Glycine and Selenium (Separately and in Combination) Reduced Bromate-Mediated Oxidative Stress and Inflammatory Response in U937 Cells

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The toxicity of bromate is ascribed to DNA damage and alteration in carbohydrate mechanism that could result to lipid peroxidation and oxidative stress. The effects of glycine and selenium on bromate-induced alterations on U937 cells and U937-derived macrophages were investigated. In the first experiment, U937 cells were incubated with or without glycine or selenium or both before subjecting cells to bromate exposure. Cell viability and production of ROS were assessed via MTT and DCHF-DA assays. In the other experiment, U937 cells were transformed to the macrophage form using phorbol 12-myristate 13-acetate before incubating with or without glycine and selenium before exposure to bromate. Secretion of nitric oxide and cytokines (tumour necrosis factor-alpha, interleukin 1 and interleukin 6) were later measured. The production of superoxide dismutase and catalase was also evaluated. The results revealed that bromate caused significant cytotoxicity and ROS production that was reduced when cells were pre-incubated by glycine and selenium (both separately and in combination). Bromate also increased macrophage secretion of nitric oxide and cytokines which was reduced by glycine and selenium. Bromate also suppressed the production of superoxide dismutase and catalase which was reversed by glycine and selenium (both separately and in combination) close to control values. The effects of glycine and selenium are ascribed to their antioxidant nature. Implications of the findings are discussed.

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1. INTRODUCTION

Bromate (as potassium bromate) is an additive in bread making and a disinfection by-product in the treatment of water via ozonation [1,2]. Bromate is used in bread because it is cheap, possesses the most efficient oxidizing property, helps bread to rise in the oven and to create a good texture [2,3]. Despite being the preferred oxidant in bread making, prolonged intake could cause adverse health effects. It degrades essential vitamins such as A2, B1, B2, E, niacin and essential fatty acids thus reduces the nutritional quality of bread [4]. Prolonged exposure to bromate could cause coughs, sore throat, abdominal pain, diarrhea, nausea, vomiting, kidney failure, hearing loss, bronchial and ocular problems [3-5]. Continuous exposure to bromate can cause various cancers thus its use has been banned in several countries [6,7].

Glycine is the smallest amino acid that is both nutritionally essential and non-essential based on growth and nitrogen balance in animals [8]. The amino acid has been reported to play significant physiological roles such as anti-inflammation, antioxidation, cytoprotection, metabolic regulation and also improves cardiovascular function [9-12].

Selenium is an essential trace element found in minute amount in the organism. Sources of the trace element include soil, water, air, plants, sea foods etc however major dietary sources are mainly plants, dietary supplements and sea foods [13,14]. Selenium plays several roles in the organism such as antioxidant defense, thyroid homeostasis, DNA synthesis, fertility, reproduction, regulation of redox status, calcium homeostasis, prevention of hydroxide-induced ferropotosis which are mediated via selenoproteins [12,14,15]. However, excessive selenium in the body is toxic (a condition known as selenosis) and could manifest as alopecia, dermatitis, non-melanoma skin cancer and type 2 diabetes [16].

The work is aimed at investigating the effect of glycine and selenium on bromate-induced cytotoxicity and production of reactive oxygen species in the cell line U937. The effect of glycine and selenium on bromate-induced alterations in the secretion of nitric oxide, cytokines and production of superoxide dismutase (SOD) and catalase in U937-derived macrophages was also evaluated.

2. MATERIALS AND METHODS

2.1 Materials

RPMI-1640 media (Roswell Park Memorial Institute), L-glutamine, penicillin-streptomycin, fetal calf serum (heat innoculated), phorbol-12-myristate-13-acetate (PMA), dimethyl sulfoxide (DMSO), sodium selenite, glycine, 2′,7′- dichlorohydrofluorescein diacetate (DCHF-DA), potassium bromate and 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma Chemicals (USA). The human macrophage cell line U937 was obtained from the European Collection of Cell Cultures (Salisbury). All antibodies and biotinylated cytokines were products from Pharmingen. All other reagents and chemicals were of analytical grade hence used without further purification. All buffers were prepared in double glass-distilled water and used immediately.

2.2 Cell Culture

The cell line U937 was cultured in RPMI-1640 medium supplemented with fetal calf serum (heat innoculated), L-glutamine, penicillin-streptomycin (hence referred to as complete medium) as described [17]. Cell numbers were maintained at 5 x 10^5 cells/ml and grown at 37°C in a humidified incubator gassing up to 5% CO₂. The Institution Research Committee gave approval for the research.

2.3 Cell Viability

Cells (in complete medium) were incubated with either glycine (300 µM) or selenium (3 µM sodium selenite) or both (300 µM glycine, 3 µM selenium) for 24 h at 37°C before exposed to 4 mM bromate (as potassium bromate). Either glycine or selenium was not added to control cells rather an equivalent quantity of RPMI-1640 was added. One hour later, cell viability was assessed via MTT reduction assay according to Zhou et al. [18] with modification. Briefly, MTT solution was added to each cell culture to a final concentration of 0.5 mg/mL and incubated at 37°C for 1 h. The MTT layer was aspirated and replaced with DMSO. Absorbance was later read at 570 nm.
2.4 Production of Reactive Oxygen Species

The production of reactive oxygen species (ROS) was determined as reported by [19] with a slight modification. This method is based on the oxidation of 2',7'-dichlorohydrofluorescein by intracellular peroxides. Cultured cells were first incubated with or without glycine or selenium or both for 24 h. Medium was removed, replaced with 50 µM DCHF-DA (prepared in RPMI-1640) and incubated for 30 min at 37°C. Medium was removed and cells washed with phosphate buffered saline (0.02 M, pH 7.4) and incubated with or without bromate (4 mM) for 1 h. Fluorescence of cells was measured at excitation and emission wavelength at 485 nm and 530 nm respectively. Production of ROS was expressed as a percentage over readings from cells treated with bromate only.

2.5 Production of Nitric Oxide and Cytokines

Cells were initially subjected to differential induction by treatment with PMA as described [17]. Media was removed and replaced with or without glycine or selenium or both and incubated for 24 h at 37°C. Subsequently, culture was supplemented with or without bromate (4 mM) and the production of nitric acid analysed according to the method of Hwang et al. [20] as modified [21] while cytokine production was analysed via cytokine capture ELISA as described [17].

2.6 Activity of Catalase (CAT) and Superoxide Dismutase (SOD)

Superoxide dismutase was assayed in cells treated as 3.3 above via the inhibition of epinephrine as described [22] while the activity of catalase was analysed according to the method of Aebi [23] with modifications. Briefly, 50 µl of culture supernatant was mixed with 100 µl of phosphate buffer (0.01M, pH 7.2) and 50 µl of distilled water. Reaction was initiated by the addition of 50 µl of hydrogen peroxide (0.01 M). The amount of hydrogen peroxide consumed was determined by measuring absorbance at 240 nm after 30 s. For both enzymes, final readings were expressed as percent production.

2.7 Statistical Analysis

Readings are presented as mean±SEM from six replicate experiments. Data were analyzed using analysis of variance followed by Duncan’s multiple range tests. Confidence level exhibited at p<0.05 was considered statistically significant.

3. RESULTS

3.1 Cell Viability and Cytotoxicity

The effect of glycine and selenium on bromate-induced cytotoxicity is shown in Fig. 1. It revealed that bromate caused significant reduction in cell viability as assessed by the MTT reduction assay. Both glycine and selenium significantly (p<0.05) reduced bromate mediated cell death when compared to cells treated with bromate alone. However, pre-incubation of cells with both glycine and selenium (GLY+Se) was better at enhancing cell viability than selenium alone (p < 0.05). There was no significant difference between effect of glycine and selenium in combination (i.e. GLY+Se) and glycine alone (p > 0.05).

3.2 Production of ROS

The production of ROS in the cells was assessed based on the oxidation of DCHF-DA. As shown in Fig. 2, bromate caused significant production of ROS in the cells which was reduced by glycine and selenium both separately and in combination. Pre-incubation of cells with glycine and selenium (i.e. GLY+Se) significantly reduced ROS production that selenium alone (p<0.05). There was no significant difference between response produced by glycine and GLY+Se (p>0.05).

3.3 Production of NO and Cytokines

The effect of glycine and selenium on the production of NO and the cytokines (TNF-α, IL-1 and IL-6) by the U937-derived macrophages is shown in Fig. 3. It revealed that incubating the macrophages with bromate alone caused significant (p<0.05) production of both NO and the cytokines when compared to untreated controls. Pre-treatment of the macrophage cells with glycine and selenium before incubation with bromate significantly reduced the production of nitric oxide and the cytokines (p < 0.05). Pre-incubation of cells with both glycine and selenium (GLY+Se) was significantly better at reducing bromate-induced alterations than selenium (p < 0.05). Glycine and selenium (GLY+Se) reduced the production of nitric oxide and TNF-α significantly more than pre-treatment of cells with glycine alone (p<0.05).
Fig. 1. Effect of glycine and selenium on bromate-induced cytotoxicity

BROMATE, cells treated with 4 mM bromate only; GLY, cells treated with 300 μM glycine before exposure to 4 mM bromate; Se, cells treated with 3 μM selenium before exposure to 4 mM bromate; GLY+Se, cells treated with both 300 μM glycine and 3 μM selenium before exposure to 4 mM bromate. Each bar represents mean ± S.E.M of six replicates expressed as % viability in comparison to control. Values having different superscript letters differ significantly (p < 0.05).

Fig. 2. Effect of glycine and selenium on bromate-induced ROS production

BROMATE, cells treated with 4 mM bromate only; GLY, cells treated with 300 μM glycine before exposure to 4 mM bromate; Se, cells treated with 3 μM selenium before exposure to 4 mM bromate; GLY+Se, cells treated with both 300 μM glycine and 3 μM selenium before exposure to 4 mM bromate. Each bar represents mean ± S.E.M of six replicates expressed as % viability in comparison to BROMATE. Values having different superscript letters differ significantly (p < 0.05).
Fig. 3. Effect of glycine and selenium on bromate-induced production of NO and cytokines in macrophages

BROMATE, cells treated with 4 mM bromate only; GLY, cells treated with 300 μM glycine before exposure to 4 mM bromate; Se, cells treated 3 μM selenium before exposure to 4 mM bromate; GLY+Se, cells treated with both 300 μM glycine and 3 μM selenium before exposure to 4 mM bromate. Each bar represents mean ± S.E.M of six replicates expressed as % viability in comparison to BROMATE. Values having different superscript letters differ significantly (p < 0.05)

3.4 Production of SOD and CAT

The effect of bromate on the production of SOD and CAT by macrophages is shown in Fig. 4. Bromate caused significant reductions in the production of both enzymes when compared to control cells (p<0.05) however, both glycine and selenium enhanced the production of the enzymes when compared to cells treated with bromate alone (p<0.05). There was no significant difference in the production of the enzymes between pre-incubation with glycine and selenium (GLY+Se) and the separate responses (p>0.05).

4. DISCUSSION

It is believed that bromate toxicity is ascribed to DNA damage which may induce apoptosis and necrosis [24-26]. Oxidative damage to DNA is a result of interaction of DNA with reactive oxygen species (ROS) which causes a lot of modifications such as generating strand breaks with various sugar modifications and release of free bases from nucleic acid [27].

Bromate mediated DNA damage is accompanied by lipid peroxidation and oxidative stress [27,28]. It has also been reported that oxidative damage appears to be the basis of bromate-induced carcinogenesis [29]. Bromate also causes alterations in carbohydrate mechanism and epigenetic changes which could be dose-dependent and can also cause cell death [29,30]. In the current experiment, bromate caused significant cell death and production of reactive oxygen species. Incubating the cell line U937 with either glycine or selenium offered protection...
Fig. 4. Effect of glycine and selenium on production of antioxidant enzymes in macrophages
BROMATE, cells treated with 4 mM bromate only; GLY, cells treated with 300 μM glycine before exposure to 4 mM bromate; Se, cells treated 3 μM selenium before exposure to 4 mM bromate; GLY+Se, cells treated with both 300 μM glycine and 3 μM selenium before exposure to 4 mM bromate. SOD, superoxide dismutase; CAT, catalase. Each bar represents mean ± S.E.M of six replicates expressed as % viability in comparison to control. Values having different superscript letters differ significantly (p < 0.05) against bromate-mediated cell death and ROS production. Glycine protect cells via various mechanisms which includes inhibition of mitochondrial permeability transition, interaction of ionophores, blocking of death channels etc thereby enhancing the integrity of the membrane [31-33]. This could also inhibit oxidative stress [34]. Selenium protects cells via selenoenzymes and other selenoproteins which are antioxidant in nature. The expression of selenoproteins is regulated by the concentration of selenium thus greater expression of these metalloproteins is associated with protection of cells against oxidative stress [35].

The production of pro-inflammatory cytokines by activated macrophages is a key event of the inflammatory response but excessive production (a condition called amplification of pro-inflammatory cytokine cascade) has been implicated in a lot of disorders such as multi-organ dysfunction, sepsis, hypotension, cancer [36,37]. Nitric oxide is also produced by activated macrophages due to the induction in the expression of inducible nitric oxide synthase (iNOS) however excessive production, which is a metabolic response to injury and inflammation, could alter redox status [38]. Nitric oxide is a substrate for peroxynitrite formation thus upregulation of NO synthesis could cause significant oxidative damage [39].

In the current study, bromate stimulated the production of nitric oxide and cytokines in the macrophages. However, incubating macrophages with glycine and selenium reduced bromate-induced elevations. Glycine could block cytokine production by activating a ligand-gated chloride channel which hyperpolarizes the plasma membrane [40]. This shows that these compounds downregulate the inflammatory response and could be potential anti-inflammatory candidates. This suggests that the anti-inflammatory effect of glycine and selenium could be partly as a consequence of their antioxidant property. This is further evidenced by the action of these antioxidant compounds in reducing the bromate-induced alterations in the production of SOD and catalase. Decrease in the level of antioxidant enzymes has been implicated in various models of bromate toxicity [41,42]. These enzymes physiologically suppress oxidative stress by removing reactive oxygen species thus their decrease will compromise the integrity of cells. Thus the cytotoxicity of bromate could be linked to the suppression of antioxidant enzyme expression.

5. CONCLUSION

From the above, both glycine and selenium are cytoprotective because they reduced bromate-induced generation of ROS and production of pro-inflammatory markers. It has been suggested
that the antioxidant effect of selenium is ascribed to the antioxidation of selenoproteins. It was observed that glycine was better at reducing the bromate-induced alterations than selenium though modifying the concentrations may alter that observation. Apart from reductions in NO and TNF-α production, the combined effort of glycine and selenium (i.e. GLY+Se) did not significantly enhance the antioxidant effects than glycine alone. Further experiments are ongoing to determine the effects of these oxidants on the expression of antioxidant genes.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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