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Antioxidant Defense Mechanisms of Endothelial Cells and Renal Tubular Epithelial Cells *In Vitro*: Role of the Glutathione Redox Cycle and Catalase[1]

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ABSTRACT. We recently demonstrated that endothelial cells are more susceptible than renal tubular epithelial cells to oxidant injury and that renal tubular epithelial cells with proximal tubular characteristics including porcine proximal tubular epithelial cells, opossum kidney proximal tubular epithelial cells, and normal human kidney cortical epithelial cells are more susceptible to oxidant injury than the distal nephron-derived Madin Darby canine kidney cell line. To determine the basis of this differential response, we evaluated several antioxidant defenses in the five cell lines. Glutathione levels were not significantly different among the five cell lines, but catalase and glutathione reductase levels were significantly ($p < 0.01$) lower in endothelial cells compared to all renal tubular epithelial cells. Among renal tubular epithelial cells, Madin Darby canine kidney cells had significantly ($p < 0.05$) higher glutathione peroxidase activity. To further evaluate the role of antioxidant defenses in limiting oxidant injury, we determined two responses to oxidant injury (ATP depletion and ^{51}Cr release) when glutathione was depleted with buthionine sulfoxamine and when catalase was inhibited with aminotriazole. Oxidant-induced ATP depletion was accentuated when catalase was inhibited as well as when glutathione was depleted with buthionine sulfoxamine. In contrast, inhibition of catalase had little or no effect on ^{51}Cr release, whereas glutathione depletion resulted in accentuated ^{51}Cr release. We conclude that the increased susceptibility of endothelial cells to oxidant injury as compared with epithelial cells is associated with lower antioxidant defenses. Disruption of the glutathione redox cycle results in accentuated ATP depletion and lytic injury, whereas inhibition of catalase results in accentuated ATP depletion with little effect on lytic injury. Augmented oxidant-induced ATP depletion without augmented cell lysis suggests that ATP depletion alone may not be a critical mediator of cell death in oxidant stress. (*Pediatr Res* 32: 360-365, 1992)

Abbreviations

BSO, buthionine sulfoxamine
LLC-PK1, porcine proximal tubular epithelial cells
MDCK, Madin Darby canine kidney cells
NHK-C, normal human kidney cortical epithelial cells
o-DD, *o*-dianisidine-dihydrochloride
OK, opossum kidney proximal tubular epithelial cells
DMEM, Dulbecco's Modified Eagle Medium
HBSS, Hanks' balanced salt solution

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Reactive oxygen molecules have been shown to participate in the pathogenesis of several renal diseases including inflammatory lesions, ischemic-reperfusion injury, toxic nephropathies, and in renal infections such as pyelonephritis (1-8). Reactive oxygen molecules arise from several sources including activated polymorphonuclear cells, monocytes, and mesangial cells, during the metabolism of exogenous drugs and toxins, and during normal and abnormal metabolic processes. When oxidant generation increases, or when antioxidant defense mechanisms are decreased, oxidant injury results from the shift in the oxidant/antioxidant balance. Oxidant stress may induce injury to proteins by oxidation of critical amino acids, may disrupt membrane integrity through lipid peroxidation, may degrade basement membranes, and may induce DNA damage by induction of strand breaks (9). When cells and organs are exposed to oxidant stress, several different antioxidant defense mechanisms exist to prevent or limit the extent of injury.

Little is known about the response of various cells within the kidney to oxidant stress. In some renal diseases, endothelial cells may be the primary target of oxidant injury, whereas renal tubular epithelial cells may be the major target of injury in other pathologic processes. We recently studied the response of human endothelial cells and four renal tubular epithelial cell lines including NHK-C (human proximal tubules), LLC-PK1 (porcine proximal tubules), OK (opossum proximal tubules), and MDCK (canine distal tubules) to oxidant stress generated with hypoxanthine-xanthine oxidase (10). In that study, we found that NHK-C, LLC-PK1, and OK cells were more easily damaged by reactive oxygen molecules than MDCK cells, whereas endothelial cells were more susceptible to oxidant injury than each of the renal tubular epithelial cell lines. Hydrogen peroxide was the reactive oxygen molecule responsible for ATP depletion and efflux of ^{3}H-adenine metabolites, whereas hydrogen peroxide and an iron-dependent radical derived from hydrogen peroxide were responsible for cell lysis and cell detachment. Superoxide dismutase had no effect in preventing oxidant-induced ATP depletion, efflux of ATP metabolites, cell detachment, or cell lysis (10).

In this study, we investigated the basis for the differential response of the five cell lines to oxidant stress and we determined the role of the glutathione redox cycle and catalase in protecting the cells from oxidant-induced ATP depletion and lytic injury. Because we previously found that hydrogen peroxide played a central role in mediating oxidant-induced endothelial and epi-

thelial cell injury, we specifically examined the activity of enzymes important in metabolizing hydrogen peroxide including the glutathione redox cycle and catalase. We found that the mechanism of increased susceptibility of endothelial cells to oxidant injury is associated with lower activities of catalase, glutathione reductase, and glutathione peroxidase, which are important in detoxifying reactive oxygen molecules. In addition, we demonstrate that the glutathione redox cycle is important in maintaining cell integrity, whereas catalase as well as the glutathione redox cycle is important in preventing oxidant-induced ATP depletion. Interestingly, we found that augmented oxidant-induced ATP depletion is not associated with augmented lytic injury, suggesting that ATP depletion alone may not be responsible for oxidant-induced cell death.

MATERIALS AND METHODS

Cell culture. Renal tubular epithelial cell lines LLC-PK1 (CRL 139) and MDCK (CCL 34) were obtained from the American Type Culture Collection (Rockville, MD) (11, 12). OK cells were provided by Professor H. Murer, Zurich, Switzerland. The MDCK, LLC-PK1, and OK cell lines are multiple-passage cell lines that have been extensively characterized and are widely used as models of proximal (LLC-PK1 and OK) and distal (MDKC) tubular epithelial cell lines. NHK-C cells were derived from fragments of human kidney cortex by progressive enzymatic dissociation; this cell line has recently been characterized as proximal tubular-like and has a limited life-span of approximately five to seven passages (13). Tubular epithelial cells were grown in DMEM and nutrient medium F12 (1:1) (JRH Biosciences, Lenexa, KS) supplemented with 100 U/mL penicillin, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, and 10% FCS (Sterile Systems, Logan, UT) at 37°C in a humidified atmosphere containing 5% CO_2 in air. Human endothelial cells were obtained from umbilical veins by collagenase digestion and identified as previously described; these cells have a limited *in vitro* life-span of approximately four to six passages (10, 14). For optimal growth, endothelial cells were first cultured in M199. For experimental studies, all cell lines including endothelial cells were subcultured in 24-well culture plates in DMEM/F12; experiments were performed 3–4 d later. Ml99 and DMEM/F12 each contain sufficient elemental iron for catalase activity (l.78 and 1.62 μM elemental iron, respectively). For each cell line, studies were performed on cultures over a range of no more than five passages.

Biochemical assays. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO), unless otherwise stated. Total glutathione levels were determined as previously described by the method of Tietze (15, 16). The cell monolayers were washed twice in HBSS (GIBCO, Grand Island, NY), then solubilized with 300 μL of 0.5% Triton X-100. Glutathione levels were determined at 37°C with 100 μL of sample or standard, 20 μL of 25 U/mL glutathione reductase, 800 μL of 0.3 mM NADPH in 125 mM sodium phosphate buffer with 6.3 mM EDTA, pH 7.5, and 100 μL of 6 mM 5'-5'-dithiobis-2-nitrobenzoic acid. The change in OD was measured at 412 nm and monitored continuously on a Pye-Unicam spectrophotometer (Unicam Instruments, Cambridge, England). The glutathione content was quantitated by comparison with a standard curve generated with known amounts of glutathione. Glutathione peroxidase and glutathione reductase activities were determined by oxidation of NADPH (17). Briefly, endothelial and epithelial cells in T25 flasks were washed free of media, dissolved in 600 μL cold 0.5% Triton, and scraped off the flask. For glutathione peroxidase activity, 100 μL and 200 μL of sample or standard were added to 50 mM sodium phosphate buffer containing 0.6 mM sodium azide, 0.25 mM glutathione, 0.125 mM NADPH, 0.4 units glutathione reductase, and 0.073 mM hydrogen peroxide. The decrease in OD was monitored continuously at 366 nm at 37°C. Glutathione peroxidase activity was quantitated by comparison with a standard curve generated with known amounts of glutathione peroxidase. Glutathione reductase was determined with 50 and 100 μL of sample or standard with 100 mM sodium phosphate buffer containing 1.3 mM glutathione and 0.1 mM NADPH. The change in OD was measured at 366 nm at 37°C. Glutathione reductase was quantitated with a standard curve generated with known amounts of glutathione reductase. Catalase was determined by metabolism of hydrogen peroxide with a spectrophotometric assay using o-DD (18). Briefly, endothelial and epithelial cells were washed with HBSS then solubilized with 0.5% Triton X-100. Twenty-five to 100 μL of sample or standard were incubated for 10 min at 25°C with 400 μL of 0.6 mM hydrogen peroxide. After 10 min, 100 μL of 6 mM sodium azide were added. The amount of hydrogen peroxide remaining was quantitated with 0.25 mM o-DD and 10 U horseradish peroxidase. The change in OD was determined at 470 nm. Catalase was quantitated with a standard curve generated with known amounts of catalase. Protein content was determined on a portion of the cell sample (19); glutathione, glutathione peroxidase, glutathione reductase, catalase, and ATP levels were each normalized per mg of cell protein.

Hydrogen peroxide metabolism. Hydrogen peroxide degradation was determined by incubating monolayers in 24-well plates with 400 nmol of hydrogen peroxide in 400 μL of HBSS containing 1% albumin. The degradation of hydrogen peroxide was determined at 37°C for 15, 30, 45, 60, 75, and 90 min. The amount of hydrogen peroxide remaining at each time was determined by reaction with o-DD as previously described (10, 18). The time required for disappearance of half the hydrogen peroxide was calculated to determine the half life.

Inhibition studies. To investigate the relative role of the glutathione redox cycle and catalase in preventing oxidant-induced ATP depletion and lytic injury, we determined ATP depletion and ^{51}Cr release in cells exposed to oxidant stress when glutathione was depleted with BSO and when catalase was inhibited with aminotriazole. BSO results in glutathione depletion through inhibition of γ-glutamylcysteine synthetase, whereas aminotriazole is a specific inhibitor of catalase by combining with compound I (hydrogen peroxide complexed with catalase) (20, 21). Endothelial and epithelial cells were preincubated overnight with 2.5 mM BSO. For the studies using aminotriazole, endothelial cells were preincubated for 30 min with 1 mg/mL aminotriazole and renal epithelial cells were preincubated with 2.5 mg/mL aminotriazole; BSO and aminotriazole were also included when cells were exposed to the reactive oxygen molecule generating system. Because of the variable susceptibility of the cells to oxidant stress, endothelial cells were exposed to 12.5 mU/mL xanthine oxidase (Boehringer Mannheim, Indianapolis, IN), NHK-C and LLC-PK1 cells were exposed to 25 mU/mL xanthine oxidase and MDCK cells and OK cells were exposed to 50 mU/mL xanthine oxidase. Soybean trypsin inhibitor, 1.0 mg/mL, was included with the xanthine oxidase to inhibit the effects of contaminating protease enzymes (22).

ATP levels. ATP levels were measured on replicate cultures of endothelial and epithelial cells with luciferin-luciferase as an early response to oxidant injury as previously described (10, 23–25). After a 1-h exposure to the oxygen radical generating system, cells were solubilized with 500 μL 0.5% Triton and acidified with 100 μL 0.6 M perchloric acid and placed on ice until assayed. At the time of assay, the cell suspension was diluted with 10 mM potassium phosphate buffer containing 4 mM $MgSO_4$ (pH 7.4); 500 μL of this were added to 1 mL of 50 mM sodium arsenate buffer containing 20 mM $MgSO_4$ (pH 7.4) to which 25 μL of 40 mg/mL luciferin-luciferase was added. Light emission was recorded precisely at 20 s with a Packard beta counter accepting signals out of coincidence (23). Protein content was determined on a portion of the cell sample and ATP was expressed as pmol/μg cell protein (19).

^{51}Cr release. Late, lytic injury and cell detachment were determined by ^{51}Cr release in cells that were radiolabeled with 2.5

Table 1. *Glutathione levels and activity of glutathione peroxidase (GPX), glutathione reductase (GR), and catalase in each cell type**

Cell line	Glutathione (nmol/mg protein)	GPX (mU/mg protein)	GR (mU/mg protein)	Catalase (U/mg protein)
LLC-PK1	21.9 ± 7.1	13.0 ± 2.2	18.8 ± 1.8†	29.2 ± 0.8‡
MDCK	25.5 ± 8.9	21.8 ± 2.9‡	11.0 ± 2.9	31.3 ± 2.1‡
NHK-C	20.2 ± 1.6	16.4 ± 2.3	9.3 ± 2.1	8.1 ± 0.5‡
OK	28.3 ± 5.9	16.1 ± 2.9	11.1 ± 1.0	15.2 ± 3.4‡
EC	26.6 ± 5.1	12.1 ± 2.7§	5.2 ± 1.8‖	3.5 ± 1.0‖

* Values represent the mean ± 1 SD of four to six separate cohorts of cells. EC, human umbilical vein endothelial cells.
† $p < 0.05$ compared with MDCK, OK, and NHK-C.
‡ $p < 0.05$ compared with all other cells.
§ $p < 0.05$ compared with MDCK, NHK-C, and OK cells.
‖ $p < 0.001$ compared with all other cells.

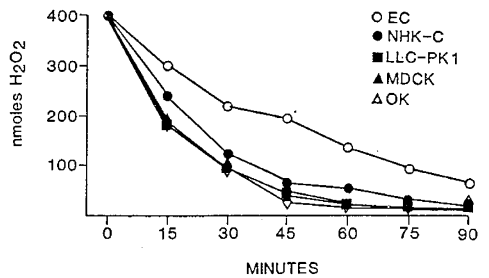

Fig. 1. The consumption of hydrogen peroxide in LLC-PK1, OK, NHK-C, MDCK, and human umbilical vein endothelial cell (*EC*) monolayers in 24-well plates when incubated with 400 nmol of hydrogen peroxide in 400 μL HBSS + 1% albumin. Metabolism of hydrogen peroxide was significantly ($p < 0.01$) slower in endothelial cells compared with epithelial cells. Among renal tubular epithelial cells, NHK-C cells metabolized hydrogen peroxide at a slightly but significantly ($p < 0.01$) slower rate. Values represent the mean of four replicates; the SD of each value is less than 7.5.

Table 2. *Glutathione levels and catalase activity in cells exposed to BSO or aminotriazole**

Cell line	Glutathione in cells exposed to BSO [nmol/mg protein (% control)]	Catalase in cells exposed to Aminotriazole [U/mg protein (% control)]
LLC-PK1	2.3 ± 1.2 (10.5%)	2.9 ± 2.5 (9.9%)
MDCK	4.5 ± 3.9 (17.6%)	2.1 ± 1.7 (7.2%)
NHK-C	5.4 ± 1.8 (26.7%)	1.2 ± 0.9 (14.8%)
OK	3.2 ± 1.3 (11.3%)	2.6 ± 2.1 (17.1%)
EC	6.7 ± 3.9 (25.1%)	0.7 ± 0.4 (20.0%)

* Cells were incubated in 2.5 mM BSO overnight or in aminotriazole (1.0 mg/mL for endothelial cells and 2.5 mg/mL for other cells) for 30 min with 500 μM hydrogen peroxide (aminotriazole inactivates catalase only in the presence of hydrogen peroxide). Glutathione levels and catalase activity were each significantly ($p < 0.01$) lower than levels in cells not exposed to BSO and aminotriazole (Table 1). The % reduction compared with control cells is also provided.

μCi/mL (1.0 μCi/well) ^{51}Cr (New England Nuclear, Boston, MA) in media overnight as previously described (10, 24). Release of ^{51}Cr may be cell associated due to detachment of cells from the culture plate in response to oxidant stress or it may represent lytic injury with release of intracellular ^{51}Cr into the media (10, 26). To differentiate these two responses to oxidant injury, the media and wash portion of ^{51}Cr-labeled cells were centrifuged for 3 min, the supernatant was removed, and the supernatant and precipitate fractions were counted separately. The remaining intracellular label in cells that remained adherent to the culture plate was released with 2.0% Triton-X100; each fraction was counted in a Beckman gamma counter for 1 min. Cell detachment, lytic injury, and total ^{51}Cr release were calculated as previously described (10); cell-associated ^{51}Cr release (representing detachment of cells from the culture plate) was calculated as cpm in the precipitate fraction/cpm in the precipitate fraction + cpm in the cell fraction that remained adherent to the culture plate, whereas release of ^{51}Cr representing lytic injury was calculated as cpm in the supernatant fraction/cpm in the supernatant fraction + cpm in the cell fraction that remained adherent to the culture plate. The sum of the release of the precipitate and supernatant fractions was the total ^{51}Cr release. Release of ^{51}Cr was determined 5 h after oxidant injury as previously described (10).

Statistical analysis. Data are presented as mean ± 1 SD. Analysis of variance with Student-Newman-Keuls multiple range test was used to detect differences in the response to oxidant stress in the presence and absence of BSO and aminotriazole, in detecting differences in the metabolism of hydrogen peroxide, and in detecting differences in glutathione levels, glutathione peroxidase, glutathione reductase, and catalase activity after log transformation to stabilize the variance.

RESULTS

Glutathione levels, glutathione peroxidase, glutathione reductase, and catalase activity. Glutathione levels, glutathione peroxidase, glutathione reductase, and catalase activity were measured in four to six different cohorts of endothelial and epithelial cells (Table 1). Among all cell lines, glutathione levels were not significantly different, ranging from 20.2 ± 1.6 nmol/mg protein for NHK-C cells to 28.3 ± 5.9 nmol/mg protein for OK cells. In contrast to minor differences in glutathione levels, the activity of catalase and glutathione reductase were each significantly ($p < 0.001$) lower in endothelial cells compared with the four tubular epithelial cell lines. In addition, the activity of glutathione peroxidase was significantly ($p < 0.05$) lower in endothelial cells compared with all tubular epithelial cells except LLC-PK1 cells. Endothelial cell catalase activity was 3.5 ± 1.0 U/mg protein compared with greater than 8.1 ± 0.5 U/mg protein for each of the tubular epithelial cells, representing a more than 2-fold difference when compared with NHK-C cells and nearly a 9-fold difference when compared with LLC-PK1 and MDCK cells (Table 1). Among renal tubular epithelial cells, glutathione peroxidase activity was significantly ($p < 0.05$) higher in MDCK cells, whereas LLC-PK1 cells had the lowest activity of glutathione peroxidase and the highest activity of glutathione reductase. Catalase activity was significantly ($p < 0.05$) higher in MDCK cells compared with all others except LLC-PK1 cells.

Metabolism of exogenous hydrogen peroxide. To confirm the role of decreased antioxidant enzymes in the increased susceptibility of endothelial cells to oxidant stress, we determined the rate of metabolism of exogenous hydrogen peroxide in the five cell lines. The consumption of exogenous hydrogen peroxide was

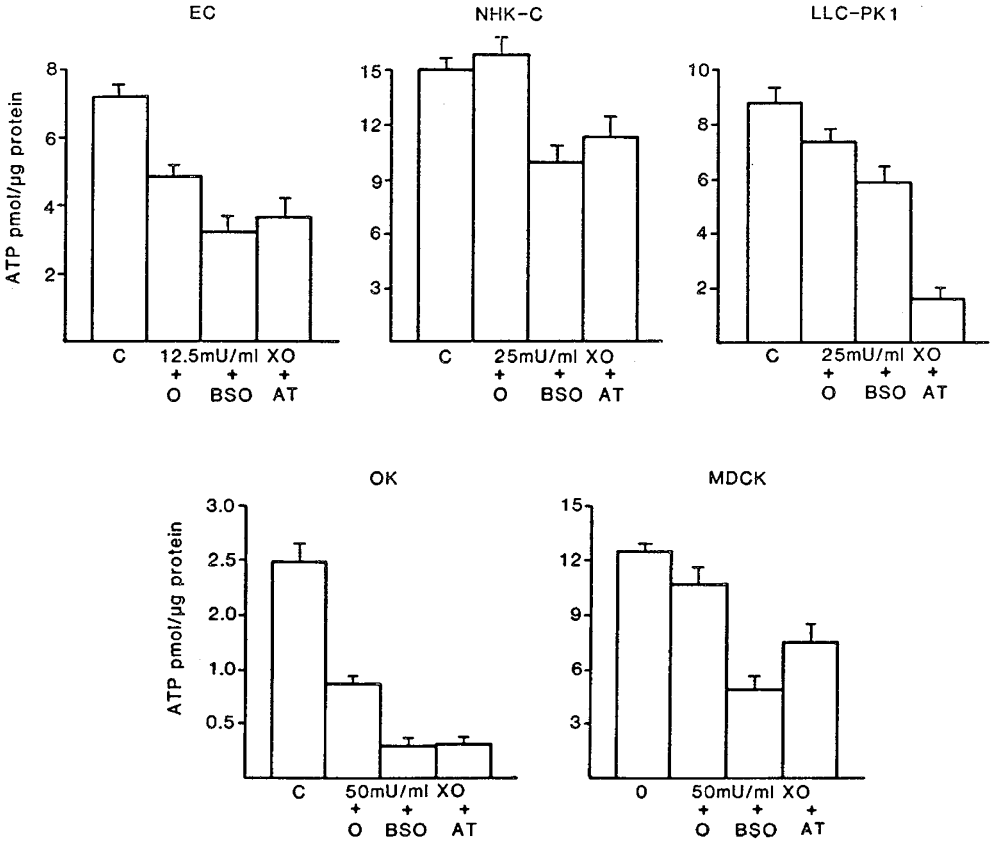

Fig. 2. ATP levels in human umbilical vein endothelial cells (*EC*), LLC-PK1, NHK-C, OK, and MDCK cells in control cells (*C*) and in cells exposed to xanthine oxidase with 5.0 mM hypoxanthine and 1.0 mg/mL soybean trypsin inhibitor with no antioxidant inhibition (*O*), with BSO, or with aminotriazole (*AT*) for 1 h. Cells were pretreated with BSO overnight or with aminotriazole for 30 min as described in Materials and Methods. When compared with ATP depletion without an antioxidant inhibitor (*O*), ATP depletion was significantly ($p < 0.01$) accelerated when cells were exposed to hydrogen peroxide generated by xanthine oxidase and hypoxanthine when glutathione was depleted with BSO and when catalase was inhibited with aminotriazole in all cell types except endothelial cells. ATP values in control cells not exposed to oxidant stress are shown (*C*). ATP values in cells exposed to BSO or aminotriazole in the absence of oxidant stress or to xanthine oxidase without hypoxanthine were greater than 90% of control values (data not shown). Values represent the mean ± 1 SD of three to four replicates.

significantly slower ($p < 0.001$) in endothelial cells compared with all tubular cells (Fig. 1). The time to metabolize half the hydrogen peroxide was 33.5 min in endothelial cells, 13.7 min in LLC-PK1 cells, 13.5 min in OK cells, 14.5 min in MDCK cells, and 18.6 min in NHK-C cells. Among the tubular epithelial cells, NHK-C consumed hydrogen peroxide slightly but significantly ($p < 0.01$) slower than LLC-PK1, OK, and MDCK cells. There was not a significant difference in the metabolism of exogenous hydrogen peroxide between LLC-PK1, OK, and MDCK cells.

Inhibition studies. When each cell line was incubated in 2.5 mM BSO overnight, glutathione levels were significantly ($p < 0.01$) reduced (Table 2) to 10–26% of levels in cells not exposed to BSO (Table 1). This reduction is similar to levels previously observed to be associated with accentuated oxidant injury (15, 26, 27). When endothelial cells and epithelial cells were exposed to aminotriazole in the presence of hydrogen peroxide, catalase activity was reduced to 10–20% of normal values (Table 2).

As a measure of an early response to oxidant stress, we determined ATP levels in each cell line with no antioxidant inhibitor, when glutathione was depleted with overnight incubation with 2.5 mM BSO, and when catalase was inhibited with aminotriazole. As shown in Figure 2, glutathione depletion and catalase inhibition resulted in accelerated ATP depletion when cells were exposed to oxidant stress. In all cell lines except endothelial cells, oxidant-induced ATP depletion was significantly ($p < 0.05$) greater when cells were exposed to BSO and aminotriazole compared with cells exposed to oxidant stress without manipulation of antioxidant defenses. These studies demonstrate that both the glutathione redox cycle and catalase are important in protecting cells from oxidant-induced ATP depletion. ATP levels remained greater than 90% of control when the cells were exposed to BSO and aminotriazole in the absence of oxidant stress, whereas ATP levels were not significantly changed in cells exposed to xanthine oxidase in the absence of hypoxanthine (data not shown).

In contrast, lytic injury as determined with ^{51}Cr release was significantly ($p < 0.05$) greater in all cell lines except MDCK when glutathione was depleted with BSO, whereas inhibition of catalase with aminotriazole resulted in accentuated lytic injury only in OK cells (Fig. 3). In endothelial cells, lytic injury was slightly greater in cells exposed to aminotriazole. In each cell line, the increase in ^{51}Cr release was found in the supernatant fraction, indicating that augmented injury was due to lytic injury rather than cell detachment. MDCK cells were resistant to detachment and lytic injury in spite of depressed endogenous antioxidant defenses. BSO and aminotriazole did not result in enhanced ^{51}Cr release in the absence of oxidant stress, and ^{51}Cr release in cells exposed to xanthine oxidase in the absence of hypoxanthine was less than 5% above control values (data not shown).

DISCUSSION

Enzymatic cellular defense mechanisms that protect organs and cells from injury mediated by reactive oxygen molecules include superoxide dismutase to dismute superoxide anion and the glutathione redox cycle and catalase to detoxify hydrogen

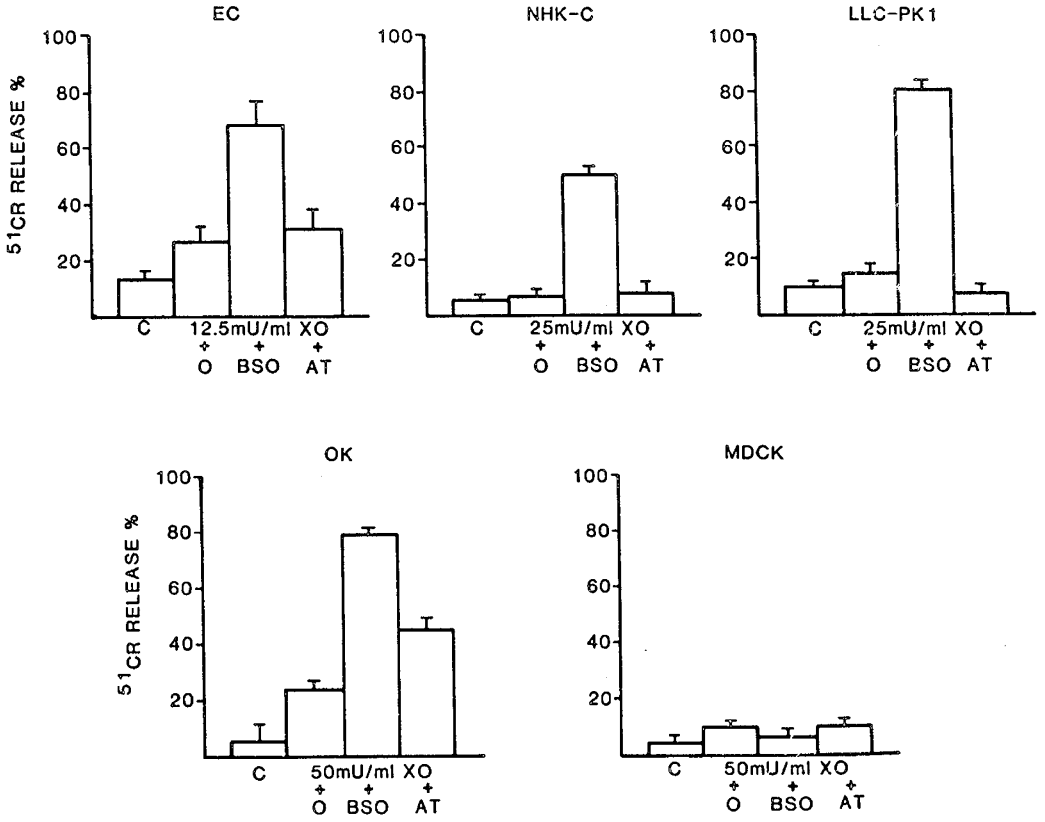

Fig. 3. Total ^{51}Cr release in human umbilical vein endothelial cells (*EC*), LLC-PK1, NHK-C, OK, and MDCK cells in control cells (*C*) and in cells exposed to xanthine oxidase with 5.0 mM hypoxanthine and 1.0 mg/mL soybean trypsin inhibitor for 5 h with no antioxidant inhibitor (*O*), with BSO, and with aminotriazole (*AT*). Cells were pretreated with BSO overnight and with aminotriazole for 30 min as described in Materials and Methods. MDCK cells were very resistant to cell lysis and cell detachment in spite of glutathione depletion and catalase inhibition. In all other cell types, ^{51}Cr release was significantly ($p < 0.01$) enhanced when glutathione was depleted with BSO. In contrast, when catalase was inhibited with aminotriazole, ^{51}Cr release was significantly ($p < 0.01$) enhanced only in OK cells. Release of ^{51}Cr in control cells not exposed to oxidant stress is shown (*C*). Values represent the mean ± 1 SD of three to four replicates.

peroxide. Catalase metabolizes hydrogen peroxide, whereas the glutathione redox cycle maintains cellular thiols, detoxifies hydrogen peroxide and other hydroperoxides and lipid peroxides generated during oxidant stress (9, 21, 27). In addition, other cellular characteristics are likely to play a role in the response of a cell to oxidant injury. In this study, we show that the enzymatic defense mechanisms important in detoxifying hydrogen peroxide are substantially lower in human endothelial cells than in the renal tubular epithelial cells. The activity of glutathione peroxidase, glutathione reductase, and particularly catalase was substantially lower in endothelial cells, suggesting a relationship between the lower antioxidant enzyme activity and their increased susceptibility to oxidant stress. Because human umbilical vein endothelial cells are derived from a relatively hypoxic environment, it is possible that the lower antioxidant enzyme activity of endothelial cells is related to a lack of oxidant stress in such an environment.

Among the renal tubular epithelial cells, differences in glutathione levels, glutathione reductase, and catalase activity did not fully explain the variable susceptibility of the renal tubular cells to oxidant stress. MDCK cells, the least susceptible of the tubular cells to oxidant stress, and LLC-PK1 cells, the most susceptible to oxidant injury, had similar levels of glutathione, catalase, and glutathione reductase activity. Because glutathione peroxidase catalyzes the regeneration of glutathione during oxidant injury, the higher activity in MDCK cells and lower activity in LLC-PK1 cells suggest that such differences may play a role in their variable susceptibility to oxidant stress. However, the metabolism of exogenous hydrogen peroxide was very similar in each of the renal tubular epithelial cells lines, suggesting that additional factors other than the glutathione redox cycle and catalase activity are likely to play a role in the resistance of MDCK cells to oxidant injury. Such factors include the composition and susceptibility of cell membranes to lipid peroxidation, the content of nonenzymatic free radical scavengers such as vitamin E and ascorbate, and the number and location of proteins that contain essential sulfhydryl groups for structural or enzymatic activity (9, 28, 29).

Previous studies using release of ^{51}Cr or lactate dehydrogenase as an indicator of oxidant injury have demonstrated an important role for the glutathione redox cycle in maintaining endothelial cell integrity (15, 17, 30). Other studies in renal tubular epithelial cells have also demonstrated an important role for the glutathione redox cycle in preventing oxidant injury (31–35). Oxidant injury mediated by tert-butyl hydroperoxide is augmented by glutathione depletion and lessened when cellular levels of glutathione are increased (31–33). *In vivo* studies in rat kidneys suggest that antioxidant enzyme activity is an important determinant of oxidant-induced renal dysfunction (36). In this study, we confirm the role of the glutathione redox cycle in preventing lytic injury and we demonstrate a role for catalase as well as the glutathione redox cycle in protecting endothelial cells and epithelial cells from oxidant injury when a different indicator (ATP depletion) of oxidant injury is determined. The mechanism of this differential response is unknown but may be related to the role of the glutathione redox cycle in detoxifying lipid peroxides generated during the propagation of lipid peroxidation after oxidant stress, the role of the glutathione redox cycle in maintaining cellular thiols, or the compartmentalization of catalase and glutathione peroxidase within the cell (8, 27, 35).

After oxidant injury, several metabolic alterations occur, including activation of the glutathione redox cycle, elevation of intracellular calcium, DNA damage, and depletion of NAD and ATP stores (24, 25, 37–39). Later changes include disruption of the cytoskeleton, resulting in blebbing, retraction, detachment, and ultimately cell lysis. Although multiple metabolic effects of oxidant injury have been elucidated, the events leading to irreversible injury and cell death are not well understood. DNA damage, NAD and ATP depletion, lipid peroxidation, elevation of intracellular calcium, and oxidation of protein thiols are each thought to be major mediators of oxidant injury by various investigators (37–42).

In this study, we found that accentuated oxidant-induced ATP depletion as a result of catalase inhibition did not result in accentuated lytic injury, suggesting that ATP depletion alone may not be a critical mediator of oxidant-induced cell death. We have previously demonstrated that oxidant-induced ATP depletion in endothelial and epithelial cells could be prevented with catalase but not with scavengers of hydroxyl radical (10). However, lytic injury was prevented with scavengers of hydroxyl radical and phenathroline, a membrane-permeable iron chelator to prevent the iron-catalyzed intracellular generation of hydroxyl radical from hydrogen peroxide (10). Similarly, a recent study in endothelial cells also demonstrated that oxidant-induced ATP depletion could not be prevented with the iron chelator deferoxamine, whereas ^{51}Cr release was prevented with deferoxamine (43). Taken together, these studies suggest that oxidant-induced ATP depletion alone does not lead to cell lysis and that other mechanisms of injury in addition to ATP depletion are likely to play a role in oxidant-induced cell death.

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