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Accumulation of α-Oxoaldehydes during Oxidative Stress: A Role in Cytotoxicity

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ABSTRACT. Glyoxal, methylglyoxal (MG), and 3-deoxyglucosone (3-DG) are physiological α-oxoaldehydes formed by lipid peroxidation, glycation, and degradation of glycolytic intermediates. They are enzymatically detoxified in cells by the cytosolic glutathione-dependent glyoxalase system (glyoxal and MG only) and by NADPH-dependent reductase and $NAD(P)^+$-dependent dehydrogenase. In this study, the changes in the cellular and extracellular concentrations of these α-oxoaldehydes were investigated in murine $P388D_1$ macrophages during necrotic cell death induced by median toxic concentrations of hydrogen peroxide and 1-chloro-2,4-dinitrobenzene (CDNB). α-Oxoaldehyde concentrations were determined by derivatization with 1,2-diamino-4,5-dimethoxybenzene. There were relatively small increases in cellular and extracellular glyoxal concentration, except that extracellular glyoxal was decreased with hydrogen peroxide. The cytosolic concentration of 3-DG and the cytosolic and extracellular concentrations of MG, however, increased markedly. Aminoguanidine inhibited α-oxoaldehyde accumulation and prevented cytotoxicity induced by hydrogen peroxide and CDNB. The accumulation of glyoxal and MG in toxicant-treated cells was a likely consequence of decreased *in situ* activity of glyoxalase I. The effect was marked for MG but not for glyoxal, suggestive of a greater metabolic flux of MG formation than of glyoxal. The accumulation of 3-DG in toxicant-treated cells was probably due to the decreased availability of pyridine nucleotide cofactors for the detoxification of 3-DG. Impairment of α-oxoaldehyde detoxification is cytotoxic, and this may contribute to toxicity associated with GSH oxidation and S conjugation in oxidative stress and chemical toxicity, and to chronic pathogenesis associated with diabetes mellitus where there is oxidative stress and the formation of glyoxal, MG, and 3-DG is increased. BIOCHEM PHARMACOL **58**;4:641–648, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. glyoxal; methylglyoxal; 3-deoxyglucosone; glyoxalase; glutathione; oxidative stress

The α-oxoaldehydes glyoxal, MG† and 3-DG are physiological metabolites. Glyoxal is formed by the slow, spontaneous oxidative degradation of glucose [1], the degradation of glycated proteins [2], and lipid peroxidation [3]. MG is formed by the non-enzymatic and enzymatic degradation of triosephosphates, the metabolism of acetone, and the catabolism of threonine [4]. 3-DG is formed by the degradation of glycated proteins [5] and by the fragmentation of fructose 3-phosphate [6]. Exposure of cells to high concentrations of α-oxoaldehydes, by addition of exogenous α-oxoaldehyde [7, 8] or inhibition of enzymatic detoxification with a specific inhibitor [9], induces growth arrest and toxicity. α-Oxoaldehydes react non-enzymatically with guanyl nucleotides to form 6,7-dihydro-6,7-dihydroxy-imidazo[2,3-*b*]purine-9(8)one derivatives [10, 11] and other adducts [12] which are associated with mutagenesis [13] and cytotoxicity [7, 14]. They also react with cysteine residues of proteins to form reversible hemithioacetal adducts and with arginine and lysine residues of proteins to form the stable adducts AGE. Some AGEs are protein cross-links, e.g. bis(lysyl)imidazolium cross-links [15], whereas others were recognition factors for AGE receptors [16]. Agonism at AGE receptors has also been associated with cell activation and cytotoxicity (reviewed in [17]). Glycation of nucleotides and proteins is suppressed in normal physiological states by the enzymatic detoxification of α-oxoaldehydes [4].

Glyoxal and MG are detoxified by the glyoxalase system in the cytosol of all cells. It catalyses the conversion of glyoxal and MG to glycolate and D-lactate, respectively, with GSH as cofactor [4]. The glyoxalase system is comprised of two enzymes, glyoxalase I and glyoxalase II, and a catalytic amount of GSH. Glycoxalase I (EC 4.4.1.5) catalyses the formation of *S*-2-hydroxyacylglutathione derivatives from the hemithioacetals formed non-enzymatically from α-oxoaldehydes and GSH, RCOCHO + GSH = RCOCH(OH)-SG → RCH(OH)CO-SG. Glyoxalase II (EC 3.1.2.6) catalyses the hydrolysis of S-2-hydroxyacylglutathione derivatives to aldonates, reforming the GSH consumed in the glyoxalase I-catalysed reaction,

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† *Abbreviations:* AGE, advanced glycated end product; CDNB, 1-chloro-2,4-dinitrobenzene; 3-DG, 3-deoxyglucosone; MG, methylglyoxal; MGB, modified Gey's buffer; and PARP, poly(ADP-ribose)polymerase.

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$RCH(OH)CO\text{-}SG + H_2O \rightarrow RCH(OH)CO_2^- + GSH + H^+$. The activity of glyoxalase I *in situ* is approximately proportional to the cytosolic concentration of GSH [4]. A decrease in the cellular concentration of GSH in oxidative stress [18] and S conjugation of GSH [19] will concomitantly decrease the *in situ* activity of glyoxalase I. This may lead to an accumulation of glyoxal and MG. 3-DG is detoxified by reduction to 3-deoxyfructose catalysed by NADPH-dependent aldehyde reductase (EC 1.1.1.2) and aldose reductase (EC 1.1.1.21) [20, 21]. This is the major route of 3-DG detoxification [22]. 3-DG may also be oxidised to 3-deoxygluconate catalysed by $NAD(P)^+$-dependent 2-oxoaldehyde dehydrogenase [23]. The *in situ* activity of 3-DG reductase may be decreased when NADPH is depleted in the regeneration of GSH from GSSG by glutathione reductase in oxidative stress [18].

In this report, we describe the changes in the concentration of glyoxal, MG, and 3-DG of $P388D_1$ cells and extracellular medium *in vitro* following exposure to median toxic concentrations of hydrogen peroxide and CDNB and the prevention of toxicity by the α-oxoaldehyde-scavenging agent aminoguanidine. This suggests that α-oxoaldehyde accumulation is a characteristic of toxicant-induced cell death and that inhibition of this may prevent cytotoxicity.

MATERIALS AND METHODS

Materials

GSH, hydrogen peroxide, CDNB, and aminoguanidine bicarbonate were purchased from Sigma. RPMI 1640 cell culture medium and fetal bovine serum were purchased from GIBCO Europe Ltd. The murine macrophage $P388D_1$ cell line [24] was purchased from the European Collection of Animal Cell Cultures. A colony of $P388D_1$ cells was maintained by seeding at a density of 80,000 cells/mL in RPMI 1640 with 10% fetal bovine serum and 2 mM glutamine in a 75-cm^2 T-flask under an atmosphere of air with 5% carbon dioxide, 100% humidity, and 37°, under aseptic conditions. The cells were cultured for 4 days to confluence. They were harvested by scraping adherent cells free and collected by centrifugation (400 *g*, 5 min). The cells were resuspended in fresh culture medium (1 mL), counted and cell viability assessed by the trypan blue exclusion technique. A portion of the cells was used to maintain the colony. The remainder was sedimented by centrifugation (400 *g*, 5 min), resuspended in MGB containing 147 mM NaCl, 5 mM KCl, 1.9 mM KH_2PO_4, 1.1 mM Na_2HPO_4, 5.5 mM glucose, 1.5 mM $CaCl_2$, 0.3 mM $MgSO_4$, and 1 mM $MgCl_2$, pH 7.4 and 37°, and used in incubations with toxicants [25].

Culture of $P388D_1$ Cells

$P388D_1$ cells (2×10^6/mL) were incubated in MGB (1 mL) with median toxic concentration TC_{50} values of toxicants hydrogen peroxide (30 mM) and CDNB (75 μM) for 3 hr at pH 7.4 and 37° [19]. At the times indicated, the cells were collected by centrifugation (400 *g*, 2 min), washed with MGB, and the cellular concentration of GSH and GSSG determined. Similar incubations were performed, 0.5 mL of 0.6 M ice-cold perchloric acid added to the cell suspension and the concentrations of glyoxal, MG, and 3-DG determined as described below. The effect of aminoguanidine bicarbonate (1 mM) and N_ω-nitroarginine (100 μM) on the viability of $P388D_1$ cells incubated with and without toxicants was investigated after incubation for 3 hr.

Assay of GSH, GSSG, and α-Oxoaldehyde Concentrations

The concentrations of GSH and GSSG in $P388D_1$ cells were determined by the microplate technique described [26]. The interbatch coefficients of variation were 4.0% and 2.0%, and the limits of detection were 3.4 pmol/10^6 cells and 1.8 pmol/10^6 cells, respectively (N = 6). The concentration of glyoxal, MG, and 3-DG in the cells and medium of $P388D_1$ cell cultures were determined by minor modification of the method previously described [27]. $P388D_1$ cells in MGB (2×10^6, 1 mL) were sedimented by centrifugation (400 *g*, 2 min), and the supernatant removed and retained. The cell pellet and aliquot of supernatant (0.5 mL) were acidified with 500 mM acetic acid (0.5 mL) and stored at −196° until analysis. For 3-DG, glyoxal, and MG, the limits of detection were 21, 11, and 14 pmol and the interbatch coefficients of variation were 15%, 11%, and 10% (N = 9), respectively. Cellular concentrations of α-oxoaldehydes are given as pmol/10^6 viable cells (it was assumed that cells that could no longer exclude trypan blue would also have leaked α-oxoaldehydes into the medium).

Statistical Analysis of the Experimental Results

The significance of changes in the experimental variables measured was assessed by Student's *t*-test.

RESULTS

Effect of Hydrogen Peroxide and CDNB on the Viability and Glutathione Concentration of $P388D_1$ Cells In Vitro

Incubation of $P388D_1$ cells with 30 mM hydrogen peroxide and 75 μM CDNB decreased cell viability by *ca.* 50% after 3 hr (Fig. 1, panels a and b; $P < 0.001$). With hydrogen peroxide, the cell viability decreased linearly with time, whereas with CDNB the cell viability was maintained near control levels for 2 hr and then decreased rapidly in the third hour of culture. The initial cellular concentration of GSH in control incubations was 4.82 ± 0.50 nmol/10^6 cells and did not change significantly during the 3-hr incubation period in control cultures ($P > 0.05$). Incubation of $P388D_1$ cells with 30 mM hydrogen peroxide gave a decrease in the cellular concentration of GSH of 28% after 15 min, which remained at this level thereafter until the

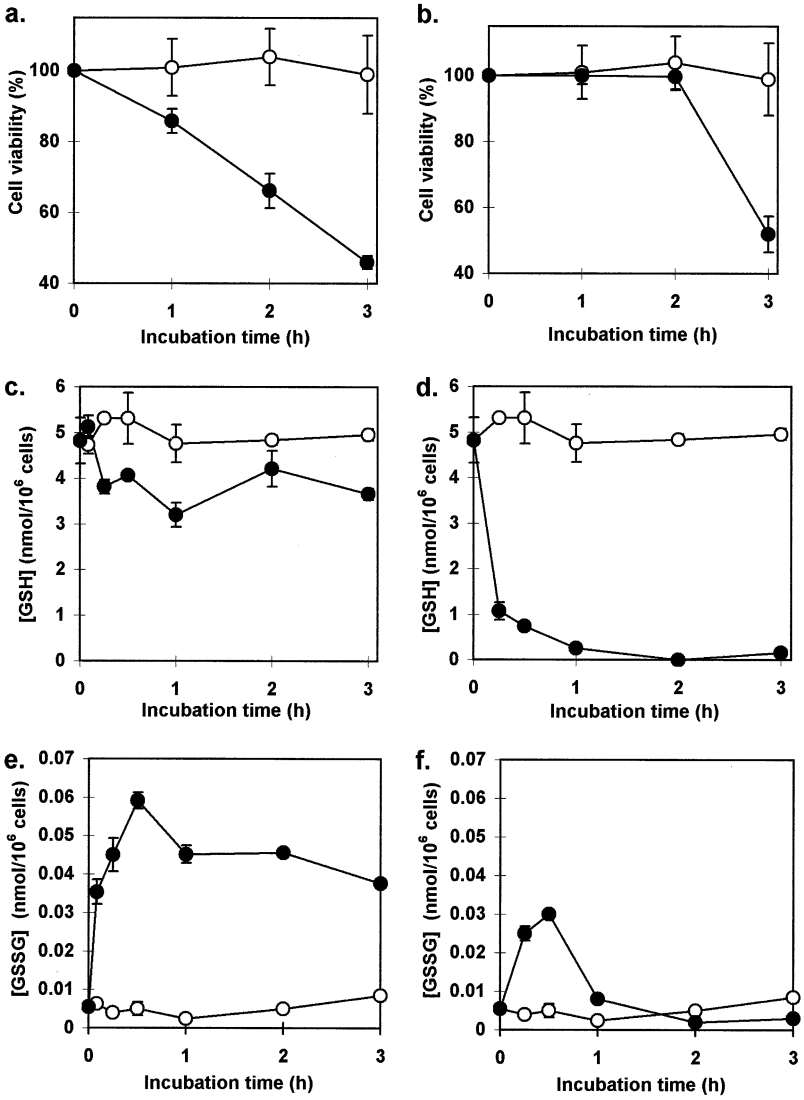

FIG. 1. Time–course of the viability, GSH, and GSSG content of P388D$_1$ macrophages incubated with (●—●) and without (○—○) 30 mM hydrogen peroxide (panels a, c, and e) and 75 μM CDNB (panels b, d, and f). Data are means ± standard deviation of 3 determinations.

end of the 3-hr incubation period (Fig. 2, panel c; $P < 0.001$). There was a concomitant increase, relative to control values, in the cellular concentration of GSSG to 0.059 ± 0.005 nmol/10^6 cells after 30 min ($P < 0.001$), which thereafter slowly decreased to 0.038 ± 0.001 nmol/10^6 cells but remained above control values ($P < 0.001$). The maximum increase in the concentration of GSSG observed, however, was < 5% of the concomitant decrease in GSH concentration (Fig. 2, panel e). Incubation of P388D$_1$ cells with 75 μM CDNB gave a rapid decrease in the cellular concentration of GSH to 22% of control levels after 15 min. This continued to decrease to 3% of control values after 3 hr (Fig. 1, panel d; $P < 0.001$). There was an initial concomitant increase in the cellular concentration of GSSG, maximising at an increase of 0.030 ± 0.001 nmol/10^6 cells after 30 min, relative to control values ($P < 0.001$). Thereafter, the concentration of GSSG declined sharply to 0.003 ± 0.001 nmol/10^6 cells after 3 hr, a concentration lower than control values ($P < 0.001$). The maximum increase in the concentration of GSSG observed, however, was < 1% of the concomitant decrease in GSH concentration (Fig. 2, panel f). The oxidative stress imposed in cells by toxicants may be judged by the cellular GSH/GSSG concentration ratio. The mean GSH/GSSG ratio in P388D$_1$ cells of control incubations was 0.115 ± 0.048% (N = 18) throughout the 3-hr incubation. This was decreased markedly in hydrogen peroxide-treated P388D$_1$ cells to 0.008 ± 0.001% ($P < 0.001$, N = 15), and in CDNB-treated P388D$_1$ cells to 0.003 ± 0.002%, $P < 0.001$ (N = 15).

Cellular and Extracellular Concentrations of α-Oxoaldehydes in Toxicant-Treated P388D$_1$ Cell Cultures

The changes in the cellular and extracellular concentrations of glyoxal, MG, and 3-DG in P388D$_1$ cell cultures during exposure to median toxic concentrations of hydrogen peroxide and CDNB were investigated. The initial concentrations of glyoxal, MG, and 3-DG in P388D$_1$ cells were 31.2 ± 8.3, 13.6 ± 9.9, and 88.2 ± 8.6 pmol/10^6 cells, respectively. The concentrations of glyoxal, MG, and 3-DG

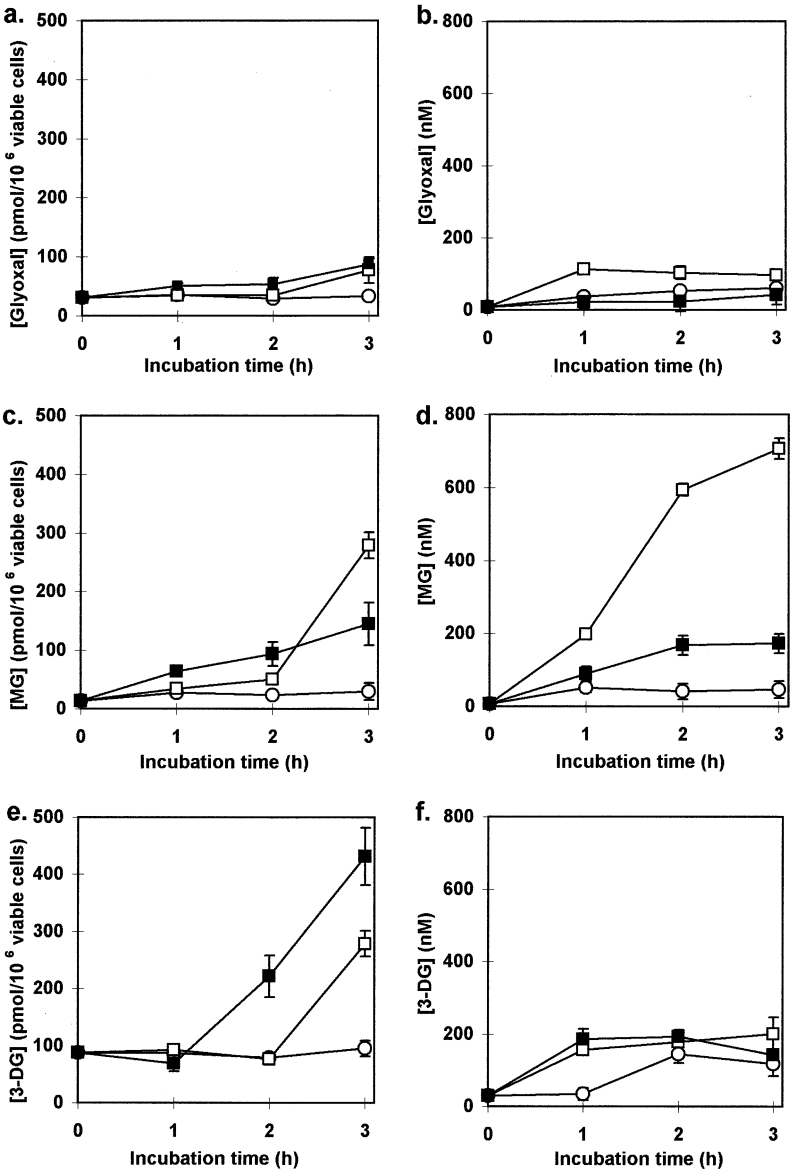

FIG. 2. Time–course of the cellular concentrations (panels a, c, and e) and extracellular medium concentrations (panels b, d, and f) of glyoxal, MG, and 3-DG in P388D$_1$ cell cultures in incubation controls (○—○) and incubations with 30 mM hydrogen peroxide (■—■) and with 75 μM CDNB (□—□). Data are means ± standard deviation of 3 determinations.

in the MGB medium were below the statistical limits of detection. In control cultures, the cellular concentrations of glyoxal, MG, and 3-DG did not change significantly during the 3-hr incubation ($P > 0.05$); the concentrations of glyoxal, MG, and 3-DG in the medium, however, increased to 61 ± 8 ($P < 0.01$), 46 ± 23 ($P > 0.05$), and 118 ± 10 nM ($P < 0.02$), respectively.

Incubation of P388D$_1$ cells with 30 mM hydrogen peroxide increased the cellular concentration of glyoxal after 3 hr ($P < 0.05$) and decreased the concentration of glyoxal in the medium ($P < 0.01$), with respect to control values (Fig. 2, panels a and b). The cellular and medium concentrations of MG, however, increased with time relative to control values, to 145 ± 37 pmol/10^6 viable cells ($P < 0.02$) and 173 ± 27 nM ($P < 0.001$), respectively, after 3 hr (Fig. 2, panels c and d). The cellular concentration of 3-DG changed little after 1 hr and then progressively increased relative to control values, to 432 ± 50 pmol/10^6 viable cells ($P < 0.001$) (Fig. 2, panel e). The concentration of 3-DG in the medium increased in the first hour relative to control values, to 186 ± 29 nM ($P < 0.001$), remained at this level in the second hour, and then decreased slightly in the third hour to 143 ± 58 nM (Fig. 2, panel f).

Incubation of P388D$_1$ cells with 75 μM CDNB increased the cellular concentration of glyoxal to 77 ± 16 pmol/10^6 viable cells ($P < 0.02$) and increased the glyoxal concentration in the medium to 96 ± 11 nM ($P < 0.01$), relative to control values (Fig. 2, panels a and b). The cellular and medium concentrations of MG increased markedly with time relative to control values, to 279 ± 22 pmol/10^6 viable cells and 707 ± 29 nM, respectively, after 3 hr ($P < 0.001$) (Fig. 2, panels c and d). The cellular concentration of 3-DG was increased significantly in the third hour of culture only, relative to control values, to 279 ± 22 pmol/10^6 cells ($P < 0.001$) (Fig. 2, panel e). The

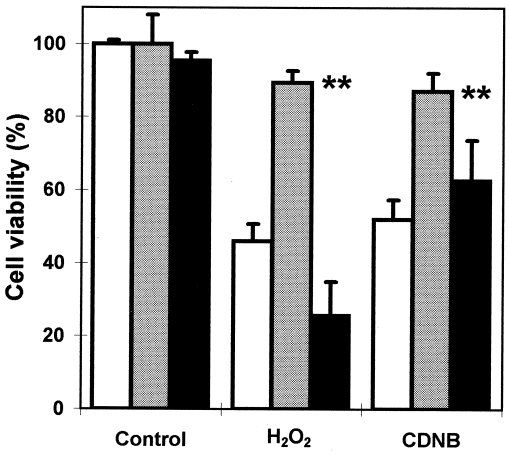

FIG. 3. Effect of aminoguanidine and N_ω-nitroarginine on the cytotoxicity of hydrogen peroxide and CDNB to $P388D_1$ cells *in vitro*. $P388D_1$ cells (2×10^6/mL) were incubated in MGB at 37° for 3 hr without toxicant (control), with 30 mM hydrogen peroxide (H_2O_2), and with 75 μM CDNB. Further additions were: (□) none, (▨) +1 mM aminoguanidine, and (■) +100 μM N_ω-nitroarginine. Data are means ± standard deviation of 3 determinations. **$P < 0.01$ with respect to toxicant control.

concentration of 3-DG in the medium increased, relative to control values, to 200 ± 47 nM after 3 hr ($P < 0.05$) (Fig. 2, panels e and f).

Effect of Aminoguanidine and N_ω-Nitroarginine on the Cytotoxicity of Hydrogen Peroxide and CDNB

The effect of the scavenging of α-oxoaldehydes by aminoguanidine on the cytotoxicity induced by hydrogen peroxide and CDNB was investigated. When $P388D_1$ cells (2×10^6/mL) were incubated for 3 hr with 30 mM hydrogen peroxide and 75 μM CDNB, the cell viability was decreased to 46.0 ± 4.7% and 52.0 ± 5.4%, respectively, relative to control values ($P < 0.001$) (Fig. 3). Aminoguanidine (1 mM) had no significant effect on cell viability in control cultures ($P > 0.05$). When $P388D_1$ cells were incubated for 3 hr with 1 mM aminoguanidine and 30 mM hydrogen peroxide, the cell viability decreased to 89.4 ± 5.0%, and when incubated with 1 mM aminoguanidine and 75 μM CDNB cell viability decreased to 87.1 ± 5%. This indicates that aminoguanidine protected $P388D_1$ cells from the toxic effects of hydrogen peroxide and CDNB ($P < 0.01$). N_ω-Nitroarginine (100 μM) did not significantly affect the decrease in viability of $P388D_1$ cells induced by 30 mM hydrogen peroxide and 75 μM CDNB ($P > 0.05$).

Effect of Aminoguanidine on α-Oxoaldehyde Concentrations during the Prevention of Cytotoxicity Induced by Hydrogen Peroxide and CDNB

The effect of 1 mM aminoguanidine on the cellular and extracellular concentrations of α-oxoaldehydes in $P388D_1$ cell cultures incubated with and without CDNB and hydrogen peroxide was investigated. Aminoguanidine did not change significantly the cellular and extracellular concentrations of α-oxoaldehydes in control cultures ($P > 0.05$) (Fig. 4). In incubations with CDNB, however, aminoguanidine decreased markedly the cellular concentrations of MG and 3-DG ($P < 0.001$), and decreased markedly the extracellular concentrations of glyoxal and MG ($P < 0.001$) and 3-DG ($P < 0.05$) (Fig. 4). In incubations with hydrogen peroxide, aminoguanidine decreased markedly the cellular concentrations of MG ($P < 0.01$) and 3-DG ($P < 0.001$), and decreased the concentration of MG in the medium ($P < 0.001$) (Fig. 4).

DISCUSSION

The cellular concentrations of glyoxal, MG, and 3-DG are expected to be increased in oxidative stress because of the decreased availability of reduced cofactors for the enzymatic detoxification of α-oxoaldehydes in this abnormal physiological state. This may also be reflected in the extracellular concentrations of the α-oxoaldehydes. Glyoxal and MG readily cross cell membranes, probably by passive diffusion of the unhydrated forms. The cytosolic and extracellular concentrations of MG and glyoxal were not equivalent, however, because of a high extent of reversible binding of these α-oxoaldehydes to cysteine, lysine, and arginine residues of cellular peptides and proteins [28]. The method of the α-oxoaldehyde assay used herein determines the sum of α-oxoaldehyde free and reversibly bound to proteins [28, 29]. The mechanism of membrane transport of 3-DG has not been reported, but it probably requires a transporter protein to cross cell membranes.

The glyoxalase system is the major pathway for the detoxification of glyoxal and MG [4], and NADPH-dependent 3-DG reductase (aldehyde reductase and aldose reductase activities) is the major pathway for the detoxification of 3-DG under normal conditions [22]. When GSH is severely depleted, however, aldehyde reductase and 2-oxoaldehyde dehydrogenase may metabolise MG and glyoxal. Similarly, when NADPH is depleted, 3-DG dehydrogenase activity may be important in the metabolism of 3-DG. The extent of accumulation of α-oxoaldehydes will depend on their rate of formation and their reactivities with cellular components and the toxicant. The flux of formation of MG in human red blood cells was *ca.* 120 nmol/mL packed cells/day [30]. Little is known of the flux of formation of glyoxal and 3-DG, except that their formation from glycation is expected to be much lower than this (*ca.* 1–2 μM/day [1–3, 5]; the flux of formation of 3-DG from fructose 3-phosphate may be much higher than this [6]. The reactivities of glyoxal and MG with proteins to form AGEs are similar, but 3-DG is *ca.* 200-fold less reactive [31]: unlike glyoxal and MG, it exists in many cyclic hemiacetal/hemiketal forms that have not yet been fully characterised [32]. There may be a similar relative reactivity of these α-oxoaldehydes with nucleotides [11, 12]. Additionally, the high concentration of hydrogen peroxide in these experiments may interact non-enzymatically with the α-oxoalde-

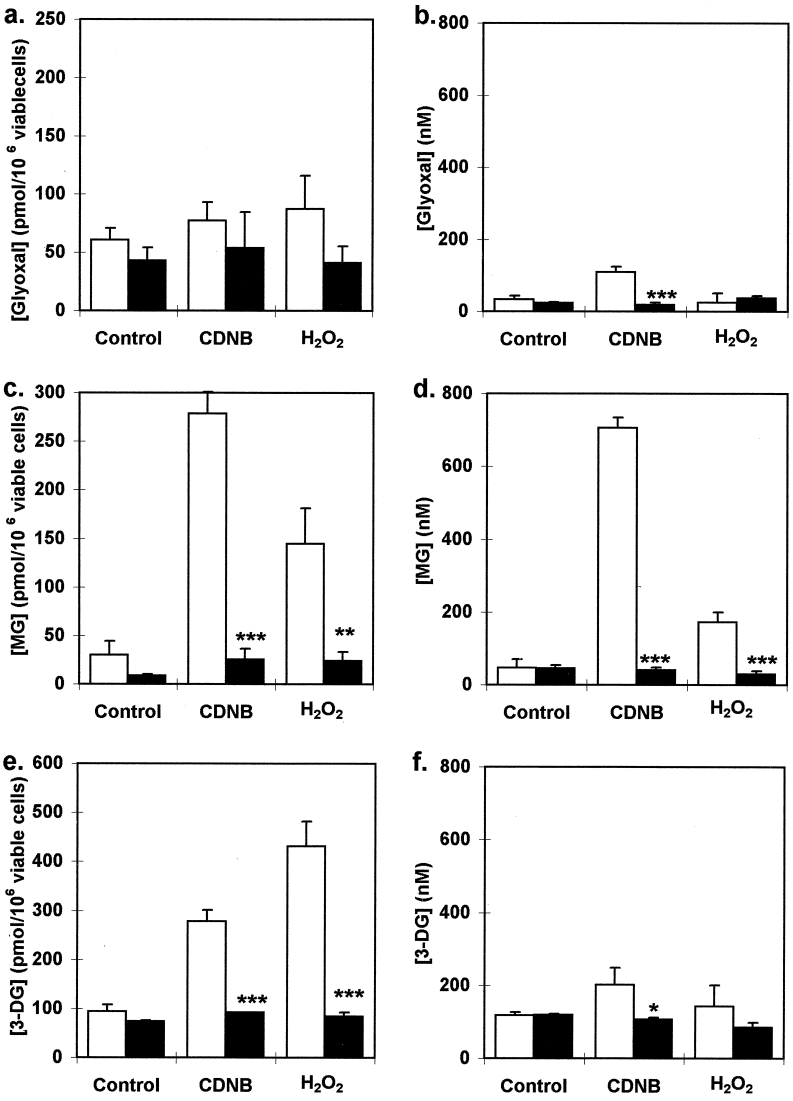

FIG. 4. Effect of aminoguanidine on cellular concentrations (panels a, c, and e) and extracellular medium concentrations (panels b, d, and f) of glyoxal, MG, and 3-DG in P388D$_1$ cell cultures during the prevention by aminoguanidine of hydrogen peroxide- and CDNB-induced cytotoxicity. P388D$_1$ cells were incubated in MGB at 37° for 3 hr without toxicant (control), with 30 mM hydrogen peroxide (H_2O_2), and with 75 μM CDNB. Further additions were: (□) none and (■) +1 mM aminoguanidine. Data are means ± standard deviation of 3 determinations. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, with respect to control.

hydes. Hydrogen peroxide induced oxidative cleavage of glyoxal and 3-DG [33].

Acute toxicity induced by hydrogen peroxide in P388D$_1$ cells is a well-characterised model of oxidant-induced cell death [25] that we have previously employed to investigate the antidote effects of GSH esters [34]. The median toxic concentrations of hydrogen peroxide and CDNB were 24 mM and 63 μM, respectively. There is initial oxidation of GSH and NADPH with concomitant activation of the hexose monophosphate shunt [19, 25]. Decreased GSH and NADPH is expected to decrease the rate of detoxification of glyoxal, MG, and 3-DG *in situ*. This is consistent with our results. There was also increased export of MG and 3-DG into the medium. The extracellular glyoxal concentration decreased in hydrogen peroxide-treated incubations, however, which may reflect non-enzymatic oxidative cleavage. The slower accumulation of glyoxal relative to MG and 3-DG is indicative of a lower flux of glyoxal formation than of MG and 3-DG. The increase in MG concentration may have been further enhanced by inhibition of glyceraldehyde-3-phosphate dehydrogenase, accumulation of triosephosphates, and consequent increased rate of MG formation [35]. Hydrogen peroxide-induced cytotoxicity was shown to induce single strand breaks in DNA with activation of PARP and depletion of cellular levels of NAD^+ of P388D$_1$ cells. A PARP inhibitor prevented cytotoxicity [36]. Modification of DNA was thought to occur by oxidation with hydrogen peroxide but given the effect of aminoguanidine herein, it is now possible that α-oxoaldehyde modification of DNA may also have been involved.

Incubation of P388D$_1$ cells with CDNB depleted GSH but more markedly than with hydrogen peroxide. Cytotoxicity induced by CDNB was characterised by an immediate, marked, and persistent decrease in the cellular concentration of GSH with little formation of GSSG [19]. Depletion of cellular GSH was a critical factor in the development of toxicity [19]. There was an 8-fold increase in the cellular concentration of MG and a 14-fold increase in extracellular concentration of MG in cells incubated with CDNB. There were much smaller increases in the extracellular and extracellular concentrations of glyoxal and a *ca.* 2-fold increase

in the cellular concentration of 3-DG in the third hour when cell viability was decreasing rapidly. The marked increase in cellular α-oxoaldehydes in the third hour may reflect the release of α-oxoaldehydes from protein thiols, as these too become oxidized. α-Oxoaldehydes are reversibly bound to protein thiols in cells *in situ* [28]. This is a major storage site for α-oxoaldehydes, suppressing the formation of irreversible adducts with lysine and arginine residues in periods of high rates of α-oxoaldehyde formation, to later release the free α-oxoaldehyde for metabolism as the enzymes of α-oxoaldehyde detoxification take effect.

The decrease in viability of $P388D_1$ cells by hydrogen peroxide and CDNB observed herein occurs by a necrotic mechanism [19]. The changes in the cellular concentrations of calcium implicated in the mechanism of hydrogen peroxide-induced cell death of $P388D_1$ cells incubated under similar conditions to those described herein may occur by oxidative inactivation of membrane ATPases [37] but also by modification of arginine residues in the nucleotide binding site by α-oxoaldehydes [38].

Aminoguanidine reacted with α-oxoaldehydes to form to isomeric 5- and 6-substituted 3-amino-1,2,4-triazines [39, 40]. The concentration of aminoguanidine that prevented half-maximal irreversible protein modification in human plasma was 203 μM [39]. The prevention of cytotoxicity by scavenging of MG with aminoguanidine in incubations of $P388D_1$ cells with hydrogen peroxide and CDNB suggests that α-oxoaldehyde accumulation may play an important role in the development of cytotoxicity. Aminoguanidine is also an inhibitor of inducible and constitutive nitric oxide synthases [41], but lack of a similar effect of N_ω-nitroarginine, an inhibitor of inducible and constitutive nitric oxide synthases but a poor scavenger of α-oxoaldehydes [41], suggests that the cytoprotective effect of aminoguanidine was not due to inhibition of inducible nitric oxide synthase. Aminoguanidine has also been found to have antioxidant activity [42], which may also contribute to the cytoprotective effect found herein. The kinetics of scavenging of aldehydes formed in lipid peroxidation, such as 4-hydroxynonenal, by aminoguanidine was much slower than those of the scavenging of α-oxoaldehydes [39, 43]. The reported pro-oxidant effect of aminoguanidine [44] was not evident.

The involvement of α-oxoaldehydes in oxidative cytotoxicity is a hitherto little-recognised aspect of mechanisms of cell death. Many studies of cell function in oxidative stress *in vitro* have investigated the effects of decreasing the cellular concentration of GSH (reviewed in [18]) and reported S phase growth arrest of proliferating blood mononuclear cells [45], inhibition of plasma membrane Ca^{2+} translocase [46], and induction of apoptosis and necrosis [47]. Under these experimental conditions, the *in situ* activity of glyoxalase I was inhibited and accumulation of α-oxoaldehydes may have contributed to the observed changes in cell function. The accumulation of glyoxal, MG, and other α-oxoaldehydes in cells may lead to the modification of DNA, giving rise to mutagenesis and apoptosis [11, 12] and modification of proteins which may lead to protein degradation, enzyme inhibition, and a cytokine-mediated immune response [15, 16]. Cellular accumulation of α-oxoaldehydes is expected to contribute to toxicity associated with GSH oxidation and S conjugation in oxidative stress and chemical toxicity [18], and in chronic pathology in diabetes mellitus where the formation of glyoxal, MG, and 3-DG is increased [48]. Increased glycation by α-oxoaldehydes is therefore generally expected to be a consequence of oxidative stress [4].

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