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Vitamin E and Lipoic Acid, but not Vitamin C Improve Blood Oxygenation after High-Energy IMPULSE Noise (BLAST) Exposure

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Exposure to high energy impulse noise (BLAST) caused by explosions, result in structural and functional damage to the hollow organs, especially to the respiratory and auditory systems. Lung damage includes alveolar wall rupture, edema and hemorrhage, and may be fatal. Previous observations at the molecular level using the rat model, suggested that secondary free radical-mediated oxidative stress occurs post exposure resulting in antioxidant depletion and hemoglobin (Hb) oxidation. This study examined whether a short period of pre-exposure supplementation with antioxidants would protect Hb from the effects of BLAST exposure. Six groups of male Sprague-Dawley rats (8/group) were gavaged with 800 IU vitamin E (VE) in 2 ml corn oil, 1000 mg vitamin C (VC) in 2 ml distilled water or 25 mg or (-lipoic acid (LA) in 2 ml corn oil for 3 days. Matched control groups were gavaged with the respective vehicles. On day 4, rats were deeply anesthetized and exposed to a simulated BLAST wave with an average peak pressure of 62 ± 2 kPa. Rats were euthanized one hour post exposure and blood samples were obtained by cardiac puncture and analyzed using a hemoximeter. Post exposure oxygenation states (HbO_2, O_2 saturation, and O_2 content) were markedly decreased, while reduced-Hb was increased. Supplementation with VE and LA reversed the trend and increased Hb oxygenation, but VC did not. This suggests that a brief dietary loading with pharmacological doses of VE or LA, but not VC shortly before BLAST exposure may be beneficial. Moreover, measurement of blood oxygenation may function as a simple, semi-invasive biomarker of BLAST-induced injury applicable to humans. **© 1998 Academic Press**

Detonation of explosives or firing of large caliber weapons as well as accidental occupational explosions produce high energy impulse noise (BLAST) waves characterized by instantaneous sharp increase in atmospheric pressure above ambient (positive phase) followed by an exponential decay to, or below, ambient level (negative phase) until finally returning to a steady state. The term high energy impulse noise (BLAST) used in this report is synonymous with blast overpressure (BOP) used in other studies. Exposure to BLAST waves can cause injury, predominantly to the gas-filled organs. Exposure of the ears may lead to hearing loss (1–6), but the lungs are the most sensitive organ to injury from BLAST and can lead to fatality. Lung injury is characterized by mechanical damage to alveolar septa and rupture of blood vessels resulting in hemorrhage and edema. This may be accompanied by airway collapse, depending on the severity of the damage (3,7,8). BLAST-induced injuries are generally divided into three classes: 1) *Primary injury*, resulting from exposure to blast overpressure waves. 2) *Secondary injury*, resulting from body exposure to flying objects. 3) *Tertiary injury*, resulting from body displacement against solid objects or structures (3,5). In this study only primary injury from low-level BLAST exposure was examined.

In humans, BLAST has been associated with physiological decrement in performance and/or endurance (9,10), cognition, and motor activity (11). Biochemically, BLAST results in increased lipid peroxidation, antioxidant depletion, and disruption of calcium transport (8,12–15). In a report from China, lipid peroxidation was found to be associated with BLAST-induced hearing loss suggesting that free radical-mediated oxidative stress may contribute to ear injury (16). Studies from our laboratory using simulated BLAST exposure suggested that hemoglobin (Hb) oxidation products may act as potential initiators of oxidative stress and lipid peroxidation (5,8,15). It is possible that antioxidant depletion and increased lipid peroxidation may

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TABLE 1

Effect of BLAST Overpressure Exposure on Blood Oxygenation States in Rats Administered 2 ml Corn Oil or 800 IU D-α-tocopheryl Acetate in 2 ml Corn Oil by Gavage, Daily for Three Days before Exposure

	Oil			Vitamin E		
Parameter	Control	Blast	Change (%)	Control	Blast	Change (%)
tHb	16.4 ± 0.21	16.9 ± 0.4	+3	16.1 ± 0.4	16.6 ± 0.2	+3
HbO_2	30.5 ± 3.3	16.7 ± 5.1	−45*	19.9 ± 4.3	30.9 ± 3.5	+55*
RHb	68.7 ± 3.4	86.4 ± 3.7	+26*	79.5 ± 4.4	68.3 ± 3.6	−14
HbO_2 SAT	30.7 ± 6.6	12.7 ± 8.7	−59*	20.0 ± 8.6	31.2 ± 6.2	+56*
HbO_2 ct	7.0 ± 1.5	2.9 ± 1.9	−58*	4.4 ± 1.7	7.2 ± 1.5	+64*
HbO_2 cap	22.6 ± 0.6	23.1 ± 1.3	+2	22.2 ± 1.0	22.9 ± 0.4	+3
MetHb	0.38 ± 0.03	0.26 ± 0.1	−25	0.25 ± 0.03	0.36 ± 0.10	+33
HbCO	0.4 ± 0.1	0.2 ± 0.1	−50*	0.4 ± 0.20	0.3 ± 0.2	−25

Note. Values are presented as mean ± SE, n = 4.
* Significantly different from control, $p<0.05$.

amplify the damage resulting from blast exposure particularly when combined with high oxygen therapy (3,17–19). Numerous reports have shown that exposure to high oxygen tension increase free radical formation and cause oxidative stress (20–24).

Based on the accumulated body of observations, it has been postulated that BLAST-induced injury has two components: i) a primary mechanical damage accompanied by release of hemoglobin (Hb) from red blood cells due to the compression/decompression cycle from the incident blast wave, and ii) a secondary free radical-mediated oxidative stress resulting from progressive Hb oxidation leading possibly to oxoferryl Hb or other heme and non-heme iron complex formation (5,8,15,25,26). Oxidation of Hb can lead to antioxidant depletion and lipid peroxidation, and to disruption of the blood's oxygen transport functions similar to carbon monoxide poisoning (27,28). With low-level BLAST, these biochemical changes are resolved over time. Conversely, irreversible changes occur with high-level BLAST, and may explain some reported cases of delayed mortality after BLAST exposure (3,5,29–32). Disruption of the blood's oxygen transport functions reflect a functional marker of damage whereas lipid peroxidation may not. In 1988, Harmon et al (33) reported that they were unable to observe any changes in blood serum after blast exposure. The present study measured the changes in oxygenation states of whole blood and examined the potential protective effect of administering pharmacological doses of some dietary antioxidants for a very short period (3 days) prior to BLAST exposure. Selection of the antioxidants to be tested was based on their reported protective efficacy and safety to animals and humans (34–38).

MATERIALS AND METHODS

Vitamin E (d-(-tocopheryl acetate; VE), vitamin C (L-ascorbic acid; VC), and (-lipoic acid (DL-6,8-Thioctic acid; LA) were purchased from Sigma Chemicals Co., St. Louis, MO. A commercially available corn oil and ultrapure deionized water were used as the respective vehicles. Animal work was conducted under a protocol approved by the Institute's Laboratory Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals (NRC 1996) in facilities that are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. Certified Virus Free (CVF), Sprague-Dawley rats, weighing 300–350g were purchased from Charles River Laboratories Inc., Wilmington, MA. The rats were acclimatized for 3 days in which they were maintained in a unidirectional filtered-air room at 22±3°C with 12/12h light/dark cycle and allowed food and water *ad libitum*. Post acclimatization, rats were randomly divided into 6 groups (8 rats/group) and gavaged daily for three days with 800 IU VE in 2 ml corn oil, 1000 mg VC in 2 ml distilled water or with 25 mg LA in 2 ml corn oil. Control rats received the respective vehicle alone. Gavage was administered to unanesthetized animals using plastic, 8 Fr, 15 inch (38.1 cm) long, infant feeding tubes (Davol Inc., Cranston, RI). After the 3-day dietary supplementaion, all rats were deeply anesthetized with sodium pentobarbital (60 mg/kg body weight) and positioned for exposure. One half the rats from each dietary group was exposed to a simulated low-level BLAST wave with 62±2 kPa peak pressure in the laboratory using a compressed air-driven shock tube as described previously (5). One hour post exposure, rats were euthanized and a thoracotomy performed. Mixed venous blood samples were collected by cardiac puncture in 1 ml plastic syringes containing 50 units of dry lyophilized lithium heparin (Aspirator, Marquest, Medical products, Inc., Engelwood, CO). Blood samples were immediately analyzed using a Hemoximeter model OSM3 (Radiometer A/S, Copenhagen, Denmark). Packed cell volumes were measured. Two samples of lung tissue homogenate from each group were analyzed with lipid peroxidation kits (Calbiochem, Calbiochem-Novabiochem Corp., San Diego, CA).

RESULTS

In this study, BLAST exposure was associated with significant ($P< 0.05$) decreases in blood oxygenation states which ranged in HbO_2 from 26–45%, in HbO_2SAT, from 26–59%, and in HbO_2ct from 31–58% (Tables 1–3). Reduced (deoxygenated) Hb (RHb) also increased post exposure, but to a lesser extent, with changes ranging from 5–26%. Administration of VE and LA appeared to reverse the trend and resulted in

TABLE 2

Effect of BLAST Overpressure Exposure on Blood Oxygenation States in Rats Administered 2 ml Corn Oil or 25 mg α-Lipoic Acid in 2 ml Corn Oil by Gavage, Daily for Three Days before Exposure

	Oil			α-Lipoic acid		
Parameter	Control	Blast	Change (%)	Control	Blast	Change (%)
tHb	15.4 ± 0.7	17.2 ± 0.8	+12	15.6 ± 0.3	17.2 ± 0.2	+10
HbO_2	18.1 ± 5.2	10 ± 3.0	−42*	14.1 ± 5.4	18.7 ± 3.1	+33*
RHb	81.3 ± 5.3	89.1 ± 3.1	+10	85.4 ± 5.4	80.7 ± 3.2	−6
HbO_2 SAT	18.2 ± 5.3	10.5 ± 3.1	−42*	14.1 ± 5.4	18.9 ± 3.2	+33*
HbO_2 ct	3.9 ± 1.1	2.6 ± 0.8	−34*	3.1 ± 1.3	4.5 ± 0.7	+44*
HbO_2 cap	21.3 ± 1.0	23.8 ± 1.0	+12	21.5 ± 0.5	23.7 ± 0.2	+11
MetHb	0.25 ± 0.03	0.20 ± 0.06	−20	0.23 ± 0.03	0.28 ± 0.02	+22
HbCO	0.25 ± 0.03	0.23 ± 0.03	−8*	0.28 ± 0.11	0.35 ± 0.06	+25

Note. Values are presented as mean ± SE, n = 4.
* Significantly different from control, $p<0.05$.

significant ($P< 0.05$) increases in blood oxygenation states (Tables 1,2) that ranged in HbO_2 from 33–55%, HbO_2SAT from 33–56%, and HbO_2ct from 44–64%. Concomitant with these increases in Hb oxygenation, RHb decreased 6–14% ($P<0.05$) suggesting increased blood oxygenation. In contrast, VC administration resulted in smaller changes (NS, $P>0.05$) that ranged from 1–10% (Table 3). Hematocrit values (46±4%) were constant throughout the study, and were neither affected by BLAST exposure nor by antioxidant supplementation. Definition of the parameters reported in this study are presented in Table 4.

DISCUSSION

High energy impulse noise (BLAST) is a health hazard to military personnel from explosion of munitions or firing of large caliber weapons during military training or operations (1–6). BLAST is also a potential occupational hazard for those employed in the mining industry, munitions manufacturing and rocket fueling. The civilian population at large has become increasingly at risk of exposure to BLAST resulting from terrorist bombings (3,5,29–32). BLAST-induced *primary injury* has been suggested to result from the mechanical compression/decompression cycle causing tissue and red blood cell damage (3,8,15). It has been recently reported that a secondary mechanism of injury in which breakage of red blood cells releasing Hb after BLAST exposure was associated with oxidative stress that resulted in rat lung antioxidant depletion including VE and VC, and by increased lipid peroxidation (5,8,12–15). This preliminary study examined the potential protection afforded by a 3-day, dietary supplementation with relatively high pharmacological doses of vitamins E and C, two well known lipid and water soluble vitamins (34,35,36,37). In addition, the effect of pretreatment with LA was also examined. LA is a lipoamide antioxidant and cofactor in several mitochondrial dehydrogenase complexes (38) that can

TABLE 3

Effect of BLAST Overpressure Exposure on Blood Oxygenation States in Rats Administered 2 ml Distilled Water or 1000 mg Ascorbate in 2 ml Distilled Water by Gavage, Daily for Three Days before Exposure

	Water			Vitamin C		
Parameter	Control	Blast	Change (%)	Control	Blast	Change (%)
tHb	17.3 ± 0.8	16.8 ± 0.3	+3	16.0 ± 0.3	17.4 ± 0.4	+9
HbO_2	18.7 ± 5.8	11.6 ± 2.1	−26*	16.0 ± 2.3	16.5 ± 5.3	+3
RHb	83.7 ± 5.8	87.9 ± 2.1	+5	83.4 ± 2.4	83.1 ± 5.3	−0.4
HbO_2 SAT	15.8 ± 5.8	11.7 ± 2.1	−26	16.1 ± 2.3	16.6 ± 5.3	+3
HbO_2 ct	3.9 ± 1.7	2.8 ± 0.5	−31*	3.6 ± 0.5	3.9 ± 1.2	+10
HbO_2 cap	23.9 ± 1.1	23.3 ± 0.4	−3	22.1 ± 0.4	24.0 ± 0.5	+9
MetHb	0.30 ± 0.06	0.25 ± 0.05	−17	0.30 ± 0.01	0.30 ± 0.04	0
HbCO	0.28 ± 0.02	0.25 ± 0.03	−9	0.33 ± 0.09	0.2 ± 0.1	−38

Note. Values are presented as mean ± SE, n = 4.
* Significantly different from control, $p<0.05$.

TABLE 4

Definitions of the Parameters Measured

Total hemoglobin (tHb), Fe = Fe(II)
tHb (mmol/l) = cRHb + $cHbO_2$ + cHbCO + cMetHb
tHb (g%) = tHb (mmol/l) × 1.6115
Oxyhemoglobin (HbO_2), Fe = Fe(II), iron associated with O_2
HbO_2 (%) = ($cHbO_2$/tHb) × 100
Reduced or deoxygenated hemoglobin (RHb), Fe = Fe(II)
RHb (%) = (1 − $cHbO_2$ − cHbCO − cMetHb) × 100
Carboxyhemoglobin (HbCO), Fe = Fe(II)
HbCO (%) = (cHbCO/tHb) × 100
Methemoglobin (MetHb), Fe = Fe(II) → Fe(III)
MetHb (%) = (cMetHb/tHb) × 100
Oxygen content (O_2ct), total O_2 concentration of Hb-bound O_2
O_2 ct (vol%) = (HbO_2 (%)/100) × tHb (g%) × 1.39 (theoretical value)
Oxygen capacity (O_2cap), total O_2 concentration when all RHb is converted to HbO_2
O_2cap (vol %) = (HbO_2 (%) + RHb (%)/100 × tHb (g%) × 1.39
Hemoglobin O_2 saturation (HbO_2 SAT, is the fraction of Hb saturated with O_2
HbO_2 SAT (%) = ($cHbO_2$/cRHb + $cHbO_2$) × 100 = (HbO_2/1 − HbCO − MetHb) × 100

function by both lipid and water soluble mechanisms. It is worth noting that no adverse effects from using such high levels of antioxidants were observed under this study's conditions.

Injury from BLAST was found to be associated with oxidative stress when Hb is released from damaged red blood cells, therefore it was postulated that oxidized Hb can be involved in Fenton-type, iron-catalyzed, free radical reactions propagating the injury after exposure (8,15). Examination of Hb oxygenation states in the rat's mixed venous blood post exposure showed that the 3-day supplementation with VE or LA, but not with VC, showed increased Hb oxygenation compared to the respective vehicle-only control rats. Mechanistically, both VE and LA are lipid soluble antioxidants that exert their protection by stabilizing cell membranes or by quenching free radicals (34). In contrast, VC is a cytosolic antioxidant that does not act upon the cell membrane and one of its functions was suggested to be regeneration of oxidized VE (35). It has been further suggested that VC and other antioxidants may act under certain conditions as a pro-oxidant (39,40). If the red blood cell membrane is the target of the injury, this may explain the greater improvement with VE and LA, but not with VC.

To examine whether this protection was extended beyond red blood cells, lipid peroxidation was measured in few samples of lung tissue homogenate. The preliminary results showed the VE group tended to have less lipid peroxidation than either the VC or LA groups; however, the results were not statistically significant.

The ease and speed by which the assay is done, suggests that assessing Hb oxygenation in whole blood has the potential to function as a semi-invasive biomarker capable of predicting internal injury due to blast overpressure and may be applicable to humans. This is in contrast with Harmon et al's report (33) in which it was stated that "the readily available serum chemical markers fail to aid in diagnosis of blast injury." The collection and speed of analysis of blood markers from BLAST victims at the site of exposure may expedite assessment of the injury. Additionally, findings in this study suggest that a brief dietary loading of VE or LA may offer at least partial protection from BLAST-induced injury which may have occupational benefits to those repeatedly at risk of exposure to high-energy impulse noise.

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