For this reason, SS was used at 5 μM in further experiments, whereas the range of HMSeBA tested was higher owing to its low toxicity in our cell cultures. Higher HMSeBA concentrations are also justified by the fact that this source has to be previously converted to SeMet (15) and that the formed SeMet could be incorporated into proteins in the place of Met nonspecifically, thus representing an endogenous Se reserve which can be later used for selenoprotein synthesis (1).

Our results indicate that Se deprivation reduced GPX activity. Because Se is the limiting factor for GPX protein synthesis (25), the reduction in GPX activity is a common feature in Se-deprived models of Caco-2 cells in which deprivation is induced by the removal of FBS from the culture medium (27–29). Similarly, GPX activity was also reduced in the colon of mice fed a Se-deficient compared with a Se-adequate diet (30). Our results also report that HMSeBA supplementation was capable of inducing GPX activity in Caco-2 cells. In this sense, Wang and Fu (31) reported similar values of GPX activity for both SS and SeMet. Regulation of the synthesis of different GPX isoforms is a complex process (32). Indeed, whereas GPX1 responds to decreased Se via a loss of protein and activity together with a marked reduction of mRNA amounts, *GPX2* mRNA is only slightly reduced or even increased by a low Se supply (25, 33, 34), according with our results in the Se-deprived model. Interestingly, it has been reported that *GPX2* and also *TXNRD1* gene expression are upregulated on nuclear factor erythroid 2-related factor 2 activation resulting from oxidative stress generated by Se deficiency (29). In fact, our results revealed an increase in ROS production in the absence of Se. In this way, the fact that TXNRD activity was not modified by Se removal could be related to the gene regulation that it shares in common with *GPX2*. Nevertheless, a reduction in the mRNA degradation rate of *GPX2* and *TXNRD1* in the absence of Se could not be discarded (35, 36). Therefore, we can assume that the mRNA of *GPX2* is increased under conditions of limited Se supply to become preferentially translated when Se supply is restored (37). The supplementation with HMSeBA was capable of increasing *GPX1* and *GPX2* gene expression. Accordingly, it has also been described that organic forms such as SeMet, SeCys, as well as HMSeBA have a greater capacity to induce mRNA expression of different GPX isoforms than SS (38–40). Moreover, HMSeBA was also capable of promoting TXNRD activity. In accordance with this, De Spirt et al. (29) demonstrated an increase in TXNRD activity in Se-supplementation conditions. Nevertheless, it has been reported that neither *TXNRD1* nor *TXNRD2* gene expression (38, 41, 42), nor their protein expression (29, 42), are affected by Se supplementation.

In Se-deficient conditions, the liver sharply reduces *GPX1* expression to maintain the synthesis of other selenoproteins, such as *SELENOP*, so that this organ can mobilize Se to other tissues (43) such as the intestinal epithelium. In Caco-2 cells we have observed a reduction of SELENOP protein expression under conditions in which Se is not available. In addition, Speckmann et al. (11) found a decrease of basolateral SELENOP secretion in Se-deprived Caco-2 cell cultures. Our results revealed that to induce SELENOP protein expression, a higher concentration of HMSeBA in comparison with SS was needed. Along the same lines, in HepG2 cells, both SS and SeMet can induce SELENOP protein expression, although the results obtained with the inorganic form are 4-fold greater than those obtained with the organic form (44). Nevertheless, only HMSeBA was capable of preparing the cell by also increasing *SELENOP* gene expression. In fact, a higher capacity to induce *SELENOP* gene expression for HMSeBA than for SS was

detected in chicken liver and muscle (40). In this sense, Yuan et al. (45) reported that the mRNA amount of *SELENOP* significantly increases upon use of organic Se, whereas no effect was found with SS. Regarding the apparent discrepancies found between SELENOP and *SELENOP* expression, Bermano et al. (46) stated that Se supply regulates in a differential manner both the activity of the selenoenzymes and the abundance of their respective mRNAs. In fact, selenoprotein expression is regulated both at a translational level and at the level of RNA stability (30) owing to the regulation by mRNA sequences and/or RNA-binding proteins (47).

The increase in *GPX1*, *GPX2*, and *SELENOP* gene expression may be of importance in terms of protection against oxidative stress because only HMSeBA was capable of protecting against H_2O_2 treatment. In fact, previous studies have demonstrated that organic Se shows better protective effects than inorganic forms under different stress conditions (25, 39, 48, 49). Although synergistic anti-inflammatory activity of GPX1 and GPX2 has been postulated, it has been demonstrated that the role of GPX2 is more important, because a single allele of *GPX2* (but not of *GPX1*) proved to be sufficient to prevent inflammation in mice (50). We have to consider that this selenoprotein is highly expressed in intestine and its upregulation appears to be part of the adaptive response, and thus a compensatory mechanism involved in the protection of intestinal epithelial homeostasis (51, 52). Moreover, Xiao et al. (53) reported that HaCaT cells overexpressing *SELENOP* are resistant to oxidative stress and toxicity, thus also highlighting the antioxidant functions of this selenoprotein, as other authors found in fibroblasts (54, 55).

In summary, our results indicate that HMSeBA, an organic form of Se which was less toxic than SS, was capable of supporting GPX and TXNRD enzyme activity as well as SELENOP protein synthesis. Moreover, this source affected the transcription levels of *GPX1*, *GPX2*, and *SELENOP*. Furthermore, in contrast to SS, HMSeBA was capable of efficiently counteracting the effects of H_2O_2. The increase in gene expression of some selenoproteins after Se incubation allows the cell to rapidly synthesize selenoproteins, resulting in enhanced protection against oxidative stress. In conclusion, the organic form HMSeBA is used as a Se source by Caco-2 cells and contributes to maintaining intestinal epithelial homeostasis by protecting against oxidative stress.

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