PLASMA AND TISSUE VITAMIN E DEPLETION BY BURN AND SMOKE INHALATION INJURY IN SHEEP

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Summary

Oxidants are involved in the pathogenesis of multiple traumas caused by burn and smoke inhalation. α and γ-tocopherols are major tissue antioxidants and their depletion can reflect oxidant injury. We hypothesized that plasma and tissue vitamin E levels in sheep would be depleted with thermal injury. Sheep were operatively prepared for chronic study and randomly divided in to following experimental groups: Sham (non-injured, non-treated , n=?), Burn and smoke exposed (B&S, received a 40% body surface area (BSA) 3rd° burn, and 48 breaths of cotton smoke, n=6), Burn (received only burn injury (40% TBSA, 3rd degree), n=6), and Smoke, (received smoke inhalation alone (48 breaths of cotton smoke), n=6). All sheep were resuscitated with Ringer's lactate solution (4ml/kg/%BSA burn) and mechanically ventilated. The sheep were sacrificed at various time intervals and vitamin E (α and γ-tocopherols) concentrations were measured plasma, liver and lung. Immediately following B&S injury, plasma α-tocopherol / lipids were significantly ($p<0.05$) depleted. (2) B&S injury depleted lung, liver, and trachea α- and γ-tocopherol concentrations, however, heart α- and γ-tocopherols were not affected by B&S injury, (3) Lung and liver vitamin E concentration were the lowest in combined burn and smoke inhalation injury, (4) Depletion of tissue γ-tocopherol appeared earlier than tissue α-tocopherol. We conclude that animals receiving a combination burn and inhalation injury have undergone marked oxidative stress. The results suggest that vitamin E might be depleted in patients with burn and smoking inhalation injury and appropriate supplementation should be evaluated.

Introduction

Oxidative stress occurs when the level of reactive oxygen intermediates (ROIs) exceeds the antioxidant defences of the host. ROIs are produced in great quantities as a result of the respiratory burst of neutrophils, other phagocytes, and endothelial cells during the inflammatory process. The pathogenesis of burn and smoke inhalation injury such as that seen in patients with acute respiratory distress syndrome (ARDS), appears to involve alterations in oxidant-antioxidant stress. α and γ-tocopherol are major endogenous antioxidants. α-tocopherol has been reported to scavenge free radicals that can propagate the lipid peroxidation chain reaction. In addition to this role γ-tocopherol can scavenge reactive nitrogen species. Large amounts of superoxide anions are known to be present in burn and smoke and are inactivated within a few minutes. Several studies on burn and smoke inhalation injury have demonstrated that lipid peroxides appear in the systemic circulation within minutes after injury and peak a second time at 24 h after injury (2). Peroxynitrite is a potent oxidant produced by the reaction of NO and superoxide anion and is produced in burn and smoke models of injury (3). This study provides the data on the tissue and plasma α-and γ-tocopherol levels after burn and smoke inhalation injury.

Materials and Methods

Animals were cared for in the Investigative Intensive Care Unit at our institution, which is approved by the American Association of Laboratory Animal Care. The experimental procedures were approved by the Animal Care and Use Committee of the University of Texas Medical Branch. The National Institutes of Health and American Physiological Society guidelines for animal care were strictly followed. Animals were studied in the awake state.

Surgical Preparation

All animals were endotracheally intubated and ventilated during the surgery while under ketamine and halothane anesthesia. Arterial catheters (16-gauge, 24 in., Intracath, Becton Dickinson, Sandy, UT) were placed in the descending aorta via the femoral artery. A Swan-Ganz thermal dilution catheter (model 131F7, Edwards Lifesciences LLC, Irvine, CA)was positioned in the pulmonary artery via the right external jugular vein. The chest was opened at the fifth intercostal space on both sides, and an efferent lymphatic from the caudal mediastinal lymph node (CMN) was cannulated (Silastic medical grade tubing, 0.025 in. ID, 0.047 in. OD, Dow Corning, Midland, MI) by a modification of the technique of Staub and colleagues (4,5). The systemic contributions to the node were removed by ligation of the tail of the CMN and cauterization of the systemic diaphragmatic lymph vessels (6). Through the fifth intercostal space, a catheter (0.062 in. ID, 0.125 in. OD; Dow Corning, Midland, MI) was positioned in the left atrium. The sheep were given 5 to 7 days to recover from the surgical procedure with free access to food and water.

Burn and smoke inhalation injury

Before the injury was produced, the surgically prepared animals, were anesthetized using ketamine (Ketalar®, Parke-Davis, Morris Plains, NJ). Then, a Foley catheter was placed in the urinary bladder to determine urine output. Next, animals received a tracheotomy and a cuffed tracheostomy tube (10-mm diameter, Shiley, Irvine, CA) was inserted. While the anesthesia was continued with halothane (source), just before the injury sheep were given 0.3mg buprenorphine. After the animal's wool had been shaved, a 20% total body surface area (TBSA) third-degree flame burn was made on one flank. The burn was produced with a Bunsen burner, applied until the skin was thoroughly contracted. We have previously determined this

degree of injury to be a full-thickness burn, i.e., including both epidermis and dermis, in which the nerve endings are destroyed by heat. Thereafter, inhalation injury was induced while the sheep was in the prone position as previously described (4,7). A modified bee smoker was filled with 50 g of burning cotton toweling, and was connected to the tracheostomy tube via a modified endothoracheal tube containing an indwelling thermistor from a Swan-Ganz catheter. During the insufflation procedure, the temperature of the smoke did not exceed 40°C. The sheep were insufflated with a total of 48 breaths of cotton smoke. After smoke insufflation, another 20% TBSA third-degree burn was made on the contra lateral flank.

Resuscitation protocol

Immediately after injury, anesthesia was discontinued, the animals were allowed to awaken and were mechanically ventilated with a Servo Ventilator 900C (Siemens-Elena, Solna, Sweden) throughout the next 48-h experimental period. Ventilation was performed with a positive end-expiratory pressure (PEEP) of 5 cm H_2O and a tidal volume of 15 mL/kg. The respiratory rate was set to maintain normocapnia. For the first 3 h after the combined injury, all animals received an inspired oxygen concentration of 100%; thereafter, it was adjusted to maintain the arterial oxygen saturation above 90%. These respiratory settings allowed a rapid disappearance of carboxyhemoglobin (CO-Hb) after smoke inhalation.

Fluid resuscitation during the experiment was performed with Ringer's lactate solution following the formula (4 mL /% burned surface area/kg body weight for the first 24 h and 2 mL/% burned surface area/kg body weight/day for the next 48 h) (8). One-half of the volume for the first day was infused in the initial 8 h, and the rest was infused in the next 16 h. Urine output volume was recorded every 6 h. Fluid balance was determined by total fluid volume infused minus urine output, and is reported as

mL/kg/day. During this experimental period, the animals were allowed free access to food, but not to water, to allow accurate determination of fluid balance.

At various times after the injury (4, 8, 24, and 48 h)Animals were anesthetized with ketamine and humanely sacrificed by administration of a saturated potassium chloride solution. Immediately after killing, the right lung was used for pathological examination and the left lung was used for measurement of wet/dry weight ratio, corrected for the content of blood, as described by Pearce and colleagues.

Measured Variables

Arterial and mixed venous blood samples were taken at different time points after the injury for measurement of blood gases (Blood gas analyzer 1302 IL, Instrumental Laboratory, Lexington, MA). The data were corrected for the body temperature of the sheep. Lung lymph flow was measured with a graduated test tube and stopwatch. Lymph and blood samples were collected in EDTA tubes.

Lung tissues were taken for measurement of lung wet-to-dry weight ratio (9).

After the blood samples were collected in ethylenediamine tetraacetic acid, plasma was obtained by centrifugation at 2000 rpm for 5 minutes at 4° C. The conjugated diene (CD)-rich lipid fraction was extracted from the plasma with chroloform-methanol, the extract dried under nitrogen flow and stored at -20° C in a nitrogen atmosphere to prevent further oxidation. When used, dienes from 4 ml plasma were dissolved in 0.4 ml 95% ethanol and brought to a volume of 1.0 ml in Dutton's balanced salt solution for injection. An aliquot of each sample was assayed for CD content by spectrophotometry at 233 nm (10).

For the analysis of α and γ-tocopherols, a modification of the method by Podda et al. (1996) was used. Briefly, ~50 mg of tissue or 100 μL of plasma was saponified

with alcoholic KOH, extracted with hexane, dried under nitrogen, resuspended in 1:1 ethanol:methanol, then injected into an HPLC system. The HPLC system consisted of a Shimadzu LC-10ADvp controller, and a SIL-10ADvp auto injector with a 50µl sample loop. Tocopherols were detected using a LC-4B amperometric electrochemical detector (Bioanalytical Systems Inc., West Lafayette, IN, U.S.A.) with a glassy carbon working electrode, and a silver chloride reference electrode. The column used was a Waters Spherosorb ODS2 C-18 column, 4.6 × 100 mm, 3 µm particle size with a Waters Spherisorb ODS precolumn, 1.0 × 4.6 mm, 5 µm. An isocratic mobile phase delivery system was used, with a total run time of 6 minutes. The mobile phase used was 99:1 (v:v) methanol:water containing 0.1% (w:v) lithium perchlorate. The electrochemical detector was in the oxidizing mode, potential 500 mV, full recorder scale at 500 nA. Peak areas were integrated using Shimadzu Scientific 4.2 Class VP software package, and tocopherols were quantitated using authentic standards.

Statistical Methods

All values are expressed as mean ± standard error of the mean (SEM). Outcome variables for physiological parameters were analyzed using a two-factor analysis of variance with repeated measures. Fisher's least-significant-difference procedure was used for multiple comparisons (or post hoc statistical analysis). Measurements at various time periods were tested at the < 0.05 level of significance.

Results

Gas exchange and Pulmonary transvascular fluid flux

The injured smoke and B&S groups showed a progressive fall in PaO2/FiO2 ratio, which was significantly lower than the baseline value 12 h after injury. There

were no significant differences in PaO2/FiO2 ratio between the burn injured group and the sham injured group (Fig.1-A).

Pulmonary transvascular fluid flux was evaluated by measurement of lung lymph flow. The injured smoke and B&S groups showed significant increases in lung lymph flow compared with the sham group. There were no significant differences in lung lymph flow between the burn injured group and the sham injured group (Fig.1-B).

At 48 h the lung wet/dry weight ratio was significantly higher in the smoke-injured (6.09 ± 0.40) and the B&S-injured (6.03 ± 0.15) groups compared with the sham injured group (5.40 ± 0.17; $p<0.05$). (Fig.1-C）.

Plasma Conjugated Dienes:

Plasma conjugated dienes remained unchanged in the sham injured groups. Plasma conjugated dienes gradually increased in both smoke and B&S injured groups throughout the entire experiment period. The B&S injured group showed significantly higher values than the sham injured group. In the burn injured group, plasma conjugated dienes increased initially, after that there were no significant differences compared with the sham injured group (Fig.2).

Plasma α-tocopherol / lipids after B&S injury

Right after B&S injury, there was a transient depletion in plasma α-tocopherol / lipids. The plasma alpha tocophreol levels were significantly lower in B&S group vs. sham group. (Fig.3).

Lung and Liver α-tocopherol after injury

Figure 4 shows lung tissue alpha- tocopherol levels corrected by lung dry weight. The lung tissue alpha-tocopherol levels were not changed at 4 h in all injury groups compared to sham animals. However, combined burn and smoke inhalation resulted in significant depletion of alpha-tocopherol 48 h after the insult. Burn or smoke alone did not alter the lung tissue alpha-tocopherol corrected by lung dry weight. (Fig.5).

After 4 h, the injuries did not deplete liver α-tocopherol however, after 48 h liver α-tocopherol was depleted in combined B&S injury.

Discussion

In the present study we report a severe depletion of both alpha and gamma-tocopherols in plasma and tissues (lung, liver,) following thermal injury. We demonstrate that more pronounced depletion of these important antioxidants was observed in combined burn and smoke inhalation injury than in burn or smoke alone injury. There have been several reports that demonstrate low plasma vitamin E levels after multiple traumas such as sepsis, and ischemia-reperfusion injury (12).

However, plasma lipid concentrations were not measured or reported in their studies. When we evaluate vitamin E status, the importance of examining of plasma lipids level was reported. For that reason, we made correction the plasma α-tocopherol data with plasma lipid. As a result, we found a fall just right after injury followed by a rapid recovery.

Tissue α-tocopherol levels fall and dramatically in the first 24 h after endotoxin infusion or cecal-ligation and puncture (13,14). Several investigators have demonstrated improved survival after α-tocopherol treatment in these animal models of sepsis (15,16,17,18). In our study, tissue (lung, liver and trachea) α tocopherol levels fell after

24 h or 48 h. Large amounts of superoxide anions are known to be present in burn/smoke and are inactivated within a few minutes. Several studies on burn/smoke inhalation injury have demonstrated that lipid peroxides appear in the systemic circulation within minutes after injury and peak a second time at 24 h after injury (2). The decreased lung tissue vitamin E concentrations and increased lipid peroxidation found in animals with injuries in the present study may represent increased free radical activity, with consequent increased utilization of antioxidant defences.

B&S injury decreased in liver endogenous antioxidants with time. Liver exported stored vitamin E to maintain vitamin E level in other tissues.

A role distinct from oxygen radical scavenging has been proposed for γ-tocopherol (19). In contrast to α-tocopherol, γ-tocopherol is a powerful nucleophile that traps electrophilic mutagens in lipophilic compartments. It thus complements glutathione, which similarly scavenges electrophilic mutagens in the aqueous phase of the cell. An electrophilic mutagen prone to react with γ-tocopherol is peroxynitrite. Thus, γ-tocopherol may protect lipids, DNA, and proteins from peroxyniterite-dependent damage (1). The reaction of γ-tocopherol with peroxynitrite yields several products, including 2, 7, 8-methyldecyl-2- (4, 8, 12-trimethyldecyl)-5-nitro-6-chromanol (NGT) was demonstrated (20). Nitric oxide (NO) produced by NO synthase (NOS) plays a major role in the pathogenesis of trauma and sepsis (21,22). Peroxynitrite is a potent oxidant produced by the reaction of NO and superoxide anion and is produced in burn and smoke models of injury (3).

Vitamin E is a lipid-soluble vitamin and a free radical-scavenging antioxidant. Because vitamin E is lipophilic, oral vitamin E should be absorbed in the small intestine, carried into the liver by the lymphatics, and distributed among lipo-proteins before transport to the other organs to manifest its therapeutic efficiency (23). Additionally,

vitamin E has been shown to have several additional biologically important effects. These include the inhibition of arachidonic acid oxidative metabolism and the inhibition of protein kinase C (PKC) activity, another important step in signal transduction. The inhibition of PKC by vitamin E does not appear to be independent on its antioxidant activity (24,25).

From the present data, we conclude that animals receiving a combination burn and inhalation injury have undergone marked oxidative stress and these results suggest that vitamin E treatment might be beneficial in burn and smoking inhalation patients.

Figure Legends

Figure 1:

Gas exchange (A; PaO_2/FiO_2). Pulmonary transvascular fluid flux (B; (% of baseline values)). Data are expressed as the mean ± SEM. Sham (❒, Squares, n=7), burn (❍ n=6), smoke (triangles, n=6), and burn and smoke (◆rhombus represent the oke group (n=10). *, p<0.05 vs. sham. Lung wet/dry weight ratio in sham (n=7), burn (n=6), smoke (n=6), and burn and smoke groups (n=10). Data are expressed as mean ± SEM. *, p <0.05 vs. sham group.

Figure 2:

Conjugated dienes levels in plasma (C; (% of baseline values)).

Figure 3:

α-tocopherol / lipids levels in plasma (% of baseline values). Data are expressed as the mean ± SEM. Circles represent the sham group (n=5), squares represent the burn and smoke group (n=9)

Figure 4:

Lung α-tocopherol / dry weight in sham, burn, smoke, and burn and smoke groups. Data are expressed as mean ± SEM. *, p <0.05 vs. sham group.

Figure 5:

Liver α-tocopherol / dry weight in sham, burn, smoke, and burn and smoke groups. Data are expressed as mean ± SEM. *, p <0.05 vs. sham group.

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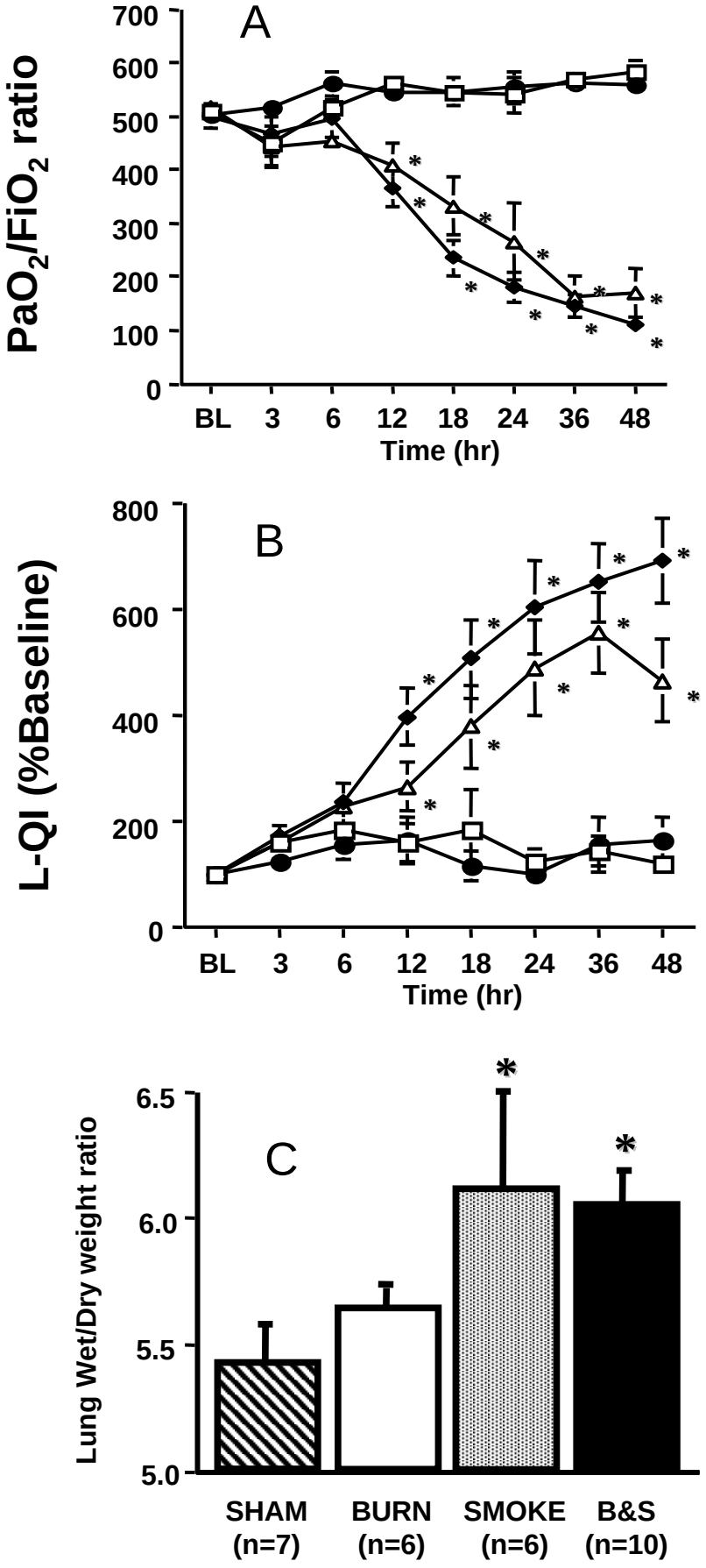
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PaO2/FiO2 ratio
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Time (hr)
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Time (hr)
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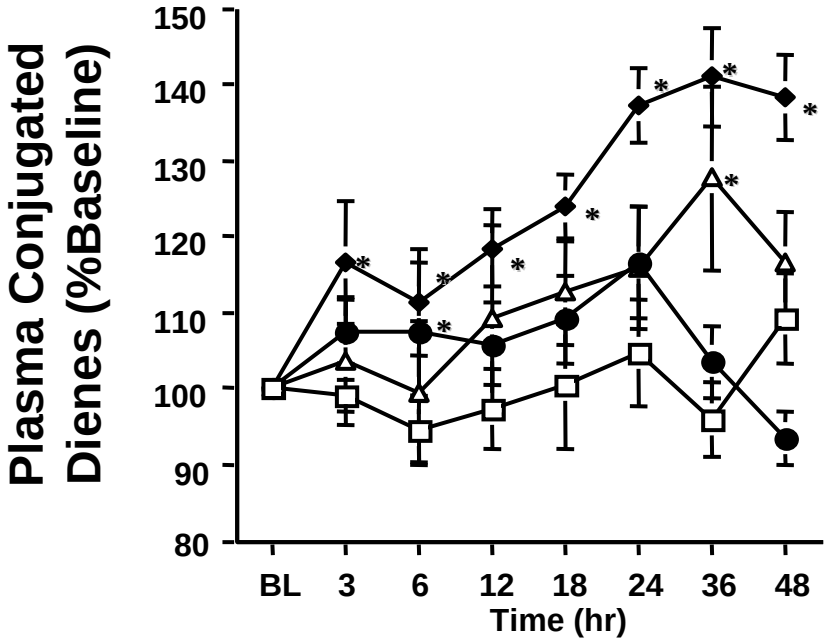
Lung Wet/Dry weight ratio
SHAM
(n=7)
BURN
(n=6)
SMOKE
(n=6)
B&S
(n=10)

Fig.1


Plasma Conjugated Dienes (%Baseline)
150
140
130
120
110
100
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80
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3
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12
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24
36
48
Time (hr)

Fig.2

*** p<0.05 vs.. Sham**

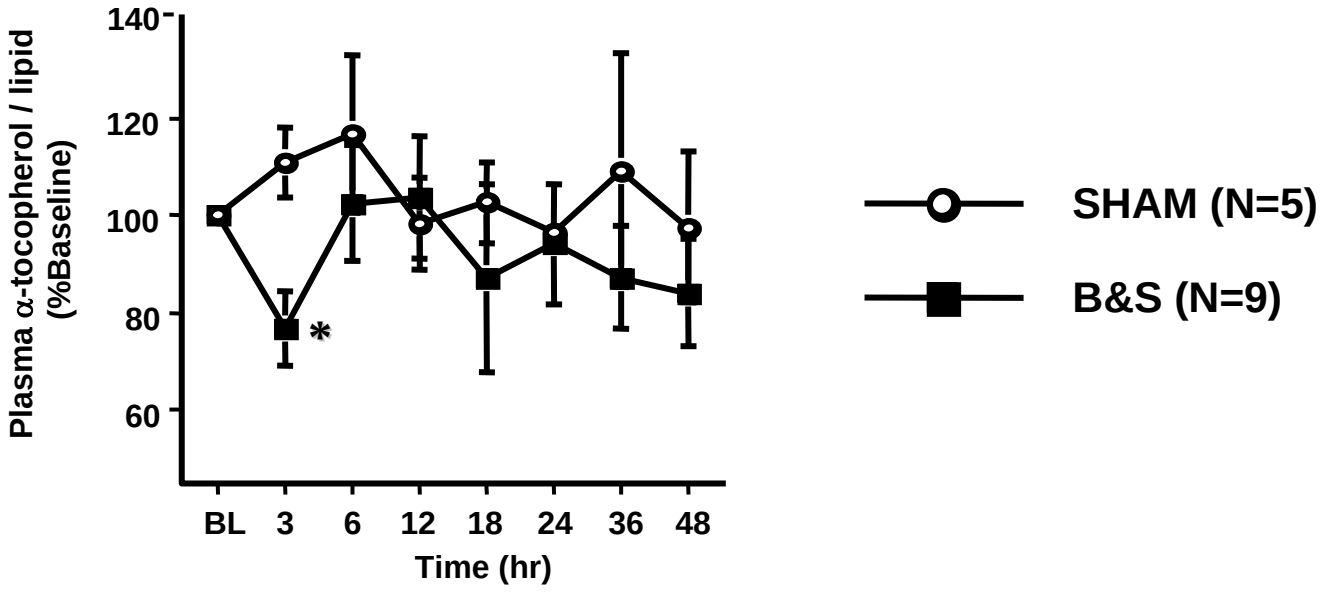

Fig.3

* p<0.05 vs.. Sham

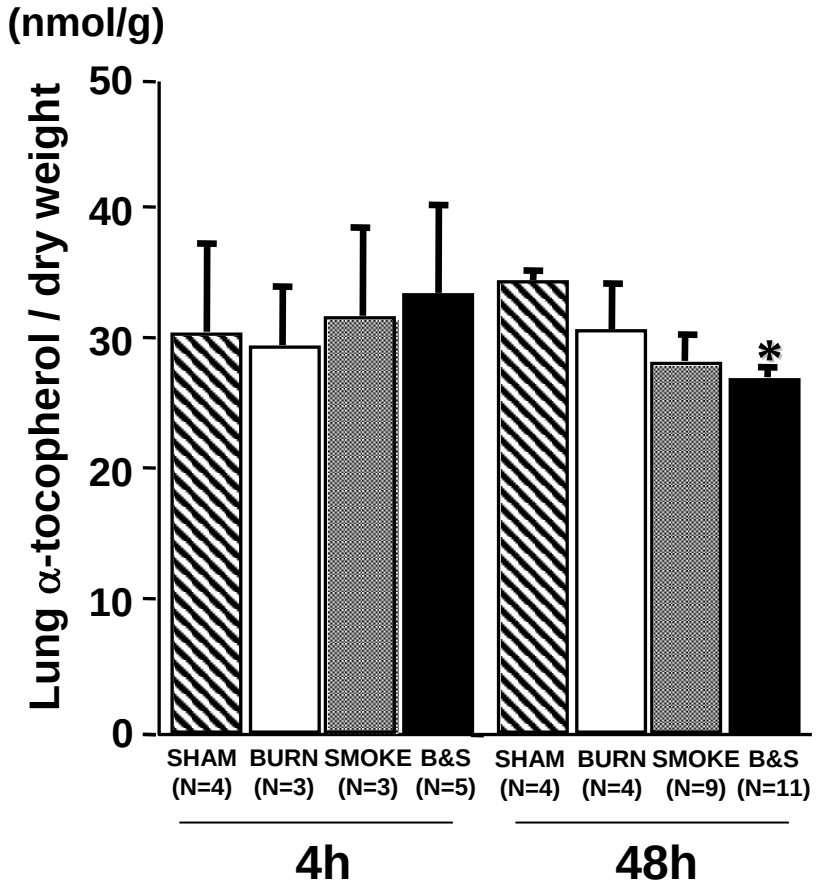

Fig.4

* p<0.05 vs.. Sham

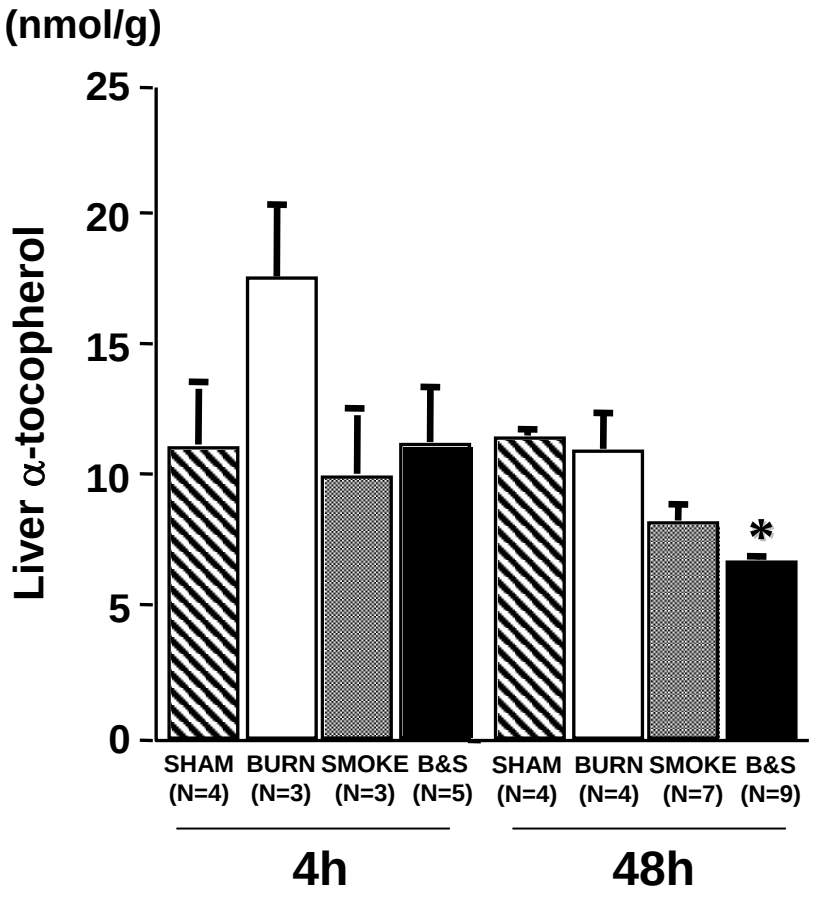

Fig.5