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Original Contribution

Burn and smoke inhalation injury in sheep depletes vitamin E: Kinetic studies using deuterated tocopherols

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Abstract

To test the hypothesis that burn and smoke injury will deplete tissue α-tocopherol and cause its faster plasma disappearance, deuterium-labeled vitamin E was administered to sheep exposed to both surface skin burn and smoke insufflation, which cause injuries similar to those of human victims of fire accidents. Two different protocols were used: (1) deuterated vitamin E was administered orally with food at time 0 (just before injury) or (2) the labeled vitamin E was administered orally with food the day before injury. The animals, which had been operatively prepared seven days before, were anesthetized and then received both 40% body surface area third-degree burn and 48 breaths of cotton smoke or sham injuries. All were resuscitated with Ringer's lactate solution (4 ml/kg/% BSA burn/24 h) and mechanically ventilated. Blood samples were collected at various times after vitamin E dosing. In both studies the depletion of plasma α-tocopherol was faster in the injured sheep. The sheep given deuterated vitamin E 24 h before injury had similar maximum α-tocopherol concentrations at similar times. The exponential rates of α-tocopherol disappearance were 1.5 times greater and half-lives were 12 h shorter ($p < 0.05$) in the injured sheep. In separate studies, various tissues were obtained from sheep that were sacrificed from 4 to 48 h after injury. The liver α-tocopherol concentrations in sheep killed at various times after injury seem to show a linear decrease at a rate of 0.1 nmol α-tocopherol/g liver per hour, suggesting that the liver is supplying α-tocopherol to maintain the plasma and lung α-tocopherol concentrations, but that this injury is so severe the liver is unable to maintain lung α-tocopherol concentrations. These findings suggest that α-tocopherol should be administered to burn patients to prevent vitamin E depletion and to protect against oxidative stress from burn injury.

Keyword: α-Tocopherol; Shock; Ovine model; Liver; Lung; Inflammation; Pharmacokinetics; Free radicals

In the United States, about 100,000 people require hospitalization and 5000 deaths occur each year because of burn injury [1]. Additionally, 70% of fire victims who die within 12 h of insult have inhalation injury [2,3]. Combined burn and smoke inhalation injury is typically associated with a systemic inflammatory response and increased reactive oxygen species (ROS) [4,5]. ROS can result in cell membrane destabilization, enzyme inactivation, increases in capillary permeability, and vascular reactivity [6,7]. All these modifications strongly resemble many of the prominent characteristics of circulatory burn shock and distant organ injury [8].

We have previously described the pathophysiology of acute lung injury in sheep subjected to the combined burn and smoke inhalation injury [9,10]. The tissue injury included an increase in pulmonary vascular permeability to both fluid and protein [11]. Although the exact mechanism of the acute lung injury in thermal damage is not completely understood, we have shown that ROS play a critical role in this pathological process [12]. Additionally, stimulated neutrophils release ROS that affect the microcirculation of the lung [13].

Both enzymatic and nonenzymatic antioxidants are important ROS defense mechanisms [14]. But given that vitamin E is a lipid-soluble antioxidant that prevents propagation of lipid peroxidation [15], it potentially plays a critical role in protection from burn and smoke injury. In burned patients, plasma

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α-tocopherol concentrations typically decline rapidly and remain depressed for more than seven days after injury [16].

We hypothesized that burn and smoke injury will deplete tissue vitamin E and thus cause its faster disappearance from the plasma. To test this hypothesis we have used stable isotope-labeled vitamin E in a well-established model of the human acute respiratory distress syndrome, which is sheep exposed to both burn injury and smoke insufflation.

Methods

Deuterated vitamin E

RRR-α-5-(C^2H_3)-tocopheryl acetate and *all rac*-α-5,7-($C^2H_3)_2$-tocopheryl acetate (d_3-*RRR*-α-α-tocopheryl acetates and d_6-*all rac*-α-tocopheryl acetates, respectively) were a gift from the Natural Source Vitamin E Association. The compounds were determined to be 96% *RRR*-α-tocopheryl acetate and 93% *all rac*-α-tocopheryl acetate by weight. Their isotopic purities at their nominal level of deuteration were 84% (d_0 4.0%, d_1 2.0%, d_2 9.7%) and 86% (d_0,d_1 <0.1%, d_2 0.1%, d_3 0.8%, d_4 1.3%, d_5 11.2%), respectively. The d_3-*RRR*- and d_6-*all rac*-α-tocopheryl acetates were encapsulated in gelatin capsules as 1:1 mixtures of 75 mg each, diluted in α-tocopherol-stripped corn oil. The *RRR*/*all rac* ratio in the capsules was determined by GC/MS to be 0.98. The two forms of vitamin E paralleled each other in the plasma with the d_6-*RRR*-α-tocopherol concentrations at roughly half those of d_3-*RRR*-α-tocopherol [17]; therefore, only d_3-*RRR*-α-tocopherol data are reported.

Animal care

Animals were cared for in the Investigative Intensive Care Unit at the University of Texas Medical Branch (UTMB; Galveston, TX), which is approved by the Association for the Assessment and Accreditation of Laboratory Animal Care. The UTMB Animal Care and Use Committee approved the experimental procedures. National Institutes of Health and American Physiological Society guidelines for animal care and use were strictly followed. The animals were fed a chow diet containing vitamin E 30 IU per pound diet (66 mg *dl*-α-tocopheryl acetate/kg) along with hay for at least two weeks before study. The vitamin E requirements of a 50-kg sheep is 15 IU (15 mg *dl*-α-tocopheryl acetate) [18].

Animals were studied while awake. The sheep had access to food and ate in a regular manner. They showed no outward signs of liver damage, nor was there histologic evidence of liver damage.

Surgical preparation and injury

Sheep were surgically prepared for chronic study as described previously [19–21]. Briefly, a Swan-Ganz thermal dilution catheter (Model 93A-1317-F; Edwards Critical Care Division, Irvine, CA, USA) was inserted through the right external jugular vein for the measurement of cardiac output and the core body temperature and to measure the central venous pressure to evaluate fluid resuscitation. An arterial catheter (16-gauge, 24-in. Intracath; Becton-Dickinson, Sandy, UT, USA) was inserted into the right femoral artery for the measurement of arterial blood gas. The caudal mediastinal lymph node was cannulated (silastic medial-grade tubing, 0.025-in. i.d., 0.047-in. o.d.; Dow Corning; Midland, MI, USA) according to a modification of the technique described by Staub and colleagues [22]. The contribution to the node was removed by ligation of the tail of the caudal mediastinal lymph node and cauterization of the systemic diaphragmatic lymph vessels [20]. After the operation, the sheep were allowed five to seven days to recover from the operative procedure and given food and water ad libitum.

The smoke and burn injury protocol was described previously [19–21]. Briefly, under induction of anesthesia with 10 mg/kg ketamine (Ketalar; Parke–Davis, Morris Plains, NJ, USA), a tracheotomy was performed, and a cuffed tracheostomy tube (10-mm diameter; Sheiley, Irvine, CA, USA) was inserted. Anesthesia was maintained with halothane. Using the Bunsen burner, a third-degree flame burn of 20% of the total body surface area was made on one flank. Thereafter, inhalation injury was induced while the sheep was in the prone position. A modified bee smoker was filled with 50 g 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 insufflations, another 20% total body surface area, third-degree burn was made on the contralateral flank.

The resuscitation protocol was described previously [19–21]. Immediately after the injury, anesthesia was discontinued and the animals were allowed to awaken and were mechanically ventilated with a Servo ventilator (Model 900C; Simens–Elena, Solna, Sweden) throughout the next 48-h experimental period. Ventilation was performed with a positive end-expiratory pressure of 5 cm H_2O and a tidal volume of 15 ml/kg. The respiratory rate was set to maintain normocapnea. For the first 3 h postinjury, all animals received an inspired oxygen concentration (F_iO_2) of 100% to expedite the removal of CO; thereafter, F_iO_2 was adjusted to maintain the arterial oxygen saturation at >90%. These respiratory settings allowed rapid carboxyhemoglobin clearance after smoke inhalation.

During the experiment, fluid resuscitation was performed with Ringer's lactate solution following the formula 4 ml/% burn surface area/kg body wt for the first 24 h and 2 ml/% burned surface area/kg body wt per day for the next 48 h [20]. During this experimental period, the animals were allowed free access to food, but not to water, to allow accurate determination of fluid balance. Fluid balance was constantly monitored.

Measured physiological variables were not considered valid until the animals were fully awake and standing, which usually occurred within 1 h postinjury. Arterial blood was measured with a blood gas analyzer (Model IL1600; Instrumentation Laboratory, Lexington, MA, USA). The data were corrected for core body temperature. Lung lymph flow was measured with a graduated test tube and stopwatch. Lymph and blood samples