Lipid Peroxidation and Acute Lung Injury After Thermal Trauma to Skin

Evidence of a Role for Hydroxyl Radical

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The authors have previously shown that thermal injury to the skin of rats results in the development of acute lung injury that is susceptible to systemic treatment of animals with catalase and dependent on the presence of neutrophils. The current studies have been expanded for exploration of the nature of the neutrophil-derived oxygen products responsible for the lung injury and have also focused on evidence of the appearance of products of lipid peroxidation (conjugated dienes). With respect to the former, treatment of rats with iron chelators (deferoxamine mesylate, 2,3-dihydroxybenzoic acid), with scavengers of hydroxyl radical (dimethyl sulfoxide, dimethyl thiourea, sodium benzoate), or with vitamin E affords a significant degree of protection from acute lung injury as assessed by changes in lung vascular permeability and by morphologic parameters. These data suggest that lung vascular injury after thermal trauma of the skin is related to the generation by neutrophils of the hydroxyl radical. Conjugated dienes have been demonstrated to appear sequentially both in the burned skin (at ¼ hour) and in the lungs (at 2 hours), as well as in the plasma (with peaks at ½ and at 3 hours) after thermal injury. The appearance of the conjugated dienes in plasma at the two intervals of time is greatly diminished if animals are pretreated with the iron chelator deferoxamine, with catalase, or with scavengers of hydroxyl radical. Furthermore, the appearance of conjugated dienes in plasma at 30 minutes and 3 hours is significantly diminished if animals are depleted of neutrophils, complement-depleted, or the burned skin is excised immediately after thermal injury. These data indicate a linkage between thermal trauma of skin, secondary injury of lung, and appearance in plasma and tissues of products of lipid peroxidation. (Am J Pathol 1985, 119:376–384)

EXPERIMENTAL THERMAL injury of rat skin (70 C, 30 seconds) results in activation of the complement system, which, in turn, leads to the appearance of C5-related chemotactic activity in the serum, transient neutropenia, and sequestration of neutrophils in pulmonary capillaries.[1] These events ultimately lead to acute lung injury, as evidenced by increases in lung vascular permeability and morphologic changes indicative of damage to lung vascular endothelium and the presence of interstitial and intraalveolar edema and hemorrhage.[1] Protection from acute lung injury following remote thermal injury (involving skin) can be achieved by depleting animals of complement or blood neutrophils or by systemic treatment of animals with a combination of catalase and superoxide dismutase (SOD).[1] The protective effects of catalase and SOD provide strong evidence that oxygen-derived free radicals released from complement-activated neutrophils are important mediators of lung injury secondary to skin burns. The nature of the oxygen species involved in lung injury following skin burns has not previously been determined. Although experimental evidence suggests that hydrogen peroxide (H_2O_2) and/or superoxide anion (O_2^-) may play a pathogenic role in the acute lung injury, there is little evidence to suggest that O_2^- or H_2O_2 are tissue toxic *per se*.

In the studies to be presented, we demonstrate that acute lung injury can be prevented by treatment of thermally injured rats with the antioxidant vitamin E or with scavengers of hydroxyl radical ($^{\cdot}OH$) such as

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dimethyl sulfoxide (DMSO) or dimethyl thiourea (DMTU). Similar protection is also afforded by treatment of animals with iron chelators (deferoxamine mesylate or 2,3-dihydroxybenzoic acid). Conversely, injection of iron-saturated deferoxamine mesylate fails to protect from tissue injury. These data suggest that an iron-catalyzed conversion product of hydrogen peroxide, probably hydroxyl radical, may be the key mediator related to acute lung injury following skin burns. The appearance in skin, lung, and plasma of products of lipid peroxidation (conjugated dienes) and the reciprocal relationship between plasma levels of conjugated dienes and protection from the effects of oxygen radicals suggest that conjugated dienes are useful markers of oxygen-radical-mediated tissue injury.

Materials and Methods

Animal Model of Thermal Injury

Specific pathogen-free Long-Evans rats (Charles River Laboratories, Portage, Mich) were anesthetized with ketamine hydrochloride (Parke-Davis, Morris Plains, NJ) and the shaved skin over the lumbosacral and flank area was then exposed to 70 C water for 30 seconds. This resulted in a nonlethal burn involving approximately 30% of total body surface area.

Lung injury was assessed morphologically by transmission electron microscopy and quantitated by measuring increases in lung vascular permeability as described in detail elsewhere.[2] Briefly, ^{125}I-labeled bovine serum albumin was injected intravenously 10 minutes prior to skin burn, and its leakage into the extravascular compartment of the lung determined at 3 hours after burning if not otherwise stated. At this time point, rats were anesthetized with ketamine and sacrificed by exsanguination from the posterior vena cava. The lungs and heart were removed *en bloc*, and 10 ml of saline was perfused through the right ventricle of the still beating heart to remove blood remaining in the pulmonary vasculature. Lung vascular permeability values were calculated by expressing, as a ratio, the content of radioactivity in the whole lung with regard to the amount of radioactivity present in 1.0 ml of blood.

Interventional Therapy

Several different agents were employed for determination of the protective effects against acute lung injury following thermal injury to skin and for investigation of whether hydroxyl radical and lipid peroxidation products could be related to the onset of lung injury. Except for vitamin E, all agents were given 10 minutes prior to burning. These agents included the hydroxyl radical scavengers dimethyl sulfoxide (1.5 ml/kg) (Fisher Scientific Co., Fair Lawn, NJ) and dimethyl thiourea (1000 mg/kg) (Alfa Products, Danvers, Mass), which were injected intraperitoneally. The other hydroxyl radical scavenger sodium benzoate (100 mg/kg) (Sigma Chemical Co., St. Louis, Mo) and the iron chelators deferoxamine mesylate (15 mg/kg) (Ciba-Geigy Corp., Summit, NJ) and 2,3-dihydroxybenzoic acid (100 mg/kg) (Aldrich Chemical Co., Inc., Milwaukee, Wis) were injected intravenously. Iron-saturated deferoxamine (approximately 90% saturation) was prepared by dissolving appropriate amounts of $FeCl_3$ in an aqueous solution of deferoxamine. Polyethylene-glycol-modified catalase (Enzon Inc., Piscataway, NJ) was also injected intravenously (1200 U/kg) 10 minutes before thermal injury. DL-α-tocopheryl acetate (vitamin E), which is suitable for intravenous infusion, was provided by O'Neal, Jones and Feldman Pharmaceuticals (Maryland Heights, Mo). Rats were given intravenous injections of 25 U.S.P. units vitamin E 18 hours and 2 hours prior to thermal injury of the skin.

Neutrophil Depletion

Depletion of circulating neutrophils (<250/cu mm) was achieved by the intraperitoneal injection of a rabbit antiserum against rat neutrophils (0.5 ml/100 g) 18 hours prior to burning, as described previously.[2,3]

Lipid Peroxidation Products

Quantitative estimations of lipid peroxidation products (defined as conjugated dienes) in plasma of rats were performed by spectrophotometric analysis.[4] Lipids were extracted from plasma with a 2:1 mixture of purified chloroform-methanol[5]; the extracts were then dried under nitrogen. Chromatographic-quality heptane was used to dissolve the lipid residue for subsequent ultraviolet absorption studies. Usually, 1.0 ml serum or plasma was mixed with 7.0 ml chloroform-methanol, and the samples were stirred for 2 minutes at 45 C and then subjected to centrifugation for 5 minutes at 1000*g*. Five milliliters of the lower layer was then carefully removed, mixed with 2.0 ml acidic (pH 2.5) water, and again subjected to centrifugation. Then, 2.0 ml of the chloroform layer was dried under nitrogen at 37 C, and the lipid residue was dissolved in 1.0 ml heptane for absorption measurements, which were carried out at 233 nm.

Tissue lipids were extracted according to the following procedure: 1.0 g (wet) tissue was added to 0.8 ml distilled water and homogenized on a Brinkman Polytron for 1 minute. Six milliliters of a 2:1 mixture of methanol-chloroform at 45 C was added to the homog-

Table 1—Effect of Iron Chelators and Scavengers of Hydroxyl Radical on Lung Injury Following Skin Burns

Treatment	No. of animals	Lung vascular permeability*		Significance (*P* value)	Protection (%)
		Individual values	Mean ± SEM		
None	5	0.20, 0.21, 0.18, 0.19, 0.22	0.20 ± 0.01		
Burn	7	0.56, 0.53, 0.66, 0.55, 0.80, 0.75, 0.94	0.68 ± 0.06		
Burn + deferoxamine (15 mg/kg)	5	0.31, 0.31, 0.33, 0.27, 0.36	0.32 ± 0.01	<0.001	75
Burn + iron-saturated deferoxamine (15 mg/kg)	4	0.69, 0.52, 0.60, 0.65	0.62 ± 0.04	NS	9
Burn + 2,3 dihydroxybenzoic acid (100 mg/kg)	5	0.32, 0.36, 0.26, 0.34, 0.35	0.33 ± 0.02	<0.001	73
Burn + DMSO (1.5 ml/kg)	7	0.34, 0.38, 0.38, 0.27, 0.21, 0.31, 0.27	0.32 ± 0.01	<0.001	75
Burn + DMTU (1000 mg/kg)	3	0.26, 0.37, 0.31	0.31 ± 0.03	<0.001	77
Burn + Benzoate (100 mg/kg)	5	0.37, 0.54, 0.48, 0.45, 0.42	0.46 ± 0.05	<0.02	46
Burn + Vitamin E†	3	0.30, 0.29, 0.42	0.34 ± 0.04	<0.01	71

* Lung vascular permeability values were determined at 3 hours after thermal injury or after saline injection in nonburned animals.

† Rats were injected intravenously with 25 U.S.P. units of DL-alpha tocopheryl acetate suitable for intravenous infusion at 18 hours and 2 hours prior to thermal injury.

enate and vortexed for 2 minutes. To this was added 2.0 ml chloroform, followed by 30 seconds of mixing. Then 2.0 ml acidic (pH 2.5) distilled water were added to the mixture, followed by another 30-second mixing. The samples were then subjected to centrifugation at 1000*g* for 10 minutes (20 C). Two milliliters of the lower chloroform layer was then removed and dried under nitrogen at 37 C, resuspended in 1.0 ml heptane, and analyzed at 233 nm for the presence of conjugated dienes.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). For determination of significance of the data, the Student *t* test was employed.

Results

Protection Against Lung Injury With Iron Chelators or Hydroxyl Radical Scavengers

We have shown previously that thermal trauma to skin can lead to lung injury, which can be prevented by treatment of thermally injured animals with a combination of catalase and SOD.[1] In a different experimental model we also demonstrated that acute lung injury following intravascular complement activation (following infusion of cobra venom factor) could be greatly attenuated by interventions that reduced availability of iron or hydroxyl radical.[6] In the present study, pretreatment of burned rats with the iron chelators deferoxamine mesylate and 2,3-dihydroxybenzoic acid,[7] greatly reduced (73–75%) the extent of acute lung injury as determined by lung vascular permeability values (Table 1). This protection failed to occur when deferoxamine that was 90% iron-saturated was employed. In view of the known potent effects of DMSO as a hydroxyl radical scavenger,[8] a series of thermally injured rats was pretreated with DMSO or with other hydroxyl radical scavengers such as DMTU[9] or sodium benzoate. As shown in Table 1, treatment with these scavengers resulted in a protection (46–77%) from acute lung injury when compared with burned animals not similarly treated. Finally, pretreatment of burned animals with the well-known antioxidant vitamin E also resulted, as anticipated, in a significant degree of protection (71%) from acute lung injury (Table 1). In control (nonther-

Table 2—Effect of Interventional Procedures on Background Values of Lung Vascular Permeability

Treatment	No. of animals	Lung vascular permeability*	
		Individual values	Mean ± SEM
Saline	4	0.12, 0.21, 0.15, 0.22	0.18 ± 0.02
Deferoxamine (15 mg/kg)	3	0.12, 0.15, 0.19	0.15 ± 0.02
Iron-saturated deferoxamine (15 mg/kg)	3	0.22, 0.19, 0.21	0.21 ± 0.01
2,3-Dihydroxybenzoic acid (100 mg/kg)	3	0.13, 0.12, 0.18	0.14 ± 0.02
DMSO (1.5 ml/kg)	4	0.13, 0.18, 0.15, 0.20	0.17 ± 0.02
DMTU (1000 mg/kg)	3	0.15, 0.27, 0.23	0.22 ± 0.04
Vitamin E (2 × 25 U.S.P. units/rat)	3	0.30, 0.25, 0.28	0.28 ± 0.01

* Lung vascular permeability values were determined 3 hours after treatment.

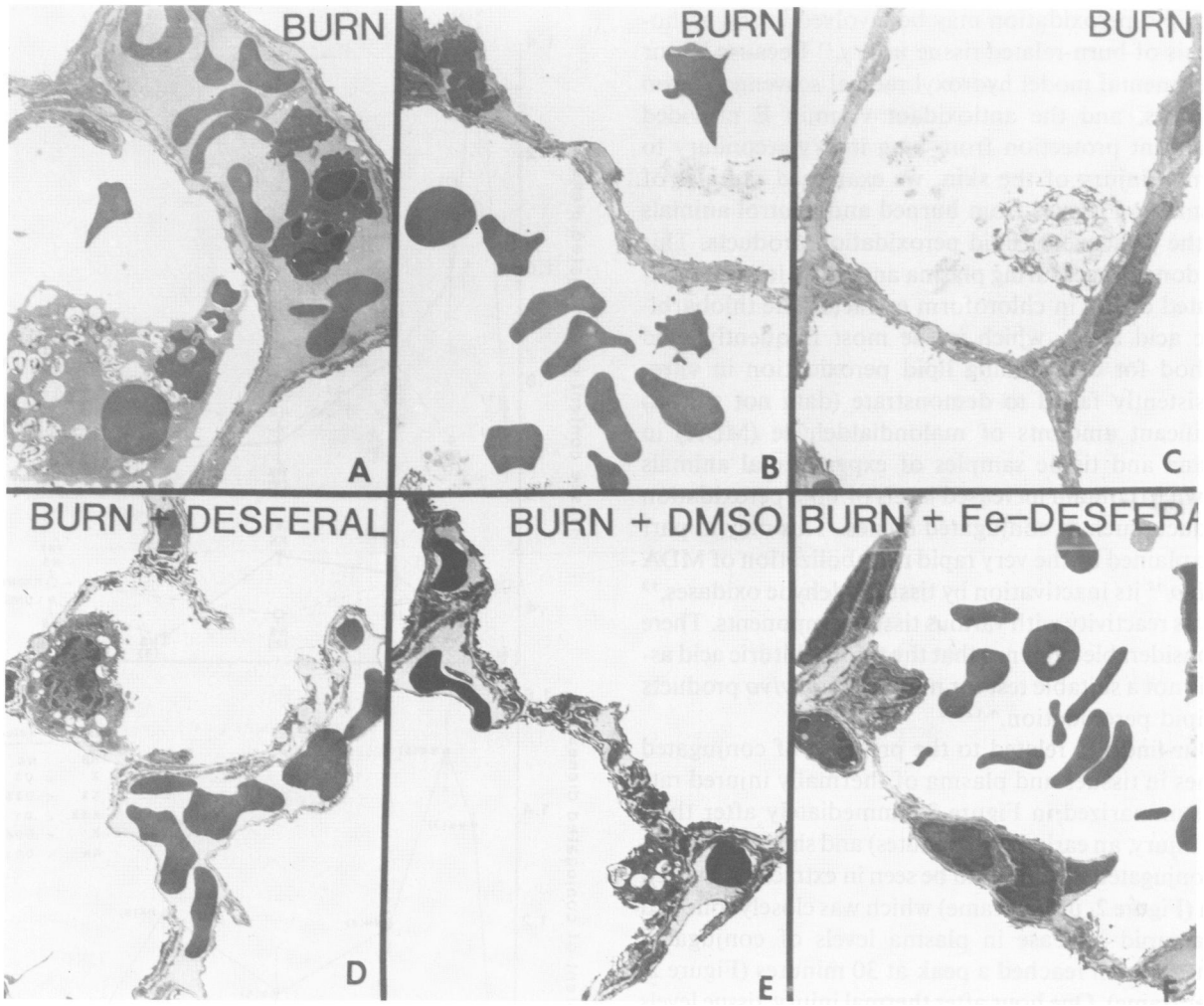

Figure 1—Transmission electron-microscopic micrographs of rat lung tissue 3 hours after skin burn. Pretreatment of thermally injured rats with Desferal (deferoxamine mesylate, 15 mg/kg) or DMSO (1.5 ml/kg) results in significant protection from lung injury (loss of intraalveolar hemorrhage and fibrin; diminished interstitial edema, and intensity of endothelial cell blebbing). Iron-saturated Desferal shows no protective effects. (×1000)

mally injured) rats, these interventional measures did not significantly change the background values for lung vascular permeability (Table 2).

Electron-Microscopic Analysis of Lung Tissues Following Skin Burns

Transmission electron microscopy was carried out on lung tissue from rats subjected to thermal injury of the skin, in the presence or absence of protective interventions (Figure 1 A–F). As shown in A, B, and C, the expected features of lung injury in thermally injured rats were noted: leukoaggregates within the pulmonary interstitial capillaries (A and B), interstitial edema (A, B, and C), blebbing of pulmonary vascular endothelial cells (A), intraalveolar hemorrhage (A and B), intraalveolar fibrin deposits (B and C), and blebbing of Type II alveolar lining cells (C). In animals treated with iron chelator (deferoxamine,* D) or with DMSO (E), there was little evidence of injury (absence of blebs involving endothelial cells or alveolar lining cells and absence of intraalveolar hemorrhage). In animals treated with iron-saturated deferoxamine (F), the morphologic changes were similar to the features found in rats in which pulmonary injury was fully expressed (A, B, and C).

Lipid Peroxidation Products in Plasma and Tissues

Oxidation of polyunsaturated fatty acids by hydroxyl radical and/or iron has been postulated as a mechanism responsible for damage and/or disruption of biologic membranes.[10] Furthermore, it has been suggested

* Also referred to as Desferal (Ciba Pharmaceutical Co., Summit, NJ).

that lipid peroxidation may be involved in the pathogenesis of burn-related tissue injury.[11] Because in our experimental model hydroxyl radical scavengers, iron chelators, and the antioxidant vitamin E provided significant protection from lung injury secondary to thermal injury of the skin, we examined samples of plasma and tissues from burned and control animals for the presence of lipid peroxidation products. This was done by measuring plasma and tissue levels of conjugated dienes in chloroform extracts. The thiobarbituric acid assay, which is the most frequently used method for determining lipid peroxidation in vitro, consistently failed to demonstrate (data not shown) significant amounts of malondialdehyde (MDA) in plasma and tissue samples of experimental animals known to contain increased levels of lipid peroxidation products such as conjugated dienes. This may in part be explained by the very rapid metabolization of MDA *in vivo*,[12] its inactivation by tissue aldehyde oxidases,[13] and its reactivity with various tissue components. There is considerable evidence that the thiobarbituric acid assay is not a suitable test for measuring *in vivo* products of lipid peroxidation.[4,12-16]

Our findings related to the presence of conjugated dienes in tissues and plasma of thermally injured rats are summarized in Figure 2. Immediately after thermal injury, an early (at 15 minutes) and short-lived peak of conjugated dienes could be seen in extracts of burned skin (Figure 2, upper frame) which was closely followed by a rapid increase in plasma levels of conjugated dienes, which reached a peak at 30 minutes (Figure 2, lower frame). One hour after thermal injury, tissue levels of conjugated dienes in the lungs were significantly increased, reaching peak values at 2 hours after burning (upper frame). Increased levels of lipid peroxidation products in the lungs were associated with a second peak of conjugated dienes in plasma 3 hours after thermal injury (lower frame). Extracts from liver and kidney and spleen (data not shown) from thermally injured rats showed no significant changes in tissue levels of conjugated dienes during the 4-hour observation period (Figure 2, upper frame). These data suggest that thermal injury is associated with the appearance of lipid peroxidation products in the burned skin and in lung, reaching peaks at ¼ and 2 hours, respectively, after burning. In addition, the conjugated dienes in plasma show two peaks, at ½ hour and 3 hours after thermal injury of skin.

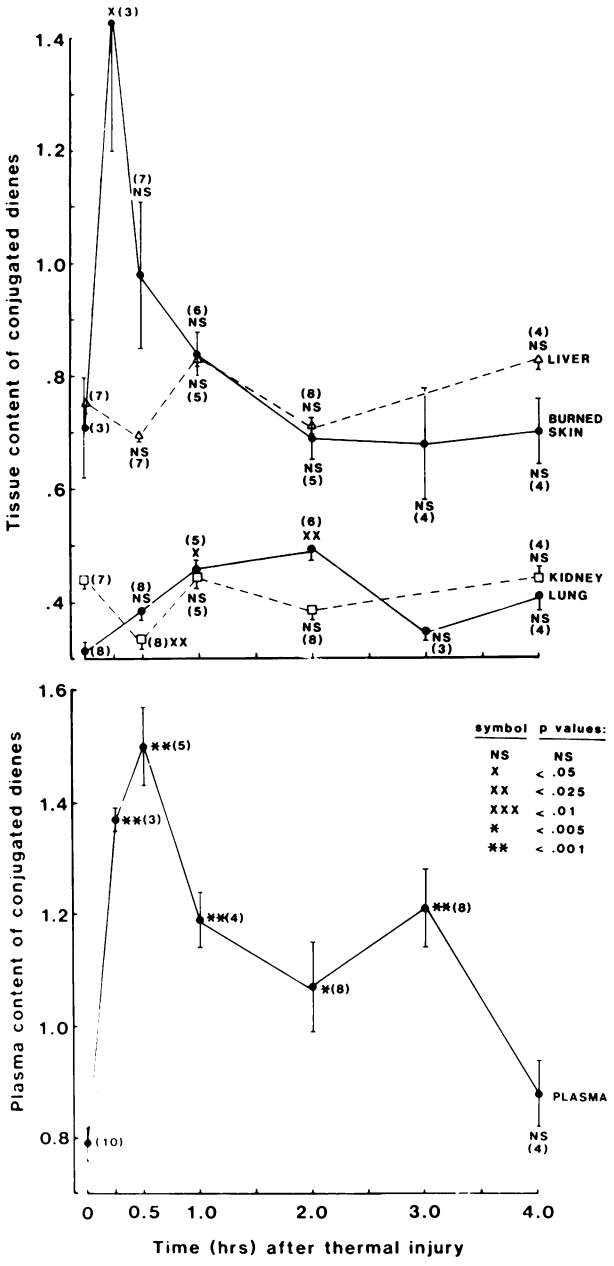

Figure 2—Kinetics of conjugated dienes in plasma and various organ tissues of thermally injured rats. Content of conjugated dienes expressed as absorbance readings at 233 nm.

Effects of Protective Interventions on Appearance of Products of Lipid Peroxidation in Plasma

In another series of experiments in which levels of conjugated dienes were measured 30 minutes after burning and compared with absorbance levels in extracts of plasma from normal rats, there was, on average, a twofold increment in absorbance values in the plasma of thermally injured rats (Table 3). In thermally injured animals treated with the iron chelator deferoxamine, the increment in absorbance was reduced by 67%. Pretreatment of thermally injured rats with DMSO ($^{\cdot}OH$ scavenger) produced similar results. We previously showed that thermally injured rats which have been catalase-treated or depleted of their neutrophils or com-

Table 3—Interventions Affecting 30-Minute Plasma Levels of Conjugated Dienes in Thermally Injured Rats

Treatment	No. of animals	Plasma diene levels* (Mean ± SEM)	Significance (*P* value)	Changes (%)
Burn	5	2.28 ± 0.05		
Burn + deferoxamine (15 mg/kg)	5	1.42 ± 0.13	<0.005	-67
Burn + DMSO (1.5 ml/kg)	3	1.22 ± 0.09	<0.001	-83
Burn + catalase (1200 U/kg)	5	1.17 ± 0.08	<0.001	-87
Burn + neutrophil depletion	3	1.27 ± 0.06	<0.001	-79
Burn + complement depletion	4	1.18 ± 0.09	<0.001	-86
Excision of burned skin†	4	1.15 ± 0.11	<0.001	-88

* Increments in plasma conjugated diene levels above those measured in normal rat plasma. Test samples were obtained 30 minutes after burning.
† Thermally injured skin was excised within 3 minutes of thermal injury of skin.

plement were protected from acute lung injury.[1] The same interventions also resulted in significantly decreased (79–86%) increments in levels of conjugated dienes in the plasma obtained 30 minutes after burning (Table 3). Furthermore, excision of the burned skin within 3 minutes after thermal injury almost completely prevented the appearance of lipid peroxidation products in plasma samples obtained at 30 minutes after thermal injury (Table 3).

When the effects of the same interventions on the 3-hour peak in plasma of conjugated dienes (Figure 2) were examined, results very similar to those described above were obtained (Table 4). Pretreatment of thermally injured rats with deferoxamine mesylate reduced the increment in levels of conjugated dienes by 62%. In companion animals treated with iron-saturated deferoxamine there was, in fact, evidence of increases in the amounts of conjugated dienes in plasma when compared with plasma from thermally injured rats that were otherwise not manipulated. This could be explained by the delivery of iron to neutrophils and facilitation of the conversion of H_2O_2 to ·OH. Treatment of rats with the ·OH scavengers DMSO or DMTU reduced by 68–92% the increased levels of conjugated dienes in plasma. Neutrophil depletion or pretreatment of rats with catalase also resulted in profound reduction (92–99%) in appearance in plasma of conjugated dienes 3 hours after burning. Interestingly, the intravenous injection of catalase 45 minutes after burning, at a time point at which the first peak of conjugated dienes had already appeared in plasma (Figure 2), completely prevented the appearance in plasma of the 3-hour peak of conjugated dienes (Table 4). This treatment also resulted in nearly complete protection from acute lung injury (lung permeability values in 3 treated rats were 0.21 ± 0.02,† similar to the negative control values in Table 1). Finally, excision of the burned skin within 3 minutes after thermal injury not only prevented the appearance of plasma conjugated dienes (Table 4) but also prevented systemic complement activation (data not shown) and development of acute lung injury (lung permeability values in 4 treated rats were 0.23 ± 0.02†).

In Figure 3 the relationship between numbers of available neutrophils and serum content of conjugated dienes at 3 hours after injury is shown. These data were taken from rats partially (1500 cells/cu mm) or fully (< 250 cells/cu mm) depleted of neutrophils after treatment with varying amounts of anti-neutrophil serum and 3 rats that were treated with normal rabbit serum. The data indicate a direct proportionality (correlation coefficient = 0.984) between appearance of conjugated dienes in thermally injured rats and numbers of available neutrophils, as reflected by blood counts. These findings support the concept that the appearance of conjugated dienes in thermally injured rats is linked to availability of neutrophils.

Discussion

The data presented above demonstrate that complement- and neutrophil-dependent lung injury developing in response to remote thermal injury of skin is greatly attenuated by experimental interventions that either decrease the availability of iron or provide for scavenging of ·OH. The ability of iron chelators or ·OH scavengers to protect against acute lung injury in animals with thermal trauma to the skin is reflected in a significant decrease in lung vascular permeability and, morphologically, by attenuation of lung damage, which, in unprotected thermally injured animals, is accompanied by interstitial and intraalveolar edema and intraalveolar hemorrhage and fibrin deposition. Treatment of thermally injured rats with the antioxidant vitamin E resulted in a similar protection from lung injury. These observations are in accord with recently published data showing that acute lung injury in rats caused by intravascular complement activation after infusion of cobra venom factor can be prevented by treatment of animals with iron chelators or with ·OH scavengers.[6] The ·OH scavenger dimethyl thiourea has also been successfully used in rabbits to prevent acute edematous

† Mean ± SEM.

Table 4—Effect of Iron Chelators and Scavengers of Hydroxyl Radical on Diene Conjugation Products in 3-Hour Plasma of Thermally Injured Rats

Treatment	Increments in serum diene levels*		Significance (*P* value)	Changes (%)
	Individual values	Mean ± SEM		
Burn	2.31, 2.56, 2.11	2.33 ± 0.13		
Burn + deferoxamine (15 mg/kg)	1.72, 1.15, 1.66	1.51 ± 0.18	<0.025	-62
Burn + deferoxamine iron saturated (15 mg/kg)	3.54, 2.12, 2.33	2.66 ± 0.44	>0.5	+25
Burn + DMSO (1.5 ml/kg)	1.46, 1.33, 1.48	1.42 ± 0.05	<0.005	-68
Burn + DMTU (1000 mg/kg)	1.08, 1.16, 1.10	1.11 ± 0.02	<0.001	-92
Burn + catalase† (1200 U/kg)	1.01, 1.00, 1.15	1.05 ± 0.04	<0.001	-96
Burn + Catalase at 45 min post-burn‡	1.00, 1.00, 1.02	1.01 ± 0.01	<0.001	-99
Burn + PMN depletion	1.00, 1.20, 1.11	1.10 ± 0.06	<0.005	-92
Excision of burned skin§	1.07, 1.01, 1.00, 1.00	1.02 ± 0.03	<0.001	-98

* Increments in plasma levels of conjugated dienes compared with those in normal rat plasma. Test samples were obtained at 3 hours after thermal injury.
† Polyethylene glycol (PEG)-modified catalase was injected intravenously 10 minutes before thermal injury.
‡ PEG-catalase (1200 u/kg) was injected 45 minutes after thermal injury.
§ Excision within 3 minutes following thermal injury of skin.

lung injury after intravenous infusion of phorbol myristate acetate (PMA),[17] in isolated rat lung preparations injured by perfusions with neutrophils and PMA,[9] and in rats exposed to 100% oxygen.[9]

The role of iron is not completely understood but may be explained by its essential role in the classic iron-dependent Fenton reaction, in which ·OH is formed from H_2O_2:

$$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {\cdot}OH \text{ (Fenton reaction)}$$

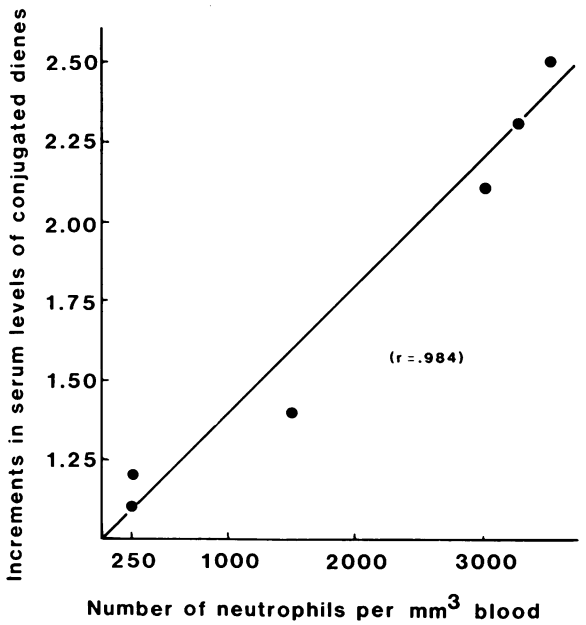

Figure 3—Correlations between appearance in serum of conjugated dienes and numbers of available neutrophils in the blood of rats subjected to thermal injury 3 hours earlier. The numbers of neutrophils in the blood were assessed in 3 rats which had not been given antibody to rat neutrophils and 3 that had received antineutrophil serum 18 hours prior to thermal injury of the skin. Serum samples for measurement of conjugated dienes were obtained 3 hours after thermal injury.

Hydroxyl radical is highly reactive and can lead to generation of lipid peroxides.[18] Our present study provides evidence that thermal injury to the skin can result in the generation of lipid peroxidation products which are detectable in burned skin, lung tissue, and plasma and may be dependent on ·OH released from complement-activated neutrophils. As shown in Tables 3 and 4, both neutrophil depletion and pretreatment with ·OH scavengers almost completely prevent the appearance of conjugated dienes in plasma of thermally injured animals. Our experimental data, showing the protective effects of iron chelators, ·OH scavengers, and vitamin E on neutrophil-dependent lung injury, and the fact that these compounds also decrease the plasma levels of conjugated dienes suggests that lipid peroxidation products are in some manner correlated with the skin injury as well as the lung injury. The conjugated dienes may be direct products of lipid peroxidation of vascular endothelial cells damaged by ·OH generated from complement-activated neutrophils. Although conjugated dienes appear in plasma in a biphasic pattern after thermal injury of skin, with the early peak (at ¼ hour) following that found in skin and the latter peak (at 3 hours) following that observed in lung, there is no proof that the two peaks of conjugated dienes in plasma are derived from the two different tissue sources. As stated above, the only unequivocal conclusion to be drawn is that interventions protective against oxygen-radical–induced injury also greatly diminish the levels of conjugated dienes appearing in the plasma.

The requirement for iron in the development of tissue injury secondary to skin burns could also be explained by a reaction involving the formation of the perferryl ion:

$$Fe^{2+} + O_2 \rightarrow Fe^{2+}O_2 \text{ (perferryl ion)} \rightleftharpoons Fe^{3+}O_2^-$$

Activated dioxygen-linked ferrous ion (perferryl ion)

has the characteristics of a free radical which can enter into reactions that may also result in lipid peroxidation.[19] It should be noted, however, that generation of the perferryl ion does not require H_2O_2, but it does have a requirement for iron, NADPH-cytochrome P-450 reductase, NADPH, and oxygen. On the other hand, H_2O_2 appears to be essential for the development of acute lung injury following skin burns. Pretreatment of thermally injured rats with catalase, which converts H_2O_2 to O_2 and H_2O, almost completely obviates the appearance in plasma of lipid peroxides. These observations, together with the effects of the $^{\cdot}OH$ scavengers, would seem to favor the role of iron via the Fenton reaction as described above.

It should be pointed out that there is abundant evidence that lipid peroxides are potentially harmful to cells and tissue.[20-23] Also, Nishigaki et al[11] have found increases in the serum of thiobarbituric-acid-reactive products within 3 hours after skin burns in rats. After these increases during the first few postburn hours, the authors observed that serum levels of lactate dehydrogenase, alkaline phosphatase, and glutamate oxalacetate transaminase increased at Days 20, 15, and 1 after thermal injury, respectively. On the basis of these observations, Nishigaki et al[11] suggested that lipid peroxidation products enter the bloodstream at the site of the skin burn directly, bringing about damage to cells of remote other organs.

As mentioned above, we have not been able to demonstrate increases in levels of thiobarbituric-acid-reactive products in skin, lung, or plasma in our experimental model. This may be a reflection of a basic difference in the two models of thermal injury of the rat. Nishigaki et al[11] used boiling water in a 5 × 7-cm shaved area of skin. Also, it is known that the thiobarbituric acid assay is unreliable for *in vivo* assessment of lipid peroxidation products because of nonspecificity of the assay, the presence in tissues of aldehyde oxidases that convert malonaldehyde and malondialdehyde, and the rapid disappearance from tissues of malonaldehyde and malondialdehyde due to metabolism and cross-linking of amino groups of proteins and amino-containing phospholipids.[16]

Nevertheless, the question remains: Can products of lipid peroxidation be directly responsible for the injury of lung noted in our model of thermal injury, or are they secondary markers of iron-dependent oxygen-radical-induced tissue injury? This question cannot easily be answered. The question is further complicated by our findings of peaks of conjugated dienes in skin and lungs at quite different time intervals (15 minutes and 2 hours, respectively) and a similar biphasic appearance in plasma of conjugated dienes, at ½ hour and 3 hours (Figure 2). What is clear is that treatment of rats with catalase at the 45-minute interval fully protects the lung from injury, even though it is long past the appearance of peak levels of conjugated dienes in the thermally injured skin and in the plasma. All that can be concluded is that the plasma conjugated dienes found at 30 minutes are not sufficient *per se* to cause lung injury. We tend to think that conjugated dienes are markers of oxygen-radical-mediated injury and do not *per se* mediate or propagate the tissue injury.

As our data show, the derivation of the plasma conjugated dienes in thermally injured rats appears to be related to neutrophils and their production of H_2O_2 and $^{\cdot}OH$. The origin and nature of these lipid peroxides are not yet known, but it appears conceivable that the conjugated dienes may originate from the cell membranes of damaged vascular endothelial cells in burned skin and lungs and/or may constitute oxidized lipids and lipoproteins of plasma and blood cell membranes.

At the present time, the question regarding the nature of the oxygen products responsible for injury to lung vascular endothelial cells cannot be answered. Our experimental data show that thermal injury of skin can result in acute lung injury and that the lung injury can be prevented by treatment of animals with iron chelators or scavengers of hydroxyl radical. These findings may provide some suggestions for interventions that might become useful in clinical situations where there is reason to believe that oxygen-derived free radicals may be responsible for tissue injury.

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