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EFFECT OF CHRONIC HYPOXIA ON DETOXICATION ENZYMES IN RAT LIVER

XIAOQIN SHAN,* TAK YEE AW,*† ELIZABETH R. SMITH,*
MAGNUS INGELMAN-SUNDBERG,‡ BENGT MANNERVIK,§
TAKASHI IYANAGI,‖ and DEAN P. JONES*¶

* Department of Biochemistry and Winship Cancer Center, Emory University School of Medicine, Atlanta, GA 30322, U.S.A.; ‡ Department of Physiological Chemistry, Karolinska Institute, S-10401 Stockholm, Sweden; § Department of Biochemistry, Biomedical Center, Uppsala University, S-75123 Uppsala, Sweden and ‖ Department of Life Science, Faculty of Science, Himeji Institute of Technology, Hyogo 678-12, Japan

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Abstract—Studies were performed to determine the effects of chronic hypoxia on enzymes that catalyze various detoxication reactions. Rats were exposed to room air or 10.5% O_2 for 10 days, and microsomes and postmicrosomal supernatants were isolated from liver. Detoxication enzyme activities were measured by radiochemical and spectrophotometric assays, and immunoreactive protein amounts were measured by Western blot analysis. Total cytochrome P450, as measured by the CO-difference spectrum, and activities of superoxide dismutase (EC 1.15.1.1), epoxide hydrolase (EC 4.2.1.63), catalase (EC 1.11.1.6), glutathione disulfide reductase (EC 1.6.4.2), and glutathione (GSH) *S*-transferase (EC 2.5.1.18) were not affected by this extent of hypoxia. In contrast, 10 days of hypoxia decreased activities or immunoreactivities (% of aerobic) of GSH peroxidase (EC 1.11.1.9) (54%), cytochrome P450EtOH2 (42%), CYP3A1 (53%), sulfotransferase (EC 2.8.2.1) (77%) and UDP-glucuronosyltransferase (EC 2.4.1.17) (65%). Activity of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), an important enzyme in NADPH production was also decreased to 56% of the aerobic value, but Western blot analysis showed that the amount of protein reactive with antibodies to glucose-6-phosphate dehydrogenase was not affected by hypoxia. Thus, hypoxia may decrease activity of enzymes by regulatory mechanisms even though the amount of immuno-detectable enzyme is unchanged. Liver cells isolated from rats exposed to hypoxia also gave lower GSH synthetic rates than cells from normoxic rats. This result, together with the effect of hypoxia on glucose-6-phosphate dehydrogenase, indicates that the GSH supply for GSH-dependent detoxication reactions may be limited due to chronic hypoxia. To test directly whether chronic hypoxia increased sensitivity to a compound normally detoxified by a GSH-dependent reaction, sensitivity to *tert*-butyl hydroperoxide (t-BuOOH) of hepatocytes from rats exposed to *in vivo* hypoxia was compared to that from normoxic rats. The results showed that the cells from the hypoxic rats were much more sensitive to injury. Taken together, these results suggest that decreases in amounts and/or activities of detoxication enzymes during chronic hypoxia may result in increased susceptibility of cells to chemical injury.

Tissue hypoxia is associated with clinical conditions under which a subnormal oxygen concentration substantially alters cellular metabolism. Of particular importance in toxicology, acute hypoxia enhances cytotoxicity of certain chemicals by increasing the rate of activation of compounds to toxic species [1]. This is particularly important for halogenated hydrocarbons, such as carbon tetrachloride, because generation of reactive species is enhanced by hypoxia. More recently, we found that acute hypoxia also decreases the capacity of cells to tolerate damage from reactive species and thus increases their susceptibility to injury [2]. For example, hypoxia and anoxia potentiate the cytotoxicity of *tert*-butyl hydroperoxide (t-BuOOH)** in hepatocytes without affecting its metabolism or activation, suggesting that cellular defense mechanisms are impaired [2].

Glutathione (GSH) can detoxify harmful active oxygen species by reduction reactions and conjugation reactions [3]. Glutathione homeostasis is maintained by a group of enzymes involved in its synthesis, regeneration, utilization, and transport. Impairment of an enzyme system in any step may render cells more susceptible to chemical-induced toxicity. Other important detoxication enzymes include superoxide dismutase, catalase, cytochrome P450, epoxide hydrolase, sulfotransferases, UDP-glucuronosyltransferases, and glucose-6-phosphate dehydrogenase [providing NADPH for mixed-function oxidations and reduction of glutathione disulfide (GSSG)].

Previous studies have shown that acute hypoxia can impair GSH-dependent detoxication systems. For instance, the NADPH supply for regeneration of GSH from GSSG [4] and the GSH synthesis rate [5] are decreased when isolated hepatocytes are incubated under anoxic or hypoxic conditions.

† Current address: Department of Physiology and Biophysics, Louisiana State University Medical Center, 1501 Kings Highway, P.O. Box 33932, Shreveport, LA 71130-3932.

¶ Corresponding author: Dr. Dean P. Jones, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322. Tel. (404) 727-5970; FAX (404) 727-2738.

** Abbreviations: GSH, glutathione; GSSG, glutathione disulfide; HEPES, *N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; SAM, *S*-adenosylmethionine; DEM, diethyl maleate; SOD, superoxide dismutase; and t-BuOOH, *tert*-butyl hydroperoxide.

However, chronic hypoxia is more clinically relevant than acute hypoxia to some diseases, e.g. obstructive lung diseases and anemias. Therefore, knowledge of the effects of chronic hypoxia on detoxication systems can potentially be useful for drug therapy of these diseases. Chronic hypoxia has been shown to induce different regulation and expression of enzyme activities [6–8]. Recent studies also showed that absorption of acetaminophen into plasma was delayed and formation of glucuronide and sulfate conjugation was decreased by chronic hypoxia [9].

To understand better the potential effect of chronic hypoxia on chemical-induced toxicity, we studied the effects of chronic hypoxia on several detoxication systems. The results showed that GSH peroxidase, glucose-6-phosphate dehydrogenase, some subtypes of cytochrome P450 (CYP2E1, CYP3A1, CYP2B1, P450EtOH2), sulfotransferase, and UDP-glucuronosyltransferase activities are decreased by 10-day exposure to 10.5% O_2. Other enzymes tested were not affected by this extent of hypoxia.

MATERIALS AND METHODS

Materials. GSH, GSSG, HEPES, iodoacetic acid, 1-fluoro-2,4-dinitrobenzene, 1-chloro-2,4-dinitrobenzene, diethyl maleate (DEM), t-BuOOH, xanthine, collagenase (type IV), xanthine oxidase, GSSG reductase, and anti-glucose-6-phosphate dehydrogenase serum were purchased from the Sigma Chemical Co. (St. Louis, MO). [7-^{3}H]Styrene oxide was purchased from Amersham (Arlington Heights, IL). All other materials were of reagent grade and purchased locally. Solvents used for high-performance liquid chromatography were of HPLC grade and were filtered prior to use. Deionized water was used throughout except for HPLC analyses, where distilled, deionized water was used.

Chronic hypoxia model. Induction of chronic hypoxia in rats was described in detail elsewhere [9]. Briefly, male rats [Kng: (SD), King Animal Laboratories, Oregon, WI] weighing between 200 and 250 g were exposed to hypoxia (100 torr for 24 hr followed by 70–80 torr) for 10 days in specially constructed plastic cages. The desired pO_2 was achieved by mixing air and nitrogen in a Matheson gas mixer (Matheson Gas Products, East Rutherford, NJ). Cages were opened daily for 5 min to change bedding and food. Control rats were housed in standard open rat cages. In this chronic hypoxia model, two parameters need to be considered: magnitude and duration of hypoxia. The magnitude of hypoxia used in the present studies does not result in irreversible cell injury [10] but is sufficient to cause major alterations in biochemical function [9].

Preparations of hepatocytes, microsomes, and postmicrosomal cytosol. Hepatocytes were isolated from rats by the collagenase perfusion procedure described by Moldeus *et al.* [11]. Routinely, > 90% excluded 0.2% trypan blue. Cell incubations (10^6 cells/mL) were carried out at 37° in modified Krebs–Henseleit buffer containing 25 mM HEPES, pH 7.4. Microsomes and postmicrosomal cytosol were prepared from homogenates of livers from normoxic and hypoxic rats by differential centrifugation as previously described [12].

Analyses. GSH peroxidase (EC 1.11.1.9) activity was measured according to Ref. 13. GSSG reductase (EC 1.6.4.2) activity was measured according to Ref. 14. Glutathione *S*-transferase (EC 2.5.1.18) was measured using 1-chloro-2,4-dinitrobenzene as the substrate [15]. Glucose-6-phosphate dehydrogenase was measured following the procedure of Deutsch [16]. The GSH synthesis rate was measured as described in Ref. 5. In this experiment, cellular GSH was first depleted by 0.25 mM DEM followed by cell incubation with 0.5 mM each of glutamate, methionine, and glycine. GSH synthesis rate was then measured by determination of the increase in cellular GSH concentration with time. A direct comparison of measurement of total non-protein thiols by this method and measurement of cellular GSH by HPLC [17] showed that greater than 90% of the increase in non-protein thiols is due to GSH synthesis. Total cytochrome P450 content was determined from the CO difference spectrum of dithionite-reduced microsome samples [18]. Catalase was measured as described in Ref. 19. Briefly, catalase concentration was determined after addition of 26 mM methanol by recording the absorbance change at 660 minus 630 nm following addition of 1.2 mM KCN, and using the extinction coefficient of 5.4 $mM^{-1}\,cm^{-1}$. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured using the method described by Crapo *et al.* [20]. In this spectrophotometric assay system, xanthine/xanthine oxidase was used as a source of superoxide radical and cytochrome *c* was used as the indicating scavenger for the radical. Epoxide hydrolase (EC 4.2.1.63) activity was measured radiometrically by determination of the conversion of [7-^{3}H]styrene oxide to [7-^{3}H]styrene glycol [21]. UDP-glucuronosyltransferase and sulfotransferase activities were determined as described in Ref. 22. In both of the measurements, acetaminophen was used as a substrate. For cytochrome P450 subtypes [7], Western-blot analysis was performed using 0.5 to 2 μg of protein per well. After electrophoresis, the proteins were blotted to nitrocellulose and the filters were blocked with 5% bovine serum albumin or 5% milk powder. Development was carried out using an alkaline-phosphatase linked goat and anti-rabbit-IgG as the secondary antibody and color was obtained by introducing 5-bromo-4-chloro-indolyl phosphate reagents as per manufacturer's instruction. The films were scanned using a Schimadzu CA-930 scanner in the reflectance mode. Relative changes in protein amounts of GSH *S*-transferase, UDP-glucuronosyltransferase [23], and glucose-6-phosphate dehydrogenase were also measured by similar Western blot analyses using specific antibodies. t-BuOOH toxicity was determined by measuring 0.5 mM t-BuOOH-induced loss in cell viability, as measured by exclusion of 0.2% trypan blue.

Statistical analyses. Analyses were performed using the paired Student's *t*-test.

RESULTS

GSH functions in protection against cell injury from oxidants and reactive electrophiles, and a major purpose of the present study was to determine the

Table 1. Effect of chronic hypoxia on GSH-dependent detoxication systems

Enzyme	Activity (nmol/min/mg protein) Hypoxia	Normoxia
GSH peroxidase	450 ± 100* (12)	830 ± 160 (12)
GSSG reductase	71.3 ± 17.7 (9)	50.6 ± 5.1 (9)
GSH *S*-transferase	855 ± 26 (6)	937 ± 62 (6)
Glucose-6-phosphate dehydrogenase	10.9 ± 1.7† (9)	19.3 ± 4.8 (9)

Microsomes and postmicrosomal cytosol were isolated from rats exposed to hypoxia for 10 days or control rats. Enzyme activities were measured under aerobic conditions as described under Materials and Methods. Values are means ± SEM; the numbers in parentheses indicate the number of experiments.

* $P < 0.05$ versus normoxic control.

† $P < 0.1$ versus normoxic control.

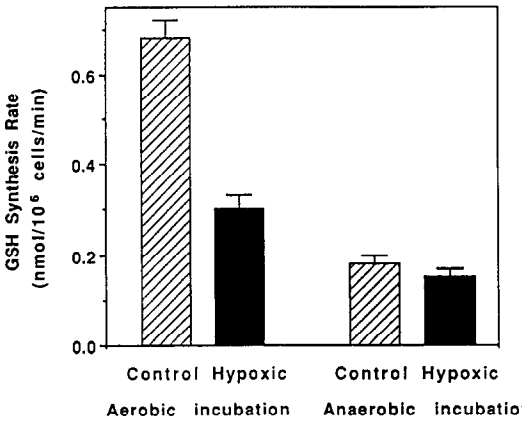

Fig. 1. Effect of chronic hypoxia on GSH synthesis rate in isolated hepatocytes. Hepatocytes isolated from chronic hypoxic rats or control rats were treated with 0.25 mM DEM for 15 min followed by the incubation with 0.5 mM each of glutamate, methionine and glycine under aerobic or anoxic conditions for 60 min. The reactions were terminated by trichloroacetic acid. GSH synthesis was determined by the increase of cellular GSH concentrations, which was measured by the method os Saville [24]. Values are means ± SEM of five cell preparations. Key: (*) $P < 0.001$ versus aerobic control.

effect of chronic hypoxia on GSH-dependent detoxication systems. The results are shown in Table 1. Although GSSG reductase and GSH-*S*-transferase did not show significant change upon hypoxia, 10-day hypoxia resulted in decreased activities of GSH peroxidase and glucose-6-phosphate dehydrogenase by 46 and 44%, respectively.

Previous studies showed that GSH synthesis rate is decreased by acute hypoxia [5]. In the present study, we addressed this question following *in vivo* chronic hypoxia. Figure 1 shows that hepatocytes isolated from chronic hypoxic rats gave much lower GSH synthesis rates under aerobic conditions than control hepatocytes. The cellular GSH content in liver from hypoxic rats was 46.0 ± 3.5 nmol/10^6 cells, which was very similar to that of control rats (41.1 ± 5.5 nmol/10^6 cells). These results suggest that in the absence of oxidative stress or xenobiotics, cellular GSH content is maintained at normal levels during chronic hypoxia even though the synthesis rate is lower. Under pathological conditions where there is a demand for GSH, GSH may not be sufficient because of lower GSH synthesis rate.

Other key enzymes in detoxication systems include cytochrome P450, SOD, catalase and epoxide hydrolase. These enzymes can catalyze reactions that protect cells against toxicity from active oxygen intermediates and other xenobiotics. The effect of chronic hypoxia on activities of these detoxication enzymes is shown in Table 2. Ten-day hypoxia did not change significantly SOD, catalase, epoxide hydrolase, or the total amount of cytochrome P450. Several forms of P450 were analyzed to reflect both constitutive and inducible forms of P450. It was found that some of the subtypes of P450 (CYP2E1, CYP2B1, CYP3A1, P450EtOH2) were decreased 50–60% by hypoxia. Measurements after 5 days on hypoxia showed no significant changes from controls, indicating that the response of P450s to hypoxia is slower than usual for induction time courses (data not shown).

UDP-glucuronosyltransferases and sulfotransferases are two central enzyme systems in detoxication systems that utilize conjugation reactions, i.e. glucuronidation and formation of sulfate esters. Our current study showed that microsomal UDP-glucuronosyltransferase and sulfotransferase activities were decreased significantly by hypoxia to 65 and 77% of their control values, respectively. The cellular values of these two enzymes were decreased to the same extent because the ratio of microsomal to cellular values was not changed by hypoxia (see Table 3).

To study whether protein amounts of those affected enzymes were decreased by hypoxia, glucose-6-phosphate dehydrogenase, GSH *S*-transferases, and UDP-glucuronosyltransferases were analyzed by Western blot analyses using specific antibodies. The results indicated that 10-day hypoxia did not change the concentrations of any of these

Table 2. Effect of chronic hypoxia on other key detoxication enzymes

Enzyme	Activity or concentration	
	Hypoxia	Normoxia
Cytochrome P450 (total) (nmol/mg protein)	0.35 ± 0.03 (12)	0.35 ± 0.05 (12)
CYP2E1 (% of control)	56 ± 39 (6)	100 ± 60 (6)
P450EtOH2 (% of control)	42 ± 13* (6)	100 ± 25 (6)
CYP3A1 (% of control)	53 ± 34† (6)	100 ± 35 (6)
CYP2B1 (% of control)	50 ± 42 (6)	100 ± 46 (6)
SOD (% inhibition of cytochrome *c* reduction/mg protein)	20.8 ± 4.0 (6)	19.6 ± 2.8 (6)
Catalase (nmol/10^6 cells)	0.38 ± 0.03 (4)	0.45 ± 0.05 (4)
Epoxide hydrolase (nmol/min/mg protein)		
Microsomes	4.50 ± 0.36 (5)	4.30 ± 0.55 (6)
Cytosol	1.55 ± 0.19 (6)	1.62 ± 0.25 (6)

Experimental conditions were the same as in Table 1. Catalase was measured in isolated cells. Cytochrome P450 was measured by Western blot analysis as described under Materials and Methods. Values are means ± SEM; the numbers in parentheses indicate the number of experiments.
* $P < 0.01$ versus normoxic control.
† $P < 0.1$ versus normoxic control.

Table 3. Effect of chronic hypoxia on UDP-glucuronosyltransferase and sulfotransferase

Enzyme	Activity		Microsomal/Cellular	
	Hypoxia (N = 9)	Normoxia (N = 6)	Hypoxia	Normoxia
UDP-glucuronosyl transferase (nmol/min/mg protein)			0.25	0.24
Cellular	0.53 ± 0.02*	0.82 ± 0.02		
Microsomal†	0.13 ± 0.01*	0.20 ± 0.02		
Sulfotransferase (nmol/min/mg protein)			0.83	0.74
Cellular	0.29 ± 0.02‡	0.42 ± 0.03		
Microsomal	0.24 ± 0.02*	0.31 ± 0.02		

Experimental conditions were the same as described in Table 1. Values are means ± SEM.
* $P < 0.01$ versus normoxic control.
† Microsomal data on UDP-glucuronosyltransferase were taken from Ref. 9.
‡ $P < 0.05$ versus normoxic control.

enzymes (data not shown). Thus, hypoxia apparently decreased the activities by enzyme regulatory mechanisms rather than changing their synthesis or degradation in the cell.

To test whether these changes in enzyme activities may be associated with increased susceptibility of cells to oxidative injury, t-BuOOH-induced cytotoxicity was studied in isolated hepatocytes from normoxic and chronically hypoxic rats. Cells from chronically hypoxic rats were much more sensitive to cell death caused by t-BuOOH (Fig. 2). Taken together with the result that chronic hypoxia decreased GSH peroxidase and glucose-6-phosphate dehydrogenase activities, the study suggests that declines in activities of enzymes such as GSH peroxidase and glucose-6-phosphate dehydrogenase, which are involved in detoxication reactions, can render cells more susceptible to chemical-induced injury.

DISCUSSION

Hypoxia is commonly associated with physiological or pathological conditions and is relevant to drug metabolism and toxicity in two general ways: it can affect the pathways and rates of drug elimination and it can alter the susceptibility of cells to injury. Many enzyme activities involved in normal metabolism of the body are known to be affected by both acute hypoxia and chronic hypoxia. However, relatively little information is available on key detoxication enzyme activities in the liver as a function of O_2 supply. In the present study, we provide evidence that chronic hypoxia can result in selective decreases in enzyme activities of detoxication systems.

This effect is in addition to the previously described dependence of detoxication upon O_2 concentration that occurs during acute hypoxia [22]. The comparison of activities was made using identical *in vitro* conditions with saturating O_2 concentrations for cells from normoxic and hypoxic rats. This comparison may not accurately reflect differences in functions *in vivo* between normoxic and hypoxic livers because these hypoxic livers have altered enzyme activities, altered cellular O_2 dependence

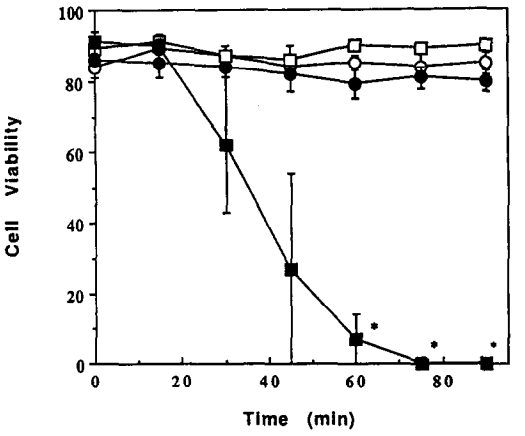

Fig. 2. Effect of chronic hypoxia on t-BuOOH-induced cytotoxicity. Cells isolated from control rats (circles) or rats exposed to hypoxia for 10 days (squares) were incubated in modified Krebs–Henseleit buffer in the absence (open symbols) or presence (closed symbols) of 0.5 mM t-BuOOH. Cell viability was measured at indicated times. Values are means ± SEM of three cell preparations. Key: (*) $P < 0.05$ versus incubation of normoxic cells with t-BuOOH at the correspondent time points.

[9], and altered cellular O_2 exposure [10]. Complete O_2 dependence studies and more accurate assessment of *in vivo* O_2 concentration will be necessary to determine which of these factors is more important in determining *in vivo* rates.

The condition of chronic hypoxia used in the present study represents a moderately severe but not life-threatening condition. In other studies using these conditions, rats gained weight at only about half the rate as controls [9], but hepatic glycogen levels were nearly normal (unpublished data). Total body clearance of acetaminophen was decreased about 35% [9]. Intestinal absorption of acetaminophen [9] and GSH [25] was decreased, as was blood plasma proteins [9] and GSH [9]. Thus, the rats were substantially affected by the degree of hypoxia in several physiological parameters yet continued to groom themselves and otherwise appeared healthy.

After exposure to 10-day hypoxia, rat liver cells showed a much lower GSH synthesis rate than cells from normoxic rats. A similar decrease occurs in cells exposed to acute hypoxia, but this appears to have a different basis. In acute hypoxia, *S*-adenosylmethionine (SAM), a very important intermediate in the cystathionine pathway for cysteine production from methionine, was shown to be decreased when O_2 concentration was decreased [5]. This decrease was due to decreased ATP concentration which limited the activity of the SAM synthetase [5]. In the present experiments, cells from chronically hypoxic rats were maintained under normoxic conditions *in vitro*. Therefore, cellular ATP concentrations were maximal and cannot account for the decreased rate of synthesis. Thus, it appears that the decreased synthesis rate during chronic hypoxia is due to decreased expression or altered regulation of enzymes required for GSH synthesis.

Although maximal GSH synthesis rate is decreased by hypoxia, the total cellular GSH content of liver from hypoxic rats is maintained at the same level as in controls. This indicates that under hypoxic conditions alone, the endogenously synthesized GSH is enough to maintain its cellular level. However, under pathological conditions, where GSH is required for detoxication reactions, GSH synthesis may not be sufficient during hypoxia and may therefore make cells more vulnerable to oxidative and chemical-induced injury.

An enhanced sensitivity to oxidative injury is also suggested by the measurements of glucose-6-phosphate dehydrogenase activity. This enzyme catalyzes oxidation of glucose-6-phosphate, producing NADPH, which is required for regeneration of GSH from GSSG. NADPH supply is decreased by acute hypoxia [4] and this effect, along with decreased GSH synthesis rates [5], contributes to an increased susceptibility of hepatocytes to oxidative stress during acute hypoxia [2]. Because cells from chronically hypoxic rats have both impaired GSH synthesis and NADPH supply, it appeared likely that cells from chronically hypoxic rats would be more vulnerable to oxidative injury and direct measurement with t-BuOOH confirmed this. Thus, altered function of GSH-dependent detoxication systems due to chronic hypoxia increases susceptibility to oxidant-induced injury.

Hypoxia at 10.5% O_2 for 10 days did not change total cytochrome P450 concentration. This is consistent with the report by Costa [26] who demonstrated that relatively moderate hypoxia did not decrease cytochrome P450 concentration. However, severe hypoxia can reduce cytochrome P450 content [26]. The present study showed that even though the total amount of cytochrome P450 was not changed under our conditions, some subtypes, i.e. CYP2B1, P450EtOH2, CYP3A1, and CYP2E1, were decreased by hypoxia. This may be explained by the fact that other P450 forms are induced or that lesser amounts of apoproteins are present in the microsomes from hypoxic rats.

Chronic hypoxia has been shown to increase a variety of enzyme activities, such as succinate dehydrogenase [27], glycogen phosphorylase [28], and pyruvate kinase [29]. Other reports indicate that hypoxia results in a decrease in enzyme activity, such as superoxide dismutase of lung and brain in mice [30]. In contrast with our results, SOD and catalase in rat liver were shown to be reduced by chronic hypoxia [26]. This may be due to the differences in chronic hypoxia models used in different studies, namely, length and severity of hypoxia. Thus, even though some enzymes are relatively more resistant to changes during hypoxia, longer or more severe hypoxia may decrease activities of these enzymes. Hypoxia affects enzyme by two ways, i.e. by changing either the enzyme concentration or the activity. For instance, studies with pyruvate kinase showed that when pyruvate kinase activity is increased by chronic hypoxia, pyruvate kinase content is increased proportionately [29]. In contrast, our current studies show that

chronic hypoxia decreased activities of some detoxication enzymes without detectably changing their concentrations. This apparently occurs by regulation of enzyme activity rather than changes in expression or degradation.

In summary, we have shown that (1) GSH homeostasis was altered by hypoxia; (2) some key enzymes in detoxication were decreased. Others were not changed significantly, but some specific isozymes were affected. Thus, chronic exposure to hypoxia *in vivo* has differential effects on expression and regulation of drug metabolism and detoxication systems; and (3) chronic hypoxia potentiates t-BuOOH-induced injury in hepatocytes. Although the mechanism responsible for this effect is not known, it may be due to reduction in GSH peroxidase and glucose-6-phosphate dehydrogenase activities during chronic hypoxia. These results therefore indicate that chronically hypoxic individuals are likely to have altered pharmacokinetics and enhanced sensitivity to drugs and other foreign compounds.

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REFERENCES

1. Shen ES, Garry VF and Anders MW, Effect of hypoxia on carbon tetrachloride hepatotoxicity. *Biochem Pharmacol* **31**: 3787–3793, 1982.
2. Tribble DL, Jones DP and Edmondson DE, Effect of hypoxia on *tert*-butylhydroperoxide-induced oxidative injury in hepatocytes. *Mol Pharmacol* **34**: 413–420, 1988.
3. Mannervik B and Danielson UH, Glutathione transferases—Structure and catalytic activity. *CRC Crit Rev Biochem* **23**: 283–337, 1988.
4. Tribble DL and Jones DP, Oxygen dependence of oxidative stress. Rate of NADPH supply for maintaining the GSH pool during hypoxia. *Biochem Pharmacol* **39**: 729–736, 1990.
5. Shan X, Aw TY, Shapira R and Jones DP, Oxygen dependence of glutathione synthesis in hepatocytes. *Toxicol Appl Pharmacol* **101**: 261–270, 1989.
6. Osada H and Nakamura A, Changes in the serum LDH isoenzymes in monkey during chronic exposure to simulated high altitude. *Aviat Space Environ Med* **56**: 362–366, 1985.
7. Tindberg N and Ingelman-Sundberg M, Cytochrome P-450 and oxygen toxicity. Oxygen-dependent induction of ethanol-inducible cytochrome-P450 (IIE1) in rat liver and lung. *Biochemistry* **28**: 4499–4504, 1989.
8. Mela L, Goodwin CW and Miller LD, *In vivo* control of mitochondrial enzyme concentrations and activity by oxygen. *Am J Physiol* **231**: 1811–1816, 1976.
9. Aw TY, Shan X, Sillau AH and Jones DP, Effect of chronic hypoxia acetaminophen metabolism in the rat. *Biochem Pharmacol* **42**: 1029–1038, 1991.
10. Jones DP, Kennedy FG, Andersson BS, Aw TY and Wilson E, When is a mammalian cell hypoxic? Insights from studies of cells versus mitochondria. *Mol Physiol* **8**: 473–482, 1985.
11. Moldeus P, Hogberg J and Orrenius S, Isolation and use of liver cells. *Methods Enzymol* **52**: 60–70, 1978.
12. Ernster L, Siekevitz P and Palade GE, Enzyme–structure relationships in the endoplasmic reticulum of rat liver. A morphological and biochemical study. *J Cell Biol* **15**: 541–562, 1962.
13. Mannervik B, Glutathione peroxidase. *Methods Enzymol* **113**: 490–595, 1985.
14. Goldberg DM and Spooner RJ, Glutathione reductase. In: *Methods of Enzymatic Analysis* (Eds. Bergmeyer HU, Bergmeyer J, and Graßl M), 3rd Edn, Vol. 3, pp. 258–264. Verlag Chemie, Weinheim, 1983.
15. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferases. *J Biol Chem* **249**: 7130–7139, 1974.
16. Deutsch J, Glucose-6-phosphate dehydrogenase. In *Methods of Enzymatic Analysis* (Eds. Bergmeyer HU, Bergmeyer J and Graßl M), 3rd Edn, Vol. 3, pp. 190–195. Verlag Chemie, Weinheim, 1983.
17. Fariss MW and Reed DJ, High-performance liquid chromatography of thiols and disulfides: Dinitrophenol derivatives. *Methods Enzymol* **143**: 101–109, 1987.
18. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. *J Biol Chem* **239**: 2379–2385, 1964.
19. Oshino N, Oshino R and Chance B, The characteristics of the "peroxidatic" reaction of catalase in ethanol oxidation. *Biochem J* **131**: 555–567, 1973.
20. Crapo JD, McCord JM and Fridovich I, Preparation and assay of superoxide dismutases. *Methods Enzymol* **53**: 382–393, 1978.
21. Oesch F, Jerina DM and Daly J, A radiometric assay for hepatic epoxide hydrase activity with [7-^{3}H]styrene oxide. *Biochim Biophys Acta* **227**: 685–691, 1971.
22. Aw TY and Jones DP, Secondary bioenergetic hypoxia. Inhibition of sulfation and glucuronidation reactions in isolated hepatocytes at low O_2 concentration. *J Biol Chem* **257**: 8997–9004, 1982.
23. Iyanagi T, Watanabe T and Uchiyama Y, The 3-methylcholanthrene-inducible UDP-glucuronosyltransferase deficiency in the hyperbilirubinemic rat (Gunn rat) is caused by a −1 frameshift mutation. *J Biol Chem* **264**: 21302–21307, 1989.
24. Saville B, A scheme for colorimetric determination of microgram amounts of thiols. *Analyst* **83**: 760–762, 1958.
25. Bai C, Physiological control and cell specificity of glutathione transport. *Ph. D. Dissertation*. Emory University, Atlanta, GA, 1992.
26. Costa LE, Hepatic cytochrome P-450 in rats submitted to chronic hypobaric hypoxia. *Am J Physiol* **259**: C654–C659, 1990.
27. Barrie SE and Harris P, Effects of chronic hypoxia and dietary restriction on myocardial enzyme activities. *Am J Physiol* **231**: 1308–1313, 1976.
28. Theen J, Gilboe DP and Nuttall FQ, Liver glycogen synthetase and phosphorylase changes *in vivo* with hypoxia and anesthetics. *Am J Physiol* **243**: E182–E187, 1982.
29. Hance AJ, Robin ED, Simon LM, Alexander S, Herzenberg LA and Theodore J, Regulation of glycolytic enzyme activity during chronic hypoxia by changes in rate-limiting enzyme content. *J Clin Invest* **66**: 1258–1264, 1980.
30. Liu J, Simon LM, Phillips JR and Robin ED, Superoxide dismutase (SOD) activity in hypoxic mammalian systems. *J Appl Physiol Respirat Environ Exercise Physiol* **42**: 107–110, 1977.