Free Radical Biology & Medicine, Vol. 20, No. 5, pp. 649–656, 1996
Copyright © 1996 Elsevier Science Inc.
Printed in the USA. All rights reserved
0891-5849/96 $15.00 + .00

ELSEVIER

0891-5849(95)02149-3

Original Contribution

GLUCOSE, GLUTATHIONE, AND CELLULAR RESPONSE TO SPERMINE OXIDATION PRODUCTS

ENZO AGOSTINELLI,* EWA PRZYBYTKOWSKI,† and DIANA A. AVERILL-BATES†

*Department of Biochemical Sciences (A. Rossi-Fanelli), University of Rome "La Sapienza" and CNR Centre of Molecular Biology, Rome, Italy; †Département de chimie, Université du Québec à Montréal and GRBM (Groupe de recherche en biothérapeutique moléculaire), Montréal, Québec, Canada

(*Received* 1 *June* 1995; *Revised* 21 *September* 1995; *Accepted* 25 *September* 1995)

Abstract—Bovine serum amineoxidase (BSAO) oxidatively deaminates polyamines, which contain primary amine groups with formation of several toxic products, H_2O_2, and aldehyde(s). We evaluated the role of glucose metabolism via the pentose phosphate cycle and the level of intracellular glutathione on cytotoxicity induced by each of the toxic products in Chinese hamster ovary (CHO) cells. Glucose protected cells against cytotoxicity in the presence of BSAO at low spermine concentrations (< 50 μM), where H_2O_2 was the only toxic species present. When catalase was present, cytotoxicity is attributed to spermine-derived aldehyde(s). Glucose did not protect cells against cytotoxicity induced by spermine-derived aldehyde(s), nor by the aldehyde acrolein. Hydrogen peroxide produced by spermine and BSAO stimulated pentose cycle activity, whereas the aldehyde(s) did not. Depletion of intracellular glutathione with L-buthionine sulfoximine (1 mM, 24 h) sensitized cells to the cytotoxic effects of both H_2O_2 and the aldehyde(s) produced by spermine and BSAO. The pentose cycle and the glutathione redox cycle have an important role in protection against H_2O_2 generated from spermine oxidation. Glutathione appears to have a role in protecting cells against cytotoxicity attributed to spermine-derived aldehyde(s), most likely by conjugation in a reaction catalyzed by glutathione S-transferase, whereas metabolism of glucose via the pentose cycle did not. The metabolism of both glucose and glutathione, affect the cellular response to H_2O_2 and aldehyde(s) derived from spermine, although different pathways are involved.

Keywords—Polyamines, Amine oxidase, Cytotoxicity, Glutathione, Pentose phosphate cycle, L-Buthionine sulfoximine, Acrolein, Hydrogen peroxide, Free radicals

INTRODUCTION

Bovine serum amineoxidase (BSAO, EC 1.4.3.6) oxidatively deaminates polyamines, which contain primary amine groups with formation of the toxic products H_2O_2 and aldehyde(s).[1–3] We have been investigating the idea that BSAO could prove to be useful as an anticancer agent. It could cause cytotoxicity in situ by oxidizing polyamines that are found in high quantities in tumors.[4] We showed that the cytotoxicity of oxidation products of spermine was markedly enhanced by 42°C hyperthermia. This enhancement was mainly attributed to the aldehydes that behave as thermosensitizers.[5]

Glucose metabolism via the pentose phosphate cycle (PC) is an important source of NADPH in animal tissues and it provides reducing equivalent for the glutathione redox cycle. The activity of the PC is stimulated under conditions of oxidative stress created by compounds such as H_2O_2, by redox cycling agents, and under conditions requiring NADPH, such as the synthesis of fatty acids.

Glutathione, a tripeptide thiol present in almost all cells, has important functions in cellular metabolism and transport as well as in cellular defenses. It is probably one of the most important cellular antioxidants. It participates in the reduction of certain molecules by glutathione peroxidase, guarding cells from the de-

Address correspondence to: Diana A. Averill-Bates, Département de chimie, Université du Québec à Montréal, CP 8888, Succursale Centre Ville, Montréal, Québec, Canada H3C 3P8.

structive effects of reactive oxygen intermediates and other free radicals. It conjugates with compounds of exogenous origin, via a reaction catalyzed by glutathione transferase, protecting cells against the toxic effects of many drugs.[6,7] As a major cellular thiol, it has been implicated in the thiol-disulfide exchange, playing an important role in processes in which mixed disulfides are involved.[8]

Buthionine sulfoximine (L-BSO) is a specific, irreversible and very effective inhibitor of γ-glutamylcysteine synthetase, the enzyme that catalyzes the rate-limiting step of glutathione synthesis.[9] It is used, both in vitro and in vivo, to cause glutathione depletion. L-BSO has been shown to sensitize cells to chemotherapy and radiation, and it is currently used in clinical trials.[7] Furthermore, several forms of resistance to anticancer agents are associated with overproduction of glutathione or with changes in glutathione metabolism.[7,10,11] Therefore, it is likely that depletion of glutathione by administration of L-BSO will sensitize cells to the enzymatic oxidation products of spermine.

Glutathione has an important role in the detoxification of H_2O_2, via the glutathione redox cycle. Depletion of cellular glutathione with L-BSO led to an increase in cytotoxicity caused by this oxidant.[12] In addition, it was suggested that conjugation by glutathione of aldehydes generated during lipid peroxidation, catalyzed by glutathione S-transferase, could be an important pathway for the detoxification of these species.[13] Furthermore, the toxic aldehyde acrolein was inactivated by human glutathione transferases.[14] It has been suggested that acrolein could be a breakdown product of the unstable dialdehyde generated during enzymatic oxidation of spermine.

We evaluated the role of glucose metabolism via the PC and the level of intracellular glutathione on the cytotoxicity induced by the each of the enzymatic reaction products of spermine. At low spermine concentrations, hydrogen peroxide was the only toxic species responsible for cytotoxicity in the presence of BSAO. Under these conditions, glucose protected cells against cytotoxicity. In the presence of catalase, cytotoxicity is attributed to other toxic products of spermine oxidation: aldehyde(s).[3,5] We show here an important role for the PC and the glutathione redox cycle in protection against hydrogen peroxide generated from BSAO and spermine. Glutathione had a role in protecting cells against cytotoxicity attributed to spermine-derived aldehyde(s), whereas the PC did not.

MATERIALS AND METHODS

BSAO purification

BSAO was purified by the method of Mondovi et al.,[15] with two additional purification steps on Q-Sepharose columns at pH 8.0 and pH 6.0.[16] The BSAO purification factor was about 1400-fold, and a single band was obtained on SDS gel electrophoresis. The enzymatic activity was assayed spectrophotometrically at 25°C by monitoring the formation of benzaldehyde at 250 nm ($E = 12500\ M^{-1}\ cm^{-1}$).[17] BSAO had a specific activity of 0.32–0.42 IU/mg, with 1 IU (International Unit) defined as the amount that catalyzes the oxidation of 1 μmol substrate/min. The protein concentration was determined from the absorbance at 280 nm, assuming an absorption coefficient of 1.74 liters $g^{-1}cm^{-1}$.[18]

Tissue culture

CHO cells (AuxB1)[19] were grown in monolayer in minimum essential medium alpha (MEM Alpha) (Gibco Canada) plus 10% FBS (Gibco Canada) and 1% penicillin (50 units/ml)-streptomycin (50 μg/ml) (Flow Laboratories), in tissue culture flasks (Sarstedt Inc, St. Laurent, Canada) in a humidified atmosphere of 5% CO_2 in a water jacketed incubator at 37°C, as previously described.[20] The cells were grown to near confluence and were then incubated for 24 h with fresh culture medium. Confluent cells were then harvested using citrated phosphate-buffered saline (0.14 M NaCl, 0.01 M sodium phosphate, 0.015 M sodium citrate), washed by centrifugation (1000 × *g*, 3 min) and resuspended in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) for experimental studies. Glucose, when present, was used at a concentration of 10 mM.

Cell survival experiments

Cell survival experiments were carried out in 1 ml of PBS-1% BSA–10 mM glucose. CHO cells (10^5/ml) were incubated in the presence or absence of the following reagents: BSAO (5.7×10^{-3} U/ml), spermine (0 to 200 μM), catalase (300 U/ml, from bovine liver), acrolein (0 to 200 μM, Sigma Chemical Co.). Spermine (Fluka Chemic, Buchs, Switzerland) was freshly prepared before each experiment and, if present, was added last. The cells were incubated in tubes in a waterbath at 37°C. After the appropriate time, the cells were washed three times by centrifugation (1000 × *g*, 2 min) to stop the incubation.[20] The cells were resuspended in culture medium, diluted to the appropriate concentration and plated in tissue culture dishes (60 × 15 mm), which were incubated at 37°C in an atmosphere of 5% CO_2 for 8 d. The dishes were then washed with PBS, fixed with 95% ethanol, and stained with methylene blue before counting macroscopic col-

onies (> 50 cells). Percent cell survival was expressed as the mean number of colonies obtained relative to the mean number of colonies obtained in the control. Pretreatment of cells with 1 mM L-buthionine-S, R-sulfoximine (L-BSO) (Sigma Chemical Co., St Louis, MO) was performed on confluent cells in monolayer during 24 h. The cells were subsequently washed three times, harvested, and cell pellets were analyzed for total glutathione (see below). For cell survival determination, cells were resuspended in PBS–1% BSA and experiments were performed as described above.

Glutathione assay

Total glutathione was determined according to Anderson, 1989.[22] CHO cell pellets (6 to 10 × 10^6 cells) were lysed by vigorous agitation with 1 ml of double distilled water and deproteinised during 15 min with 5-sulfosalicylic acid (3% w/v). The suspension was centrifuged (14,000 × *g*, 5 min) in a microfuge. An aliquot (20 to 100 μl) of the supernatant was added to a 1 ml cuvette containing 0.7 ml of a solution of 0.143 M sodium phosphate containing 6.3 mM EDTA (pH 7.5) and NADPH (0.248 mg/ml), and 0.1 ml of 6 mM 5,5′-dithiobis(2-nitrobenzoic acid). The assay was initiated by addition of 10 μl of glutathione reductase (266 U/ml). The rate of formation of 5-thio-2-nitrobenzoic acid, which is proportional to total glutathione concentration, was followed at 412 nm during 2 min at 30°C in a jacketed spectrophotometer compartment. Sample values were calculated from a standard curve of nmol glutathione vs. rate and expressed as nmol/10^6 cells.

Activity of the pentose phosphate cycle

PC activity was determined as the amount of $^{14}CO_2$ liberated from samples incubated with [1-^{14}C]-D-glucose, as previously described.[12,22] CHO cells (5 × 10^6/ml), in a final volume of 2 ml in PBS–1% BSA-2 mM glucose, were introduced into 25 ml Erlenmeyer flasks. A quantity of 0.2 μCi of [1-^{14}C]-D-glucose (55.4 mCi/mmol, Du Pont Corp.–New England Nuclear, Boston, MA) per flask was added and, when present, 200 μM spermine and BSAO (28 × 10^{-3} U/ml) with or without catalase (300 U/ml), or 200 μM acrolein, or 2 mM hydrogen peroxide (4 μmol/10^7 cells) (Fisher Scientific Co., Montreal, Canada). The flasks were immediately sealed with serum stoppers and incubated under agitation. A plastic tube containing a glass filter saturated with 0.1 ml of 5% KOH was attached inside each flask to the serum stopper. After 1 h at 37°C, 0.2 ml of 6 N H_2SO_4 was injected into the flasks. After 30 min, the filters were placed into scintillation vials containing Scinti Verse II (Fisher Scientific Co.). The radioactivity was determined 12 h later.

Glutathione level after exposure to aldehydes

CHO cells (5 × 10^6/ml), in a final volume of 2 ml in PBS–1% BSA–2 mM glucose, were placed in 25 ml Erlenmeyer flasks. Samples contained 200 μM spermine and BSAO (28 × 10^{-3} U/ml) with or without catalase (300 U/ml), or 200 μM acrolein, or 400 μM hydrogen peroxide. Control samples were incubated under agitation for 1 h at 37°C without addition of other reagents. After the incubation, cell pellets were analysed for total glutathione as described above.

RESULTS

Figure 1 shows the effect of glucose on the clonogenic cell survival of CHO cells exposed to 3.8 μM exogenous spermine and purified BSAO (5.7 × 10^{-3} U/ml). There was no cytotoxicity when glucose (10 mM) was present in the medium during a 60-min incubation. However, there were two logarithms of cell killing after a 40-min incubation, when glucose was absent from the medium. Glucose protected the cells from cytotoxicity induced by oxidation products of spermine.

The effect of glucose on cytotoxicity induced by BSAO (5.7 × 10^{-3} U/ml) during 60 min, as a function

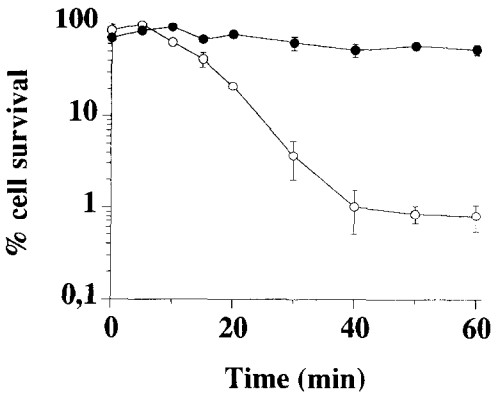

Fig. 1. Time course for cytotoxicity induced by BSAO and 3.8 μM spermine, with and without glucose. CHO cells (10^5/ml) in 1 ml of PBS–1% BSA were incubated with BSAO (5.7 × 10^{-3} U/ml) and 3.8 μM spermine with (●) and without (○) 10 mM glucose. The data represent mean and SEM from three independent experiments.

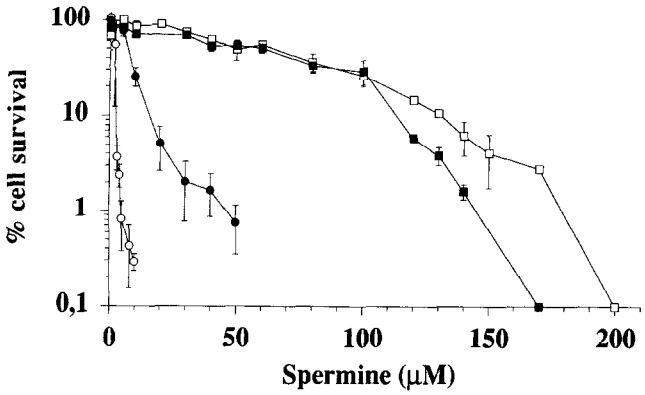

Fig. 2. Effect of glucose on cytotoxicity induced by BSAO as a function of spermine concentration, with and without catalase. CHO cells (10^5/ml) in 1 ml of PBS–1% BSA were incubated (1 h, 37°C) with BSAO (5.7×10^{-3} U/ml) and spermine with (closed symbols) and without (open symbols) 10 mM glucose, in the presence (■,□) and absence (●,○) of exogenous catalase (300 U/ml). The data represent mean and SEM from three independent experiments.

of spermine concentration, is shown in Fig. 2. The cytotoxicity data is shown both with and without addition of exogenous catalase, an enzyme that eliminates hydrogen peroxide. The plating efficiency decreased to 41% for control cells that were not exposed to spermine and BSAO, when incubated for 1 h without glucose. Curves for cytotoxicity in the absence of glucose were normalized to the control value (41%) obtained without glucose, which is designated as 100%, to factor out the cytotoxicity caused by the absence of glucose. Cytotoxicity due to BSAO was apparent at 5 μM spermine, and this increased steadily up to 50 μM spermine when glucose was present. When cells were exposed to BSAO without glucose, there was a marked increase in cytotoxicity. Cytotoxicity increased with spermine concentration to reach almost three logarithms of cell killing at 10 μM spermine. Glucose afforded considerable protection to the cells against cytotoxicity induced by BSAO and spermine. Catalase (300 U/ml) afforded marked protection to the cells against cytotoxicity due to BSAO for spermine concentrations up to 100 μM, whether or not glucose was present in the incubation medium (Fig. 2). This means that the cytotoxicity observed for spermine concentrations below 50 μM, in the absence of exogenous catalase, with and without glucose, was due to hydrogen peroxide alone.

We considered the effect of glucose on cytotoxicity caused by aldehyde(s), generated by enzymatic oxidation of spermine, in relation to its metabolism via the PC. When catalase was present, cytotoxicity due to BSAO occurred for spermine concentrations above 100 μM, regardless of whether glucose was present or not (Fig. 2). There was no protection afforded by glucose against cytotoxicity. Moreover, glucose appeared to sensitize CHO cells to the toxic effects of aldehyde(s). Cell viability decreased more rapidly when glucose was present in the incubation medium.

An essential role for glucose, metabolized via the PC, was reported for the detoxification of H_2O_2 via the glutathione cycle.[12] We compared the ability of BSAO and spermine to stimulate activity of the PC, relative to exogenously added hydrogen peroxide (Table 1). The quantity of $^{14}CO_2$ released from untreated control CHO cells, incubated with 1-^{14}C-labeled glucose for 1 h at 37°C, was 23.00 nmol/10^7 cells. When cells were exposed to BSAO (28×10^{-3} U/ml) and spermine (200 μM), the activity of the PC increased almost threefold, relative to control cells (Table 1). This effect was similar to that observed when cells were exposed to H_2O_2 (4 μmol/10^7 cells). When catalase was present, the activity of the PC was not stimulated by BSAO and spermine (Table 1). Furthermore, exogenously added acrolein did not have any marked effect on the level of PC activity.

Figures 3 and 4 show how depletion of glutathione can modulate the response of CHO cells to the enzymatic oxidation products of spermine. Total glutathione was reduced to 0.27 (SD 0.086) nmol/10^6 cells, from an initial value of 3.30 (SD 0.19) nmol/10^6 cells, by pretreatment of cells during 24 h with 1 mM L-BSO. This treatment decreased plating efficiency to 71.2%. Cell survival curves for L-BSO–treated cells were normalized to the control value obtained with L-BSO treatment (71.2%), which is designated as 100%, to factor out the cytotoxicity caused by the L-BSO treatment. Figure 3A shows cytotoxicity induced by BSAO and low concentrations of spermine (< 50 μM) in L-BSO–treated cells, under conditions where cytotoxicity was attributed to H_2O_2 alone (Fig. 2). When

Table 1. Activity of the Pentose Phosphate Cycle in CHO Cells Exposed to Enzymatic Reaction Products of Spermine

Samples[a]	Activity of PC[d] (nmol CO_2/10^7 Cells)
Control	23.00 ± 7.69
BSAO + spermine (200 μM)[b]	63.25 ± 9.87
BSAO + spermine + catalase (300 U/ml)	30.90 ± 10.32
Exogenous acrolein (200 μM)	39.49 ± 13.19
Exogenous H_2O_2[c]	72.61 ± 11.36

[a] CHO cells (5×10^6/ml) in 2 ml of PBS-1% BSA during 1 h at 37°C.
[b] 28×10^{-3} U/ml.
[c] 4 μmol/10^7 cells.
[d] Mean and SD from two independent experiments.

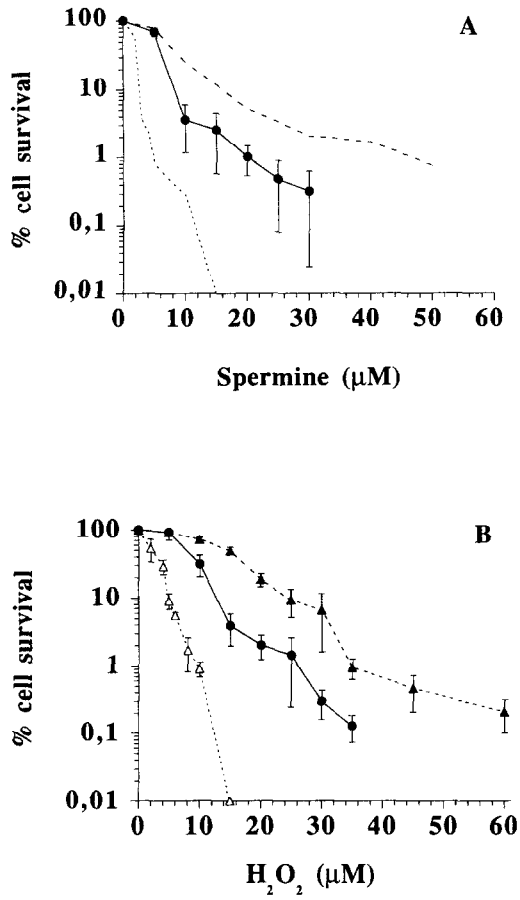

Fig. 3. Effect of glutathione depletion with L-BSO on (A) cytotoxicity induced by BSAO and spermine and (B) cytotoxicity of exogenous H_2O_2. (A) CHO cells (10^5/ml), pretreated with L-BSO (24 h, 1 mM), were incubated (1 h, 37°C) in 1 ml of PBS–1% BSA–10 mM glucose with BSAO (5.7×10^{-3} U/ml) and spermine. The curves from Fig. 2 for cytotoxicity induced by BSAO and spermine, either with (----) or without (. . . . .) 10 mM glucose, are shown for sake of comparison in cells without pretreatment with L-BSO. (B) CHO cells (10^5/ml), pretreated with L-BSO, were exposed to hydrogen peroxide for 1 h in 1 ml of PBS–1% BSA–10 mM glucose (●). Cytotoxicity induced by hydrogen peroxide in cells without pretreatment with L-BSO is shown with (▲) or without (△) 10 mM glucose. The data represent mean and SEM from three independent experiments.

glucose was present, glutathione depletion sensitized cells to the cytotoxic effects of H_2O_2 produced during the oxidation of spermine by BSAO, relative to cells without pretreatment with L-BSO and, thus, containing normal levels of glutathione (Fig. 3A, dashed line). Cells, in the absence of glucose (Fig. 3A, dotted line), were still more sensitive to the cytotoxic effects of the oxidant than cells with severe depletion of glutathione (glucose present). Figure 3B shows equivalent data obtained when cells were exposed to exogenous hydrogen peroxide. The data in Figs. 3A and 3B are very similar. This confirms further that at low spermine concentrations cytotoxicity induced by BSAO resembles the cytotoxicity of exogenous H_2O_2.

Figure 4 shows how depletion of glutathione can modulate the response of CHO cells to other oxidation products of spermine, aldehyde(s). Figure 4A shows cytotoxicity induced by BSAO and spermine, when catalase was present in the incubation mixture. Glutathione depletion sensitized the cells to the cytotoxic effects of aldehyde(s) produced during oxidation of spermine by BSAO, relative to cells without L-BSO

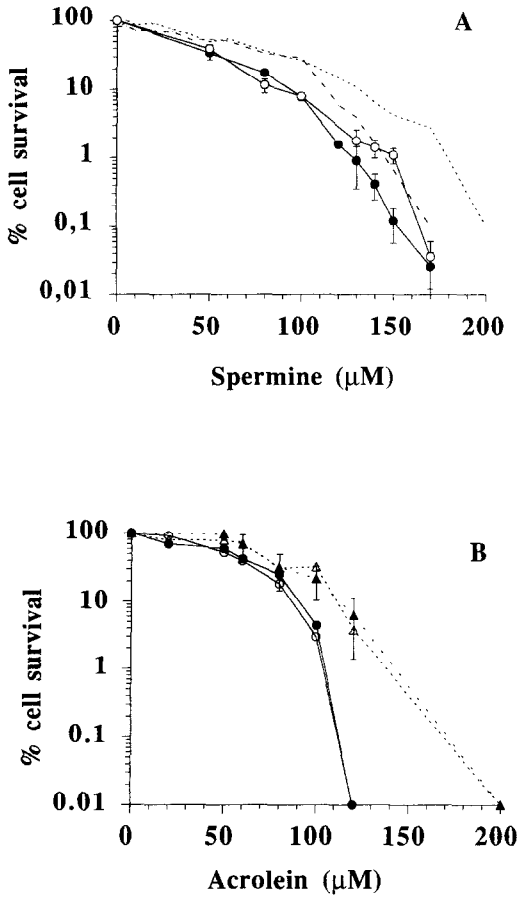

Fig. 4. Effect of glutathione depletion with L-BSO on (A) cytotoxicity induced by BSAO and spermine in the presence of exogenous catalase and (B) cytotoxicity of exogenous acrolein. (A) CHO cells (10^5/ml), pretreated with L-BSO (24 h, 1 mM), were incubated (1 h, 37°C) with BSAO (5.7×10^{-3} U/ml) and spermine in the presence of exogenous catalase (300 U/ml), with (●) or without (○) 10 mM glucose, in 1 ml of PBS–1% BSA. The curves from Fig. 2 for cytotoxicity under equivalent conditions are shown for sake of comparison, in cells without pretreatment with L-BSO, with (----) or without (. . . . .) glucose. (B) CHO cells (10^5/ml), pretreated with L-BSO, were exposed to acrolein (1 h, 37°C), with (●) or without (○) 10 mM glucose, in 1 ml of PBS–1% BSA. Data for cytotoxicity under equivalent conditions, in cells without pretreatment with L-BSO, are shown with (▲----▲) or without (△. . . .△) glucose. The data represent mean and SEM from three independent experiments.

treatment (Fig. 4A, dashed and dotted lines). This effect occurred regardless of whether glucose was present or not. Figure 4B shows equivalent data obtained with exogenous acrolein. Cells with severe depletion of glutathione were more sensitive to acrolein than cells with normal levels of glutathione (Fig. 4B, dashed and dotted lines). The response of cells to exogenous acrolein was not affected by the level of glucose in the incubation medium.

The effect of exposure to the enzymatic reaction products of BSAO during 1 h at 37°C on the level of intracellular glutathione is shown in Table 2. The total glutathione content decreased markedly when CHO cells were exposed to BSAO and spermine (200 μM), both with and without catalase. Exposure to exogenous acrolein (200 μM) also caused a marked decrease in the level of glutathione in cells. However, exposure to exogenous hydrogen peroxide (400 μM) did not alter glutathione content. The data shown in Fig. 4 and Table 2 suggest that aldehyde(s) produced from enzymatic oxidation of spermine could be detoxified by conjugation to glutathione.

Table 2. Total Glutathione Content of CHO Cells Exposed to Enzymatic Reaction Products of Spermine

Sample[a]	Total Glutathione[c] (nmol/10^6 Cells)
Control	2.67 ± 0.16
BSAO + Spermine (200 μM)[b]	0.0604 ± 0.029
BSAO + spermine + catalase	0.0496 ± 0.023
Exogenous acrolein (200 μM)	0.0239 ± 0.0013
Exogenous H_2O_2 (400 μM)	2.99 ± 0.18

[a] CHO cells (5×10^6/ml) in 2 ml of PBS-1% BSA during 1 h at 37°C.
[b] 28×10^{-3} U/ml.
[c] Mean and SD from two independent experiments.

DISCUSSION

We show here that glucose present in the incubation media protected CHO cells from cytotoxicity induced by oxidation products of exogenous spermine generated by BSAO. This effect was observed at low spermine concentrations, below 50 μM, where H_2O_2 was the sole enzymatic oxidation product responsible for cytotoxicity. Therefore, for low spermine concentrations, the protective effect of glucose against cytotoxicity induced by oxidation products of spermine, could be explained as a protective effect against H_2O_2 produced by the enzymatic reaction. At higher spermine concentrations, glucose no longer protected the cells, but essentially complete protection was afforded by exogenous catalase up to 100 μM spermine. It was previously demonstrated that glucose, metabolized via the PC, has an essential role for the detoxification of H_2O_2 via the glutathione redox cycle, and it may also have a role in the function of endogenous catalase.[12] Therefore, the absence of glucose from the cellular medium, or severe depletion of intracellular glutathione with L-BSO, deprives cells of the major antioxidant defence systems against H_2O_2, one of the oxidation products of spermine. As expected, either deprivation of glucose or depletion of glutathione led to considerable sensitization to the effects of BSAO and spermine, under conditions where only hydrogen peroxide was present. Furthermore, stimulation of PC activity by BSAO and spermine occurred only under conditions where hydrogen peroxide was generated. Our data suggest that stimulation of PC activity was involved in the protective effect of glucose against cytotoxicity due to hydrogen peroxide generated from spermine by BSAO. The sensitization to peroxide cytotoxicity in L-BSO–treated cells is most likely explained by inhibition of glutathione peroxidase, a major cellular pathway for detoxification of hydrogen peroxide, due to a lack of GSH.

Glucose did not protect CHO cells from cytotoxicity in the presence of exogenous catalase, under conditions where the aldehyde(s) was responsible for the cytotoxicity of BSAO and spermine. Under these conditions, there was no stimulation of PC activity. Similarly, glucose did not protect cells against cytotoxicity of the exogenous aldehyde acrolein and there was no appreciable stimulation of PC activity. Moreover, glucose increased the cytotoxic effect of the spermine-derived aldehyde(s). This effect was not related to the metabolism of glucose via the PC and the explanation is not known. Glucose is required for active transport processes of many molecules into cells. Enhancement of cytotoxicity of spermine-derived aldehyde(s) by glucose would be expected if these relatively large molecules were transported into cells via an active process. The mechanism(s) of transport involving the spermine-derived aldehyde(s) is not known, and this is difficult to determine given the controversy concerning the chemical nature of the aldehyde(s) generated.[23,24] Other possible explanations are that metabolism of glucose could enhance reactions involved in the cytotoxicity of the aldehyde(s), or that it could interfere with the detoxifying reactions against aldehydes.

Depletion of cellular glutathione with L-BSO sensitized cells to the cytotoxic effects of spermine-derived aldehyde(s) and also to exogenous acrolein. Therefore, depletion of glutathione with L-BSO deprived CHO cells of an important detoxifying system that protects the cells

against aldehydes. It is known that acrolein is a substrate for glutathione transferases and that it is detoxified by conjugation with glutathione.[14,25] This is a likely explanation for the sensitizing effect of glutathione depletion on the cytotoxicity of acrolein in our system. Glutathione S-transferase (GST) may also have an important role in cellular protection against other aldehydes.[13,26] We postulate that spermine-derived aldehyde(s) could be detoxified by conjugation to glutathione. This could occur either by the direct reaction with glutathione or it could be catalysed by GST. Such an effect could be analogous to that reported for the detoxification of acrolein.[14,25] Consistent with this idea, the spermine-derived aldehyde(s) caused depletion of cellular glutathione levels. Furthermore, our findings suggest that the glutathione redox cycle and metabolism of glucose via the PC were not involved in the detoxification of this aldehyde(s). Although glutathione depletion sensitized the cells to the cytotoxic effects of both the spermine-derived aldehyde(s) and exogenously added acrolein, glucose only affected cytotoxicity during exposure to spermine-derived aldehyde(s). This difference in cellular response to these different aldehyde(s) suggests that the effect of glutathione depletion and the effect of glucose are not related. In contrast, hydrogen peroxide generated from spermine and BSAO was detoxified via the glutathione redox cycle requiring an elevated level of glucose metabolism via the PC. This means that sensitization by L-BSO to the toxicity of aldehyde(s) occurred via a different mechanism than that involved in the sensitization by L-BSO to hydrogen peroxide.

In conclusion, both the metabolism of glucose via the PC and the metabolism of glutathione affect the cellular response to hydrogen peroxide and aldehyde(s) derived from spermine. However, cells respond to these cytotoxic species in different ways, suggesting that different pathways are involved. H_2O_2 is detoxified via the glutathione redox cycle with participation of GSH and glucose. The aldehyde(s) is most likely detoxified by conjugation to GSH, in a reaction catalyzed by GST. Glucose metabolism via the PC is not required for this detoxification pathway. In contrast to the protective effect of glucose against cytotoxicity of hydrogen peroxide, glucose enhanced cytotoxicity in cells exposed to spermine-derived aldehyde(s).

Acknowledgements — This work was supported by the NCIC (D.A.B.), by Italian CNR contract No. 94.00510.CT11, and Special Project ACRO contract No. 92.02224.39 (E.A.), by grant MURST (E.A.) and by joint project CNR-Canada (E.A., D.A.B.).

REFERENCES

1. Agostinelli, E.; Bates, D. A.; Przybytkowski, E.; Mateescu, M. A.; Mondovi', B. Cytotoxicity of polyamines in Chinese hamster ovary (CHO) cells in the presence of purified serum amine oxidase. *Life Chem. Rep.* **9:**193–204; 1991.
2. Averill-Bates, D. A.; Agostinelli, E.; Przybytkowski, E.; Mateescu, M. A.; Mondovi', B. Cytotoxicity and kinetic analysis of purified bovine serum amine oxidase in the presence of spermine in Chinese hamster ovary cells. *Arch. Biochem. Biophys.* **300:**75–79; 1993.
3. Averill-Bates, D. A.; Agostinelli. E.; Przybytkowski, E.; Mondovi', B.Aldehyde dehydrogenase and cytotoxicity of purified bovine serum amine oxidase and spermine in Chinese hamster ovary cells. *Biochem. Cell Biol.* **72:**36–42; 1994.
4. Bachrach, U.; Heimer, Y. M., eds. *The physiology of polyamines.* Boca Raton, FL: CRC Press; 1989.
5. Agostinelli, E.; Przybytkowski, E.; Mondovi', B.; Averill-Bates, D. A. Heat enhancement of cytotoxicity induced by oxidation products of spermine in Chinese hamster ovary cells. *Biochem. Pharmacol.* **48:**1181–1186; 1994.
6. Meister, A. Glutathione deficiency produced by inhibition of its synthesis and its reversal; Applications in research and therapy. *Pharmacol. Ther.* **51:**155–194; 1991.
7. Meister, A. Glutathione, ascorbate and cellular protection. *Cancer Res. Suppl.* **54:**1969s–1975s; 1994.
8. Brigelius, R. Mixed disulfides: Biological functions and increase in oxidative stress. In: Sies, H., ed. *Oxidative stress.* London: Academic Press Inc.; 1985:243–272.
9. Griffith, O. W.; Meister, A. Potent and specific inhibition of glutathione synthesis by prothionine sulfoximine (S-n-butyl homocysteine sulfoximine). *J. Biol. Chem.* **254:**7558–7560; 1979.
10. Kramer, R. A.; Zakher, J.; Kim, G. Role of the glutathione redox cycle in acquired and de novo multidrug resistance. *Science* **241:**694–696; 1988.
11. Hamilton, T. C.; Masuda, H.; Ozols, R. F. The multidrug-resistant phenotype and its relationship to glutathione. In: Kessel, D., ed. *Resistance to antineoplastic drugs.* Boca Raton, FL: CRC Press; 1989:49–61.
12. Averill-Bates, D. A.; Przybytkowski, E. The role of glucose in cellular defences against cytotoxicity of hydrogen peroxide in Chinese hamster ovary cells. *Arch. Biochem. Biophys.* **312:**52–58; 1994.
13. Spitz, D. R.; Sullivan, S. J.; Malcolm, R. R.; Roberts, R. J. Glutathione dependent metabolism and detoxification of 4-hydroxy-2-nonenal. *Free Radic. Biol. Med.* **11:**415–423; 1991.
14. Berhane, K.; Mannervik, B. Inactivation of the genotoxic aldehyde acrolein by human glutathione tranferases of classes alpha, mu, and pi. *Mol. Pharmacol.* **37:**251–254; 1990.
15. Mondovi', B.; Turini, P.; Befani, O.; Sabatini, S. Purification of bovine plasma amine oxidase. *Methods Enzymol.* **94:**314–318; 1983.
16. Janes, S. M.; Mu, D.; Wemmer, D.; Smith, A. J.; Kaur, S.; Maltby, D.; Burlingame, A. L.; Klinman, J. P. A new redox cofactor in eukaryotic enzymes: 6-Hydroxydopa at the active site of bovine serum amine oxidase. *Science* **248:**981–987; 1990.
17. Tabor, C. W.; Tabor, H.; Rosenthal, S. M. Purification of amine oxidase from beef plasma. *J. Biol. Chem.* **208:**645–661; 1954.
18. Suzuki, S.; Sakurai, T.; Nakahara, A.; Manabe, T.; Akuyama, T. Effect of metal substitution on the chromophore of bovine serum amine oxidase. *Biochemistry* **22:**1630–1635; 1983.
19. Ling, V.; Thompson, L. H. Reduced permeability in CHO cells as a mechanism of resistance to colchicine. *J. Cell Physiol.* **83:**103–116; 1974.
20. Bates, D. A.; Mackillop, W. J. Hyperthermia, adriamycin transport, and cytotoxicity in drug-sensitive and -resistant Chinese hamster ovary cells. *Cancer Res.* **46:**5477–5481;1986.
21. Anderson, M. E. Tissue glutathione. In : Greenwald, R. A., ed. *Handbook of methods for oxygen radical research.* Boca Raton, FL: CRC Press; 1989:283–284.
22. Varnes, M. E.; Tuttle, S. W.; Biaglow, J. E. Nitroheterocycle metabolism in mammalian cells. Stimulation of the hexose

monophosphate shunt. *Biochem. Pharmacol.* **33**:1671–1677; 1984.

23. Alarcorn, R. A. Acrolein IV. Evidence for the formation of the cytotoxic aldehyde acrolein from enzymatically oxidised spermine or spermidine. *Arch. Biochem. Biophys.* **137**:365–372; 1970.
24. Tabor, C. W.; Tabor, H.; Bachrach, U. Identification of the aminoaldehydes produced by the oxidation of spermine and spermidine with purified plasma amine oxidase. *J. Biol. Chem.* **239**:2194–2203; 1964.
25. Tsuchida, S.; Sato, K. Glutathione transferases and cancer. *Crit. Rev. Biochem. Mol. Biol.* **27**:337–384; 1992.
26. Spitz, D. R.; Malcolm, R. R.; Roberts, R. J. Cytotoxicity and metabolism of 4-hydroxy-2-nonenal and 2-nonenal in H_2O_2-resistant cell lines. *Biochem. J.* **267**:453–459; 1990.

ABBREVIATIONS

BSAO—bovine serum amine oxidase
CHO—Chinese hamster ovary
FBS—fetal bovine serum
MEM—minimum essential medium
BSA—bovine serum albumin
BSO—buthionine sulfoximine
GSH—reduced glutathione
PBS—phosphate-buffered saline
PC—pentose phosphate cycle
SEM—standard error of mean