Elevation of lung glutathione by oral supplementation of L-2-oxothiazolidine-4-carboxylate protects against oxygen toxicity in protein-energy malnourished rats

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ABSTRACT **The objectives of this study were to investigate whether oral supplementation of L-2-oxothiazolidine-4-carboxylate (OTC) is effective for increasing tissue glutathione (GSH) concentrations in rats fed a diet very low (0.5%) in protein—a model of wasting malnutrition—and to determine the efficacy of OTC for protection against pulmonary oxygen toxicity. Weanling rats, fed a 0.5 or 15% protein diet for 2 wk, were given an oral supplement of OTC, and tissue GSH concentrations were measured over a 24 h period. OTC supplementation to rats fed 0.5% protein significantly increased GSH concentrations in liver and lung, but not in kidney and blood, when compared with the 0.5% protein unsupplemented group. The liver GSH concentration in the 0.5% protein OTC-supplemented group was higher than the 15% control group. Daily supplementation of OTC protected rats from pulmonary oxygen toxicity during 4 days of 85% oxygen exposure as determined by lung-to-body weight ratios and in vivo proton magnetic resonance imaging. Although hyperoxia exposure increased lung GSH concentrations in all groups, OTC supplementation was effective for increasing lung GSH concentration in rats fed the 0.5% protein diet. This study demonstrated that oral administration of OTC to wasting malnourished rats is an effective procedure to increase GSH concentration rapidly in target organs such as lung, and that daily supplementation of a low dose of OTC has a sustained effect to protect against pulmonary oxygen toxicity during 4 days of hyperoxia exposure.—Taylor, C. G.; Bauman, P. F., Sikorski, B., Bray, T. M. Elevation of lung glutathione by oral supplementation of L-2-oxothiazolidine-4-carboxylate protects against oxygen toxicity in protein energy malnourished rats. *FASEB J.* 6: 3101-3107; 1992.**

Key Words: glutathione • L-2-oxothiazolidine-4-carboxylate • hyperoxia • magnetic resonance imaging • malnutrition

GLUTATHIONE (GSH,[3] γ-GLUTAMYLCYSTEINYLGLYCINE), a substrate for GSH transferase and GSH peroxidase, plays important roles in cellular defense against xenobiotic compounds, reactive oxygen species, and free radicals (1). The concentration of GSH in various tissues reflects the potential for detoxification in the body. For example, depletion of GSH with diethyl maleate increases the toxicity of many xenobiotics whereas treatment with cysteine, methionine, or N-acetylcysteine, precursors for GSH synthesis, decreases their toxicity (1). Tissue GSH concentrations can be influenced by dietary manipulation, e.g., hepatic GSH is decreased during fasting or by feeding diets low in protein or limiting in sulfur amino acids (2, 3).

Wasting malnutrition is a worldwide problem. Protein energy malnutrition (PEM) affects millions of children in developing countries. In affluent countries, a large number and variety of patients also suffer PEM secondary to, for example, burns, chronic digestive diseases, alcoholism, AIDS, or cancer (4, 5). Decreases in tissue and blood GSH concentrations occur in malnutrition (6) and are associated with an increased toxicity to drug or hyperoxia therapy (7, 8). Enhancing detoxification by the antioxidant defense system during the early stabilization phase of the rehabilitation of wasted patients is becoming recognized as being important to the survival of the most debilitated subjects (9).

Prolonged high concentration oxygen therapy is often used with infants of low birth weight (LBW) to increase their chance of survival (10). Several factors, including precarious nutritional status and a compromised antioxidant defense system, may contribute to the high morbidity of these infants (11). Rapid and noninvasive procedures for establishing the defense system in LBW infants are also required at the early stabilization stage.

Cysteine is the limiting amino acid for GSH synthesis, and hepatic GSH responds to the availability of cysteine and cysteine precursors in the diet (3). However, it is difficult to supplement cysteine because cysteine can be toxic and is rapidly oxidized to cystine, which has low solubility at neutral pH (12, 13). L-2-Oxothiazolidine-4-carboxylate (OTC), a stable derivative of cysteine, is converted intracellularly to cysteine by the enzyme 5-oxoprolinase (14). Oral administration of OTC is able to elevate hepatic GSH in protein-deficient rats within 4 h (15). Thus, the objectives of this study are: *1*) to test the hypothesis that oral OTC supplementation is effective for increasing tissue GSH concentrations, especially in the lung, in rats fed a very low protein (0.5%) diet as a model of wasting malnutrition, and *2*) to determine the efficacy of OTC for protecting PEM rats from pulmonary oxygen toxicity.

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[3]Abbreviation: GSH, glutathione; PEM, protein energy malnutrition; OTC, L-2-oxothiazolidine-4-carboxylate; LBW, low birth weight; DTNB, 5,5'-dithiobis(2-nitrobenzoid acid); MRI, magnetic resonance imaging; TE, echo time; TR, delay time; VOSY, volume-selective spectroscopy.

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MATERIALS AND METHODS

Chemicals

5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB), NADPH, GSH reductase (type III from yeast), and OTC were obtained from Sigma Chemical (St. Louis, Mo.). GSH was obtained from Boeringer-Manheim Canada (Dorval, Quebec). All other chemicals were obtained from Fisher Scientific (Toronto, Ontario).

Animals and diets

The animal care protocol for experiments conducted in this paper was reviewed and approved by the University of Guelph Animal Care Committee. Male weanling Wistar rats (Charles River Canada, St. Constant, Quebec) with initial weight of 55–65 g were housed individually in suspended stainless steel mesh cages in a temperature- and humidity-controlled room with light from 0800 to 2000 h. Rats had free access to casein-based purified diets containing either 0.5 or 15% protein (**Table 1**) for 2 wk.

Experimental design

In the first experiment, the effect of oral supplementation of OTC on tissue GSH concentration over 24 h was studied in rats fed 0.5 or 15% protein diet for 2 wk. Before OTC supplementation, feed was removed during the light period. At the beginning of the dark period (time 0) and 4 and 8 h later, each rat was given by gavage either OTC (0.035 g dissolved in 0.5 ml water) or vehicle (water). The dose of OTC was chosen such that rats fed the 0.5% protein diet would receive an amount of sulfur amino acid equivalent to the amount available to rats fed the 15% protein diet, taking into account the average amount of feed consumed and the methionine and cyst(e)ine content of the diet. Feed was returned at time 0 and was available during the entire 24 h experimental period. Rats were killed by CO_2 intoxication, followed by decapitation beginning at time 0 and every 4 h for 24 h. Liver, lung, and kidney were removed, frozen in liquid nitrogen, and stored at −80°C until analysis. Trunk blood was collected in heparinized tubes and 50 μl was immediately aliquoted into 1 ml 5% trichloroacetic acid, centrifuged, and the supernatant fraction was stored frozen at −80°C until analysis for total GSH.

To study the effect of oral supplementation of OTC on pulmonary oxygen toxicity, a similar design was carried out in the second experiment. At the end of a 2 wk feeding regimen, each dietary treatment group (0.5 or 15% protein) was given a daily supplement of OTC (0.035 g in 0.25 ml water) or vehicle for 4 days during the hyperoxia exposure. OTC supplementation and the hyperoxia exposure started at the beginning of the dark period (time 0). Rats were exposed to 85% oxygen or air for 4 days in Plexiglas chambers. The chambers were opened once per day for approximately 15 min to supply fresh feed and administer OTC or vehicle. For 85% oxygen chamber, compressed oxygen and air were mixed with a Bird 3800 Microblender, and the oxygen concentration was monitored continuously using a Servomex Oxygen Analyzer OA570. The air flow rate for both chambers was controlled at 8–10 chamber changes/h.

TABLE 1. *Diet composition*

Ingredient	Dietary protein, g/100 g	
	0.5%	15%
Casein[a]	0.59	17.6
DL-methionine[b]	0.01	0.30
Cornstarch[c]	84.7	67.4
Corn oil[c]	5.0	5.0
Cellulose[d]	5.0	5.0
Vitamin mix[e]	1.0	1.0
Mineral mix[f]	3.5	3.5
Choline bitartrate	0.2	0.2

[a]Casein—Vitafree [85.2% protein on air-dried basis, protein contains 2.5% methionine and 0.3% cystine (U.S. Biochemical product information)], U.S. Biochemical (USB), Cleveland, Ohio. [b]USB. [c]St. Lawrence Starch, Mississauga, Ontario. [d]Celufil—non-nutritive bulk, USB. [e]AIN-76A (16, 17). [f]AIN-76 (17).

GSH assay

Tissue was homogenized in 5% trichloroacetic acid for 10 s (Ultra Turrax disperser with stainless steel shaft, Terochem Laboratories, Mississauga, Ontario) and centrifuged at 10,000 × *g* for 15 min at 4°C. The supernatant fraction was analyzed for total GSH by the enzymatic method of Tietze (18). The formation of a conjugate with DTNB was measured at 412 nm.

Assessment of lung damage

To assess the severity of hyperoxia-induced lung damage, lung-to-body weight ratios, in vivo proton magnetic resonance imaging (MRI), and histopathology were used. Rats were imaged by in vivo proton MRI on day 0 and after 4 days of hyperoxia exposure using a SIS 2.0 tesla/31 cm bore imaging system (Spectroscopy Imaging Systems Corporation, Fremont, Calif.). For the imaging sequences, a respiratory gating device was used to trigger data acquisition and thus eliminate motion artifacts. Multiple proton image slices were taken in the transverse plane using an echo time (TE) of 25 ms and a delay time (TR) of 1.0 s. The image slices were 2.4 mm thick, field of view was 8 × 6 cm^2 with 128 phase-encoding steps, 4 acquisitions per step, and had 512 frequency encoding points. To verify that the high-intensity proton signal in the lung region of the images represented protons in water molecules and not lipid accumulation, localized spectra (4.7 × 4.7 mm^3 voxels) were obtained using the volume-selective spectroscopy (VOSY) technique (19, 20). The sequence for the VOSY determinations was a 20 ms delay after the first and third 90° pulses and a 65 ms delay after the second 90° pulse. Immediately after MRI lungs were removed, placed in 10% phosphate-buffered formalin, processed for histology using H & E staining, and assessed by light microscopy.

Statistical analysis

Data were analyzed using ANOVA within the General Linear Model procedure of SAS (21) with a factorial model. When an overall F ratio was significant, the least-squares means were used pairwise to determine significant differences. When the probability of getting a larger *t* value was less than 0.05, the differences were considered significant. The preplanned comparisons made in each time period were between 15% treatment and each other treatment and between 0.5 and 0.5% + OTC. Values are expressed as means ± SEM.

RESULTS

The model of wasting malnutrition was successfully established by feeding a diet very low in protein. Rats fed the 0.5% protein diet consumed 134.7 ± 3.4 g of feed but lost

9.1 ± 0.2 g over 2 wk, whereas rats fed the 15% protein diet for 2 wk consumed 213.7 ± 4.8 g and gained 97.7 ± 1.1 g.

The effect of oral supplementation of OTC on tissue and blood concentration of GSH over 24 h is shown on **Fig. 1.** OTC supplementation had a significant effect on increasing liver GSH concentration at all time points in rats fed the 0.5% protein diet but not in rats fed 15% protein diet (Fig. 1*A*). Rats fed the unsupplemented 0.5% protein diet had significantly lower concentrations of GSH in the liver at all time points than rats in the other treatment groups. OTC supplementation had a significant effect on lung GSH concentration (Fig. 1*B*). Supplementation of OTC to rats fed the 0.5% protein diet significantly increased lung GSH compared with rats fed the 0.5% protein diet but not supplemented (except at 20 h). OTC also elevated lung GSH at 4 and 8 h in rats fed 15% protein. However, lung GSH in rats fed 0.5% protein, regardless of OTC supplementation, was significantly lower than lung GSH in rats fed the 15% protein diet. Kidney GSH concentrations were lower than in liver and lung, and there were no significant differences in kidney GSH concentrations among the treatment groups (Fig. 1*C*). Giving oral supplementation of OTC did not have a significant effect on GSH concentration in whole blood (blood GSH). Blood GSH in rats fed the 0.5% protein diets (supplemented with OTC or unsupplemented) was lower at all time points than blood GSH in rats fed the 15% protein diets (Fig. 1*D*).

In experiment 2, the efficacy of OTC supplementation for protecting against hyperoxia-induced lung damage was evaluated. **Figure 2** demonstrates the effect of OTC and

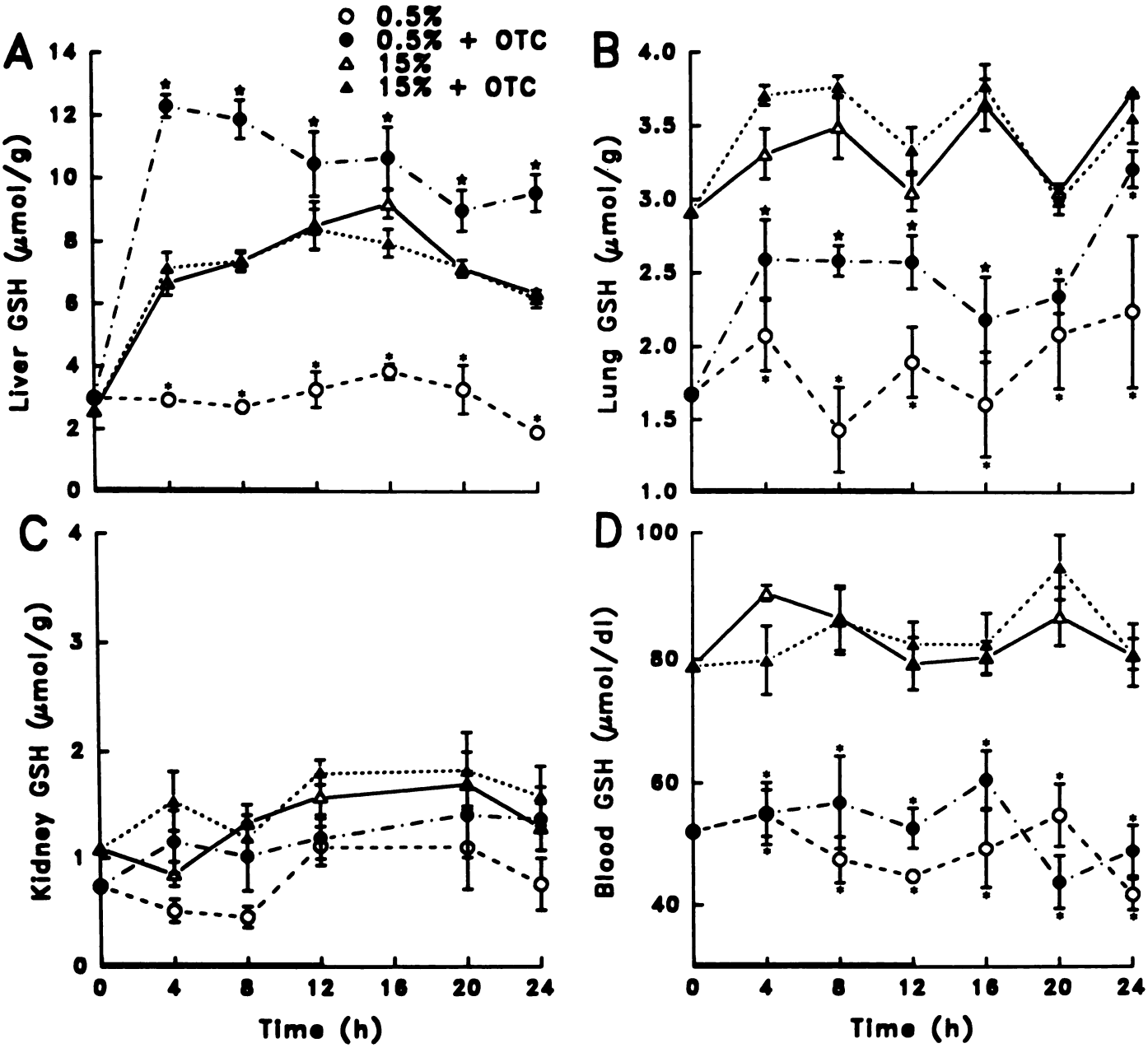

Figure 1. Effect of oral supplementation of OTC on organ GSH concentration over a 24 h period in rats fed 0.5 or 15% protein diets. Values are expressed as means ± SEM, n = 5 or 6. Significant ($P < 0.05$) main effects were: diet (all organs), time (liver, lung, and kidney), and time × diet interaction (liver and kidney). Values marked with a star are significantly different from values in both the 0.5 and 15% groups. Values marked with an asterisk are significantly different from values in either the 0.5 or 15% group.

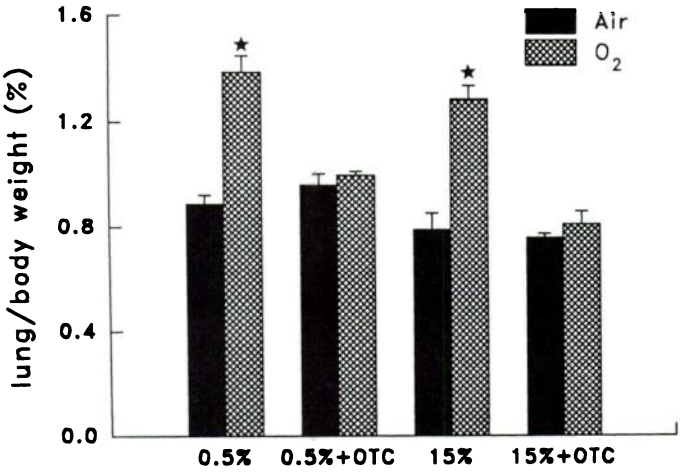

Figure 2. Effect of oral supplementation of OTC and hyperoxia exposure on lung-to-body weight ratios in rats fed 0.5 or 15% protein diet. Values are expressed as means ± SEM, n = 3 or 4. Significant ($P < 0.05$) main effects are diet, hyperoxia exposure, and diet × hyperoxia exposure interaction. Values marked with a star are significantly different from both the value in the respective air control group and the value in the respective OTC-supplemental group.

hyperoxia on the lung-to-body weight ratio as an index of pulmonary oxygen toxicity in rats fed the 0.5 and 15% protein diets. Exposure to 85% oxygen for 4 days significantly increased the percent of lung/body weight ratio in unsupplemented groups regardless of dietary protein level. The lung-to-body weight ratio increased from 0.89 to 1.39% in rats fed a 0.5% protein diet and from 0.79 to 1.29% in rats fed a 15% protein diet. OTC supplementation reduced the increase in lung-to-body weight ratio significantly from 1.39 to 1.00% in rats fed the 0.5% protein diet and from 1.29 to 0.81% in rats fed the 15% protein diet.

The severity of pulmonary oxygen toxicity was also evaluated by in vivo proton MRI. Proton MRI is a technique for noninvasively monitoring lung damage due to an increased water content such as edema. Increased concentrations of protons will appear as areas of high intensity or "bright spots" in the image. In vivo proton MRI images of transverse section of the upper chest cavity of rats after exposure to hyperoxia for 4 days are shown in **Fig. 3.** In all treatment groups, the absence of high-intensity proton signals in the lung region before hyperoxia (images not shown) indicated that there was no lung damage detectable by MRI. After 4 days of 85% oxygen exposure, the 0.5% protein group without the OTC supplementation (Fig. 3*A*) had severe lung damage as indicated by the large area of increased signal intensity that appeared to outline the area occupied by the

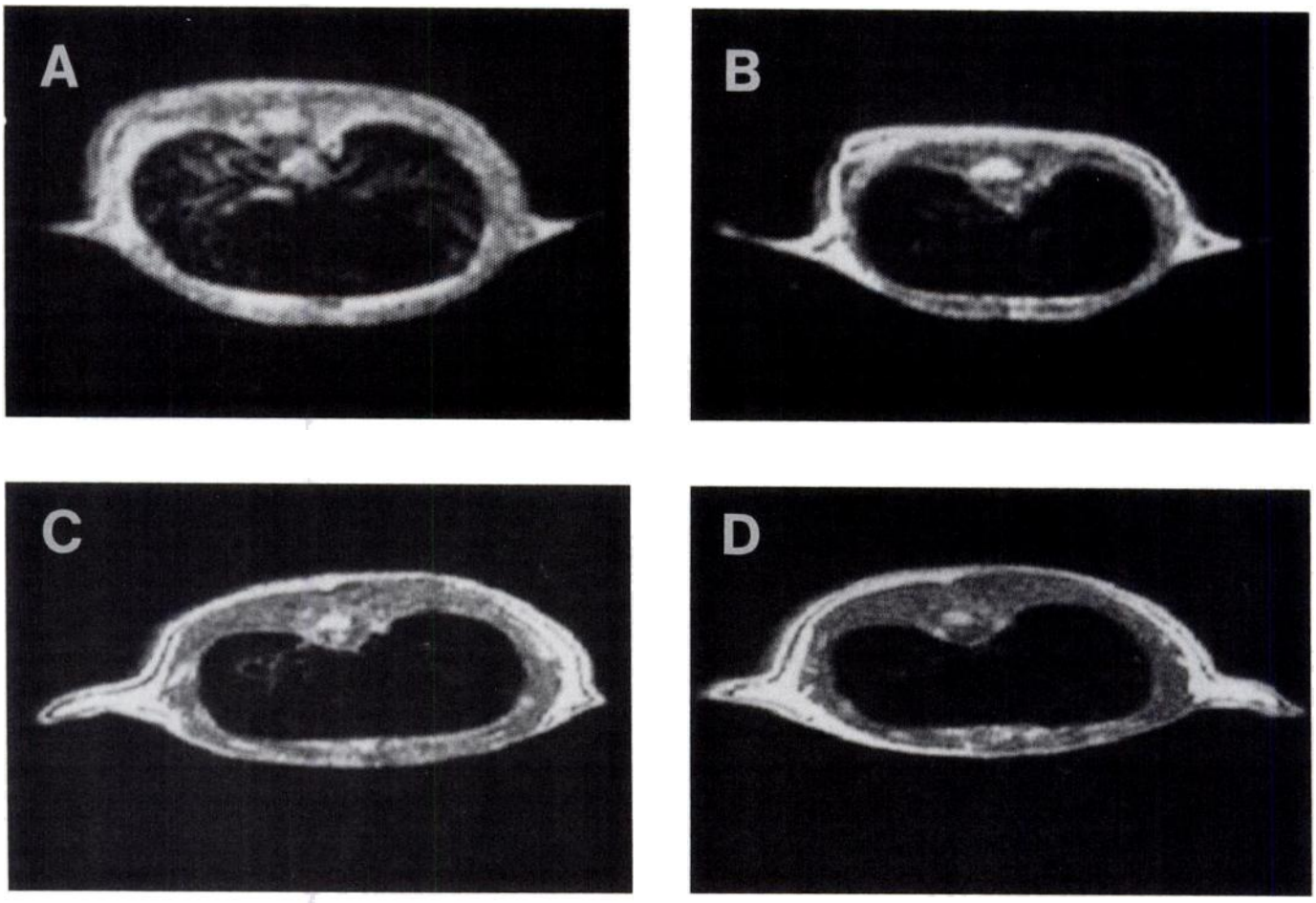

Figure 3. In vivo proton MRI transverse images of the lung region of rats after 4 days of hyperoxia exposure (85% oxygen). Respiratory gating was used to prevent motion artifacts caused by anatomic motion from respiration. At the top of the image or the dorsal side of the rat, the spinal cord is represented as a bright region. The heart is not visible in these images because cardiac gating was not used. The most severe lung damage, as indicated by the presence of increased proton signal intensity in the lung region, is observed in *A* (0.5% protein) when compared with the other dietary treatments: 0.5% protein + OTC (*B*), 15% protein (*C*), and 15% protein + OTC (*D*).

lungs. VOSY was used to verify that the high intensity signals were from protons in water molecules (chemical shift was 4.5 ppm) and not lipids. When a daily supplement of OTC was given to rats fed 0.5% protein diet, it appeared to prevent proton MRI-detectable, hyperoxia-induced lung damage (Fig. 3*B*). By comparison, rats fed the 15% protein diet showed minimal lung damage as monitored by proton MRI (Fig. 3*C*). Daily supplementation of OTC to rats fed the 15% protein diet may have offered some protection on MRI-detectable, hyperoxia-induced lung damage (Fig. 3*D*). Histopathological assessment by light microscopy confirmed the evaluation of the lung damage detected by MRI (data not shown).

Lung GSH concentrations at 0, 2, and 4 days after exposure to 85% oxygen in rats fed the 0.5 or 15% protein diet with or without OTC supplementation are shown in **Table 2.** Exposure to hyperoxia for 4 days elevated lung GSH in all treatment groups when compared with day 0 values. Daily OTC supplementation to 0.5% protein group was effective in elevating GSH concentration after 2 days, with a further increase after 4 days of hyperoxia. Daily OTC supplementation to the 15% protein group significantly increased lung GSH concentration after 2 days of hyperoxia exposure but no further increase was observed after 4 days of hyperoxia.

DISCUSSION

This study demonstrated that oral administration of OTC to wasting malnourished animals is an effective procedure to increase GSH concentration rapidly in target organs such as lung, and that daily supplementation of a low dose of OTC has a sustained effect to protect against pulmonary oxygen toxicity during 4 days of hyperoxia exposure. It has been previously shown that OTC, a prodrug of cysteine, can be absorbed readily and that a peak concentration can be reached in plasma between 45 and 60 min after a single oral dose in healthy humans (22). It has also been shown that OTC can increase hepatic GSH concentration, but only in animals in which GSH has been previously depleted (14, 15). In the current study we demonstrated that in wasting malnourished animals, hepatic GSH can be restored within 4 h and reaches a concentration beyond the reported normal physiological maximum (8–10 μmol/g) (23, 24) with oral supplementation of a single substrate (Fig. 1), without the complete nutritional support of amino acids and energy. This overshoot phenomenon of hepatic GSH synthesis has been observed in weanling rats fed a marginally protein-deficient (7.5%) diet for 2 wk that gained 121% of their initial body weight (15). However, we were surprised that weanling rats fed a diet containing practically no protein for 2 wk that lost 20% of their initial body weight were still able to respond rapidly to OTC supplementation and maintain the ability to synthesize GSH.

There are at least three possibilities for this overshoot phenomenon. First, it seems that there is a high priority to maintain the enzymes for GSH synthesis, such as γ-glutamylcysteinyl synthetase, even in a severe catabolic state. Tateishi et al. (25) demonstrated that treatment of actinomycin D or cycloheximide did not inhibit the recovery of hepatic GSH level after refeeding of rats starved for 1 or 2 days. They suggested that the rapid increase in the hepatic GSH level upon feeding starved animals did not require de novo formation of GSH-synthesizing enzymes. In addition, they have shown a declined GSH-degrading activity in the liver upon refeeding rats after 24 h starvation (25). Second, it is possible that hepatic GSH efflux is sensitive to the dietary protein level and that the efflux mechanism is impaired during dietary protein or energy deprivation. On the contrary, it has been shown in rats that, during fasting, hepatic GSH efflux into plasma has increased and the clearance rate of plasma GSH has also increased (26). Third, the feedback inhibition of γ-glutamylcysteinyl synthetase by GSH (27) may be delayed upon refeeding of rats after protein or energy deprivation, but there are no direct experimental data to support this speculation.

In addition to the increase in hepatic GSH concentration, oral supplementation of OTC enhanced pulmonary GSH concentration in wasting animals. However, GSH concentration in the lung was influenced mainly by the long-term dietary protein regimen; e.g., rats fed the 0.5% protein diet for 2 wk had lower lung GSH concentration than rats fed the 15% protein diet (Fig. 1*B*). This increased GSH concentration in the lung by OTC reflects the possible combined effects of increased influx of GSH from blood and increased endogenous synthesis of GSH. Similarly, blood GSH concentration is influenced mainly by protein nutritional status (Fig. 1*D*). As erythrocyte does not contain oxoprolinase (28), OTC supplementation should not supply substrate directly for endogenous synthesis of GSH in red blood cells. From the clear differences between 0.5 and 15% protein diets (Fig. 1*D*), blood GSH may be a good index for long-term GSH status affected by protein nutritional status. In fact, consistently low erythrocyte GSH concentration has been used to differentiate between kwashiorkor (predominantly protein deficiency) and marasmus (mainly energy deficiency) in children (6).

There were no significant differences in kidney GSH concentrations among the treatment groups (Fig. 1*C*). The values for kidney GSH concentration in this experiment are lower than values in the literature, which range from 2.5 to 3.5 μmol/g (29). Perhaps this is because of the method of handling, i.e., freezing and thawing, all tissue samples in this experiment. The kidney, along with the pancreas, contains

TABLE 2. *Effect of oral supplementation of OTC and hyperoxia exposure on lung GSH concentration in rats fed 0.5 or 15% protein diets*

	GSH, μmol/g lung weight[a]		
	Day 0	Day 2	Day 4
0.5%	2.20 ± 0.12^{AX}	2.10 ± 0.09^{AX}	3.00 ± 0.35^{BX}
0.5% + OTC	3.23 ± 0.12^{AY}	4.78 ± 0.17^{BY}	5.93 ± 0.13^{CY}
15%	4.20 ± 0.07^{AZ}	4.53 ± 0.06^{AY}	5.00 ± 0.04^{BZ}
15% + OTC	3.95 ± 0.09^{AZ}	5.15 ± 0.06^{BZ}	5.20 ± 0.11^{BZ}

[a]Values are expressed as means ± SEM, $n = 3$ or 4. Significant ($P < 0.05$) main effects are diet, hyperoxia exposure, and diet × hyperoxia exposure interaction. Values within a row not sharing the same A, B, or C are significantly different. Values within a column not sharing the same X, Y, or Z are significantly different.

high levels of γ-glutamyl transpeptidase that readily cleave GSH as the tissue is thawing. In other tissues with low γ-glutamyl transpeptidase activity, such as the lung, liver, and blood, GSH is stable when stored at −80°C (30).

It has been proposed that the clinical symptoms of kwashiorkor result from a weakened defense system unable to detoxify an increased production of free radicals from endogenous noxious metabolites, infection, and toxins contaminating the food supply such as aflatoxin (6). Antioxidant nutrient status—for example, vitamin E, A, carotene, Zn, and Se—in children with kwashiorkor is generally low (6, 31) and erythrocyte GSH concentration is characteristically low (6, 32). Children who die soon after admission to the hospital have the lowest levels of vitamin E, Zn, and GSH and the highest levels of plasma ferritin and hepatic Fe (6). When children with kwashiorkor are treated with a high-energy diet containing high polyunsaturated fatty acids, iron, or protein hydrolysate, there is often a high mortality rate (6, 33). Thus, immediate rehabilitation with a high-energy diet could be contraindicated. In addition to its antioxidant function, GSH is also reported to be important in the initiation of lymphocyte activation (34) and thus essential for host immunological defense. Supplementation of OTC before treatment with a high-energy diet or drugs, to rapidly restore GSH for both antioxidant and immune defense systems, may improve the survival and recovery rate of wasted patients.

Adults with acute or chronic pulmonary diseases who require treatment with hyperoxia are often unable to maintain an adequate food intake and show evidence of protein and caloric malnutrition (35). Deneke et al. (36) have shown that adult rats fed a low-protein or low-energy diet for 6 days exhibit an increased susceptibility to 98% oxygen compared with animals fed a 25% protein diet. Supplementation of the low-protein diet with sulfur-containing amino acids cystine, cysteine, and methionine prevented the increased oxygen toxicity. Similarly, premature infants often require the use of elevated oxygen concentrations in incubators due to immaturity of the lung. This increased oxidative stress requires adequate defense and repair systems to prevent chronic lung damage. However, LBW infants are at high risk due to their limited energy reserve and low antioxidant status. The availability of the limiting substrate for GSH synthesis in premature infants may especially be reduced because the cystathionase pathway that converts methionine to cysteine is not fully developed (37). Our study showed that hyperoxia exposure is a stress to weanling rats fed either the 0.5 or 15% protein diet (Fig. 2 and Fig. 3). In this and other studies (8), it has been demonstrated that hyperoxia also induces lung GSH concentration in response to the oxidative stress. Daily supplementation of a low dose of OTC during the hyperoxia exposure was capable of further increasing lung GSH concentration (Table 2) and protecting lung from oxygen toxicity (Fig. 2 and Fig. 3).

Various methods such as supplementation of prodrug, precursor, or GSH itself have been used to increase GSH in different tissues for protection against oxidative toxicity (38–41). This may be clinically relevant, especially for those diseases in which decreased tissue GSH is implicated. For example, it is reported that the GSH concentrations in plasma and lung epithelial lining fluid of HIV-seropositive subjects are about 30 and 60%, respectively, of those in normal individuals (42). OTC has been shown to increase GSH concentration in the liver (15), lung (Fig. 1), brain (43), and lymphocytes (22). It is also effective in prevention of hepatotoxicity of bromobenzene (44), acetaminophen (14, 45), and pulmonary toxicity of hyperoxia (Fig. 2 and Fig. 3). Optimizing GSH status in target organs or tissues could be an important strategy for the enhancement of detoxification and immune functions to prevent and treat the clinical manifestation of various diseases. FJ

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REFERENCES

1. Meister, A., and Anderson, M. E. (1983) Glutathione. *Annu. Rev. Biochem.* **52,** 711–760
2. Cho, E. S., Sahyoun, N., and Stegink, L. D. (1981) Tissue glutathione as a cyst(e)ine reservoir during fasting and refeeding of rats. *J. Nutr.* **111,** 914–922
3. Bauman, P. F., Smith, T. K., and Bray, T. M. (1988) The effect of dietary protein and sulfur amino acids on hepatic glutathione concentration and glutathione-dependent enzyme activities in the rat. *Can. J. Physiol. Pharmacol.* **66,** 1048–1052
4. Bistrian, B. R., Blackburn, G. L., Hallowell, E., and Heddle, R. (1974) Protein status of general surgical patients. *J. Am. Med. Assoc.* **230,** 858–860
5. Bistrian, B. R., Blackburn, G. L., Vitale, J., Cochran, D., and Naylor, J. (1976) Prevalence of malnutrition in general medical patients. *J. Am. Med. Assoc.* **235,** 1567–1570
6. Golden, M. H. N., and Ramdath, D. (1987) Free radicals in the pathogenesis of kwashiorkor. *Proc. Nutr. Soc.* **46,** 53–68
7. Mitchell, J. R., Jollow, D. J., Potter, W. Z., Gillette, J. R., and Brodie, B. B. (1973) Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. *J. Pharmacol. Exp. Ther.* **187,** 211–217
8. Deneke, S. M., Lynch, B. A., and Fanburg, B. L. (1985) Effects of low protein diets or feed restriction on rat lung glutathione and oxygen toxicity. *J. Nutr.* **115,** 726–732
9. Woodward, B., and Filteau, S. M. (1990) Immunoenhancement in wasting protein-energy malnutrition: assessment of present information and proposal of a new concept. In *Advances In Nutritional Research* (Draper, H. H., ed) Vol. 8, pp. 11–34, Plenum Press, New York
10. Northway, W. H., Jr. (1990) Bronchopulmonary dysplasia: then and now. *Arch. Dis. Child.* **65,** 1076–1081
11. Frank, L., and Sosenko, I. R. S. (1988) Undernutrition as a major contributing factor in the pathogenesis of bronchopulmonary dysplasia. *Am. Rev. Resp. Dis.* **138,** 725–729
12. Birnbaum, S. M., Winitz, M., and Greenstein, J. P. (1957) Quantitative nutritional studies with water-soluble, chemically defined diets. III. Individual amino acids as sources of "nonessential" nitrogen. *Arch. Biochem. Biophys.* **72,** 428–436
13. Olney, J. W., and Ho, O.-L. (1970) Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. *Nature (London)* **227,** 609–611
14. Williamson, J. M., Boettcher, B., and Meister, A. (1982) Intracellular cysteine delivery system that protects against toxicity by promoting glutathione synthesis. *Proc. Natl. Acad. Sci. USA* **79,** 6246–6249
15. Bauman, P. F., Smith, T. K., and Bray, T. M. (1988) Effect of dietary protein deficiency and L-2-oxothiazolidine-4-carboxylate on the diurnal rhythm of hepatic glutathione in the rat. *J. Nutr.* **118,** 1048–1054
16. American Institute of Nutrition (1977) Report of the AIN Ad Hoc Committee on standards for nutritional studies. *J. Nutr.* **107,** 1340–1348
17. American Institute of Nutrition (1980) Second report of the Ad Hoc Committee on standards for nutritional studies. *J. Nutr.* **110,** 1726
18. Tietze, F. (1969) Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione:

applications to mammalian blood and other tissues. *Anal. Biochem.* **27,** 502–522
19. Frahm, J., Merboldt, K. D., Hanicke, W., and Haase, A. (1985) Stimulated echo imaging. *J. Magn. Res.* **64,** 81–93
20. Granot, J. (1986) Selected volume excitation using stimulated echoes (VEST): applications to spatially localized spectroscopy and imaging. *J. Magn. Res.* **70,** 488–492
21. SAS Institute (1985) SAS *User's Guide: Statistics.* SAS (Statistical Analysis System) Institute, Cary, North Carolina
22. Porta, P., Aebi, S., Summer, K., and Lauterburg, B. H. (1991) L-2-Oxothiazolidine-4-carboxylic acid, a cysteine prodrug: pharmacokinetics and effects on thiols in plasma and lymphocytes in human. *J. Pharmacol. Exp. Ther.* **257,** 331–334
23. Jaeschke, H., and Wendel, A. (1985) Diurnal fluctuation and pharmacological alteration of mouse organ glutathione content. *Biochem. Pharmacol.* **34,** 1029–1033
24. Williamson, J. M., and Meister, A. (1981) Stimulation of hepatic glutathione formation by administration of L-2-oxothiazolidine-4-carboxylate, a 5-oxo-L-prolinase substrate. *Proc. Natl. Acad. Sci. USA* **78,** 936–939
25. Tateishi, N., Higashi, T., Shinya, S., Naruse, A., and Sakamoto, Y. (1974) Studies on the regulation of glutathione level in rat liver. *J. Biochem.* **75,** 93–103
26. Kaplowitz, N., Eberle, D. E., Petrini, J., Touloukian, J., Corvasce, M. C., and Kuhlenkamp, F. (1983) Factors influencing the efflux of hepatic glutathione into bile in rats. *J. Pharmacol. Exp. Ther.* **224,** 141–147
27. Richman, P. G., and Meister, A. (1975) Regulation of γ-glutamyl-cysteine synthetase by nonallosteric feedback inhibition by glutathione. *J. Biol. Chem.* **250,** 1422–1426
28. Van Der Werf, P., and Meister, A. (1975) The metabolic formation and utilization of 5-oxo-L-proline (L-pyroglutamate, L-pyrrolidine carboxylate). *Adv. Enzymol.* **43,** 519–556
29. Davies, M. H., Birt, D. F., and Schnell, R. C. (1984) Direct enzymatic assay for reduced and oxidized glutathione. *J. Pharmacol. Methods* **12,** 191–194
30. Anderson, M. E. (1985) Determination of glutathione and glutathione disulfide in biological samples. In *Methods in Enzymology* (Meister, A., ed) Vol. 113, pp. 548–555, Academic, San Diego, California
31. Thurnham, D. I. (1990) Antioxidants and prooxidants in malnourished populations. *Proc. Nutr. Soc.* **49,** 247–259
32. Jackson, A. A. (1986) Blood glutathione in severe malnutrition in childhood. *Trans. R. Soc. Trop. Med. Hyg.* **80,** 9111–9113
33. McFarlane, H., Reddy, S., Adcock, K. J., Adeshina, H., Cooke, A. R., and Akene, J., and McFarlane, H. (1970) Immunity, transferrin, and survival in kwashiorkor. *Br. Med. J.* **4,** 268–270
34. Hamilos, D. L., and Wedner, H. J. (1985) The role of glutathione in lymphocyte activation. I. Comparison of inhibitory effects of buthionine sulfoximine and 2-cyclohexene-1-one by nuclear size transformation. *J. Immunol.* **135,** 2740–2747
35. Tremper, J. C. (1981) Nutritional assessment of individuals with chronic obstructive pulmonary disease. *Am. Rev. Respir. Dis.* **123,** 118S
36. Deneke, S. M., Gershoff, S. N., and Fanburg, B. L. (1983) Potentiation of oxygen toxicity in rats by dietary protein or amino acid deficiency. *J. Appl. Physiol.* **54,** 147–151
37. Zlotkin, S. H., and Anderson, G. H. (1982) The development of cystathionase activity during the first year of life. *Pediatr. Res.* **16,** 65–68
38. Meister, A., Anderson, M. E., and Hwang, O. (1986) Intracellular cysteine and glutathione delivery systems. *J. Am. Col. Nutr.* **5,** 137–151
39. Moore, W. R., Anderson, M. E., Meister, A., Murata, K., and Kimura, A. (1989) Increased capacity for glutathione synthesis enhances resistance to radiation in *Escherichia coli:* a possible model for mammalian cell protection. *Proc. Natl. Acad. Sci. USA* **86,** 1461–1464
40. Mårtensson, J., Jain, A., Frayer, W., and Meister, A. (1989) Glutathione metabolism in the lung: inhibition of its synthesis leads to lamellar body and mitochondrial defects. *Proc. Natl. Acad. Sci. USA* **86,** 5296–5300
41. Buhl, R., Vogelmeier, C., Critenden, M., Hubbard, R. C., Hoyt, R. F., Jr., Wilson, E. M., Cantin, A. M., and Crystal, R. G. (1990) Augmentation of glutathione in the fluid lining epithelium of the lower respiratory tract by directly administering glutathione aerosol. *Proc. Natl. Acad. Sci. USA* **87,** 4063–4067
42. Buhl, R., Holroyd, K. J., Mastrangeli, A., and Cantin, A. M. (1989) Systemic glutathione deficiency in symptom-free HIV-seropositive individuals. *Lancet* **2,** 1294–1298
43. Mesina, J. E., Page, R. H., Hetzel, F. W., and Chopp, M. (1989) Administration of L-2-oxothiazolidine-4-carboxylase increases glutathione levels in rat brain. *Brain Res.* **478,** 181–183
44. Brodeur, J., and Goyal, R. (1987) Effect of a cysteine prodrug, L-2-oxothiazolidine-4-carboxylic acid, on the metabolism and toxicity of bromobenzene: an acute study. *Can. J. Physiol. Pharmacol.* **65,** 816–822
45. Hazelton, G. A., Hjelle, J. J., and Klaassen, C. D. (1986) Effects of cysteine prodrugs on acetaminophen-induced hepatotoxicity. *J. Pharmacol. Exp. Ther.* **237,** 341–349

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