Biochemical Pharmacology, Vol. 60, pp. 1297–1303, 2000.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(00)00440-8

Peroxide-like Oxidant Response in Lungs of Newborn Guinea Pigs Following the Parenteral Infusion of a Multivitamin Preparation

*Jean-Claude Lavoie, Sophie Laborie, Thérèse Rouleau, Monika Spalinger and Philippe Chessex**

Perinatal Service and Research Center, Hôpital Ste-Justine, Department of Pediatrics, University of Montreal, Montreal, Quebec, Canada

ABSTRACT. The multivitamin solution is a major component responsible for the photo-induced generation of peroxides in parenteral nutrition. The lung is a target of oxidant injury; however, the specific role of infused peroxides is unknown. The aim of this study was to determine if parenteral multivitamins induce in the lung an oxidant challenge similar to that of peroxides. Newborn guinea pigs were infused with dextrose plus relevant concentrations of H_2O_2 (0, 250, 500 μM) or multivitamins (0, 1%), as well as parenteral nutrition supplemented with multivitamins (0, 1%). After 4 days, total glutathione, glutathione-related enzymes, and oxidant-sensitive eicosanoids were measured in the lungs. Peroxides as well as multivitamins led to a significant decrease in glutathione and the activity of glutathione synthase, indicating that infused peroxides were not entirely transformed into free radicals, which would have stimulated glutathione synthesis. The multivitamin solution induced a response in oxidant-sensitive eicosanoids similar to the response to peroxides, suggesting an oxidant stress that was not alleviated by the antiradical properties of its components. The effects on prostaglandins occurred independently from the stimulation in glutathione levels induced by parenteral nutrition. The multivitamin solution carries an oxidant load and causes effects similar to those of peroxides in the lungs of newborn guinea pigs. BIOCHEM PHARMACOL **60**;9:1297–1303, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. peroxides; glutathione; newborn; guinea pig; parenteral nutrition; prostaglandins; vitamins

Premature infants often require intravenous nutrition during the first days of life because of feeding problems related to their immaturity. Solutions of TPN† are contaminated mainly with H_2O_2 [1, 2], as well as with organic peroxides [3]. The generation of these peroxides is the result of reactions between oxygen dissolved in the solutions and different electron donors (ascorbate, amino acids, lipids) catalyzed by riboflavin when exposed to light [3]. H_2O_2 is regarded as a key element in the oxygen toxicity of the cell, as H_2O_2 can accumulate to high concentrations owing to its stability [4]. Infused peroxides can cause oxidant stress on their own or be converted to oxygen free radicals in the presence of trace metals [5]. These reactive substances can disrupt normal cell function in isolated organs [6, 7]. This may occur when the antioxidant defenses are insufficient to protect against this oxidant load, which results in oxidant stress. Although hyperoxia can stimulate antioxidant defenses [8], it is not known whether peroxides cause oxidant stress in an organism protected by its antioxidant systems.

To determine if peroxides infused with TPN produce oxidant stress *in vivo*, the antioxidant response and markers of oxidant stress need to be determined simultaneously in the target tissue. Hydroperoxides and free radicals interfere with eicosanoid metabolism [9, 10], which serves as a sensitive marker of oxidant stress. Glutathione, which has antiradical as well as antiperoxide properties, is a key element in the antioxidant defense of the cell. The central role of glutathione is underlined by the observation that its supplementation protects the lungs of preterm rabbits from oxygen-induced lung injury [11].

The lung is a target of oxidant injury following hyperoxia; however, the specific role of peroxides in the pathogenesis of neonatal pulmonary complications is unknown. The transcription of the gene coding for the first enzyme for GSH formation (γ-glutamylcysteine synthetase) is induced by free radicals [12], accounting for the stimulation of tissue glutathione levels by oxygen [8, 13]. In contrast, perfusion with peroxides leads to consumption of glutathione, as shown with TBH in the myocardium [14] and in endothelial cells [15]. This raises the question as to whether an intravenous oxidant load, such as H_2O_2 infused in the lung

* Corresponding author: Philippe Chessex, M.D., Research Center, Hôpital Ste-Justine, 3175 Chemin Côte Ste-Catherine, Montreal, Quebec, Canada H3T 1C5. Tel. (514) 345-4730; FAX (514) 345-4801; E-mail: brassard@justine.umontreal.ca

† *Abbreviations*: C, control; γ-GT, γ-glutamyltranspeptidase; GPx, glutathione peroxidase; GSSG-R, glutathione reductase; Gsynth, synthetic activity of glutathione; MVP, multivitamin preparation; PG, prostaglandin; PN, parenteral nutrition; TBH, *tert*-butylhydroperoxide; TPN, total parenteral nutrition; and Tx, thromboxane.

Received 21 February 2000; accepted 6 April 2000.

on first passage, stimulates pulmonary glutathione responses differently from an oxidant load such as hyperoxia. This might be the case if infused peroxides are not entirely transformed by the Fenton reaction into free radicals.

Infused MVP is the major inducer of the generation of peroxides in neonatal TPN regimens exposed to light [2, 3]. However, MVP provides several molecules and vitamins with antiradical properties, but they do not have efficient antiperoxide activities [2]. As light protection helps conserve several vitamins in solution [16], peroxides could be responsible for the alteration of the composition of multivitamins. Therefore, it was hypothesized that either the peroxides contaminating the intravenous solutions or oxidized components of the MVP represent an oxidant load that is not completely quenched by the antioxidant defenses. The aim of this study was to determine if MVP is associated with oxidant stress similar to that caused by peroxides in the lungs of guinea pig pups.

MATERIALS AND METHODS

The experiments were carried out to compare the effects of various intravenous regimens providing concentrations of H_2O_2 (250–500 mM) similar to those measured in preparations used in clinical practice [1, 2, 17]. The newborn guinea pig was chosen because we have previous experience with this animal model [13], and the pups have a glutathione status at a developmental stage [18] similar to that of the human neonate [19]. Furthermore, this newborn animal model has been used by others to study lung injury [20, 21]. As dietary amino acids are associated with the generation of peroxides [1] and they also serve as a substrate for glutathione in enterally fed animals [22], studies were performed in the presence and the absence of these parenteral nutrients to isolate the effects of MVP from those of the TPN regimen.

Experimental Model

One-day-old guinea pig pups (Charles River) were fitted under anesthesia (ketamine/xylazine) with a 0.4 mm polyurethane catheter (Luther Medical Products) in the external jugular vein as previously described [13]. The catheter, which was exteriorized in the scapular region, was connected to a flow-through system permitting mobility of the animals, which were housed in plastic boxes with wire mesh bottoms. Environmental conditions were thermo-controlled with a 12-hr alternation between light and dark. The animals were fed exclusively intravenously. After animals were killed on day 4, lungs were minced, aliquoted, and stored at −70° until analysis. The duration of the present study was justified by previous experience with guinea pig pups treated parenterally in oxygen [13]. The study was approved by the Institutional Review Board for the care and handling of animals. The animals were handled in accord with the guidelines of the Canadian Council of Animal Care.

Parenteral Solutions

To determine the effect of infused peroxides, a set of animals was infused at 240 mL/kg/day with one of the following solutions:

C (control): 50 g dextrose/L + 4.5 g NaCl/L + 1 U heparin/mL.

C + 250 μM H_2O_2: control + 250 μM H_2O_2 (Aldrich Chemical Co.).

C + 500 μM H_2O_2: control + 500 μM H_2O_2.

To compare the effects of MVP with those of peroxides, and to separate the effects of MVP from those of other substrates known to contribute to the generation of peroxides in the parenteral solution (amino acids, bisulfite, lipids), a further set of animals was infused at 240 mL/kg/day with one of the following regimens:

C (control): 50 g dextrose/L + 4.5 g NaCl/L + 1 U heparin/mL.

C + MVP: control + 1% (v/v) MVI ® Pediatric [Multi-vitamins for infusion, Rhône-Poulenc Rorer; the 5-mL solution contains Vit A, 2300 IU; Vit D, 400 IU; α-tocopherol, 7 IU; Vit K, 200 μg; ascorbate, 80 mg; thiamine, 1.2 mg; riboflavin, 1.4 mg; pyridoxine, 1.0 mg; pantothenate, 5 mg; folate, 0.14 mg; cyanocobalamin, 1 μg; nicotinamide, 17 mg; biotin, 20 mg; and the additives butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and mannitol, as well as polysorbates as emulsifiers]. One percent MVI is used frequently to cover parenteral allowances for newborn infants.

PN: 76 g dextrose/L + 19 g amino acids/L (Travasol 10% Blend C®, Clintec-Baxter, which contains no cysteine but 26.8 mM methionine as a limiting precursor for glutathione synthesis)+ 28 mM Na + 28 mM K + 36 mM Cl + 14 mM SO_4 + 40 mM acetate + 4.5 mM Ca + 1.9 mM P+ 1.78 mg Zn/L + 0.24 mg Cu/L + 59 μg Mn/L + 36 μg I/L + 12 μg Se/L + 2.4 μgCr/L.

PN + MVP: PN + 1% (v/v) MVI.

Handling of all parenteral solutions was carried out in a similar fashion, namely, no photoprotection was applied.

Peroxide Determination

The concentrations of peroxides were measured in the infused solutions using the FOX technique [23]. According to this method, peroxides oxidize Fe^{2+} into Fe^{3+}, which reacts with xylenol orange to form a chromophore absorbing at 560 nm. This chemical assay measures organic as well

TABLE 1. Characteristics of the studied animals and peroxide concentrations of intravenous feeding regimens

Feeding regimens	Number of animals	Initial body weight (g)	Δ Body weight (% of initial weight)	[ROOH] (μM)
C	5	101 ± 4	−12 ± 1	8 ± 1
C + H_2O_2 (250 μM)	6	100 ± 4	−16 ± 1	253 ± 1
C + H_2O_2 (500 μM)	6	119 ± 7	−23 ± 3	512 ± 16
C	5	96 ± 3	−9 ± 3	9 ± 5
C + MVP	5	99 ± 4	−15 ± 3	295 ± 8
PN	4	104 ± 2	−14 ± 2	26 ± 1
PN + MVP	5	104 ± 3	−9 ± 2	212 ± 7

Results are expressed as means ± SEM. Δ Body weight = change in body weight over the 4 days of the protocol; [ROOH]: peroxide concentration; C: control = 0.45% NaCl + 5% dextrose; MVP: 1% multivitamin solution; PN: fat-free parenteral nutrition.

as inorganic peroxides [24] and has been validated for TPN solutions using an enzymatic assay [25]. Results were expressed in equivalents of TBH.

Glutathione Determination

The sample was homogenized in a buffer (100 mM Tris + 1 mM EDTA + 10 mM l-serine + 10 mM borate, pH 7.6). After centrifugation at 5000 *g*, the supernatant was divided for protein and total glutathione determinations. Glutathione was assayed [19] in the supernatant after precipitation by sulfosalicylic acid, according to the method of Griffith [26]. Briefly, 0.6 mM 5,5′-dithiobis(2-nitrobenzoic acid) (Aldrich Chemical Co.) and 0.2 mM NADPH (Boehringer Mannheim) were added to the sample in the buffer. The reaction was started by adding 10 μg GSSG-R (Boehringer Mannhein) to 1 mL of final volume. The absorbance was read at 412 nm over 10 min. The slope of the increase in optical density was compared with that of a standard GSSG curve. The results were reported as GSH equivalents. The threshold of detection was 40 pmol.

Activities of Glutathione-Related Enzymes

The activity of GSSG-R was measured according to the method of Becker and Wilkson [27]. The NADPH molar extinction coefficient was used to quantify the activity (NADPH oxidized per minute per milligram protein). The level of detection was 80 pmol. To measure the activity of GPx, the sample was mixed with 1 mM GSH, 1 mM TBH, 0.1 mM NADPH, and 5 mg of purified GSSG-R in a buffer (250 mM Tris, 0.1 mM EDTA, pH 7.6, 30°). The disappearance of NADPH was recorded as described for the determination of the activity of GSSG-R. The activity reflected the amount of NADPH oxidized per minute per milligram protein. The limit of detection was 2 nmol. The overall Gsynth was determined using a technique described previously [28]. Results were reported in nanomoles GSH formed per hour per milligram protein. The threshold of the assay was 70 pmol GSH. The activity of γ-GT was measured according to the method described previously [28]. The activity reflected the amount of *p*-nitroaniline formed per minute per milligram protein. The threshold of the assay was 50 pmol.

Eicosanoid Determination

The tissue was homogenized in a buffer (100 mM Tris + 1 mM EDTA, pH 7.4). Eicosanoids were extracted on a reverse C_{18} Sep-Pak cartridge (Waters) [29]. PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2), and TxB_2 (the stable metabolite of TxA_2) were measured by radioimmunoassay [15] using antisera from PerSeptive Diagnostics. Briefly, after a 2-hr 30 min incubation at 22–25° with antisera, the free ^3H-tracer was removed by precipitation with charcoal–dextran [14]. An aliquot of supernatant, reflecting the bound fraction, was counted. Since the activity of the first enzyme responsible for the catabolism of PGE_2 and $PGF_{2\alpha}$ in the lung is inhibited by an oxidant challenge [10], an increase in (PGE_2 + $PGF_{2\alpha}$) normalized for total PG was used as a marker of imbalance in the redox equilibrium. An increase in the (PGE_2 + $PGF_{2\alpha}$)/PG ratio, therefore, was used as an index of oxidant stress in the lungs.

Protein Determination

Proteins were measured using the Bradford colorant (Bio-Rad). Bovine serum albumin was used for the standard curve.

Statistical Analysis

All results are presented as means ± SEM. The comparison of C vs (H_2O_2: 250 vs 500 μM) was performed by ANOVA; the effects of MVP and PN were separated by a multifactorial ANOVA. The effect of the oxidant load on total prostaglandins was analyzed by ANOVA [C vs (H_2O_2 vs MVP)]. The level of significance was set at $P < 0.05$.

RESULTS

There was no statistical difference in the body weight or its rate of change between the two sets of animals (Table 1). Compared with controls, there was no effect of peroxides or of MVP ± PN on the relative lung weight (1.1 ± 0.1 vs 1.1 ± 0.2% body weight). During the 4-day experiments, no animals died in the control groups or following infusion

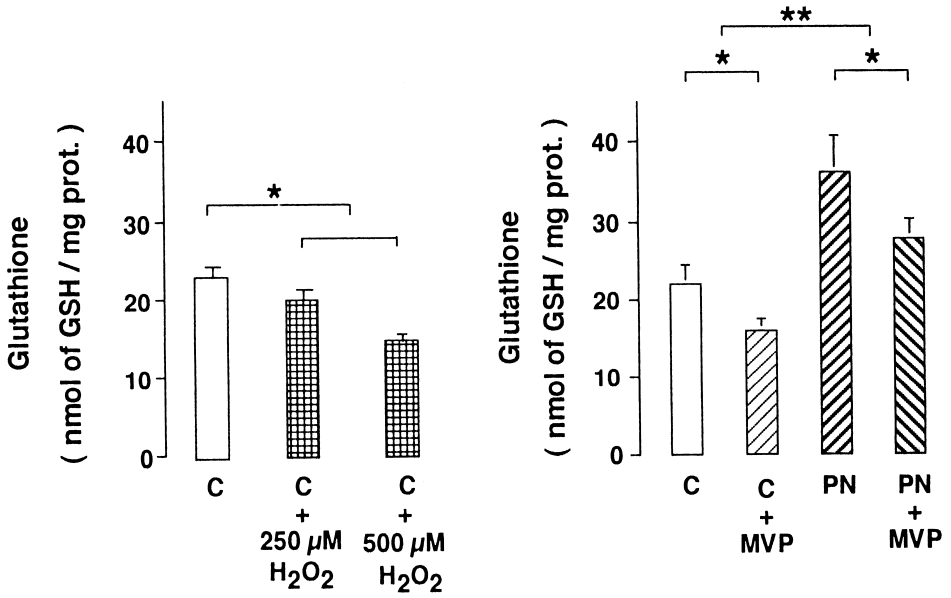

FIG. 1. Effect of H_2O_2 as well as of multivitamins and fat-free PN on lung glutathione. Total glutathione content in lungs of newborn guinea pigs infused with the following solutions: in the left panel, 5% dextrose + 0.45% NaCl (Control: C) with or without H_2O_2; in the right panel, C or PN with or without clinically relevant concentrations (1%) of MVP. The admixture of H_2O_2 and MVP was associated with a significant (* $P < 0.05$) drop in lung glutathione, whereas PN induced a significant rise ($P < 0.001$) in glutathione levels. Data represent means ± SEM, N = 4–6 (see Table 1).**

of peroxides, while two animals died with MVP (C + MVP: N = 1; PN + MVP: N = 1). The deceased animals were not included in the study population.

The pulmonary concentrations of total glutathione in animals receiving H_2O_2 (Fig. 1) were significantly lower than the control [$F_{(1, 12)} = 4.8$, $P < 0.05$]. Similarly, the level of glutathione dropped by 25% [$F_{(1, 15)} = 6.2$, $P < 0.05$] in the presence of MVP, whereas PN was associated with a 41% increase in glutathione content compared with the control [$F_{(1, 15)} = 27.5$, $P < 0.001$]. The drop in pulmonary glutathione content observed with peroxides and with C + MVP was associated with a significantly lower Gsynth [$F_{(1,15} = 5.3$, $P < 0.05$], while the activities of the other enzymes involved in modulating glutathione content (GSSG-R, GPx, and γ-GT) were not affected significantly (Fig. 2). On the other hand, PN and PN + MVP, which induced an increase in glutathione levels, were associated with an activity of Gsynth (3.7 ± 1.0 and 3.8 ± 0.8 nmol/hr/mg protein) that did not differ from the control (4.0 ± 0.5 nmol/hr/mg protein). Because of limited

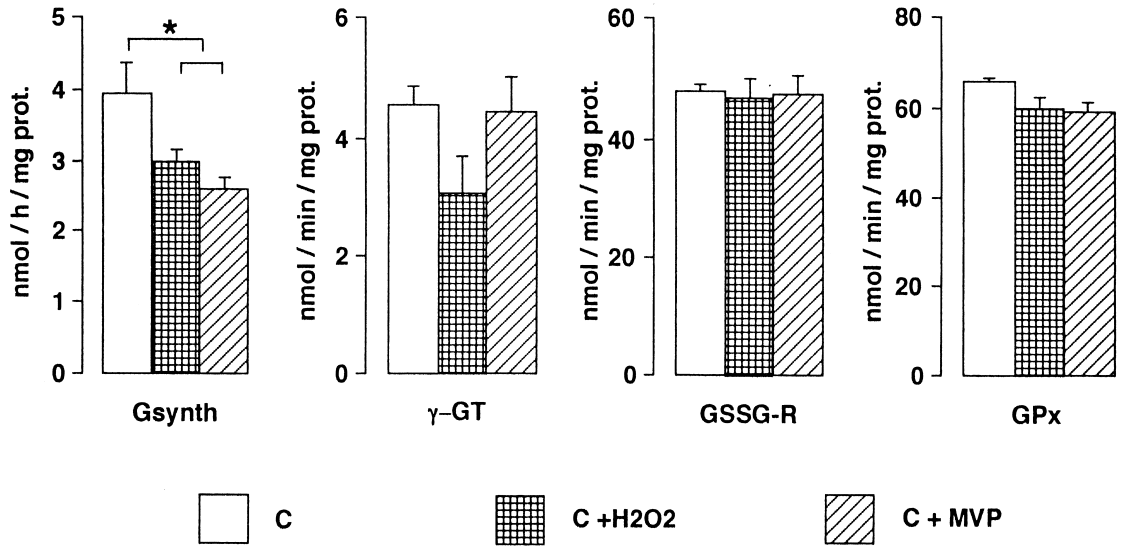

FIG. 2. Effect of peroxides and multivitamins on pulmonary activities of enzymes involved in maintaining the intracellular concentration of glutathione. Enzymatic activities in lungs of newborn guinea pigs infused with 5% dextrose + 0.45% NaCl (Control: C) with or without relevant concentrations of H_2O_2 (250 mM H_2O_2) (C + H_2O_2) and multivitamins (C + MVP). The activity of Gsynth was significantly (* $P < 0.05$) lower in response to H_2O_2 and MVP. Data represent means ± SEM, N = 4–6 (see Table 1).

TABLE 2. Effect of intravenous feeding regimens on lung eicosanoid levels

Feeding regimen	Eicosanoid (ng/mg protein)				
	PGI_2	PGE_2	$PGF_{2\alpha}$	TxA_2	Total PGs
C	1.02 ± 0.26	1.42 ± 0.39	6.79 ± 0.34	16.68 ± 5.42	25.92 ± 5.55
C + H_2O_2 (250 μM)	1.09 ± 0.32	1.87 ± 0.43	10.04 ± 1.47	12.57 ± 2.15	25.57 ± 3.63
C + H_2O_2 (500 μM)	0.46 ± 0.16	0.51 ± 0.11	3.10 ± 2.44	2.39 ± 0.82	5.36 ± 1.94
C	0.93 ± 0.23	1.57 ± 0.40	7.63 ± 1.14	16.11 ± 3.44	26.26 ± 4.80
C + MVP	1.15 ± 0.23	2.08 ± 0.25	13.23 ± 1.86	13.46 ± 3.13	29.92 ± 4.50
PN	0.95 ± 0.31	1.12 ± 0.38	6.02 ± 2.30	30.60 ± 13.9	38.69 ± 16.3
PN + MVP	0.49 ± 0.03	1.65 ± 0.34	8.94 ± 3.92	7.74 ± 1.40	18.82 ± 5.08

Results are expressed as means ± SEM, N = 4-6 (see Table 1). C: control = 0.45% NaCl + 5% dextrose; MVP = 1% multi-vitamin solution; PN = solution of fat-free parenteral nutrition; PG = prostaglandin.

sample availability, activities of GSSG-R and GPx as well as γ-GT were not determined with the PN and PN + MVP regimens.

The effect of peroxides as well as MVP and PN on individual PGs is presented in Table 2. It appeared that TxA_2 and PGE_2 + $PGF_{2\alpha}$ responded differently. TxA_2 decreased significantly in response to H_2O_2 [$F_{(1, 14)} = 6.7$, $P < 0.05$] as well as MVP [$F_{(1, 15)} = 5.7$, $P < 0.05$]. A trend towards a decrease in total PG was observed: H_2O_2 [$F_{(1, 14)} = 4.9$, $P < 0.05$]; MVP [$F_{(1, 15)} = 1.3$]. The effect attributed to H_2O_2 was accounted for by the higher (500 μM) concentration [$F_{(1, 14)} = 13.7$, $P < 0.005$]. On the other hand, the ratio (PGE_2 + $PGF_{2\alpha}$)/PG was increased significantly in response to H_2O_2 [$F_{(1, 14)} = 9.9$, $P < 0.01$] as well as MVP [$F_{[1, 15]} = 43.3$, $P < 0.001$] (Fig. 3), but not in response to PN [$F_{(1, 15)} = 4.09$]. A ratio was used to control for the variations in PGs, especially those observed with 500 μM H_2O_2 (Table 2). The modifications observed in the (PGE_2 + $PGF_{2\alpha}$)/PG ratio (Fig. 3) are accounted for by both an increase in the numerator and the trend towards a decrease in the denominator (Table 2). Indeed, the level of PGE_2 + $PGF_{2\alpha}$ in the groups with comparable peroxide contents (250 mM H_2O_2, C + MVP, PN + MVP) (Table 1) was higher than in the controls (C and PN) [$F_{(1, 28)} = 5.95$, $P < 0.05$].

DISCUSSION

The results of the present study show that infused peroxides induce a modulation in eicosanoids compatible with an oxidation of lung tissue. The oxidant load carried by peroxides produces a specific antioxidant response, as it does not lead to the stimulation of glutathione seen with hyperoxia in the same animal model [13]. Although the multivitamin solution has components with antioxidant properties, it induces responses similar to those induced by

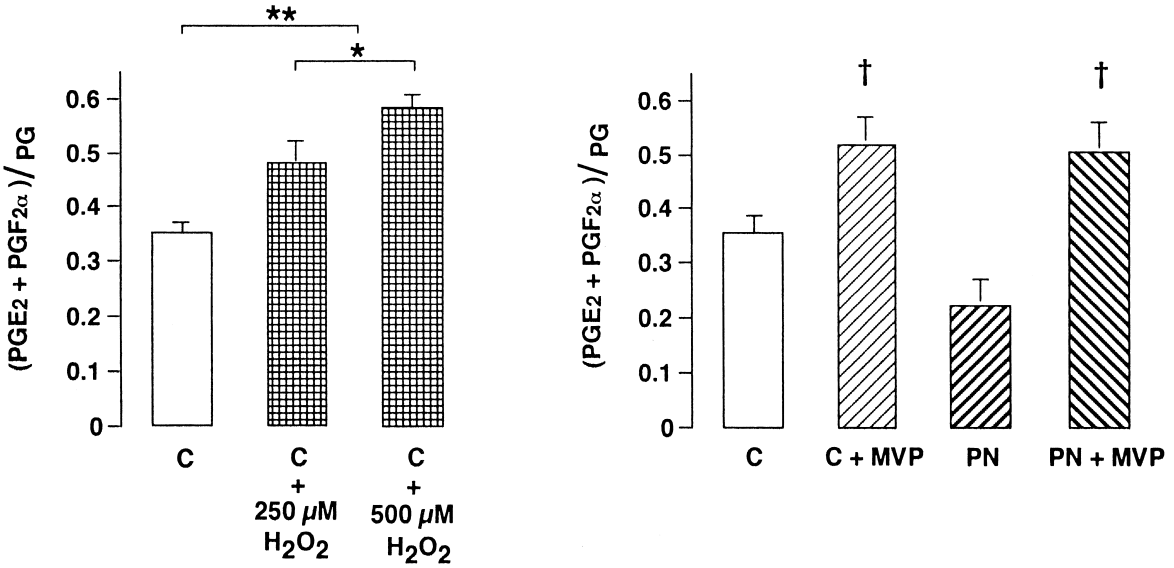

FIG. 3. Effect of H_2O_2 as well as of multivitamins and fat-free PN on an oxidant-sensitive eicosanoid marker. The ratio of the sum of prostaglandins PGE_2 and $PGF_{2\alpha}$ over total prostaglandins [(PGE_2 + $PGF_{2\alpha}$)/PG] determined in lungs of newborn guinea pigs receiving intravenously the following solutions: in the left panel, 5% dextrose + 0.45% NaCl (Control: C) with or without H_2O_2; in the right panel, C or PN with or without clinically relevant concentrations (1%) of MVP. A significant increase in the ratio was induced by H_2O_2 (* $P < 0.05$; ** $P < 0.01$) as well as MVP († $P < 0.001$), suggesting an oxidant challenge in the lungs. Data represent means ± SEM, N = 4–6 (see Table 1).

peroxides, confirming that it does not have complete antiperoxide activity [2], thereby inducing oxidant responses.

The drop in glutathione could be interpreted as reflecting consumption by GPx, the principal enzyme involved in the detoxification of peroxides, or inhibition of synthesis (Fig. 2) by the oxidant load. A direct inhibitory effect of peroxides or MVP on the activity of Gsynth is less probable, since it was not observed with PN + MVP. On the other hand, one might argue that the difference in Gsynth between C + MVP and PN + MVP is related to the lower levels of infused peroxides observed with PN + MVP (Table 1). These lower levels are explained by the amino acids in the solution, which quench part of the peroxides generated in the TPN regimen [2].

To protect the cell against large modifications in the redox ratio, GSSG can be recycled by GSSG-R and/or exported actively from the cell, thereby keeping the ratio within a narrow range. Indeed, we observed in the lungs of guinea pig pups that the GSH/GSSG ratio did not differ significantly after 4 days of exposure to room air or 95% oxygen [13]. Therefore, we preferred to measure eicosanoids, as their metabolism is sensitive to peroxides [30]. Depending on cellular conditions, the oxidant load, and the duration of exposure, cyclooxygenase and prostacyclin synthase are either activated [31, 32] or inhibited by peroxides [10, 30]. An inhibition of the enzymes of the arachidonic acid cascade above PGH_2 would have led to a decrease in all of the PGs. This was observed with the higher peroxide load of 500 μM H_2O_2 (Table 2). Under the present experimental conditions, H_2O_2 and MVP had a significant inhibitory effect on TxA_2 production. For the possible reasons stated above, this finding is at variance with reports in human tissues, where organic [15] as well as lipid peroxides stimulate Tx levels [31]. This raises the suggestion that H_2O_2 has a specific effect on eicosanoid metabolism, which is supported by our observation, under the same experimental conditions and in the same animal model, that 350 μM TBH induced 100% higher lung Tx levels (33.97 ± 5.51 ng/mg protein, N = 5) compared with controls (data not shown).

On the other hand, the significant increase in the ratio (PGE_2 + $PGF_{2\alpha}$)/PG (Fig. 3) documents that H_2O_2 as well as MVP had an effect on PGE_2 and $PGF_{2\alpha}$ that was separate from the other PGs. PGE_2 and $PGF_{2\alpha}$ are catabolized mainly in the lungs. The relative increase in PGE_2 + $PGF_{2\alpha}$ suggests pulmonary oxidant stress, as the activity of 15-hydroxyprostaglandin dehydrogenase, the enzyme responsible for over 80% of the pulmonary catabolism of these PGs [33], is inhibited by oxidants [10]. One should be cautious, however, when extrapolating the biological significance of these animal findings to humans, as the levels of individual eicosanoids are specific to organs and species. For instance, TxA_2 is the major eicosanoid produced in the lungs of guinea pigs, whereas PGI_2 is predominant in human lungs. However, independently from the specificities of eicosanoids, the effects of peroxides and MVP on these markers were similar, and they suggest that the antioxidant defenses were overwhelmed by the oxidant load.

MVP and peroxides both led to a drop in glutathione levels, emphasizing that they induce a similar oxidant challenge. If infused peroxides were converted to free radicals, we would have found an increase in the levels of glutathione [12, 13]. On the other hand, if these peroxides were converted to free radicals and completely quenched by the antioxidants in MVP, glutathione levels would have remained at control levels. Although peroxides and hyperoxia are often confounded in the overall oxidant challenge observed in clinical practice, the results from this animal study suggest that they elicit different responses. MVP and H_2O_2 both affected an oxidant sensitive marker in a similar fashion, confirming the hypothesis that either the peroxides contaminating the intravenous solutions (Table 1) or oxidized components of the multivitamin solutions are not completely quenched by the antioxidant defenses. This is supported by the demonstration that the admixture of MVP to the TPN solution of preterm infants is associated with a significant increase in urinary peroxide excretion [34].

Based on the report that glutathione supplementation protects preterm rabbits from hyperoxic lung disease [11], we tested whether the provision of substrates for glutathione synthesis by the PN regimen modified the response to the oxidant load associated with MVP. Although PN led to a significantly higher glutathione level (Fig. 1), it did not protect the lung from the effect of MVP (Fig. 3). As glutathione is involved in protecting cells in culture from peroxides [35], we speculate that, in the present study, the levels of glutathione reached in the lungs were not sufficient to protect against the oxidant stress. The liver is a net glutathione producer from sulfur-containing amino acids, and glutathione originating from the liver is the main source of this substrate for peripheral tissues such as the lung. Therefore, we suspect that peroxides could produce hepatic injury limiting glutathione production [36].

In conclusion, the increase in glutathione induced by parenteral nutrition was not sufficient to protect lung tissue from oxidation by peroxides and/or oxidized components of the multivitamin solution. The present study suggests strongly that it is of biological importance to find ways to prevent the generation of peroxides in multivitamin solutions that otherwise remain an essential nutrient admixture to PN [37]. Since the developing antioxidant system [18] was unable to protect the pups of this precocious animal model, these findings could be of particular relevance to human subjects with a compromised antioxidant capacity [38, 39].

This work was supported by grants from the Clintec Nutrition Division of Baxter Canada, Toronto, Ontario, Canada, the Medical Research Council of Canada (UI 14011), and the Toronto Hospital for Sick Children Foundation (XG97–025), Toronto, Ontario, Canada.

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