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# Difference in $H_2O_2$ Toxicity Between Intact Renal Tubules and Cultured Proximal Tubular Cells

*Yong Keun Kim,* Sun Hee Ko, Jae Suk Woo, Sang Ho Lee and Jin Sup Jung*

Department of Physiology, College of Medicine, Pusan National University, Pusan, 603-739, Korea

**ABSTRACT.** The present study was undertaken to examine the response to $H_2O_2$ and *t*-butylhydroperoxide (*t*-BHP) in various *in vitro* model systems of renal proximal tubules: rabbit renal cortical slices, freshly isolated rabbit proximal tubules, rabbit primary cultured proximal tubular cells, and opossum kidney (OK) cells. *t*-BHP increased lactate dehydrogenase release and lipid peroxidation in a concentration-dependent manner over the concentration range of 0.2 to 3 mM in cortical slices, whereas $H_2O_2$ caused a similar concentration-dependent increase in both parameters at 5–100 mM. The sensitivity of isolated tubules to both peroxides was similar to that of cortical slices. In primary cultured cells and OK cells, however, the cytotoxicity of $H_2O_2$ was identical to that of *t*-BHP. The cytotoxicity of *t*-BHP was not different among all the systems examined. The specific activity of catalase in cortical slices was similar to that of isolated tubules, but it was much higher than that of primary cultured cells or opossum kidney cells. Glutathione (GSH) peroxidase activity was not different among all the systems examined. The expression of catalase mRNA in cortical slices and isolated tubules was higher than that in primary cultured cells, whereas those of superoxide dismutase, glutathione peroxidase, or β-actin were not different among the systems. These results indicate that intact proximal tubules are more resistant to $H_2O_2$ than are cultured proximal tubular cells, and the resistance is due to a higher specific activity of catalase resulting from the increased expression of its mRNA. BIOCHEM PHARMACOL **56**;4:489–495, 1998. © 1998 Elsevier Science Inc.

**KEY WORDS.** oxidant cell injury; $H_2O_2$; *t*-butylhydroperoxide; catalase; renal proximal tubular cells

ROS† play a role in acute tubular necrosis caused by a number of renal insults including ischemia/reperfusion injury and chemical nephrotoxicity [1–5]. Under normal conditions, all aerobic cells generate, both enzymatically and nonenzymatically, ROS such as superoxide anions, $H_2O_2$, and possibly hydroxyl radicals. At the same time, cells have antioxidant defense systems that prevent cell injury induced by ROS. The defense systems include antioxidant compounds such as GSH, tocopherol, and ascorbic acid, and antioxidant enzymes such as SOD, GSH peroxidase, and catalase [2, 6–8]. Therefore, the ability to generate intracellular ROS and the levels of intracellular antioxidants would determine the overall sensitivity to oxidant injury. A number of studies have demonstrated that higher levels of intracellular antioxidants decrease the sensitivity to oxidant-induced cell injury [9–12].

$H_2O_2$ and *t*-BHP are peroxides that have been used frequently as model agents to study the mechanisms of cell injury resulting from acute oxidative stress in various cell types [6, 13–15]. In previous studies, we observed that renal cortical slices are more resistant to $H_2O_2$ than are OK cells, an established proximal tubular cell line, whereas the cytotoxicity of *t*-BHP is similar in both systems [16, 17]. Similar results in renal proximal tubular cells were reported by Schnellmann [18]. These results suggest that intact proximal tubules may have a greater ability to counteract the cell injury induced by $H_2O_2$ but not *t*-BHP when compared with cultured proximal tubular cells. Thus, the present study was undertaken to examine the response to $H_2O_2$ and *t*-BHP in various *in vitro* model systems of intact proximal tubules and cultured proximal tubular cells and to explore the underlying mechanisms of the differences in the susceptibility of cells to $H_2O_2$. We employed rabbit renal cortical slices and freshly isolated rabbit proximal tubules as intact proximal tubule models and rabbit primary cultured cells and OK cells as cultured proximal tubular cell models. These have been widely used as *in vitro* model systems to study mechanisms of the nephrotoxicity induced by hypoxia and chemicals in renal proximal tubular cells [19–23].

## MATERIALS AND METHODS

### *Slice Preparation*

New Zealand white male rabbits weighing 1.5 to 2 kg were killed, and their kidneys were removed rapidly. The kidneys were perfused immediately through the renal artery with an ice-cold isotonic saline solution containing 140 mM NaCl, 10 mM KCl, and 1.5 mM $CaCl_2$ to remove as much blood

* Corresponding author: Yong Keun Kim, Ph.D., Department of Physiology, College of Medicine, Pusan National University, Pusan, 602-739, Korea. FAX 82-51-246-6001.

† *Abbreviations:* DMEM, Dulbecco's Modified Eagle's medium; F12, Ham's F12; GSH, glutathione; LDH, lactate dehydrogenase; MDA, malondialdehyde; OK, opossum kidney; ROS, reactive oxygen species; SOD, superoxide dismutase; and *t*-BHP, *t*-butylhydroperoxide.

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as possible. Thin slices (0.25 to 0.35 mm) of renal cortex were prepared using a Stadie-Riggs microtome and were stored in an ice-cold modified Cross-Taggart medium containing 130 mM NaCl, 10 mM KCl, 1.5 mM $CaCl_2$, 5 mM glucose, and 20 mM Tris–HCl (pH 7.4).

### *Isolation and Culture of Proximal Tubules*

Proximal tubules were isolated by the method of Brendel and Meeanz [24] and prepared for cultures as described by Chung *et al.* [25] with some modifications. In brief, adult male New Zealand white rabbits were killed by cervical dislocation. Their kidneys were removed immediately, cleaned of fat and debris, and washed with sterile antibiotic-supplemented medium. The kidneys were perfused with phosphate-buffered saline (pH 7.4) through the renal artery and subsequently with DMEM/F12 (/F12, Sigma Chemical Co.) containing 0.5% (w/v) iron oxide until the kidneys turned grey-black in color. The cortex was removed and homogenized with 4 strokes of a sterile glass homogenizer. The homogenate was passed through a series of sterile nylon mesh sieves (254 and 85 μm; TETCO, Inc.). Tubules and glomeruli retained on the 85-μm sieve were suspended in a tube containing DMEM/F12 medium and a magnetic stirring bar. Glomeruli containing iron oxide were attracted to the magnetic stirring bar. The stirring bar was removed from the solution.

To make a preparation of primary cultured proximal tubular cells, the isolated proximal tubules were incubated briefly in DMEM/F12 medium containing 80 μg/mL of collagenase A and 0.025% soybean trypsin inhibitor. Then the dissociated tubules were washed by centrifugation, resuspended in DMEM/F12 medium, and transferred into tissue culture plates. Proximal tubule cells were grown on 24-well tissue culture plates in DMEM/F12 medium supplemented with bovine insulin (5 μg/mL), human transferrin (5 μg/mL), and hydrocortisone ($5 \times 10^{-8}$ M). The cultures were maintained in a humidified 95% air/5% $CO_2$ incubator at 37°. Culture medium was changed every 48 or 72 hr and 24 hr before the beginning of each experiment. All experiments started 7 days after plating when a confluent monolayer culture was achieved.

OK cells were obtained from the American Type Culture Collection and were maintained by serial passages in 75-$cm^2$ culture flasks (Costar). The cells were grown in DMEM/F12 medium containing 5% fetal bovine serum at 37° in a 95% air/5% $CO_2$ incubator. When the cultures reached confluence, subculture was prepared using a 0.02% EDTA–0.05% trypsin solution. The cells were grown on 24-well tissue culture plates in DMEM/F12 medium containing 5% fetal bovine serum. All experiments started 3–4 days after plating when a confluent monolayer culture was achieved. Cells were treated with oxidants in a medium without serum.

### *Induction of Oxidant Injury*

Cortical slices, isolated proximal tubules, and cultured cells were treated with peroxides of the indicated concentration in the incubation medium for 60 min at 37°. The composition of the incubation medium was as follows (mM): 130 NaCl, 10 KCl, 1.5 $CaCl_2$, 5 glucose, and 20 Tris–HCl (pH 7.4) for slices, and 115 NaCl, 5 KCl, 25 $NaHCO_3$, 2 $NaH_2PO_4$, 1 $MgSO_4$, 1 $CaCl_2$, and 5 glucose (pH 7.4) for isolated tubules and cultured cells. Following oxidant stress, cell viability and lipid peroxidation were measured as described below.

### *Measurement of Cell Viability*

Cell viability in cortical slices and isolated tubules was estimated by measuring LDH release. Renal cortical slices were homogenized in 2 mL of distilled water, and the tissue homogenate was centrifuged at 150 *g* for 5 min. The pellet was discarded, and the supernatant was used. LDH activity was determined in the supernatant and the incubation medium, using an LDH kit (Iatron Lab.). For measurement of LDH release in isolated tubules, 1 mL of tubule suspension was centrifuged to separate the tubule from the medium. The remaining tubule pellet was lysed by homogenation in 0.2% Triton X-100. LDH activity was determined in the supernatant and lysed tubules as described for the slices.

The viability in cultured cells was determined by a trypan blue exclusion assay, since this assay was a more sensitive indicator of cell death than LDH release in cultured cells, as demonstrated by Aleo *et al.* [26]. The cells were harvested using 0.025% trypsin, incubated with 4% trypan blue solution, and were counted using a hemacytometer under light microscopy. Cells failing to exclude the dye were considered nonviable, and the data were expressed as a percentage of nonviable cells.

### *Measurement of Lipid Peroxidation*

Lipid peroxidation was estimated by measuring the tissue content of MDA according to the method of Uchiyama and Mihara [27]. Tissues were homogenized in ice-cold 1.15% KCl. A 0.5-mL aliquot of homogenate was added to 3 mL of 1% phosphoric acid and 1 mL of 0.6% thiobarbituric acid. The mixture was heated for 45 min in a boiling water bath. After the addition of 4 mL of *n*-butanol, the contents were vortexed vigorously and centrifuged at 2000 *g* for 20 min. The absorbance of the upper, organic layer was measured at 535 and 520 nm with a diode array spectrophotometer (Hewlett Packard, 8452A), and was compared with results obtained using freshly prepared MDA tetraethylacetal standards. MDA values were expressed in picomoles per milligram of protein. Protein was measured by the method of Bradford [28].

**TABLE 1. Sequences of PCR primers**

| DNA | Primers (S, sense; AS, antisense) | Size* (bp) |
|---|---|---|
| Catalase | S: 5′-TGAAGCAGTGGAAGGAGCAGC-3′<br>AS: 5′-GCCATAGCCATTCATGTGCCG-3′ | 645 |
| Mn-SOD | S: 5′-CCTCAGCAATGTTGTGTCGG-3′<br>AS: 5′-CGGAAGGGCTTCACTTCTTG-3′ | 701 |
| GSH peroxidase | S: 5′-ACCATGTGTGCGGCTCGTATGG-3′<br>AS: 5′-GCTTGGATGTCGGGCTCGATGT-3′ | 575 |
| β-Actin | S: 5′-TTGTAACCAACTGGGACGATATGG-3′<br>AS: 5′-GATCTTGATCTTCATGGTGCTAGG-3′ | 764 |

*Refers to the size of the PCR products.

### *Measurement of Antioxidant Enzyme Activity*

Renal cortical slices and tubules were homogenized in 50 mM phosphate buffer (pH 7.0) and treated with 1% Triton X-100. The cultured cells were washed twice with phosphate buffer, scraped from the plate, centrifuged, resuspended in the buffer, and lysed by sonication on ice. Cell lysates were treated with 1% Triton X-100 in phosphate buffer prior to measurement. The composition of the phosphate buffer was as follows (mM): 137 NaCl, 2.7 KCl, 1.5 $CaCl_2$, 8.0 $NaH_2PO_4$, and 1.5 $KH_2PO_4$ (pH 7.4).

The catalase activity was determined by measuring the decomposition of $H_2O_2$ according to the method of Aebi [29]. Absorbance at 240 nm was measured for 1 min after the addition of sample in a buffer containing 50 mM phosphate (pH 7.0) and 30 mM $H_2O_2$.

The GSH peroxidase activity was determined by measuring the oxidation of NADPH using $H_2O_2$ as a substrate at 340 nm [30]. The assay was performed in a buffer containing 1 mM EDTA, 100 mM potassium phosphate (pH 7.0) with 0.5 mM $H_2O_2$, 150 μM NADPH, 10 mM GSH, and 0.24 U of GSH reductase. Sodium azide (1 mM) was added to the reaction solution to inhibit the catalase activity. The $H_2O_2$-independent consumption of NADPH was measured in reaction solution without $H_2O_2$ and was corrected for all data.

### *Expression of Catalase mRNA*

Total RNA was isolated from cortical slices, freshly isolated tubules, and primary cultured proximal tubules, and reverse-transcribed with Moloney murine leukemia virus transcriptase (Promega) and oligo-dT primers. PCR was carried out using primers for catalase, Mn-SOD, GSH peroxidase, and β-actin in the following profile: 94° 1 min, 50° 1 min, 72° 1 min for 30 cycles. PCR primers used in the experiments are shown in Table 1. PCR primers were designed from the rat cDNA sequences.

### *Statistical Analysis*

Data are expressed as means ± SEM. Comparison between two groups was made using the unpaired *t*-test. Multiple group comparison was done using one-way ANOVA followed by the Tukey *post hoc* test. $P < 0.05$ were considered statistically significant.

## RESULTS

Figure 1A depicts effects of $H_2O_2$ and *t*-BHP on LDH release in renal cortical slices. Both peroxides increased LDH release in a concentration-dependent manner, but the effective concentrations of $H_2O_2$ were much higher than those of *t*-BHP. *t*-BHP caused a significant increase in LDH release at 0.2 to 3 mM, whereas $H_2O_2$ produced similar effects at 5–100 mM. Both peroxides also increased lipid peroxidation in a concentration-dependent fashion, but the difference in potency between the two agents was similar to

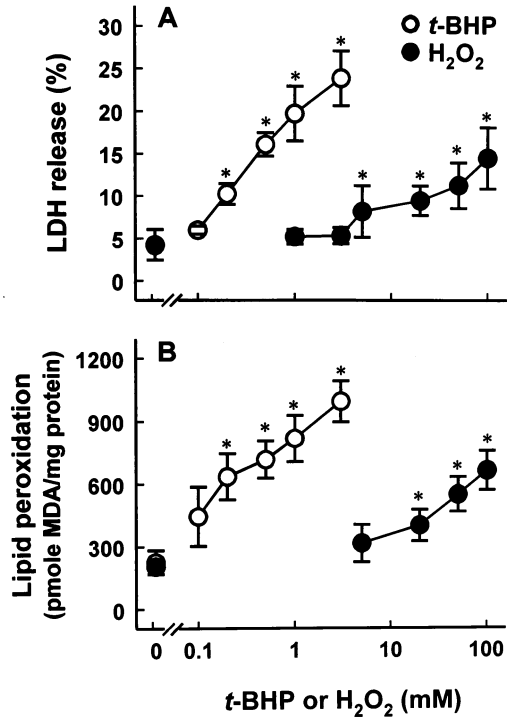


**FIG. 1. Effect of various concentrations of *t*-BHP and $H_2O_2$ on LDH release (A) and lipid peroxidation (B) in renal cortical slices. Slices were treated with various concentrations of *t*-BHP or $H_2O_2$ for 60 min at 37°, and LDH release and lipid peroxidation were measured as described under Materials and Methods. Values are means ± SEM of four experiments. *P < 0.05 compared with the control (0 oxidants).**

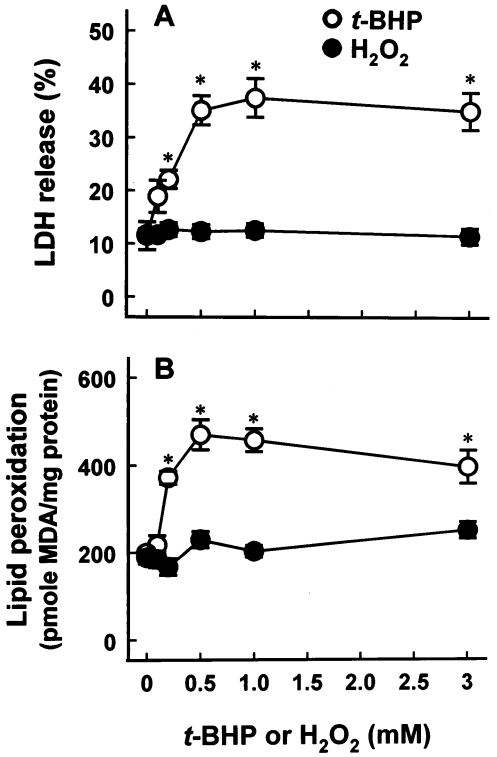


**FIG. 2. Effect of various concentrations of *t*-BHP and $H_2O_2$ on LDH release (A) and lipid peroxidation (B) in freshly isolated proximal tubules. Tubules were treated with various concentrations of *t*-BHP or $H_2O_2$ for 60 min at 37°, and LDH release and lipid peroxidation were measured as described under Materials and Methods. Values are means ± SEM of five experiments. *P < 0.05 compared with the control (0 oxidants).**

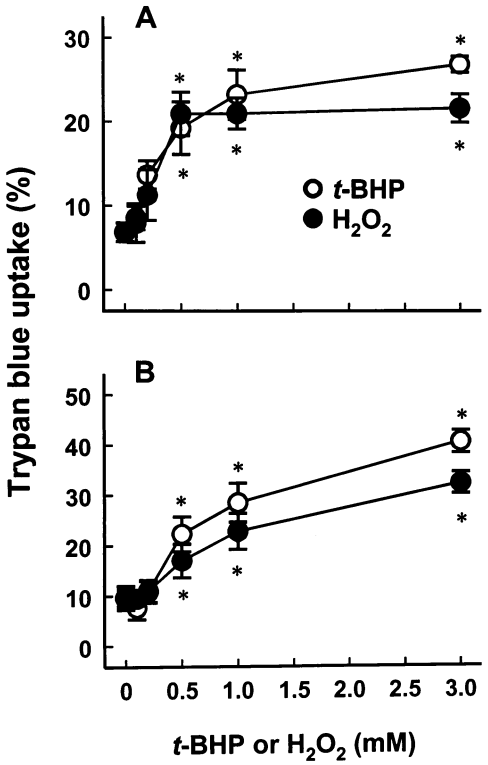


**FIG. 3. Effect of various concentrations of *t*-BHP and $H_2O_2$ on cell injury in primary cultured proximal tubular cells (A) and OK cells (B). Cells were treated with various concentrations of *t*-BHP or $H_2O_2$ for 60 min at 37°, and cell viability was measured by the trypan blue exclusion assay as described under Materials and Methods. Values are means ± SEM of six experiments. *P < 0.05 compared with the control (0 oxidants).**

that observed in LDH release (Fig. 1B). These results are consistent with reports of others that the renal cell injuries induced by peroxides are attributed to lipid peroxidation [13, 14].

Differences in the cytotoxicity of both peroxides on LDH release and lipid peroxidation were also observed in freshly isolated proximal tubules (Fig. 2). *t*-BHP increased the LDH release and lipid peroxidation over the concentration of 0.2 to 3.0 mM, but $H_2O_2$ did not produce significant changes at the corresponding concentrations. We used $H_2O_2$ concentrations up to 3 mM in isolated tubules, while higher concentrations (5–100 mM) were used in slices. Use of the same concentrations in the two systems would provide better results. However, the tubules could not be treated with higher concentrations of $H_2O_2$ because they floated in the medium during incubation with concentrations higher than 3 mM.

Figure 3 depicts the cell injury induced by $H_2O_2$ and *t*-BHP in primary cultured proximal tubular cells and OK cells. Both peroxides caused significant cell injury as determined by trypan blue exclusion over the concentration range of 0.5 to 3.0 mM. Their cytotoxic potencies were similar, unlike those in slices or isolated tubules.

To evaluate if the lower susceptibility of intact tubules (slices and isolated tubules) to $H_2O_2$ cytotoxicity was attributed to a higher ability to detoxify $H_2O_2$, catalase and GSH peroxidase activities were measured. These enzymes are major intracellular enzymes scavenging $H_2O_2$ [7, 31, 32]. The specific activities of catalase in cortical slices and isolated tubules were approximately 5 times higher than those of primary cultured cells and OK cells (Fig. 4). However, the specific activities of GSH peroxidase were

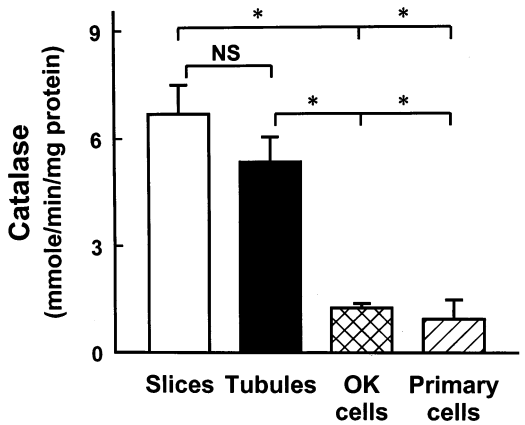


**FIG. 4. Specific activity of catalase in various preparations. The enzyme activity was measured as described under Materials and Methods. Values are means ± SEM of five experiments. *P < 0.05 compared with the control (0 oxidants).**

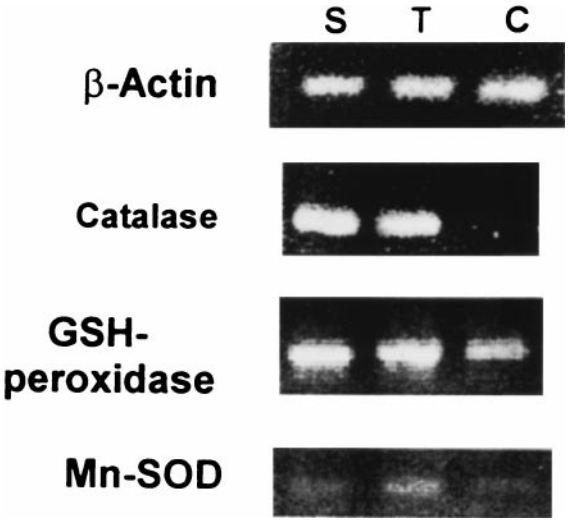


**FIG. 5. Expression level of catalase, GSH peroxidase, and Mn-SOD mRNA in rabbit cortical slices (S), freshly isolated tubules (T), and primary cultured proximal cells (C). Total RNAs were isolated from cortical slices, freshly isolated tubules, and primary cultured cells. Total RNA (1 μg) was reverse-transcribed and amplified using the specific primers for catalase, Mn-SOD, GSH peroxidase, and β-actin primers. Polymerase chain reaction products were electrophoresed in 2% agarose gel and photographed.**

not significantly different among the systems (6.62 ± 1.25, 6.10 ± 1.26, 6.80 ± 1.85, and 6.50 ± 0.30 nmol/mg protein in slices, tubules, primary cultured cells, and OK cells, respectively).

In the last series of experiments, reverse transcription-polymerase chain reaction analysis was performed to examine if the differences in catalase activity between intact tubules and cultured cells were due to differential expressions of catalase mRNA. As shown in Fig. 5, the expression level of catalase in cortical slices was similar to that of freshly isolated tubules, but higher than that of primary cultured cells, whereas the levels of Mn-SOD, GSH peroxidase, and β-actin were not significantly different among the systems.

## DISCUSSION

In previous studies, we observed that renal cortical slices are approximately 100-fold more resistant to $H_2O_2$ than to *t*-BHP, whereas cultured OK cells exhibited comparable sensitivity to both peroxides [16, 17]. Sandström and Marklund [33] also reported that $H_2O_2$ is less cytotoxic than *t*-BHP in human colon carcinoma cells. However, the underlying mechanisms of such differences have not been clarified. In the present study, we compared the cytotoxicity of both peroxides in various *in vitro* model systems of renal proximal tubular cells: cortical slices, isolated proximal tubules, primary cultured proximal tubules, and OK cells. In slices and isolated tubules, $H_2O_2$ did not produce significant increases in LDH release and lipid peroxidation at concentrations lower than 3 mM (Figs. 1 and 2), whereas it induced significant increases in both parameters at a concentration as low as 0.2 mM in cultured cells (Fig. 3). However, *t*-BHP induced cell injury at concentrations of 0.5 to 3 mM in all the systems examined. It is apparent, therefore, that intact tubules are more resistant to $H_2O_2$ than are cultured cells, whereas the sensitivity to *t*-BHP is comparable in all the preparations examined.

Catalase and GSH peroxidase are major cellular defense systems against $H_2O_2$ [7, 31, 32]. Even though $H_2O_2$ is not a radical species, it can be reduced to the potent radical species hydroxyl radical in the presence of transition metals such as iron by an iron-catalyzed Haber–Weiss reaction [34]. Thus, the cytotoxic potential of $H_2O_2$ is, in large part, a function of intracellular catalase and GSH peroxidase activities that scavenge $H_2O_2$. A number of studies have demonstrated that higher or lower levels of endogenous antioxidants decrease or increase the sensitivity to cell injury concomitantly [8–12]. Thus, we measured the endogenous activities of catalase and GSH peroxidase in all the preparations. Interestingly, the activity of catalase in cortical slices and freshly isolated proximal tubules was much higher than that observed in cultured cells (Fig. 4), whereas the activity of GSH peroxidase was comparable in all the preparations. These results strongly suggest that the lower susceptibility of intact tubules to $H_2O_2$ is attributed to the higher activity of catalase. Since *t*-BHP is not a substrate for catalase, unlike $H_2O_2$ [16], no difference between intact tubules and cultured cells in its cytotoxicity could be anticipated, as evidenced by the results of the present study. Varani *et al.* [35] reported that the sensitivity of $H_2O_2$ decreased rapidly when human umbilical vein endothelial cells were maintained in culture, and that such changes were attributed to increased levels of intracellular total thiols but not to GSH peroxidase or catalase activity. In the present study, we did not measure changes in the levels of GSH or total thiols. However, similar changes seem unlikely under our experimental models, since differences in the susceptibility of cells to *t*-BHP were not observed.

Chung *et al.* [25] observed that the activities of brush-border enzymes (γ-glutamyl transpeptidase, leucine aminopeptidase, and alkaline phosphatase) in primary cultures were lower than in freshly isolated purified proximal tubules. Sandström and Marklund [33] also reported in human mesothelioma cells that the cells lose as much as 80% of their initial GSH peroxidase activity during 5 months of culture. However, the mechanisms underlying decreases in specific activities of various enzymes in cultured cells compared with intact cells have not been defined. In the present study, the expression level of catalase mRNA in slices or isolated tubules was higher than that in cultured cells, suggesting that decreased specific activity of catalase in cultured cells results from a diminished expression of mRNA. Although the factors responsible for the lower expression of catalase mRNA remain to be determined, differences between the environments of cell culture and *in vivo* conditions could be responsible for such changes.

Cell culture is a powerful technique for studying physiological, biochemical, and toxicological processes *in vitro*. The validity of a cultured model relies on the ability of the cultured cells to accurately reflect the functional properties of the *in vivo* tissue. However, since cultured cells have lower catalase activity than intact tubules, exposure of the cells to chemicals that generate ROS or pathological conditions that induce oxidative stress could produce distinct patterns of cell injury. Thus, the sensitivity of cultured cells to oxidative stress, especially endogenous generation of ROS, may not reflect the sensitivity of cells in the *in vivo* situation. In this context, cortical slices and freshly isolated tubules may be suitable *in vitro* models, which reflect the sensitivity of the *in vivo* cells in studies to define the involvement of ROS in conditions such as hypoxia/reoxygenation injury.

In summary, the cytotoxic potency of $H_2O_2$ was much higher in cortical slices and freshly isolated tubules than in cultured cells, whereas the potency of *t*-BHP was similar in all preparations. The expression of catalase mRNA was higher in intact tubules than in cultured cells. These results indicate that intact renal tubules have a greater ability to counteract $H_2O_2$-induced cell injury than do cultured cells, due to a higher specific activity of catalase.

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## References


1. Weinberg JM, The cell biology of ischemic renal injury. *Kidney Int* **39:** 476–500, 1991.
2. Baud L and Ardaillou R, Reactive oxygen species: Production and role in the kidney. *Am J Physiol* **251:** F765–F776, 1986.
3. Shah SV and Walker PD, Reactive oxygen metabolites in toxic acute renal failure. *Ren Fail* **14:** 363–370, 1992.
4. Walker PD and Shah SV, Gentamicin enhanced production of hydrogen peroxide by renal cortical mitochondria. *Am J Physiol* **253:** C495–C499, 1987.
5. Nath KA, Croatt AJ, Likely S, Behrens TW and Warden D, Renal oxidant injury and oxidant response induced by mercury. *Kidney Int* **50:** 1032–1043, 1996.
6. Farber JL, Kyle ME and Coleman JB, Biology of disease: Mechanisms of cell injury by activated oxygen species. *Lab Invest* **62:** 670–679, 1990.
7. Ross D and Moldeus P, Antioxidant defense systems and oxidative stress. In: *Membrane Lipid Oxidation* (Ed. Vigo-Pelfrey C), Vol. II, pp. 151–170. CRC Press, Boca Raton, FL, 1993.
8. Turrens JF, Crapo JD and Freeman BA, Protection against oxygen toxicity by intravenous injection of liposome-entrapped catalase and superoxide dismutase. *J Clin Invest* **73:** 87–95, 1984.
9. Beckman JS, Minor-RLJ, White CW, Repine JE, Rosen GM and Freeman BA, Superoxide dismutase and catalase conjugated to polyethylene glycol increases endothelial enzyme activity and oxidant resistance. *J Biol Chem* **263:** 6884–6892, 1988.
10. Freeman BA, Young SL and Crapo JD, Liposome-mediated augmentation of superoxide dismutase in endothelial cells prevents oxygen injury. *J Biol Chem* **258:** 12534–12542, 1983.
11. Markey BA, Phan SH, Varani J, Ryan US and Ward PA, Inhibition of cytotoxicity by intracellular superoxide dismutase supplementation. *Free Radic Biol Med* **9:** 307–314, 1990.
12. Lash LH and Tokarz JJ, Oxidative stress in isolated rat renal proximal and distal tubular cells. *Am J Physiol* **259:** F338–F347, 1990.
13. Schnellmann RG, Mechanisms of *t*-butyl hydroperoxide-induced toxicity to rabbit renal proximal tubules. *Am J Physiol* **255:** C28–C33, 1988.
14. Sheridan AM, Fitzpatrick S, Wang C, White MD and Lieberthal W, Lipid peroxidation contributes to hydrogen peroxide induced cytotoxicity in renal epithelial cells. *Kidney Int* **49:** 88–93, 1996.
15. Salahudeen AK, Role of lipid peroxidation in $H_2O_2$-induced renal epithelial (LLC-$PK_1$) cell injury. *Am J Physiol* **268:** F30–F38, 1995.
16. Kim YK and Kim YH, Differential effect of $Ca^{2+}$ on oxidant-induced lethal cell injury and alterations of membrane functional integrity in renal cortical slices. *Toxicol Appl Pharmacol* **141:** 607–616, 1996.
17. Kim YK, Park KM, Ko SH and Woo JS, Role of protein kinase C in increase of transepithelial permeability induced by oxidants in opossum kidney cells. *Cell Physiol Biochem* **7:** 61–68, 1997.
18. Schnellmann RG, Intracellular calcium chelators and oxidant-induced renal proximal tubule cell death. *J Biochem Toxicol* **6:** 299–303, 1991.
19. Reimer KA and Jennings RB, Alterations in renal cortex following ischemic injury. I. PAH uptake by slices of cortex after ischemia or autolysis. *Lab Invest* **25:** 176–184, 1971.
20. Phelps JS, Gandolfi AJ, Brendel K and Dorr RT, Cisplatin nephrotoxicity: *In vitro* studies with precision-cut rabbit renal cortical slices. *Toxicol Appl Pharmacol* **90:** 501–512, 1987.
21. Ruegg CE, Gandolfi AJ, Nagle RB and Brendel K, Differential patterns of injury to the proximal tubule of renal cortical slices following *in vitro* exposure to mercuric chloride, potassium dichromate, or hypoxic conditions. *Toxicol Appl Pharmacol* **90:** 261–273, 1987.
22. Edelstein CL, Ling H and Schrier RW, The nature of renal cell injury. *Kidney Int* **51:** 1341–1351, 1997.
23. Cotterill LA, Gower JD, Clark PK, Fuller BJ, Thorniley MS, Goddard JG and Green CJ, Reoxygenation following hypoxia stimulates lipid peroxidation and phosphatidylinositol breakdown in kidney cortical slices. *Biochem Pharmacol* **45:** 1947–1951, 1993.
24. Brendel K and Meezan E, Isolation and properties of a pure preparation of proximal kidney tubules without collagenase treatment. *Fedn Proc* **34:** 803, 1975.
25. Chung SD, Alavi N, Livingston D, Hiller S and Taub M, Characterization of primary rabbit kidney cultures that express proximal tubule functions in a hormonally defined medium. *J Cell Biol* **95:** 118–126, 1982.
26. Aleo MD, Taub ML and Kostyniak PJ, Primary cultures of rabbit renal proximal tubule cells. III. Comparative cytotoxicity of inorganic and organic mercury. *Toxicol Appl Pharmacol* **112:** 310–317, 1992.
27. Uchiyama M, and Mihara M, Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem* **86:** 271–278, 1978.
28. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72:** 248–254, 1976.
29. Aebi H, Catalase *in vitro*. *Methods Enzymol* **105:** 121–126, 1984.
30. Flohe L and Gunzler WA, Assays of glutathione peroxidase. *Methods Enzymol* **105:** 114–121, 1984.
31. Ursini F, Maiorino M and Gregolin C, The selenoenzyme

phospholipid hydroperoxide glutathione peroxidase. *Biochim Biophys Acta* **839:** 62–70, 1985.
32. Duan YJ, Komura S, Fiszer SB, Szafarz D and Yagi K, Purification and characterization of a novel monomeric glutathione peroxidase from rat liver. *J Biol Chem* **263:** 19003–19008, 1988.
33. Sandström BE and Marklund SL, Effects of variation in glutathione peroxidase activity on DNA damage and cell survival in human cells exposed to hydrogen peroxide and *t*-butyl hydroperoxide. *Biochem J* **271:** 17–23, 1990.
34. Samokyszyn VM, Miller DM, Reif DW and Aust SD, Iron-catalyzed lipid peroxidation. In: *Membrane Lipid Oxidation* (Ed. Vigo-Pelfrey C), Vol. I, pp. 101–127. CRC Press, Boca Raton, FL, 1993.
35. Varani J, Dame MK, Gibbs DF, Taylor CG, Weinberg JM, Shayevitz J and Ward PA, Human umbilical vein endothelial cell killing by activated neutrophils. Loss of sensitivity to injury is accompanied by decreased iron content during *in vitro* culture and is restored with exogenous iron. *Lab Invest* **66:** 708–714, 1992.