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Protective Effects of Anethole Dithiolethione against Oxidative Stress-induced Cytotoxicity in Human Jurkat T Cells

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ABSTRACT. The protective effects of anethole dithiolethione (ADT) against H_2O_2- or 4-hydroxynonenal (HNE)-induced cytotoxicity in human Jurkat T cells were investigated. Jurkat T cells were pretreated with ADT (10–50 μM) for 18 hr and then challenged with H_2O_2 or HNE for up to 4 hr. Cytotoxicity was assessed by measuring: 1) leakage of lactate dehydrogenase from cells to medium; and 2) exclusion of the DNA intercalating fluorescent probe propidium iodide by viable cells. Pretreatment of cells with ADT (10 or 25 μM) for 18 hr significantly protected cells against H_2O_2- or HNE-induced cytotoxicity. Treatment of cells with ADT (10–50 μM) for 72 hr significantly increased the activities of catalase and glutathione reductase. The maximum effect of ADT treatment on the activity of these enzymes was observed when cells were treated with 25 μM of ADT for 72 hr. A significant increase in cellular GSH was observed in cells that were treated with ADT for 72 hr. Using monobromobimane as a thiol probe, we consistently observed that cells pretreated for 18 hr with ADT (25 or 50 μM) had also increased total thiol content. Exposure of Jurkat T cells to H_2O_2 or HNE resulted in a time-dependent decrease in cellular GSH. ADT (10–50 μM, 18 hr) pretreatment circumvented H_2O_2-dependent lowering of cellular GSH. In conclusion, ADT proved to be a potent cytoprotective thiol antioxidant with multifaceted mechanisms of action, suggesting that the drug has a remarkable therapeutic potential. BIOCHEM PHARMACOL **56**;1:61–69, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. redox; thiol; glutathione; antioxidant; free radical; reactive oxygen species

Oxidation-reduction (redox)-based regulation of signal transduction and gene expression is emerging as a fundamental regulatory mechanism in cell biology [1, 2]. Our current knowledge of redox-regulated signal transduction has led to the unfolding of the remarkable therapeutic potential of cellular thiol-modulating agents. ADT‖ has been used since 1947 as choleretic agent without any clinically adverse effects [3]. Dithiolethiones occur naturally in several edible plant products. Cabbage, brussel sprouts, and other cruciferous vegetables have been found to contain significant amounts of dithiolethiones [4, 5]. One major characteristic property of dithiolethiones is that they may influence the overall cellular redox state by bolstering cell or tissue GSH levels [6–11]. In support of this, we have observed that ADT pretreatment of cells suppresses oxidant-induced activation of the redox-sensitive transcription factor NF-κB in human Wurzburg T cells [11]. Thus, among the several thiol agents tested for their efficacy to modulate cellular redox status, ADT, similar to *N*-acetyl-L-cysteine and α-lipoic acid [12], holds promise for clinical use.

Dithiolethiones have been established as anticarcinogens [13–17], and protect against radiation injury [18] and hepatotoxicity induced by carbon tetrachloride and acetaminophen [18, 19]. All of these pathologies are suspected to have an oxidative stress-based etiology. This suggests that the protective effect of ADT under those conditions may be related to an antioxidant function of the drug. A protective effect of ADT against oxidative lipid damage has been clearly evident [8, 11, 20, 21]. This effect, in combination with the favorable effect of ADT on cellular GSH level, suggests that this thiol-based drug may have potent antioxidant functions [22].

In T lymphocytes, thiols regulate proliferation and programmed cell death. T cell thiol status is compromised in situations such as HIV infection [23–26], and several studies have indicated that restoration of cellular GSH levels by glutathione-replenishing drugs may markedly modulate the effect of inflammatory cytokines and inhibit the stimulation of HIV in both acute and chronic infec-

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‖ *Abbreviations:* ADT, anethole dithiolethione; CDNB, chlorodinitrobenzene; EC, electrochemical; G/GO, glucose/glucose oxidase; HNE, 4-hydroxynonenal; LDH, lactate dehydrogenase; MBB, monobromobimane; and PI, propidium iodide.

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tions [25, 26]. In the present study, we sought to investigate the cytoprotective antioxidant properties of ADT in an *in vitro* experimental system involving Jurkat T cells exposed to: 1) an enzymatic system generating low concentrations of H_2O_2 at a steady rate; or 2) HNE, a lipid peroxidation metabolite. Because of properties such as high diffusibility and a long half-life, the reactive oxygen species H_2O_2 is regarded as a physiological metabolite that triggers oxidative damage. HNE has been identified to be a rather long-living lipid peroxidation metabolite that is able to diffuse from the site of its generation to surrounding tissues [27]. HNE has genotoxic, cytotoxic, and mutagenic activities and produces a multitude of effects such as depletion of cellular GSH, disturbance of intracellular calcium homeostasis, inhibition of DNA, RNA, and protein syntheses, initiation of lipid peroxidation, and inhibition or activation of a number of enzymes and genes [27]. Both H_2O_2 and HNE are considered to be physiological messenger molecules signaling for various cellular responses including inflammation, activation of NF-κB, and apoptosis.

MATERIALS AND METHODS

Cell culture

Cell line and culture conditions. Human Jurkat T cells clone E6-1 (American Type Culture Collection) were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/mL of penicillin and 100 μg/mL of streptomycin, 110 mg/L of sodium pyruvate and 2 mM L-glutamine (University of California, San Francisco, CA). Cells were maintained in a standard culture incubator with humidified air containing 5% CO_2 at 37°.

Cell treatments. ADT (Solvay Pharma, L. T. M. Laboratories) was dissolved in DMSO for treatment to cells. Control samples were treated with the corresponding amount of DMSO. For the treatment of cells with ADT, the 18- and 72-hr time points were chosen because we wanted to have sufficient time to monitor that ADT did not affect cell proliferation. The doubling time of Jurkat T cells in culture is approximately 18 hr. H_2O_2 was generated in a system using GO (1.4 U/mL; Sigma) and additional glucose (5 mM; Fisher Scientific) [28]. HNE (50 μM, Cayman Chemical) was added to the cell culture medium and incubated for time intervals indicated in the respective figures.

For HPLC analyses, cells were pelleted (400 *g* × 5 min), and the pellet and medium were deproteinized separately by treatment with 4% monochloroacetic acid (Fisher Scientific). Following acid treatment, the mixtures were snap-frozen in liquid nitrogen and stored at −80° for HPLC determination of ADT or GSH content in cells and medium. HPLC assay was done within 1 week of storage. Immediately before the assay, samples were thawed, vortexed, and then centrifuged at 15,000 *g* for 2 min. The clear supernatant was used for injection.

HPLC–EC Detection of ADT

ADT was analyzed using a HPLC–EC detection method as described below. A coulometric detector (ESA, Coulochem II) was used.

HPLC settings. The electrodes of the coulometric detector were set as follows: electrode 1, +0.45 V; electrode 2, +0.85 V; and guard cell, +0.90 V. ADT was separated on an Altima C18 (250 × 4.6 mm, 5-μm pore size; Alltech) column. The mobile phase, consisting of 80% methanol and 20% of 0.05 M of monochloroacetic acid, was delivered using an isocratic solvent delivery module (ESA). The flow rate was maintained at 1 mL/min throughout the analysis.

Performance of the detection method. Using cell extracts spiked with a known amount of ADT, the recovery of ADT in the above-mentioned assay was estimated to range between 85 and 90%. Each biological sample was injected several times, and a less than 3% variation of results was noted. The present method is more sensitive compared with the previously reported HPLC method that utilized a UV detection system [29]. Previously, it was described that for the HPLC detection of thiols in biological samples EC detection had several advantages over UV detection [30]. The EC method used in this work utilizes the redox property of ADT for its identification and does not rely only on UV absorbance, which is more likely to include nonspecific substances in biological samples with interfering UV absorbance properties. HPLC chromatograms and calibrations curves of ADT standards are illustrated in Fig. 1, A–E.

HPLC–EC Detection of Cellular GSH

GSH measurements were performed using a HPLC system coupled with an EC coulometric detector (ESA). A C-18 column (150 mm × 4.6 mm, 5-μm pore size; Alltech) was used for GSH separation. GSH levels were expressed as nanomoles per milligram of protein. Protein was determined using the Pierce BCA protein assay kit.

Flow Cytometric Determination of Thiols Using MBB

MBB (Molecular Probes) was dissolved in acetonitrile to obtain an 8-mM stock concentration. MBB (final concentration 40 μM) was loaded into cells as described earlier [31]. MBB-loaded cells were excited using a 20 mW powered UV line of an Innova 90-4 argon ion laser (Co-herent) set at 350 nm in an EPICS Elite (Coulter) flow cytometer. Fluorescent emission from cellular sulfhydryl reacted MBB was recorded using a 450 nm band pass filter as described earlier [31]. A morphometrically homogeneous cell population, typically representing ≈90% of the total population, was gated. Data were collected from at least 10,000 cells at a flow rate of 250–300 cells per sec.

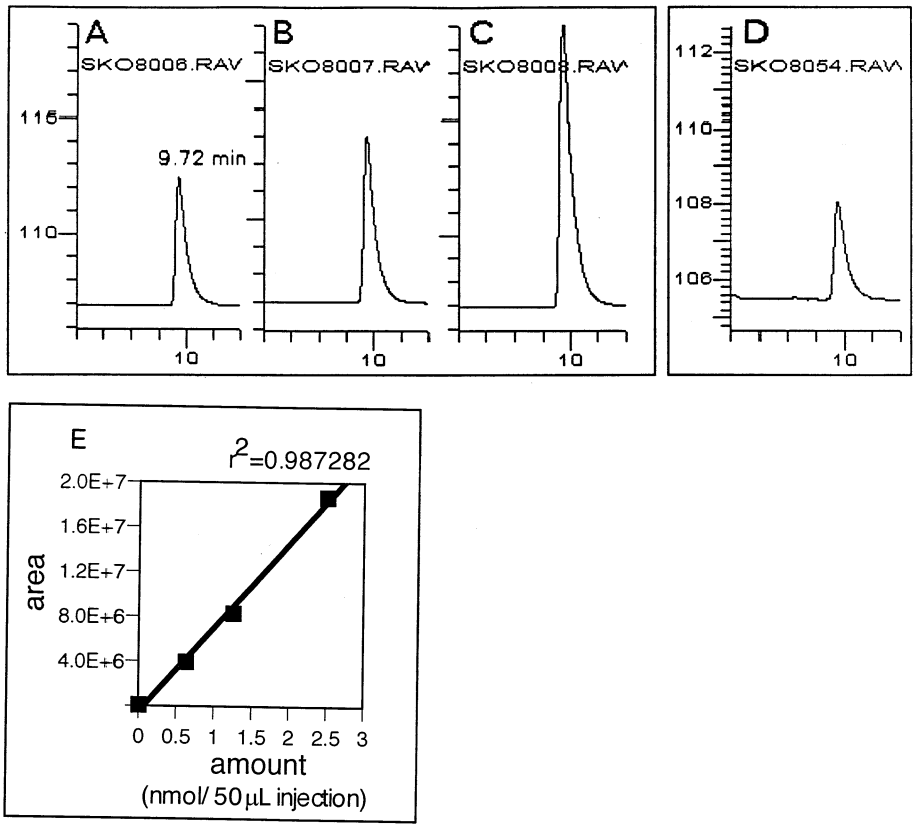

FIG. 1. HPLC-coulometric detection of ADT. Panels A, B, and C represent chromatograms of standard ADT stock solution in methanol, 50 µL of which was injected. The typical retention time was 9.72 min. (A) 0.625 nmol, (B) 1.25 nmol, and (C) 2.5 nmol per injection. Panel D represents the ADT peak detected in an extract of Jurkat T cells treated with 50 µM ADT for 18 hr. The ADT peak was not detected in extracts of ADT nontreated cells (data not shown). Panel E represents the standard curve. For details, see Materials and Methods. The y-axis represents the area under the curve defined by the peak; 2.0E + 7 = 2.0×10^7.

Determination of Cell Viability

LDH RELEASE IN MEDIUM. Cell viability was assayed on the basis of LDH leakage from cells as described by Murphy *et al.* [32]. After each experiment, cells were pelleted (400 $g \times 5$ min). The supernatant was collected and mixed with an equal volume of bovine serum albumin solution [5% (w/v) in PBS] to help stabilize LDH activity in the solution for storage at 4° [33]. LDH activity was measured from the samples spectrophotometrically [34] within 2 days of storage.

CELL VIABILITY ASSAY USING FLOW CYTOMETRY. Cell membrane integrity was also monitored by flow cytometry (EPICS Elite or XL, Coulter) as a measure of cell viability. For this assay, the nonpermeant DNA intercalating dye PI (Molecular Probes) that is generally excluded by viable cells [35] was used. A 15 mW powered argon ion laser was used for excitation at 488 nm, and the emission signal was collected at 575 nm as described earlier [31]. PI negative cells were interpreted as viable cells.

Enzyme Assays

Cells in suspension were centrifuged (200 $g \times 5$ min) at 4°, and the pellet was washed three times with ice-cold PBS (pH 7.4). After washing, the pellet was resuspended in a lysis buffer (PBS + 0.05% Triton X-100). Then the suspension was ultrasonicated in short bursts on ice for 90 sec. The cell lysate was centrifuged at 15,000 *g* for 5 min at 4°. The supernatant was collected and stored in aliquots at −80°. Glutathione reductase activity was assayed spectrophotometrically by monitoring oxidation of NADPH at 340 nm [36]. Glutathione *S*-transferase activity was assayed using CDNB (Sigma) as substrate [37]. Catalase activity was assayed by monitoring the loss of absorbance of H_2O_2 at 240 nm. An extinction coefficient of 39.4 $M^{-1} \cdot cm^{-1}$ was used for the calculation of catalase activity [38]. Protein contents of homogeneous cell suspensions were determined by a Pierce BCA protein assay kit.

Statistical Analyses

Results are presented as means ± SD of at least three separate experiments. Differences between the means of groups were compared by Student's *t*-test and ANOVA with $P < 0.05$ set as the minimum level of significance.

RESULTS

HPLC–EC Detection of ADT from Cells and Medium

ADT content in cells and medium was measured following exposure of Jurkat T cells to 10–50 µM of ADT for 18 hr. Exposure of the cells to higher concentrations of ADT resulted in a higher accumulation of the compound in the cells (Fig. 2). The maximum level of ADT detected in cells was ~5 nmol/mg protein following 50 µM of ADT expo-

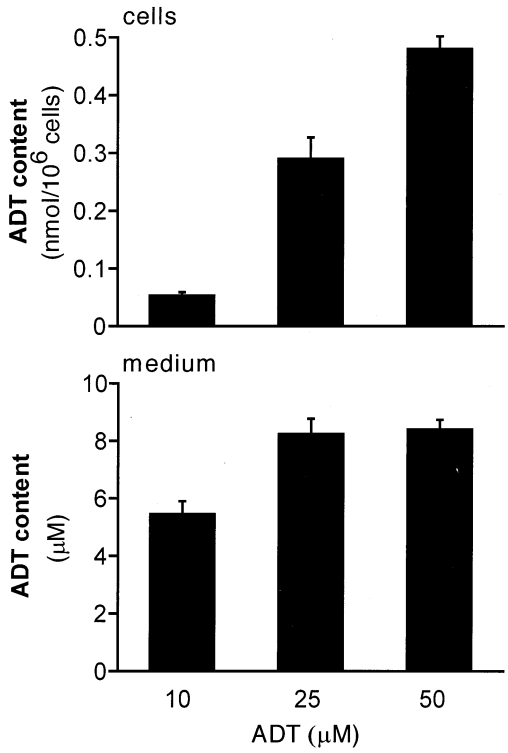

FIG. 2. ADT content in Jurkat T cells and culture medium. Jurkat T cells were treated with different concentrations of ADT for 18 hr. ADT content was measured from cell extracts, and medium by HPLC-coulometric detection. Values represent means ± SD of at least three separate experiments.

sure for 18 hr. Because of the poor solubility of ADT in aqueous medium [11], only up to 10 μM of ADT could be detected in the culture medium to which 25 or 50 μM of ADT was added (Fig. 2).

Time Course of Cytotoxicity in Response to Oxidative Stress

Treatment of Jurkat T cells with G/GO (H_2O_2 generating system) or HNE resulted in a time-dependent decrease in cell viability as measured by LDH release from cells and the PI exclusion assay (see Figs. 3 and 5, A1–A4; for details, see Materials and Methods). Following 4 hr of exposure of cells to oxidants, the release of LDH from cells was higher in cells treated with HNE (50 μM) than in cells treated with G/GO (Figs. 3 and 4). Following 4 hr of HNE treatment, almost all of the cells in the ADT-untreated group were PI positive, suggesting loss of membrane integrity (Fig. 5, A1 vs A4).

Protection of Jurkat T Cells Against H_2O_2 and HNE-induced Cytotoxicity

Pretreatment of cells with ADT (10 or 25 μM) for 18 hr significantly protected cells against H_2O_2- or HNE-induced cytotoxicity (Figs. 4 and 5). In cells that were exposed to

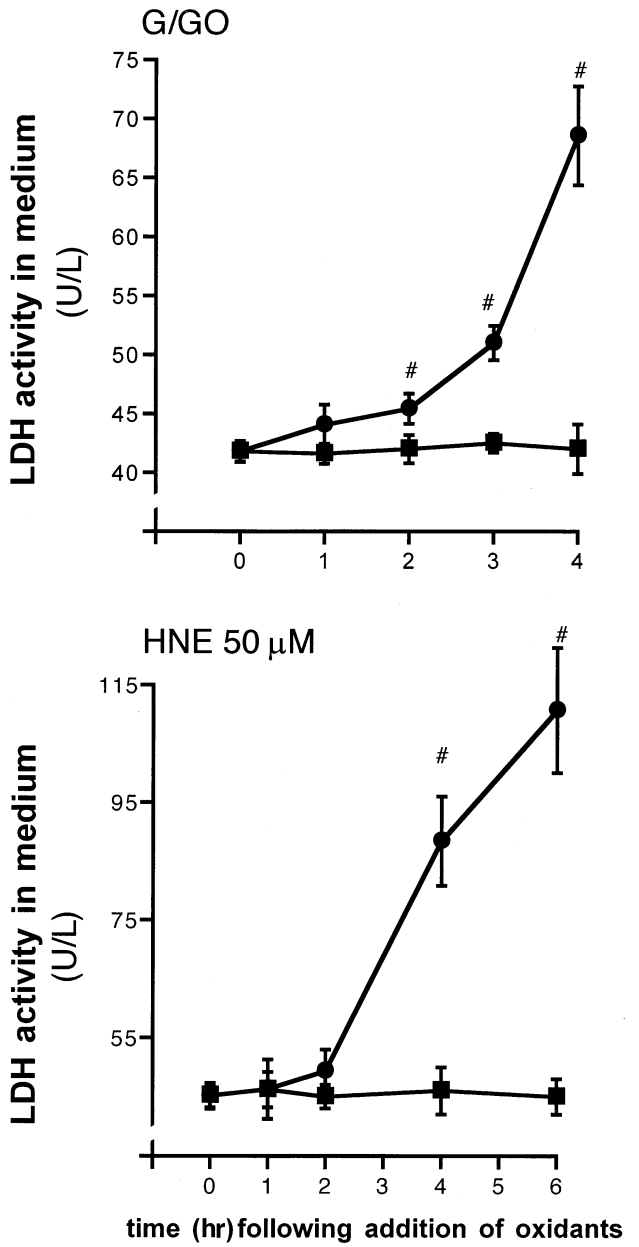

FIG. 3. Kinetics of H_2O_2- and HNE-induced leakage of LDH from Jurkat T cells to the culture medium. Jurkat T cells (1.5×10^6 cells/mL) were exposed to an H_2O_2 generating system (5 mM of glucose + 1.4 U/mL of glucose oxidase, G/GO), or HNE (50 μM). Leakage of LDH from the cells to the medium was measured as an indicator of cell viability. Squares represent controls without G/GO or HNE treatment, and circles represent cells treated with G/GO or HNE. Values represent means ± SD of at least three separate experiments. #$P < 0.01$, compared with G/GO- or HNE-nontreated cells.

H_2O_2, maximum protection against LDH leakage was observed following 25 μM of ADT pretreatment for 18 hr (Fig. 4A). Complete protection against HNE-induced LDH leakage was observed in cells that were treated with 10 μM of ADT for 18 hr (Fig. 4B). ADT pretreatment also protected against HNE-induced loss of membrane integrity as measured by PI exclusion (Fig. 5).

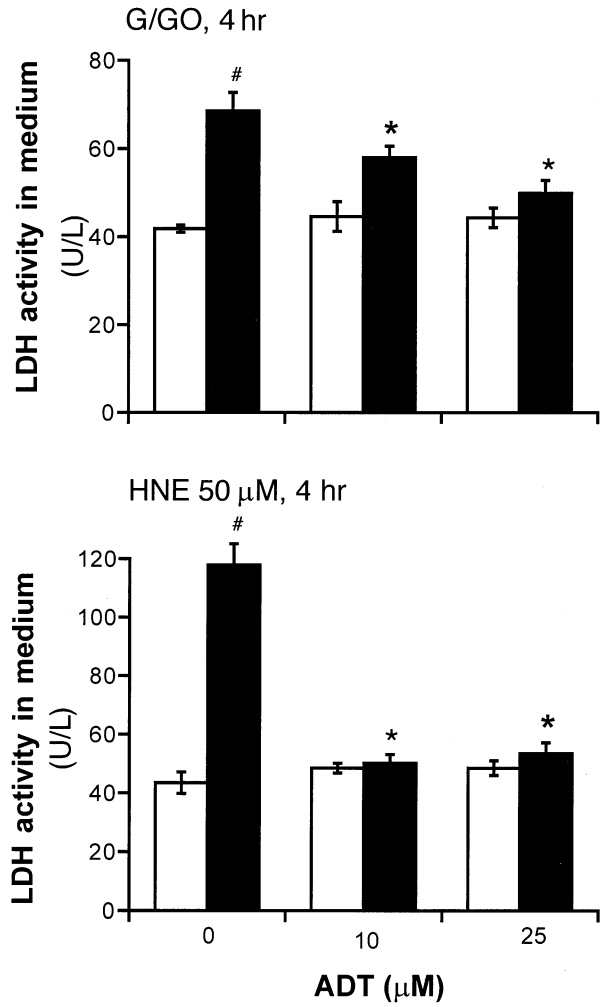

FIG. 4. Protective effect of ADT against H_2O_2- and HNE-induced leakage of LDH. Jurkat T cells (1.5×10^6 cells/mL) were pretreated with ADT for 18 hr and then either not exposed (open bars) to or exposed (closed bars) to an H_2O_2 generating system (5 mM of glucose and 1.4 U/mL of glucose oxidase, G/GO), or HNE (50 μM) for 4 hr. Leakage of LDH induced by G/GO or HNE from the cells to the medium was measured as an indicator of cell viability. Values represent means ± SD of at least three separate experiments. #$P < 0.01$, compared with G/GO- or HNE-untreated cells, and *$P < 0.01$, compared with control ADT-nontreated cells.

Effect of ADT on the Activity of Catalase and GSH-dependent Enzymes in Jurkat T Cells

Treatment of cells with ADT (10–50 μM) for 72 hr significantly increased the activities of catalase and glutathione reductase (Table 1). No effect of ADT exposure on the activities of catalase or glutathione reductase was observed in these cells following 18 hr of exposure (data not shown). Treatment of cells with ADT (10–50 μM) for 18 (data not shown) or 72 hr had no effect on glutathione *S*-transferase activity (Table 1). The cited effects of ADT on antioxidant enzyme activity were significant for catalase (~15%, $P < 0.01$) and glutathione reductase (~25%, $P < 0.01$) in cells pretreated with 10 μM of ADT compared with nontreated cells (Table 1). The maximum effect of ADT treatment on the activity of these enzymes was observed when cells were treated with 25 μM of ADT for 72 hr. With a higher concentration (50 μM) of ADT treatment, no further increase in the activity of these enzymes was noted (Table 1).

Effect of ADT on Cellular GSH and Total Thiol Content in Jurkat T Cells

A significant increase in cellular GSH was observed in cells that were treated with ADT for 72 hr (Fig. 6A). Following 18 hr of ADT (25 μM) treatment, a small (12%) but significant ($P < 0.01$) increase in cell GSH was observed. The increase in cellular GSH was most pronounced (51%) in cells that were treated with 25 μM of ADT for 72 hr. Using MBB as a thiol probe, we consistently observed that cells pretreated for 18 hr with ADT (25 or 50 μM) also had increased total thiol content (Fig. 6B). A maximum increase of 25% in cellular total thiols was observed in cells that were treated with 25 μM of ADT for 18 hr.

Prevention by ADT of Loss of Cellular GSH Following H_2O_2 Treatment

Exposure of Jurkat T cells to H_2O_2 (G/GO system) or HNE (50 μM) resulted in a time-dependent decrease in cellular GSH (data not shown). This loss in cellular GSH was more severe in HNE-treated cells than in G/GO-treated cells. Compared with controls, an approximately 80% decrease in cell GSH was observed within 1 hr of HNE treatment of cells (data not shown). Treatment of cells with G/GO resulted in a 39% decrease in cellular GSH compared with the nontreated controls (Fig. 7). ADT (10–50 μM, 18 hr) pretreatment circumvented H_2O_2-dependent lowering of cellular GSH (Fig. 7).

DISCUSSION

ADT added to the cell culture medium was taken up by cells in a concentration-dependent manner, although solubility of the compound in the culture medium reached maximum at 25 μM. This is consistent with our previous report that ADT crystallizes when added to the cell culture medium at 100 μM [11]. Based on these observations, it may be thought that the undissolved ADT in the culture medium may serve as a reservoir from which ADT is slowly solubilized in the culture medium as the compound is taken up by the cells.

Treatment of Jurkat T cells with H_2O_2 or HNE caused remarkable cell damage, as indicated by LDH leakage from cells to the medium and disruption of cell membrane integrity as shown by the PI exclusion assay. Both H_2O_2 and HNE are highly reactive molecules that have been reported to be cytotoxic [28, 39]. We reported previously that ADT is a potent inhibitor of lipid peroxidation and oxidant-induced NF-κB activation [11]. Results of this

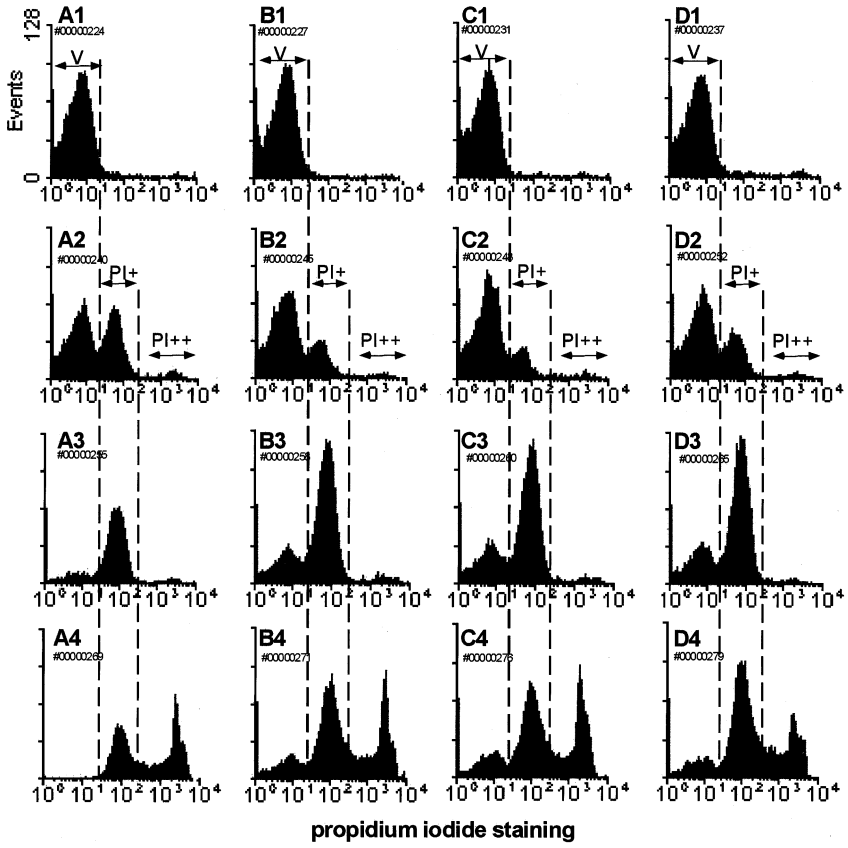

FIG. 5. Protective effect of ADT against HNE-induced loss of membrane integrity. Jurkat T cells (1.5 × 10^6 cells/mL) were pretreated with ADT for 18 hr and then exposed to HNE (50 μM) for 4 hr. Loss of membrane integrity was measured by a PI assay as described in Materials and Methods. Key: A1, control; A2, control + HNE, 1 hr; A3, control + HNE, 2 hr; A4, control + HNE, 4 hr; B1, ADT (25 μM); B2, ADT (25 μM) + HNE, 1 hr; B3, ADT (25 μM) + HNE, 2 hr; B4, ADT (25 μM) + HNE, 4 hr; C1, ADT (50 μM); C2, ADT (50 μM) + HNE, 1 hr; C3, ADT (50 μM) + HNE, 2 hr; C4, ADT (50 μM) + HNE, 4 hr; D1, ADT (100 μM); D2, ADT (100 μM) + HNE, 1 hr; D3, ADT (100 μM) + HNE, 2 hr; D4, ADT (100 μM) + HNE, 4 hr; V, viable cells; PI+, PI positive cells with loss of membrane integrity; PI++, strongly PI positive cells in which marked loss of membrane integrity has led to intense intercalation of PI with exposed DNA. ADT treatment was not cytotoxic (A1–D1). In the 1-hr time-point (A2–D2), the PI+ cell population was clearly smaller compared with the corresponding control HNE-treated cells. After 2 hr (A3–D3) or 4 hr (A4–D4) of HNE treatment, almost no viable cells were detected. In HNE-treated cells that were pretreated with ADT, however, a distinct viable cell population was noted in both the 2- and 4-hr time points.

study further demonstrate the potent cytoprotective properties of ADT in two experimental systems involving two inducers of oxidative stress, H_2O_2 and HNE. Oxidative damage of cell lipids may disrupt the organization of cellular membranes. Inhibition of lipid peroxidation by ADT may be one mechanism by which it protects against cytotoxicity induced by oxidative stress situations [11, 20, 21].

Up-regulation of cellular GSH and total thiol levels may be considered as one major mechanism that accounts for the cytoprotective effects of ADT observed in this study.

TABLE 1. Effect of ADT treatment on the activity of catalase and GSH-dependent enzymes in human Jurkat T cells*

Enzyme	Nontreated control	ADT (μM)		
		10	25	50
Catalase (U mg protein^{-1})	9.69 ± 0.22	10.92 ± 0.28† (13)	11.51 ± 0.22‡ (19)	11.58 ± 0.36‡ (20)
Glutathione reductase (nmol · min^{-1} · mg protein^{-1})	68.74 ± 4.85	84.38 ± 1.75† (23)	91.10 ± 3.42‡ (33)	87.04 ± 3.85† (28)
Glutathione S-transferase (nmol · min^{-1} · mg protein^{-1})	392.81 ± 25.94	378.92 ± 24.38	371.01 ± 20.39	363.00 ± 11.92

*Enzyme activity was measured following 72 hr of ADT treatment. Data are means ± SD of three separate experiments. Values in parentheses are percent increase compared with the corresponding untreated control.

†‡Significantly different from respective nontreated controls: †$P < 0.01$ and ‡$P < 0.001$.

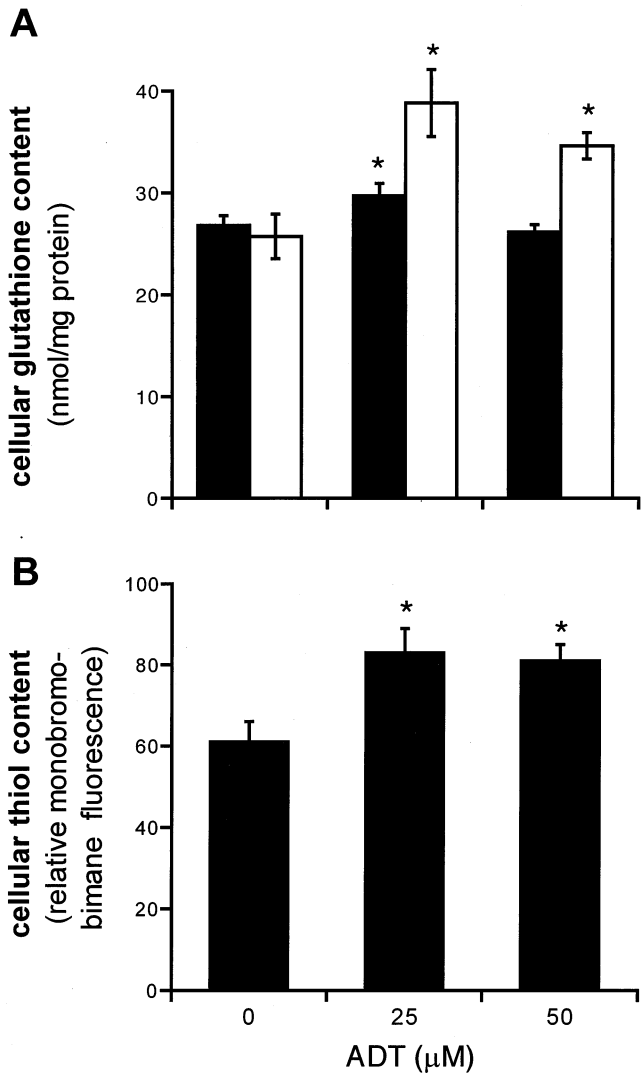

FIG. 6. Regulation of cellular GSH and total thiol content by ADT. Jurkat T cells (1.5×10^6 cells/mL) were pretreated with the indicated concentrations of ADT for 18 hr (solid bars) or 72 hr (open bars). (A) Cellular GSH content was measured from acidified cell extract by HPLC-coulometric detection. (B) Total cellular thiol content was determined after 18-hr pretreatment of ADT by flow cytometry using MBB as the reduced thiol probe. Values represent means ± SD, N = 3. *P < 0.01 compared with control ADT-nontreated cells.

Previously, we and others have consistently observed that ADT increases the GSH content of various cells [6, 11]. It has been suggested that ADT increases cell GSH by stimulating GSH synthesis and not by decreasing catabolism of the thiol [40]. GSH plays an important role in the circumvention of cellular oxidative stress and the maintenance of intracellular thiol redox status [2, 41, 42]. GSH contributes to antioxidant defense by interacting with other major antioxidants such as vitamins E and C [43]. A decreased level of cellular GSH has been observed to be associated with a number of pathologies including diabetes, liver cirrhosis, drug poisoning, age-related disorders, and AIDS [12]. Correction of T cell thiol status appears to be of central importance in the treatment of AIDS [12, 31].

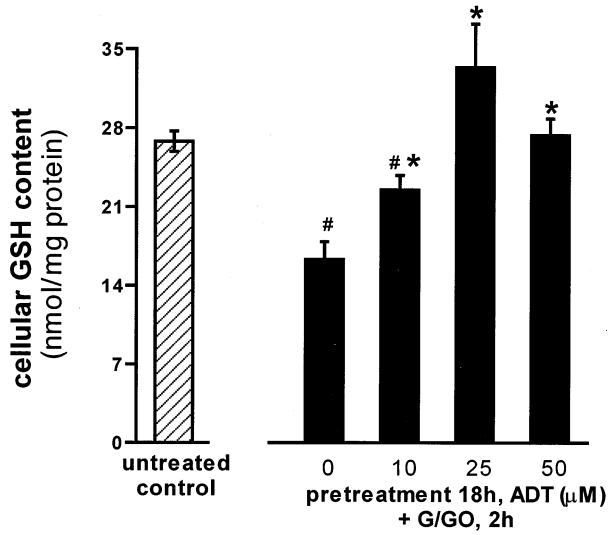

FIG. 7. Sparing of H_2O_2-induced loss of cellular GSH by ADT. Jurkat T cells were pretreated with the indicated concentrations of ADT for 18 hr and then exposed to an H_2O_2 generating system (5 mM of glucose + 1.4 U/mL of glucose oxidase, G/GO) for 2 hr. Cell GSH content was measured from acidified cell extracts by HPLC-coulometric detection as described in Materials and Methods. Values represent means ± SD of at least three separate experiments. #P < 0.01 compared with the untreated control, and *P < 0.01 compared with G/GO-treated ADT-nontreated cells.

Another major effect of ADT that was observed in this study was the sparing of oxidative stress-induced loss of intracellular GSH. Both H_2O_2 and HNE exposure to cells resulted in a severe loss of cellular GSH. H_2O_2-induced loss of cellular GSH is likely to be because of oxidation of GSH to glutathione disulfide [44]. However, HNE-induced loss in cellular GSH has been reported to be primarily because of an HNE–GSH adduct formation [39]. ADT pretreatment completely prevented H_2O_2-induced loss of cellular GSH, suggesting that ADT may have directly detoxified H_2O_2 or rapidly stimulated GSH synthesis or recycling of GSSG to compensate for the oxidation in an oxidative stress situation. The former possibility may be considered negligible because it has been evident previously that ADT is unable to directly detoxify H_2O_2 [40]. Apart from increasing cell GSH levels, the cytoprotective effects of ADT observed in this study may be explained by the effect of the drug on strengthening enzymatic antioxidant defenses in the cell. ADT significantly increased the activities of catalase and glutathione reductase in Jurkat T cells. Both of these enzymes are major components of cellular antioxidant defenses that contribute to peroxide decomposition and recycling of GSSG to GSH, respectively. Acute and subchronic administrations of dithiolethiones are known to increase the activity of several enzymes such as glutathione *S*-transferase [6, 13, 19], glutathione reductase, and quinone reductase [6]. In our experimental system, however, we did not observe any significant effect of ADT treatment on glutathione *S*-transferase activity.

Results from studies in mice show that intraperitoneal

treatment of ADT results in a concentration of over 50 μM conjugated desmethylADT and over 25 μM ADT in the plasma [45]. Among the three concentrations (10, 25, and 50 μM) of ADT studied, almost maximum effect of ADT was observed already at 25 μM and no concentration-dependent protective effect was observed. This suggests that low amounts of ADT in the cell, as obtained following exposure to a 25 μM concentration of the compound, were sufficient to provide maximal beneficial effect. ADT has been shown previously to be safe for clinical use. In this study, ADT showed cytoprotective effects against direct oxidant (H_2O_2) challenge as well as against HNE, a deleterious by-product of lipid peroxidation. A distinct pro-GSH effect of ADT was evident in human Jurkat T cells. In addition, ADT increased cellular total reduced thiol status and the activity of antioxidant enzymes. In conclusion, consistent with previous studies [46], ADT proved to be a potent cytoprotective thiol antioxidant with multifaceted mechanisms of action that suggest a remarkable therapeutic potential of the drug.

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