Laboratory Reports

Systemic thermal injury in anesthetized rabbits causes early pulmonary vascular injury that is not ablated by lazarooids

Duncan Miles MD,*
Thomas S. Hurst MVSC,†
A. Saxena MD,‡
Irvin Mayers MD,§
David H. Johnson MD†¶

Purpose: To study the effects of a systemic thermal injury on the pulmonary vasculature with and without inhibitors of lipid peroxidation (U74389G).

Methods: In a prospective, placebo control, randomized, and blinded multi-group study, burn shock was induced by scalding thermal injury (65°C) to 35% body surface area in rabbits (n=28). Hemodynamics and gas exchange were followed for 240 min post burn in four groups; No Burn, Burn-Control, Burn-U74 (10 mg·Kg⁻¹ U74389G), No Burn-U74 (10 mg·Kg⁻¹ U74389G).

Results: Scald resulted in early pulmonary injury as measured by increased pulmonary vascular resistance in the pooled Burn group compared with the No Burn groups (942 ± 358 vs 605 ± 255 dynes·sec⁻¹·cm⁻⁵ respectively, *P* < 0.05). These pulmonary changes were associated with alveolar sequestration of leukocytes (4.8 ± 2.9 vs 17.7 ± 6.0 cells × 10⁹·L⁻¹, *P* < 0.05) in the No Burn and Burn groups respectively. Histological evidence of decreased neutrophil sequestration after scald injury was present in U74 treated animals (3⁺ vs 2⁺, *P* < 0.05 in the Burn and No Burn groups respectively and 2⁺ vs 2⁺, *P* > 0.05 in the Burn-U74 and No Burn-U74 groups respectively) although bronchial alveolar lavage still demonstrated neutrophil sequestration (5.3 ± 2.5 vs 12.2 ± 3.3 cell 10⁹·L⁻¹, *P* < 0.05 in No Burn-U74 and Burn-U74 groups respectively). Similarly, circulating white blood cells were increased in the Burn group but not Burn-U74 group four hours post burn. The increase in pulmonary vascular resistance after burn was not altered by administration of U74.

Conclusions: Systemic burn results in early pulmonary vascular changes associated with leukocyte sequestration. After scald injury administration of lazarooids (U744389G) did not lessen pulmonary vascular resistance changes but did reduce neutrophil sequestration.

Objectif : Étudier les effets d'une lésion thermique généralisée, associée ou non à des inhibiteurs de peroxydation lipidique (U74389G), sur le système vasculaire pulmonaire.

Méthode : Dans une étude multigroupe contre placebo, prospective, randomisée et à l'insu, le choc des brûlés a été induit par un ébouillement traumatique (65°C) sur 35 % de la surface corporelle de lapins (n = 28). Les caractéristiques hémodynamiques et les échanges gazeux ont été enregistrés pendant 240 min après la brûlure dans quatre groupes : Non brûlés, Brûlés-témoins, Brûlés-U74 (10 mg·Kg⁻¹ U74389G), Non brûlés- U74 (10 mg·Kg⁻¹ U74389G).

Résultat : Le fait d'ébouillanter a produit une lésion pulmonaire précoce prouvée par l'accroissement de la résistance vasculaire pulmonaire dans les groupes Brûlés comparés aux groupes Non brûlés (942 ± 358 vs 605 ± 255 dynes·sec⁻¹·cm⁻⁵ respectivement, *P* < 0,05). Ces changements pulmonaires étaient associés à une séquestration alvéolaire de leucocytes (4,8 ± 2,9 vs 17,7 ± 6,0 cellules × 10⁹·L⁻¹, *P* < 0,05) dans les groupes Non brûlés et Brûlés respectivement. Après le traumatisme thermique, une évidence histologique de la baisse de la séquestration des polynucléaires neutrophiles était présente chez les animaux traités au U74 (3⁺ vs 2⁺, *P* < 0,05 chez les Brûlés et les Non brûlés respectivement et 2⁺ vs 2⁺, *P* > 0,05 chez les Brûlés-U74 et les Non brûlés- U74 respectivement, bien que le lavage broncho-alvéolaire indiquait toujours la séquestration des polynucléaires neutrophiles (5,3 ± 2,5 vs 12,2 ± 3,3 cellules 10⁹·L⁻¹, *P* < 0,05 chez les Non brûlés-U74 et les Brûlés-U74, respectivement). De même, après les brûlures, les leucocytes circulants ont augmenté dans le groupe d'animaux Brûlés, mais non dans celui des Brûlés-U74, et ce, pendant quatre heures. L'augmentation de la résistance vasculaire pulmonaire après une brûlure n'était pas changée par l'administration de U74.

Conclusion : Une brûlure généralisée provoque des changements vasculaires pulmonaires hâtifs associés à une séquestration de leucocytes. Après le traumatisme thermique, l'administration de lazaroïdes (U74389G) n'a pas diminué les changements de résistance vasculaire pulmonaire, mais a réduit la séquestration de polynucléaires neutrophiles.

From the Departments of Surgery,* Medicine,† Anaesthesia,‡ and Pathology,§ University of Saskatchewan, Saskatoon, Saskatchewan, Canada, and Department of Medicine,§ University of Alberta, Edmonton, Alberta, Canada.

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Address correspondence to: Dr. David H. Johnson, 103 Hospital Drive, Saskatoon, Saskatchewan
S7N 0W8. Phone: 403-403-0350; Fax: 306-966-8021; E-mail: cujec@v-wave.com

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RECENT evidence has suggested that thermal injury induces a shock state similar to other causes of shock and that secondary mediators are involved. The end organ damage produced by secondary mediators can be documented as early as a few hours after the burn injury.¹⁻³ Stopping the production or release of secondary mediators within the first hours of injury may be crucial in preventing later multiple system organ failure.²⁻⁵ Free radical inhibitors in the lazaroid family (U74389G) prevent progressive burn ischemia and expansion of primary tissue loss.⁶ Even in the absence of smoke inhalation, lung changes also occur early in the post burn period following a systemic body burn.⁷⁻¹⁰ We hypothesized that, similar to sepsis, neutrophils would be a facilitator in subsequent lung damage after thermal injury and that the administration of lazaroids would diminish the early pulmonary deterioration.¹⁰

Methods

Twenty eight rabbits (3.0-3.5 kg) were sedated with 0.1 mg·kg⁻¹ morphine *iv* and anesthetized with 20 mg·kg⁻¹ pentobarbital *iv*. A Doppler flow probe (Transonic Instruments, Utica, NY) measured continuous cardiac output (Qt). A catheter was introduced into the pulmonary artery and measured pulmonary artery pressure (P_{PA}). The animals were hydrated (Ringer's Lactate 15 mL·Kg⁻¹·hr⁻¹). Rectal temperature was maintained near normal using a heating blanket.

A standardized thermal injury (c. 35% body surface area) was produced using a continuous flow of water, heated to 65°C, and circulated through a hollow copper plate. The plate was applied to the shaved and debilitated dorsal surface of the rabbit for 90 sec.

Experimental Protocol

Baseline measurements include: hemodynamics (P_{PA}, and Qt); systemic arterial and venous blood gases (CIBA-Corning 238 pH - Blood Gas Analyzer, Midfield, MA); systemic and venous oxyhemoglobin saturation (CIBA-Corning Co-oximeter #5000, Midfield, MA). A thermal injury was produced in 14 rabbits (burn groups) while 14 animals were similarly exposed to 37°C water (control group). All measurements were repeated after exposure at 1, 2, 3, and 4 hr post injury.

The 14 animals of the burn group and 14 animals of the non-burn group were randomly and in blinded fashion divided to receive drug or vehicle. U74389G (5 mg·kg⁻¹, Upjohn Company Kalamazoo, MI), dissolved in CS-4 buffer (0.02 M citric acid monohydrate, 0.032 M sodium citrate dehydrate, and 0.077 M NaCl) to a volume of 2 mL·Kg⁻¹ buffer which was infused over 5

min after the burn injury. U74389G (5 mg·Kg⁻¹) was also administered by constant *iv* infusion over the subsequent 30 min. The remaining 14 animals (7 Control, 7 Burn-Control) received Ringer's Lactate without added drug. Left atrial pressure was increased to at least 5 mm Hg at the start of experimental protocol by bolus infusion of Ringer's Lactate. After death, by thiopentone overdose, the lungs were expanded by insufflation then placed in formalin 10%. The isolated right lung was repetitively (× 5) washed with saline 0.9% and counted for white blood cells. The right lung was weighed and dried to constant weight. A 1.5 × 1.5 cm of burn scar was excised and placed in formalin 10%.

Histology

Specimens were stained with hematoxylin-eosin and interpreted blindly. Five fields (0.05 mm²) in each lung were assessed for peribronchial edema and scored 1 - present and 0 - absent (total score 0-5). Extravascular leukocytes were scored 1 - present and - absent. Scald injury was scored from 1 to 3: 1 - intact epithelium, 2 - denuded epithelium, 3 - deep dermal injury.

Statistics

A two-way ANOVA with repeated measurements determined differences between No Burn and Burn groups and between U74-No Burn and U74-Burn groups. If the F statistic was significant, a Student-Newman-Keuls multiple comparison test was used. Pooled groups compared over time refer to the combination of burn and no burn or burn-U74 and no burn-U74 animals contrasted at different time periods. Histological variables were analyzed using sign rank test. A two-sided level of 0.05 was significant. A sample size of seven animals was chosen as previous experiments of this sample size had demonstrated significant differences in hemodynamics and hypoxic pulmonary vasoconstriction.¹⁰ All values are represented as mean ± standard deviation.

Results

Hemodynamics and Gas Exchange (Tables I-II)

Mean pulmonary artery pressure was lower and pulmonary vascular resistance was less in the initial pooled pre-burn period than in all other pooled time periods. Pulmonary vascular resistance was increased at all times postburn in the Burn-Control and U74-Burn groups when compared with the preburn period in the No Burn and U74-No Burn groups respectively. Directly comparing equivalent time periods between groups by t test was not statistically significant.

Arterial PaO₂ was not different in any period or group. The PaCO₂ was decreased and pH was

TABLE I

<i>No burn group (N=7)</i>					
	Pre-Burn	1 Hr	2 Hr	3 Hr	4 Hr
Pulmonary Artery Mean Pressure (mmHg)	12.0 ± 1.8*	12.0 ± 1.2	14.4 ± 1.9	14.0 ± 1.9	14.5 ± 1.9
Pulmonary Vascular Resistance (dynes·sec·cm ⁻⁵)	350 ± 97*	409 ± 105	622 ± 173	569 ± 18	683 ± 239
PaO ₂ (mmHg)	308 ± 77	346 ± 98	326 ± 90	396 ± 88	245 ± 117
PaCO ₂ (mmHg)	26 ± 6*	28 ± 5	34 ± 6	39 ± 8	40 ± 8
pH (mmHg)	7.57 ± .06*	7.54 ± .02	7.45 ± .05	7.40 ± .08	7.38 ± .10
PvO ₂ (mmHg)	48 ± 9	56 ± 10	54 ± 10	53 ± 8	49 ± 11
Qs/Qt (%)	15.1 ± 6.5	14.5 ± 6.3	13.7 ± 5.0	14.0 ± 5.1	18.1 ± 15.2
<i>Burn group (n=7)</i>					
	Pre-Burn	1 Hr	2 Hr	3 Hr	4 Hr
Pulmonary Artery Mean Pressure (mmHg)	12.0 ± 2.1*	15.1 ± 6.5	14.9 ± 5.5	15.3 ± 3.7	14.5 ± 2.9
Pulmonary Vascular Resistance (dynes·sec·cm ⁻⁵)	372 ± 171*	814 ± 432†	834 ± 354†	1024 ± 340†	942 ± 358†
PaO ₂ (mmHg)	360 ± 137	254 ± 123	299 ± 103	324 ± 117	304 ± 115
PaCO ₂ (mmHg)	25 ± 5*	36 ± 9	36 ± 4	31 ± 9	37 ± 6
pH (mmHg)	7.57 ± .06*	7.40 ± .1	7.39 ± .06	7.40 ± .11	7.34 ± .06
PvO ₂ (mmHg)	43 ± 2	46 ± 7	44 ± 7	36 ± 6	38 ± 5
Qs/Qt (%)	10.5 ± 4.0	10.3 ± 2.9	9.4 ± 3.0	4.2 ± 10.2	9.0 ± 2.8

* $P < 0.05$ comparing pooled (burn and no burn) groups by time (initial vs all other times).

† $P < 0.05$ comparing all individual periods at any post burn time in the burn group with initial control period in no burn group.

TABLE II

<i>U74 - No burn group (n=7)</i>					
	Pre-Burn	1 Hr	2 Hr	3 Hr	4 Hr
Pulmonary Artery Mean Pressure (mmHg)	12.7 ± 2.4*	12.7 ± 2.0	14.1 ± 2.0	15.1 ± 2.0	14.0 ± 2.6
Pulmonary Vascular Resistance (dynes·sec ⁻¹ ·cm ⁻⁵)	410 ± 214*	452 ± 217	563 ± 340	608 ± 305	605 ± 255
PaO ₂ (mmHg)	387 ± 75	361 ± 75	354 ± 85	300 ± 138	355 ± 69
PaCO ₂ (mmHg)	26 ± 5*	28 ± 5	32 ± 5	33 ± 10	32 ± 12
pH (mmHg)	7.62 ± .05*	7.50 ± .06*	7.43 ± .07	7.39 ± .08	7.38 ± .09
PvO ₂ (mmHg)	43 ± 13	45 ± 11	49 ± 15	50 ± 18	49 ± 13
Qs/Qt (%)	10.9 ± 5.6	12.0 ± 6.5	14.4 ± 7.9	12.1 ± 19.6	14.6 ± 6.4
<i>U74 - Burn group (n=7)</i>					
	Pre-Burn	1 Hr	2 Hr	3 Hr	4 Hr
Pulmonary Artery Mean Pressure (mmHg)	12.6 ± 2.2*	20.4 ± 12.0	22.1 ± 11.0	20.8 ± 7.0	20.9 ± 4.4
Pulmonary Vascular Resistance (dynes·sec ⁻¹ ·cm ⁻⁵)	405 ± 169*	1730 ± 115†	1851 ± 1532†	1737 ± 1245†	1817 ± 1201†
PaO ₂ (mmHg)	332 ± 77	275 ± 151	303 ± 217	248 ± 116	308 ± 124
PaCO ₂ (mmHg)	27 ± 5*	34 ± 8	42 ± 14	43 ± 12	37 ± 12
pH (mmHg)	7.54 ± .07*	7.39 ± .10*	7.28 ± .18	7.21 ± .15	7.16 ± .21
PvO ₂ (mmHg)	48 ± 9	49 ± 5	50 ± 5	48 ± 9	43 ± 8
Qs/Qt (%)	14.4 ± 5.6	11.2 ± 4.0	19.7 ± 2.1	17.7 ± 8.4	12.0 ± 4.3

* $P < 0.05$ comparing pooled (U74 burn and U74 no burn) groups by time (initial with all other times).

† $P < 0.05$ comparing all post burn time periods in the Burn U74 group with the U74 no burn initial control group.

TABLE III White blood cells

<i>Burn group (n=7)</i>				
	Pre Burn	Time 60	Time 240	BAL
% Neutrophils	10.3 ± 6.5	12.4 ± 8.5	53.9 ± 29.2*	39.6 ± 55‡
WBC	2.1 ± 0.7	2.0 ± 0.9	4.9 ± 2.5†	17.7 ± 6‡
<i>No burn group (n=7)</i>				
	Pre Burn	Time 60	Time 240	BAL
% Neutrophils	10.0 ± 5.3	6.3 ± 3.4	51.9 ± 17.7*	14.3 ± 9.9
WBC	2.0 ± 0.7	1.7 ± 0.9	2.6 ± 1.1	4.8 ± 2.9
<i>U74 Burn group (n=7)</i>				
	Pre Burn	Time 60	Time 240	BAL
% Neutrophils	8.0 ± 4.6	24.4 ± 9.4	47.9 ± 22.8*	19.0 ± 8.7‡
WBC	2.1 ± 0.6	1.5 ± 0.4	3.2 ± 1.5	12.2 ± 3.3‡
<i>U74 No burn group (N=7)</i>				
	Pre Burn	Time 60	Time 240	BAL
% Neutrophils	7.9 ± 3.6	6.5 ± 4.7	37.0 ± 21.0*	9.0 ± 5.4
WBC	1.7 ± 0.6	1.7 ± 0.3	2.8 ± 0.5	5.3 ± 2.5

Where WBC are leukocyte cells × 10⁹·L⁻¹.

*P < 0.05 comparing pooled periods (burn and no burn or burn-U74 and no burn-U74) at 240 min to pooled pre burn period.

†P < 0.05 compared with initial period in the No Burn Group.

‡P < 0.05 Burn group vs No-Burn group or U74 Burn group vs U74 No-Burn group.

TABLE IV Histology

	<i>Burn</i>	<i>No Burn</i>	<i>U74 Burn</i>	<i>U74 No Burn</i>
Wet/Dry Weight (%)	8.66 ± .98	8.52 ± 1.13	9.49 ± .81	8.62 ± .85
Edema	4*	2	4*	2
(median;quartiles)	(.5 - 4.75)	(.25 - 3)	(1.25 - 4.75)	(.5 - 4)
PMN	3*	2	2	2
(median; quartiles)	(2 - 4.75)	(.25 - 2)	(1 - 3.5)	(.25 - 2)
Skin Burn	2*	1	2*	1
(median; quartiles)	(2 - 3)	(1 - 1)	(2 - 3)	(1 - 1)

*P < 0.05 comparing Burn with No-Burn group or U74 Burn with U74 No-Burn group.

increased in the pooled initial preburn period compared to all other pooled periods. The pH was different in the pooled U74-Burn group (combining all times) than in the pooled U74-Control group (all times). Mixed venous oxygen and shunt (Qs/Q_t) were similar in all groups and periods.

Neutrophils (Table III)

White blood cell count was increased at 240 min post burn in the Burn group but not in the U74-Burn group. The percentage of neutrophils at 240 min increased in all groups. The bronchio-alveolar lavage demonstrated increased white blood cell count and percentage neutrophils in the U74-Burn group and Burn group when compared with the U74-No Burn group and No Burn group respectively.

Histology (Table IV)

The ratio of wet/dry weight was similar between all groups. Histological edema was similar between the two burn and two no-burn groups. There was significant increased leukocytes in the Burn groups compared to the No Burn group but not the U74-Burn group compared to the U74 No-Burn group. Skin burn was histologically demonstrated in both burn groups and was absent in both No Burn groups.

Discussion

We created an animal model with acute thermal injury. Effects are most likely the result of the scald rather than non-specific surgical trauma, anesthesia, or infused heparin, because our non-burn control group did not show similar magnitude of change over time.

Burn temperature of 65°C applied to constant surface area with copper as a conduction material resulted in deep thermal injury as demonstrated by our histological assessment.

The lung injury caused by the burn was mild in terms of its acute gas exchange effects. Arterial PaO₂ and intrapulmonary shunt were not significantly different after burn suggesting minimal alveolar flooding. Estimates of lung edema (histology but not wet/dry weights) showed a significant difference comparing burn to non-burn groups. The absence in alterations of gas exchange, as measured by PaO₂, is not unexpected¹⁰ as this develops within the first 48 hr after burn injury.⁹⁻¹³

Thermal injury results in activation of neutrophils¹⁴ and increased lipid peroxidation.¹⁴⁻¹⁶ Early use of antioxidants within hours of the injury attenuates the degree of burn shock and loss in plasma volume.¹⁷⁻²⁰ Lazaroids (potent lipid peroxidase inhibitors) preserve vascular patency, restore blood flow, and prevent the rise in tissue conjugated dienes with the end result of maintaining greater tissue viability.¹³ In this present study, we note no improvements in pulmonary vascular resistance the physiological variable that demonstrated the greatest change with burn. When using multiple physiological endpoints it is difficult to conduct an overall power calculation. Physiological outcomes that were not statistically different in some variables compared to those that demonstrated significant differences may be reflective of larger variation or smaller effect size in that outcome rather than true biological equivalence (i.e. type 2 error). U74 did not diminish the increased leukocyte count in the bronchiolar lavage (despite the decrease in lung tissue and systemic circulation) suggesting that the activation of leukocytes may still be occurring despite the administration of U74. Even if alveolar neutrophil sequestration was successfully blocked, this in itself may be still insufficient to limit the subsequent pulmonary deterioration.

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