主論文

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Dear Dr. Tasaki:

Your revised manuscript #97-0340 entitled "The effect of Sulfo Lewis C on smoke inhalation injury in an ovine model," has been accepted for publication and is being edited to conform to the journal format and style. When your manuscript is assigned to an issue of *Critical Care Medicine*, it is important that we have current information so we can send you the galleys. If your numbers change, please notify us as soon as possible.

You will be receiving galleys from the publisher in the next few months. They must be corrected and returned to our office by special air mail or fax within 48 hours of receipt. With the publisher's schedule, revisions cannot be included if they are not received in time.

Thank you for submitting your fine study to *Critical Care Medicine.*

Sincerely,

Joseph E. Parrillo, MD, FCCM
Editor-in-Chief

JP:cc

Title: The Effect of Sulfo Lewis C on Smoke Inhalation Injury in an Ovine Model

A shortened running title: Effect of Sulfo Lewis C on Smoke Injury

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Abstract

Objective: This study evaluates the effect of Sulfo Lewis C(SO_3-3βGal1-3βGlcNAc-O$(CH_2)_8$-COOMe), a putative ligand of selectins, on smoke inhalation injury.

Design: Prospective animal study with concurrent controls.

Setting: An animal laboratory.

Subjects: Twelve one year old female sheep(weight range 24 to 33 kg)

Interventions: Twelve sheep received nine exposure units of smoke generated by thermolysis of pine woodchips(80g). Group 1(n=6) was untreated. Group 2(n=6) was treated with an intravenous infusion of Sulfo Lewis C following smoke exposure. Animals were sacrificed 48 hours post-injury.

Measurements and main results: Cardiopulmonary variables and blood gases were measured serially. Granulocyte free-radical production was measured pre-smoke and at 4 and 48 hours post-injury. Ventilation perfusion distribution(V_A/Q) was analyzed using the multiple inert gas elimination technique(MIGET). Granulocyte free-radical production was elevated after smoke exposure in both groups. Oxygenation was significantly improved by the administration of Sulfo Lewis C. V_A/Q analysis demonstrated significantly less blood flow to low V_A/Q lung segments in treated animals.

Conclusion: Selectin blockade attenuated lung injury following smoke exposure. These data support the hypothesis that polymorphonuclear leukocytes(PMNs) play a pivotal role in smoke

inhalation injury.

Key words: smoke inhalation, Sulfo Lewis C, ovine, selectin, adhesion molecule, lung injury, multiple inert gas elimination technique, free radical, granulocyte, hypoxemia

Introduction

Smoke inhalation injury is a significant comorbid factor in patients with major thermal trauma. Noxious chemicals generated by incomplete combustion not only directly injure exposed airways, but appear to cause a release of chemotactic factors which may attract and activate leukocytes. Activated polymorphonuclear leukocyte(PMNs) are considered to be significant effectors in the progressive airway inflammation following smoke injury (1). Neutrophil recruitment into tissues is a multistep process involving sequential engagement of adhesion molecules, including selectins, integrins and the immunoglobulin supergene family of adhesion molecules(ICAMs)(2). These processes result in the initial slow rolling of neutrophils along the endothelial surfaces, leading to subsequent firm attachment to the vascular endothelium and migration into the endothelium. It has been shown that treatment of adhesion molecules with antibodies reduces lung injury following ischemia-reperfusion (3-9), administration of Pseudomonas aeruginosa(10-11) and bacterial lipopolysaccharide(12-13), intratracheal instillation of IgG or IgA immune complex (14-18) and infusion of cobra venom factor (17-19).

Sialyl Lewis X is an oligosaccharide ligand for the selectin family of adhesion molecules (20) and has been shown to reduce lung injury and diminish tissue accumulation of neutrophils after cobra venom factor infusion(21) and intratracheal instillation of IgG immune complex(22).

Sulfo Lewis C, a sulfated oligosaccharide, is a putative

ligand of selectins and has been reported to bind to E-selectins(23). The objective of this study was to determine the pulmonary effect of selectin inactivation with Sulfo Lewis C following smoke inhalation.

Material and Methods

Animals and Preparations

Twelve female sheep weighing 24kg to 33kg and free of antibodies to "Q" fever rickettsia were used in this study. The animals were housed in covered outdoor runs, treated for parasites(IVERMECTIN, 0.2 mg/kg, IM) and fed commercial chow and water ad libitum. The animals were divided into two groups. Group 1(n=6) received only vehicle following smoke exposure. Group 2(n=6) received treatment with Sulfo Lewis C for 48 hours after smoke exposure. This study was approved by our institutional animal use committee. The care of all animals was in accordance with the guidelines set forth by the Animal Welfare Act and other federal statutes and regulations relating to animals and studies involving animals and by the *Guide for the Care and Use of Labolatory Animals*, National Institute of Health Publication 86-23.

On the day before smoke exposure, all animals were instrumented while anesthetized with sodium pentobarbital (25 mg/kg, IV). Polyethylene cannulae were placed in a femoral artery and vein. One radiopaque sheath introducer, through which a Swan-Ganz catheter was placed, was inserted into an external jugular vein. The animals were then awakened, extubated, and returned to their

cages.

Smoke exposure methods

Twenty four hours after instrumentation, the animals were re-anesthetized, intubated with a 7.5 mm orotracheal tube and exposed to inhalation injury as described previously(24). Smoke was generated by thermolysis of pine woodchips(80g) in a crucible furnace at a constant temperature of 400°C and airway flow of 6.0 L/minute. The smoke was delivered into a 20 L reservoir and mixed with a 2.0 L/minute flow of 100 % oxygen. Animals received nine exposure units of this mixture; one exposure unit consisted of five breaths(tidal volume:30mL/kg, and a breathhold of 6 seconds), with a 5-second rest between exposure units.

Following smoke exposure, the animals were housed in individual cages in a climate-controlled facility, and observed for 48 hours while breathing room air spontaneously. The animals received a maintenance intravenous infusion of 5% dextrose in lactated Ringer's solution(2mL/kg/h) during the experiment.

Protocol

Sheep were randomized to one of two groups. Group 1(n=6) received smoke inhalation injury and vehicle, while Group 2(n=6) received smoke inhalation injury and treatment with Sulfo Lewis C(Alberta Research Council, Alberta, Canada) postinjury. Group 2 animals received a bolus injection of 10mg/kg body weight of Sulfo Lewis C immediately after smoke exposure followed by a

continuous infusion of Sulfo Lewis C. The Sulfo Lewis C was resolved in lactated Ringer's solution was infused at 1mg/kg/hour for 48 hours.

Measurements

Cardiopulmonary variables and blood gases were measured before smoke inhalation injury and at 2, 4, 8, 12, 24, 36, and 48 hours after smoke exposure. Pulmonary artery pressure, pulmonary capillary wedge pressure(PCWP), and systemic arterial pressure were measured using a pressure monitor(Model 78354A, Hewlett-Packard, Waltham, MA) and quartz transducer (Model 1290A, Hewlett-Packard). Cardiac output was measured by the thermodilution technique (Cardiac Output Computer Model 9520A, American Edwards Laboratories, Santa Ana, CA). Blood gas analyses were performed using an IL1303 pH/blood gas analyzer (Instrumentation Laboratories, Inc., Lexington, MA) and an IL482 CO-oximeter (Instrumentation Laboratories, Inc.).

Alveolar-arterial oxygen tension gradient($P(A\text{-}a)O_2$) was calculated by the following equation:

$P(A\text{-}a)O_2 = PAO_2 - PaO_2$. PAO_2 was calculated according to the following equation:

$$PAO_2 = FiO_2 \times BP - paCO_2 \times ((FIO_2) + (1 - FIO_2)/R)$$

where:

BP=(Barometric pressure)-(Vapour pressure)

(Vapour pressure)=0.4+antilog(0.024T+0.7659)

T=absolute temperature in ˚C

FIO_2=fraction of inspired oxygen

R=0.8=respiratory quotient

Superoxide production by circulating granulocytes were measured presmoke and at 4 and 48 hour postsmoke using the modified method of Pick (25). Whole blood(15ml) was diluted with an equal volume of isotonic saline. Five milliliters of Ficoll-Paque™ (Pharmacia LKB, Biotechnology AB, Uppsala, Sweden) were added to a centrifuge tube, onto which 10 ml of each diluted blood sample were layered and then centrifuged at 1300 g for 50 min. The upper part of the lowest layer containing granulocytes was resuspended in 33 ml of sterile distilled water and mixed to perform hypotonic lysis of red blood cells. After 15 sec, 11 ml of 3.5% NaCl solution was added and mixed to stop the lysis process. Samples were centrifuged at 1300 g for 10 min at 24°C and the supernatant and ghost cells were removed. Hypotonic lysis was repeated once. The pellet was resuspended in 5 ml of phosphate buffered saline and diluted to 1 X 10^6 cells/ml. One hundred microliters of the solution were added to the assay buffer in each well of a 96-well flat-bottom plate. The plate was placed on the ELISA reader (340 ATTC, SLT Lab Instruments, Research Triangle Park, NC) for 15 minutes at 39°C. The assay for measurement of superoxide is based on the reduction of cytochrome C by superoxide generated from granulocytes stimulated by phorbol 12-myristate 13-acetate.

At the end of 48 hours, the animals were anesthetized with sodium pentobarbital (25 mg/kg IV), orally intubated, paralyzed

with pancuronium bromide (0.1-0.15 mg/kg, Pavulon^{R}, Organon Pharmaceuticals, West Orange NJ), and mechanically ventilated. During mechanical ventilation, the tidal volume was set at 15 ml/kg and the respiratory rate at 8 breaths/minute . PEEP at 5 cmH_2O and an FIO_2 of 0.21 were maintained throughout the remainder of the study period.

Measurement of ventilation perfusion distribution(V_A/Q) was performed using the multiple inert gas elimination technique(MIGET). A lactated Ringer's solution containing six inert gases (sulfur hexafluoride, ethane, cyclopropane, halothane, diethyl ether, and acetone) was infused at a rate of 0.1 ml/kg/minute. After 40 minutes, arterial and mixed-venous blood samples(10 cc each) were simultaneously drawn anaerobically into preweighed, heparinized syringes. Mixed-expired gas was collected from a temperature-controlled(40°C) copper coil (outer diameter= 3.5 cm, length= 550 cm) about 1 min after blood sampling, compensating for the delay of the mixing chamber. Blood and expired gas samples were immediately analyzed by gas chromatography. V_A/Q on a 50 compartment scale was calculated based upon retention ratios using a special computer program(26).

After MIGET study, bronchoalveolar lavage(BAL) was performed. Twenty milliliters of 0.9% sterile saline was injected into the left lower lobe and the fluid was immediately aspirated. This process was repeated three times. White blood cell count was determined using a hemocytometer. Differential

cell counts were performed on Wright-Giemsa-stained cytocentrifuge preparations.

All animals were sacrificed with sodium pentobarbital(25 mg/kg IV) and an overdose of a 20% potassium chloride. The right lung was excised for the determination of wet to dry lung weight ratio(W/D) and the left lung was excised for histological evaluation. W/D was determined by the method described previously(27). Segments of midtrachea(the middle portion of trachea), distal trachea(2 centimeters above carina), proximal bronchus(the middle portion of the second division) and distal bronchus(the middle portion of the fourth division) of the left lung were obtained for histological analysis. Two specimens per site were taken. Histologic evaluation of the tracheobronchial injury was performed, using light microscopy and graded by the following criteria based on the previous study(26):

Tracheobronchoepithelial Damage Score

0=normal

1=some loss of cilia, loss of apical epithelium and flattening of epithelium

2=marked attenuation of epithelium, single layer of epithelium

3=<50% segmental/focal ulceration of epithelium

4=>50% ulceration of epithelium

Lung tissue blocks were taken from the left apical lobe. Randomly selected fields for quantification of PMN in the alveolar spaces were evaluated. The numbers of PMN per field at 400X magnification were counted in 10 randomly selected fields. These evaluation were done by a pathologist without knowledge of treatment.

Statistical Analysis

Statistical analysis was performed using repeated measures ANOVA with post hoc Scheffe's test for comparison between groups. Mann-Whitney U test was used for histological evaluation. Data are shown as mean ± standard error of the mean(SEM); significance was assigned at $p<0.05$.

Results

The arterial carboxy-hemoglobin levels(%) immediately after smoke exposure were 89.8±2.2 in Group 1 and 87.8±3.2 in Group 2. The difference was not statistically significant. All animals survived the 48 hour observation period.

Figure 1, *top*, depicts the serial $P(A-a)O_2$. $P(A-a)O_2$ increased progressively following smoke exposure in Group 1. Although the increase in $P(A-a)O_2$ in Group 2 was similar to that observed in Group 1 until 12 hours postinjury, the increase during the second 24 hours was significantly blunted($p<0.05$).

Figure 1, *middle*, depicts the serial mean pulmonary arterial pressure(MPAP). Although there was not statistically significant difference between two groups, significant pulmonary hypertension occurred in Group1 (baseline:16±0.5 vs 48hr:24±2.4mmHg) but was not documented in Group 2(baseline:16±0.3 vs 48hr:18±2.0mmHg).

Table 1 contains other physiologic measurements. Progressive hypoxemia was seen in Group 1. The decrease in PaO_2 was significantly attenuated in Group 2 during the second 24

hour($p<0.05$). $PaCO_2$ increased significantly in both groups and there was no difference between the two groups. Neither mean arterial pressure(MAP) nor total peripheral resistance index(TPRI) changed significantly in either group and did not differ between groups. Although mean values of pulmonary capillary wedge pressure(PCWP), pulmonary vascular resistance index(PVRI), and cardiac index(CI) appear to be lower in Group 2 than in Group 1, the differences between the groups were not statistically significant.

Table 2 shows the results of the MIGET analysis. The percentage of blood flow to true shunt or very low V_A/Q area($V_A/Q<0.1$) was significantly greater in Group 1 than in group 2. The mean V_A/Q of blood flow distribution was smaller in Group 1, and the logarithmic standard deviation of blood flow distribution, an index of V_A/Q mismatching, was greater in Group 1 compared to Group 2 although the differences were not statistically significant.

Figure 1, *bottom*, depicts PMA-stimulated superoxide production of granulocytes. There was no difference between the groups, suggesting that Sulfo Lewis C had no effect on the free-radical production. Although the superoxide production was elevated after injury, the changes were not significantly different because of great variability among animals. However, the increase in all animals was statistically significant($p<0.05$ by ANOVA repeated measures), which suggests that granulocytes were primed after smoke injury.

Table 3 contains the histologic scores at the level of mid trachea, distal trachea and segmental bronchus. Although the damage scores at each level did not differ significantly between groups, there was a significant difference observed in the summed histological damage score from Mann-Whitney U test($p=0.03$).

PMN count in an alveolar space per field in Group 1 was 23±1.5 /field, whereas that in Group 2 was 17±1.1 /field, which was significantly smaller than in Group 1.

There was no statistically significant difference between two groups in PMN counts in BAL fluid. W/D in Group 1 was 5.3±1.8 and 5.1±0.8 in Group 2. There was not significant difference between two groups.

Discussion

Smoke exposure results in acute airway inflammation which is histologically characterized by a loss of cilia, erosion and sloughing of bronchoepithelium, and pseudomembrane formation with small airway occlusion and atelectasis(28). Although the mechanism of the inflammatory process caused by smoke exposure has not been defined, activated neutrophils have been thought to be important in this process. Several studies have identified interventions that exert beneficial effects on smoke inhalation injury. Nitrogen mustard-mediated leukocyte depletion attenuated the subsequent increase in lung microvascular permeability, pulmonary hypertension, and hypoxia after smoke inhalation injury(1). In addition, nebulized dimethyl sulfoxide(DMSO)(29) and administration of a synthetic

antiprotease(30) reduced lung injury. We have previously reported that treatment with pentoxifylline decreased airway damage and pulmonary edema and was associated with fewer PMNs in bronchoalveolar lavage fluid(31). These findings support the hypothesis that PMNs play a significant role in the process of smoke injury.

Selectin blockade or binding may attenuate lung injury following smoke inhalation by blocking adhesion of neutrophils to vascular endothelial cells. The selectin family plays an important role in the rolling stage of PMN adhesion while the later stages are associated with the integrin family(CD11, CD18) and ICAMs(2).

In the present study oxygenation was significantly improved by the administration of Sulfo Lewis C. This finding is supported by the MIGET analysis which demonstrated significantly less blood flow to low V_A/Q lung segments in treated animals. Guha et al. reported that anti-CD18 antibodies(R15.7) did not reduce pulmonary tissue PMN numerical density in an ovine model of smoke injury after 24 hours of observation(32). In the present model of smoke injury, there was a significant difference in oxygenation during the second 24 hours of observation, which suggests that PMN adherence is essential in the development of airway inflammation. Goldman et al. reported in a rabbit model of localized acid aspiration injury that although treatment with the R15.7 had no effect on neutrophil accumulations in the aspirated segment, it attenuated the remote inflammatory response in the opposite lung as shown by

neutrophil accumulation and protein content of BAL fluid, neutrophil sequestration in the lung, and pulmonary wet to dry weight ratios(33). In smoke inhalation as well as acid aspiration, the initial airway injury is produced by direct cytotoxicity. Sulfo Lewis C is thought to protect the lung from secondary injury induced by PMNs. As shown in Figure 1, *bottom*, free radical production is elevated both 2 hours and 48 hours postinjury, indicating that smoke injury results in priming of circulating PMNs, which may be associated with secondary lung injury. The findings of the smaller number of PMN in an alveolar space and the attenuated airway injury are suggesting that Sulfo Lewis C attenuated lung injury by PMNs with the blockade of neutrophil migration. The results are indicating that adhesion molecules play an important role in the pulmonary circulation as well as in the systemic circulation. Several studies reported that neither L-selectin nor P-selectin played an important role in neutrophil migration into the pulmonary circulation(34-36). These findings are inconsistent with our results. Sulfo Lewis C has been reported to bind to E-selectins(23). E-selectin may be required for neutrophil migration into the lung. Moreover, the nature of leukocyte traffic in the bronchial vessels has not been clarified yet. As described above, smoke injury is primarily a bronchoepithelial injury and the alveolar damage is not severe in our model, although PMN infiltration into an alveolus is observed. The bronchial artery supplies the major branches of the bronchial tree. Selectins may participate in the neutrophil migration from the bronchial vascular system into the airway.

The reason why there was no difference in neutrophil counts in BAL fluid may be due to airway occlusion by cast. Cast formation occurs following smoke inhalation injury. If the cast occludes the airways, alveolar lavage can not be performed well and cells are not collected very much. There was not significant difference in W/D between two groups either. In smoke inhalation injury, the tracheobronchial epithelium sloughs into the airway lumen and the sloughed cells and protein-rich fluid exudate form intraluminal casts of the airways. Sulfo Lewis C attenuated the cast formation leading to less blood flow to low VA/Q area lung segments but had no beneficial effects on the edema formation. The different mechanism may be involved between cast and edema formation. However, the exact mechanism remains unclear in the present study.

Mileski et al. reported that administration of monoclonal antibodies against CD 18(R15.7) and against CD 11a(R7.1) increased susceptibility to infection with Pseudomonas aeruginosa, whereas antibody to L-selectin did not(37-38). However, in congenital hereditary leukocyte adhesion deficiency(LAD) syndromes bacterial infections occur often. Although bacterial infection is more severe in LAD I, in which the ß-subunit of the integrin molecule is absent, than in LAD II, in which there is a deficiency of Sialyl Lewis X(39), the administration of Sulfo Lewis C might well increase the risk of infection.

In summary Sulfo Lewis C significantly improved oxygenation following smoke exposure. These findings are complemented by the MIGET analysis, which demonstrated significantly less blood

flow to low VA/Q area lung segments in treated animals. The data suggest that selectin blockade attenuates lung injury following smoke exposure and support the hypothesis that PMNs play a significant role in the process of smoke inhalation injury.

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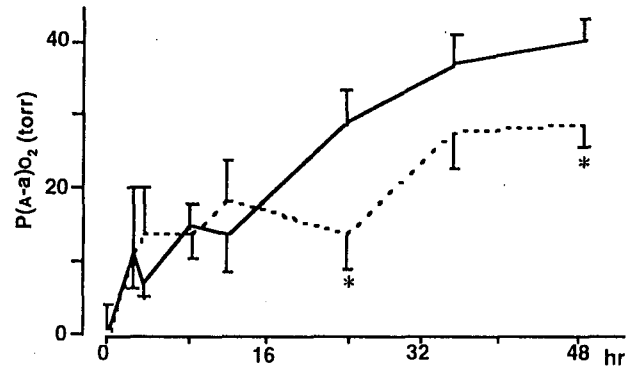
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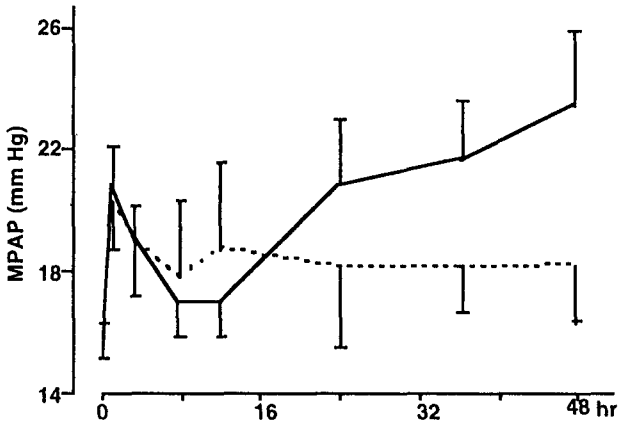
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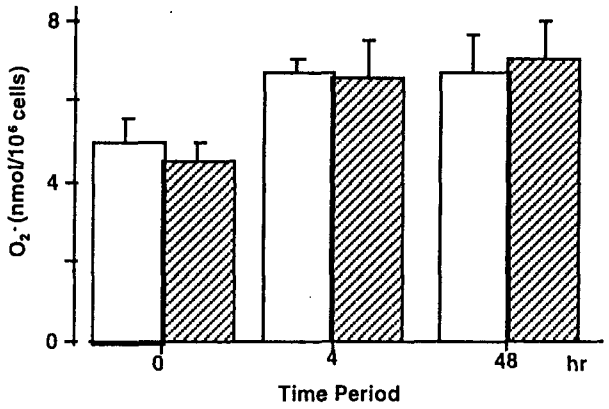
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Figure legends

Figure 1, top: Serial alveolar-arterial oxygen gradient(AaDO2) following smoke exposure. AaDO2 increased progressively following smoke exposure in G 1. Although the increase in AaDO2 in G 2 was similar to that observed in G 1 until 12 hours postinjury, the increase during the second 24 hours was significantly blunted($p<0.05$ by ANOVA repeated measures). Solid line shows G1; dotted line shows G2. *$p<0.05$ vs G 1.

Figure 1, middle: Serial mean pulmonary arterial pressure(MPAP) following smoke exposure. Significant pulmonary hypertension observed in Group 1($P<0.05$ by ANOVA repeated measures) was not seen in Group 2. Solid line shows G1; dotted line shows G2. * $p<0.05$ vs baseline.

Figure 1, bottom: Granulocyte superoxide(O_2^-) production following smoke exposure stimulated by PMA. There was no difference between the two groups. Although O_2^- production was elevated after injury, the changes were not significantly different in either group because of great variability among animals. However the increase in all animals was statistically significant($p<0.05$ by ANOVA repeated measures). Open bars show G1; shaded bars show G2.

Table 1. Cardiopulmonary data following smoke inhalation injury

		times(hr) pre	2	4	8	12	24	36	48
PaO2	G1	112 ± 5	96 ± 10	105 ± 4	98 ± 6	97 ± 7	78 ± 9	68 ± 6	62 ± 6
(torr)	G2*	113 ± 3	100 ± 6	98 ± 8	97 ± 6	87 ± 8	93 ± 8	79 ± 6	78 ± 5
PCWP	G1	7.3 ± 0.4	8.5 ± 1.1	7.3 ± 0.8	6.5 ± 0.3	7.0 ± 0.7	8.0 ± 0.6	8.0 ± 0.9	9.2 ± 0.7
(mmHg)	G2	7.2 ± 1.1	6.2 ± 0.9	6.3 ± 0.8	6.5 ± 1.5	5.8 ± 1.5	6.8 ± 1.3	7.8 ± 1.4	6.5 ± 1.1
PVRI	G1	106 ± 6	144 ± 28	124 ± 20	119 ± 12	137 ± 20	150 ± 21	136 ± 10	159 ± 28
	G2	104 ± 14	176 ± 26	153 ± 17	190 ± 55	219 ± 61	153 ± 27	113 ± 11	142 ± 9
PaCO2	G1	29.4 ± 2.5	31.5 ± 9.0	31.0 ± 1.8	30.6 ± 1.3	30.9 ± 1.3	33.8 ± 3.8	35.3 ± 3.9	36.3 ± 5.0
(mmHg)	G2	30.3 ± 1.2	31.2 ± 1.3	31.2 ± 1.7	31.5 ± 1.2	35.0 ± 2.2	33.9 ± 3.1	34.2 ± 1.6	34.3 ± 1.4
MAP	G1	93 ± 4	100 ± 4	90 ± 5	89 ± 4	88 ± 4	94 ± 7	93 ± 5	97 ± 8
(mmHg)	G2	92 ± 4	95 ± 7	97 ± 6	93 ± 3	88 ± 3	85 ± 4	94 ± 5	94 ± 8
TPRI	G1	1097 ± 52	1075 ± 99	870 ± 68	968 ± 104	1136 ± 107	1033 ± 84	854 ± 63	984 ± 171
	G2	1155 ± 146	1226 ± 129	1153 ± 121	1459 ± 272	1372 ± 227	1112 ± 123	1004 ± 85	1157 ± 173
CI	G1	6.5 ± 0.3	7.6 ± 0.9	8.1 ± 0.7	7.3 ± 0.7	6.1 ± 0.5	6.8 ± 0.3	8.1 ± 0.8	7.7 ± 1.1
	G2	6.7 ± 0.7	6.4 ± 0.5	6.8 ± 0.5	5.5 ± 0.5	5.5 ± 0.6	6.1 ± 0.5	7.3 ± 0.3	6.8 ± 0.8

PCWP, pulmonary capillary wedge pressure; PVRI (dyne.sec/cm^5/m^2), pulmonary vascular resistance index; MAP, mean arterial pressure; TPR(dyne.sec/cm^5/m^2), total peripheral resistance index; CI(l/min/m2), cardiac index; G1, Group 1; G2, Group 2; PRE, presmoke measurement.
Values are means ± SEM. * shows the serial change pattern was significantly different between the two groups($p<0.05$, ANOVA repeated measures)

Table 2
V_A/Q distribution by MIGET analysis

	Group 1	Group 2
Mean V_A/Q of Q	0.32 ± 0.06	0.42 ± 0.03
Log SDQ	1.03 ± 0.18	0.71 ± 0.09
$V_A/Q<0.1$(%Q)	22.4 ± 7.00	3.75 ± 1.43*

Q:pulmonary blood flow; V_A:ventilation; Mean V_A/Q:mean value of Q distribution; Log SDQ:Q dispersion on log V_A/Q axis; $V_A/Q<0.1$(%Q), percentage of Q distribution with V_A/Q less than 0.1. * $p<0.05$ vs Group 1.

Table 3. Tracheobronchoepithelial Damage Score

	Group 1	Group 2 *
Midtrachea	3.33 ± 0.21	2.67 ± 0.42
Distal Trachea	2.83 ± 0.48	2.17 ± 0.60
Proximal Bronchus	2.83 ± 0.54	2.17 ± 0.60
Distal Broncus	2.83 ± 0.54	1.83 ± 0.48

*The summation of the four site-specific damage scores in Group 2 is significantly smaller than in Group 1.

参考論文
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＜原　著＞

広範囲熱傷患者の耐糖能障害に関する検討

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広範囲熱傷患者において，耐糖能障害は非常に重要な問題である．受傷後14日間の検討では56例の熱傷患者のうち16例で高血糖をコントロールするためにインスリンを必要とした．インスリン投与量はburn indexと相関し，耐糖能障害が熱傷の重症度と関連していた．インスリンを必要とした患者では，1日インスリン投与量とカロリー摂取量の比（インスリン・カロリー比：U/Cal）は，カロリー摂取量が増加するとともに増加した．そのためこれらの多くの患者でカロリーの補いをいったん低下させる必要があった．インスリン・カロリー比の最大値は，第8病日前後となった．この結果は，加速度的に悪化する熱傷患者の耐糖能障害が，熱傷受傷早期のカロリーの補いと関連していることを示している．一例で，インスリン，グルカゴン，コルチゾルの血中濃度および尿中カテコールアミン排泄量を2週間の間測定しえたが，インスリン・カロリー比の上昇は，血中インスリン濃度および尿中カテコールアミン排泄量の著しい上昇と一致していた．報告されているように著しい高インスリン血症は，交感神経を刺激し，分泌されたノルアドレナリンが血糖を上昇させる．われわれは，このメカニズムが，熱傷急性期にカロリーの補いとともに加速度的に耐糖能が悪化する原因になっているのではないかと考えた．

Key Words：広範囲熱傷，耐糖能障害，高インスリン血症，インスリン

はじめに

広範囲熱傷患者に認められる耐糖能障害は古くから認識されてきたが，現在にいたるまで臨床上大きな問題である[1,2]．すなわち，こうした患者では激しい代謝亢進とともに著しい異化状態にあり，十分な栄養的サポートが必要とされる．しかし，強引な栄養投与は熱傷患者の耐糖能障害をしばしば悪化させ，栄養管理上大きな問題となる．さらにこうした患者にみられる耐糖能障害は，安定した血糖管理が可能な多くの糖尿病患者とは異なり不安定であり，時にはおびただしい量のインスリンを必要とする．この研究は，特に受傷後栄養投与を開始する時期の耐糖能低下に注目し，この時期のインスリン投与に関する問題について検討したものである．

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対象および方法

昭和60年1月から平成5年4月までの8年間に，当科で治療した糖尿病の既往のない熱傷患者56例（男36例，女20例）を対象とした．年齢は30.7±20.0歳（平均±標準偏差，以下同様），Burn Index（＝II度熱傷面積(%)×1/2＋III度熱傷面積）は37.1±20.5であった．電撃症，化学熱傷は除外した．

1．熱傷の重症度と耐糖能障害との関係を明らかにするために，対象56例について各症例の受傷時のBurn Indexと14病日までの累積インスリン投与量の関係を検討した．

2．対象56例のうちインスリンを投与した16例で，インスリン投与量とカロリー投与量（総カロリー量）をそれぞれ経日的にプロットした．さらに耐糖能の指標として，1日投与カロリーあたりのインスリン投与量（U/Cal：以下インスリン・カロリー比）を求め，その経日

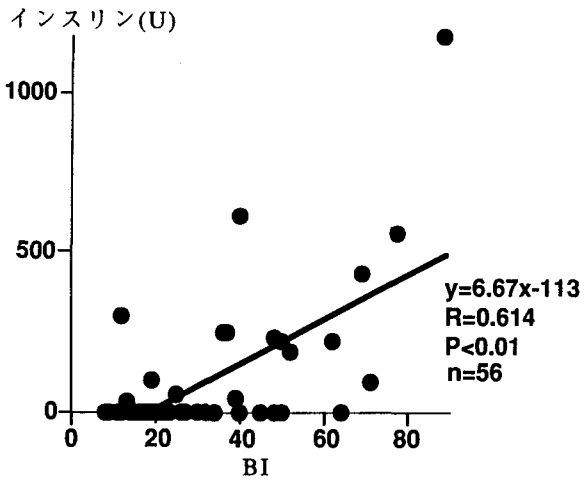

図1　受傷後14日間の累積インスリン投与量．
Burn Index 40未満では，41例中7例しかインスリンを必要としなかったのに対して，Burn Index 40以上では15例中9例がインスリンを必要とした．またインスリン投与量は，熱傷の重症度と相関した．

的推移を検討した．インスリンは，1単位/mlの濃度で，微量注入ポンプを用いて経静脈的に持続投与した．投与量は，主として血糖値を測定しながら決定し，血糖値が250 mg/dl以下となるように，調節した．ただし，尿糖の測定値や浸透圧利尿の程度も参考にした．なお，これらのインスリン投与例における，経静脈的カロリー投与量の総カロリー投与量に占める割合は，78.5±25.6%であった．

3．インスリンを必要とした16例中，インスリン必要量増大のため，1日のカロリー投与量を最大値から1000 Cal以上減少させた症例（16例中8例）と減少させなかった症例（16例中8例）に分けて，カロリー投与量とインスリン・カロリー比の経日的推移をそれぞれ検討した．

4．上記のカロリー投与量を減少させた8例中1例において，内分泌学的因子の推移も含めた詳細な検討を行った．

結　果

1．各症例のBurn Indexに対する14病日までの累積インスリン投与量を図1に示した．Burn Index 40未満では，41例中7例しかインスリンを必要としなかったのに対して，Burn Index 40以上では15例中9例がインスリンを必要としており，重症熱傷では多くの症例でインスリン投与を必要とした．またインスリン投与量は熱傷の重症度と相関していた（$p<0.01$）．なお，年齢に関しては，インスリン投与量と有意な関係を認めなかった（$R=0.031$，$N=56$）．

2．インスリンを投与した16例でインスリン投与量の推移を検討したところ，インスリン投与量は，カロリー投与量を上昇させるとともに急速に増加していた（図2）．またインスリン・カロリー比は，第7病日まではほとんどの症例で経日的に上昇しており，耐糖能は急速に低下していた（図3）．その後，第8病日前後をピークにインスリン・カロリー比が減少している症例が多くみられたが，これらの症例では，耐糖能の急激な悪化に対応して主治医の判断でカロリー投与量を減少させたことが，反映されていると考えられる．なお，16例中7例が受傷後14日間に植皮術などの手術をうけた．一般に術前には，栄養投与はひかえられ，カロ

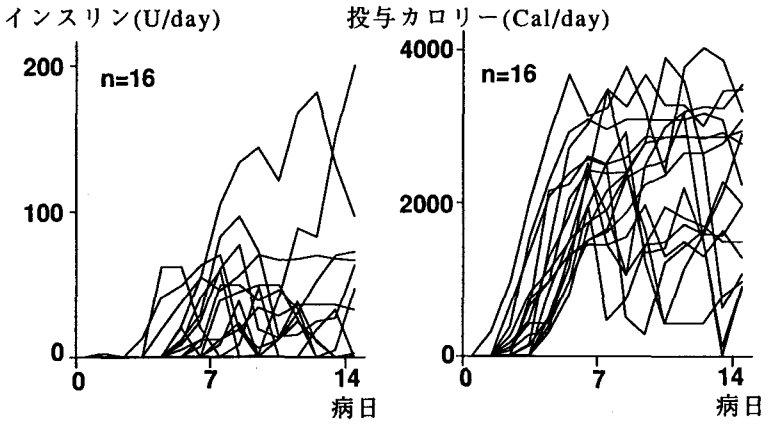

図2　インスリン投与量とカロリー投与量の推移
インスリン投与量は，カロリー投与量を上昇させるとともに急速に増加している．

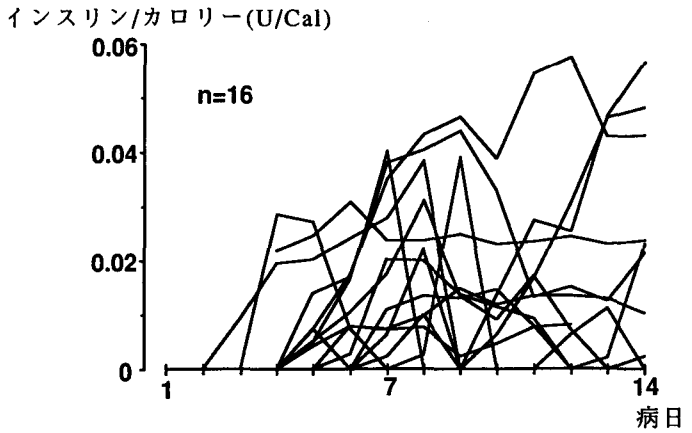

図3　インスリン・カロリー比の推移．
第7病日までは多くの症例で経日的に上昇しており，耐糖能は急速に低下している．その後，第8病日前後をピークに耐糖能が改善している例が多くみられる．

リー投与量は減少するが，手術に伴うこのようなカロリー投与量の減少と，インスリン投与量を減少させる目的でのカロリー投与量の減少を分離して検討することができなかった症例は，7例中1例のみであった．

3．カロリー投与量を減少させた症例で，カロリー投与量とインスリン・カロリー比を経日的に検討した．カロリーを減少させた症例では，カロリー負荷によるインスリン・カロリー比の上昇は急速であること，カロリー負荷の軽減に伴うインスリン・カロリー比の低下もすみやかであることが明らかである．したがってインスリン・カロリー比の推移は尖鋭なピークを形成していた（図4）．これに対して，投与カロリーを減少させなかった症例では，その推移は異なっており，尖鋭なピークを形成しなかった．まず8例中5例では，インスリン・カロリー比は急速に上昇することなく，経過を通じ

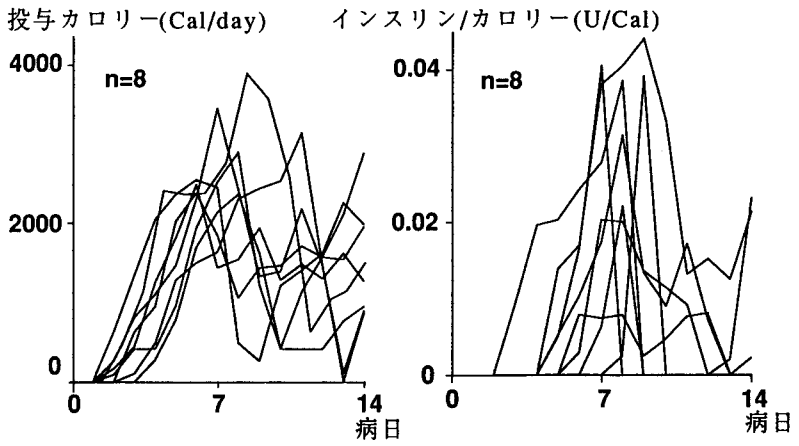

図4　カロリーを減少させた症例のカロリー投与量とインスリン・カロリー比．
投与カロリーの減少に伴いインスリン・カロリー比は著明に低下し，耐糖能が改善している．

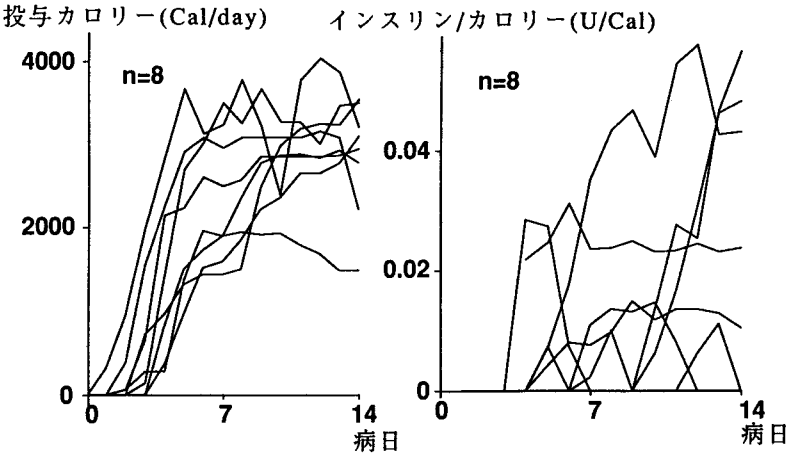

図5　カロリーを減少させなかった症例のカロリー投与量とインスリン・カロリー比．
8例中5例では，インスリン・カロリー比は急速に上昇することなく，経過を通じて安定している．しかし，残り3例ではインスリン・カロリー比は上昇し続けており，インスリン大量投与にいたっている．

て安定していた．すなわち，これらの症例ではカロリー負荷に伴う耐糖能の悪化は急速ではなく，カロリー投与量を減少させる必要がなかったといえる．しかし残り3例では，インスリン・カロリー比は上昇し続けておりインスリン大量投与にいたっている（図5）．

4．症例は50歳の男性で，両上肢，胸部，背部を中心に全体表面積の44%（Burn Indexは37）のII度からIII度の火炎熱傷を負った．インスリン投与は受傷後第5病日より始まり，

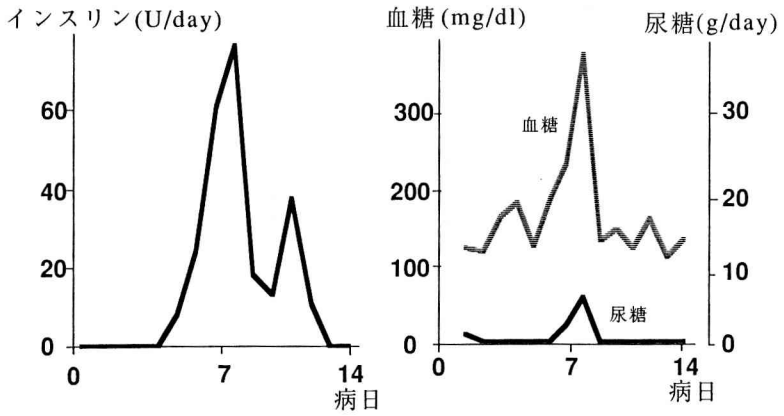

図6　血中インスリン，血糖および尿糖排泄量の推移．（50歳男性）
インスリン投与は受傷後第5病日より始まり，第8病日まで投与量は急速に増加している．しかし血糖値や尿糖排泄量は，むしろインスリン投与量が増えるとともに増加している．

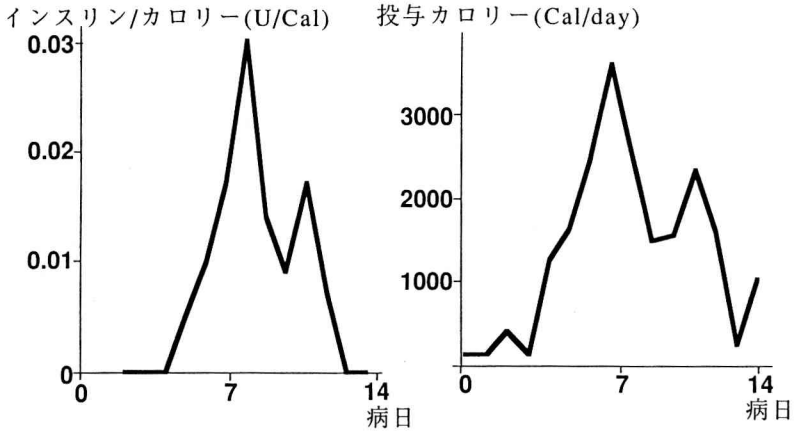

図7　インスリン・カロリー比とカロリー投与量の推移．（50歳男性）
インスリン・カロリー比は著しく上昇している．またインスリン・カロリー比の推移は投与カロリー量の推移と一致している．

第8病日まで投与量は急速に増加していたが，それにもかかわらず血糖値や尿糖排泄量はむしろインスリン投与量が増えるとともに増加していた（図6）．インスリン・カロリー比も著しく上昇しており，この時期に耐糖能が急速に悪化していることが明らかであった．またインスリン・カロリー比の推移は投与カロリー量の推移と一致していた（図7）．血中インスリン濃度は耐糖能が極端に悪化した第8病日にピークとなり108 μU/mlに達したが，その後急速に低下した（図8）．抗インスリンホルモンの中ではコルチゾル，グルカゴンの血中濃度は，血中インスリン濃度の推移と明らかには関連していなかったが，ノルアドレナリンの1日尿中排泄量の推移は血中インスリン濃度の推移と一致していた．なお，インスリン感受性の急速な低下

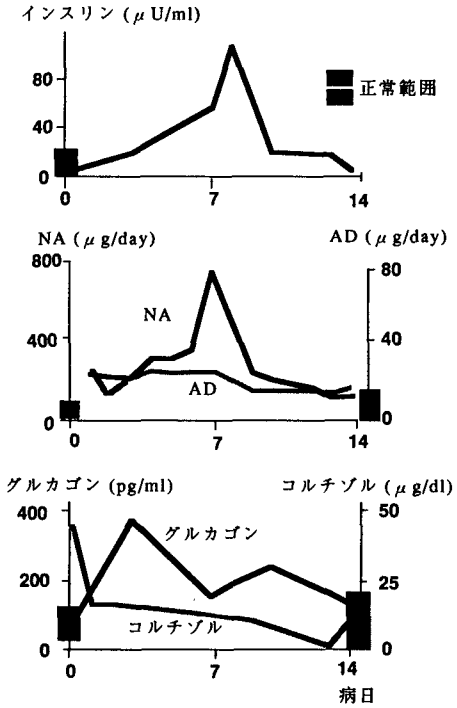

図8　インスリンおよび抗インスリンホルモンの推移．（50歳男性）

著明な高インスリン血症と一致して，著しい尿中ノルアドレナリン排泄量の増加が認められる．これに対し，アドレナリン，コルチゾル，グルカゴンなどの他の抗インスリンホルモンの推移と高インスリン血症との関連はみられない．

の背景に感染を疑い，第6，7，8病日に血液，創部，喀痰，尿等の細菌検査を行ったが，細菌感染は否定的であった．

考　察

激しい代謝変化を生ずる広範囲熱傷患者の治療上，高血糖などの代謝障害に対する対策は重要な問題である．われわれの対象とした56例は，いずれも平時，まったく耐糖能障害が指摘されていないにもかかわらず，16例が血糖の管理のためにインスリンを必要としており，いかに熱傷に伴う糖代謝の変化が大きいかをものがたっている．このうち，Burn Index 40以上の重症患者では，過半数の患者がインスリンの投与を必要としており，熱傷の重症度が大きくなれば耐糖能障害に対する対策が不可避であることが明らかである．しかし，この耐糖能障害は実際には，臨床上はなはだ困難な問題をはらんでいる．

その1つは，広範囲熱傷患者の糖代謝に関する知見が必ずしも十分ではなく，至適な糖負荷とインスリン使用の関係にいたるまでは理論的な解答が得られていないことである．熱傷に伴う糖代謝の変化については，従来より多くの研究がなされてきた．熱傷を受けた生体では，内因性のグルコースの産生は増加しturnover rateは亢進するが，多くは末梢組織で嫌気的代謝によりC 3 unit（乳酸，アラニンなど）に代謝され，好気的代謝により酸化される割合は著明に低下するといわれている[3]．その原因はインスリン受容体の感受性低下や，細胞内のグルコース代謝障害（postreceptor defect）であることが指摘されているが，確証は得られていない[4~6]．このように，熱傷に伴う糖代謝の変化のメカニズム自体が明らかではないため，激しい代謝変化時にグルコース負荷とインスリン投与をいかなる指針に基づいて行うべきか，理論的見地から具体的な回答をえることは現時点ではむずかしい．

また，熱傷患者の耐糖能はさまざまな要因をうけて変動し，経過中一定ではない．この耐糖能の不安定性が，さらに問題を複雑にしている．しかし，これは臨床上さけて通れない問題である．耐糖能が不安定になる時期としては，受傷後栄養投与を開始する時期と，経過中敗血症にいたる時期が特に問題になるであろう．なかでも，受傷後，初期のresuscitationが終了して栄養投与を負荷する時期の耐糖能の悪化は，臨床上とりわけ重要である．というのは，これは栄養管理上の問題と不可分であるからである．

Curreriによれば，受傷後7日以内に患者の摂取エネルギー量が目標値に達するように，栄養的サポートを行うことが必要であるという[7]．しかし，この時期には，実際上耐糖能の急激な悪化のため，きわめて重症な熱傷例では

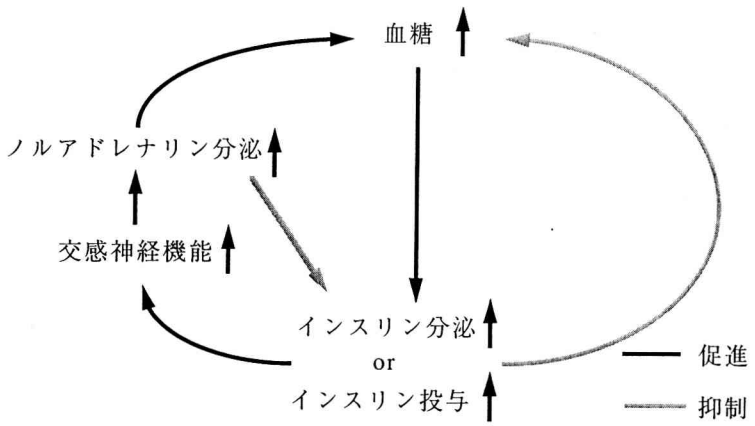

図9　インスリンと交感神経機能との関係．
カロリー負荷とインスリンの投与による著明な高インスリン血症により，交感神経機能が亢進し耐糖能が悪化するという悪循環が形成されている．

栄養負荷は容易ではない．われわれの結果では，インスリンを必要とした16例中，安定して栄養負荷が可能であったのはわずかに5例であり，残りの症例はインスリン必要量の急速な増量を余儀なくされた．

橋本らは，広範囲熱傷患者において状態が安定していれば，総投与カロリー40 Calに対しおおむね1単位のインスリンの投与で血糖管理が可能であると報告している[8]．これは，インスリン・カロリー比としては0.025 U/Calとなるが，われわれの結果では，栄養投与開始後インスリン投与量は急増し受傷後7病日付近では多くの症例がこのラインを越えている．このように，この時期の血糖管理は，これらの症例ではまことに困難であり，臨床上特に注意が必要であることが明らかである．

急速に悪化する熱傷患者の耐糖能の背景因子については，系統的な検討を行った報告をみいだせないが，臨床の現場ではもっぱら経験則に基づいた管理が行われている．たとえば，インスリン投与量が著しく増加する場合には，糖負荷量を一旦減少させてインスリン量を調節し，あらためて糖負荷を増量するとなめらかな血糖管理が可能になることはしばしば経験する．めまぐるしく変動する耐糖能障害か認められる場合，投与インスリンの上限を一日100単位とすることも，その数値の理論的根拠はともかく，臨床医にはわかりやすい指標である．ただし，加速度的な耐糖能障害をきたすメカニズムは明らかではない．

われわれは，そのメカニズムとして抗インスリンホルモンの動態に注目している．詳細な内分泌学的検討が行えた一例では，インスリンの血中濃度の上昇に伴い，特にノルアドレナリンの尿中排泄量が明らかに上昇していた．高インスリン血症が，交感神経機能を亢進させカテコラミンの分泌を刺激するという知見は，よくコントロールされた研究で報告されているが，近年，これが肥満者の高血圧のメカニズムにも関与しているのではないかと考えられている[9~14]．熱傷患者の場合，高インスリン血症がノルアドレナリン分泌を刺激すれば，図9に示すように血糖値は上昇し，一方ノルアドレナリンは内因性のインスリン分泌を抑制するため[15]，投与インスリンの必要量がさらに増加するという悪循環が形成されることが考えられる．この悪循環を絶ち切るために，糖負荷を一旦減少させ高インスリン血症を解除することが，合理的だと結論つけられる．

まとめ

広範囲熱傷急性期の耐糖能障害について検討した．

1．インスリン投与量は熱傷の重症度と相関していた．

2．インスリン・カロリー比は第8病日前後にピークとなる症例が多く，耐糖能障害はカロリー投与量の増加と関連していた．

3．不安定な耐糖能障害の一因として，著明な高インスリン血症が交感神経機能を亢進させ，さらに耐糖能を悪化させている可能性が示唆された．

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Study of Glucose Intolerance in Extensively Burned Patients

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In extensively burned patients, glucose intolerance is a very serious problem. Fifty six burned patients (burn index 37.1±20.0, age 30.7±20.0) were studied for 14 days after injury. Sixteen patients required insulin to control hyperglycemia. Administered insulin doses were correlated with the burn index ($R=0.614$, $P<0.01$) showing that glucose intolerance is related to the severity of the burn injury. In these patients, the ratio of daily administered insulin dose to calorie intake (insulin・Calorie ratio：U/Cal) increased as the calorie supplement increased. In most of these patients, the insulin・Calorie ratio decreased with the decrease in the calorie supplement. The maximum insulin・Calorie ratio of each patient was observed around the 8 th day after the injury. Our results indicate that accelerated glucose intolerance in burn patients is related to the calorie supplement in the early postburn period. In one case we could measure the plasma concentration of insulin, glucagon and cortisol and urinary excretion of catecholamines for two weeks. The rise in the insulin・Calorie ratio paralleled the marked increase in the plasma insulin concentration and urinary catecholamine excretion. It is possible that the marked hyperinsulinemia stimulates sympathetic activity as reported elsewhere and secreted noradrenaline increases the plasma glucose concentration. We speculate that this mechanism is the cause of accelerated glucose intolerance in accordance with the calorie supplement in the early postburn period.

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Effects of Burns on Inhalation Injury

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Background: **There are few studies of smoke injury combined with thermal burn.** ***Methods:*** **Seven sheep (G1) received smoke injury alone; eight (G2) received a 40% full-thickness scald burn immediately after smoke injury. All animals were resuscitated with lactated Ringer's solution and killed 48 hours after injury. Cardiopulmonary variables and blood gases were measured serially. Ventilation perfusion distribution was analyzed using the multiple inert gas elimination technique. Lung wet to dry weight ratio and malondialdehyde levels were determined.** ***Results:*** **G2 resulted in early significant hemodynamic changes. Serum total protein concentration was significantly lower and malondialdehyde significantly higher in G2. However, Pao_2, lung wet to dry weight ratio, and ventilation perfusion mismatching in G2 did not differ from those in G1.** ***Conclusions:*** **Although the addition of burn injury exaggerated the lung lipid peroxidation and hypoproteinemia in the presence of more pronounced hemodynamic changes, the pulmonary dysfunction was not accentuated.**

Key Words: **Smoke inhalation, Thermal injury, Malondialdehyde, Hypoproteinemia, Multiple inert gas elimination technique (MIGET).**

Smoke inhalation injury remains a primary determinant of burn mortality and the mortality rate of patients with burn and smoke inhalation injury is reported to be much higher than that of patients with either injury alone.[1] Although the pathophysiologic changes after smoke injury have been investigated in many animal models,[2] there are few studies of the pathophysiology of smoke injury combined with thermal burn. Because most patients with smoke injury also have a cutaneous thermal injury, it is important to clarify the effect of combined injury on lung damage. Demling et al. reported that the addition of a 15% total body surface burn did not accentuate the degree of lung dysfunction 24 hours after inhalation injury even though lung malondialdehyde (MDA), an indicator of lipid peroxidation, was significantly higher in animals with combined injury.[3,4] Moreover, Matsumoto et al. have reported that lung damage was minimized when thermal injury preceded smoke inhalation injury.[5] The purpose of this study was to evaluate the effects of a 40% total body surface burn combined with smoke inhalation injury on pulmonary function over a 48-hour study period.

MATERIALS AND METHODS

Animals and Preparations

Fifteen female sheep, each weighing between 24 kg and 35 kg and devoid of antibodies to Q fever, were used in this study. The animals were housed in covered outdoor runs, treated for parasites (Ivermectin, St. Louis, Mo, 0.2 mg/kg IM) and fed commercial chow and water ad libitum. The animals were randomized to two groups: group 1 (G1; n = 7) received smoke inhalation injury alone; group 2 (G2; n = 8) received a 40% full-thickness scald burn immediately after smoke injury. This study was approved by our institutional animal use committee. The care of all animals was in accordance with the guidelines set forth by the Animal Welfare Act and other federal statutes and regulations relating to animals and studies involving animals and with the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, DC, 1996).

On the day before smoke exposure, all animals were instrumented while anesthetized with sodium pentobarbital (25 mg/kg IV, Sigma Chemical Co., St. Louis, Mo). Polyethylene tubing was placed into a femoral artery and vein. One radiopaque sheath introducer, through which a Swan-Ganz catheter was placed, was inserted into an external jugular vein. On the dorsum of each animal, 40% of the total body surface area was shaved. Total body surface area was calculated by Meeh's formula: $A = 0.09W^{0.67}$, where A was surface area in meters squared and W is body weight in kilograms.

Smoke Exposure Methods

Twenty-four hours after instrumentation, the animals were intubated with 7.5-mm orotracheal tube and exposed to inhalation injury as described previously.[6] Smoke was generated by thermolysis of pine woodchips (100 g) in a crucible furnace at a constant temperature of 400°C and air flow of 6.0 L/min. The smoke was delivered into a 20-liter reservoir and mixed with a 2.0 L/min flow of 100% oxygen. Animals received 15 exposure units of this mixture; one exposure unit consisted of five breaths (tidal volume, 30 mL/kg, with a breathhold of 6 seconds) and a 5-second pause between exposure units.

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Address for reprints: Library Branch, US Army Institute of Surgical Research, 2322 Harney Road, Fort Sam Houston, San Antonio, TX 78234-6315.

Burn Injury

Immediately after smoke exposure, a 40% third-degree burn was inflicted on the G2 animals. Additional pentobarbital (8 mg/kg IV) was administered, and the shaved area of each animal in G2 was immersed in 100°C water for 30 seconds, whereas animals in G1 were immersed in 24°C water. After injury, the animals were housed in individual cages in a climate-controlled facility (24°C), and observed for 48 hours in the awake state while breathing spontaneously.

Fluid Resuscitation

G1 received lactated Ringer's solution at a rate of 2 mL/kg/h. G2 was resuscitated according to weight and burn size using 4 mL/kg/% burn for the first 24 hours. During the second day of the study, all animals were administered lactated Ringer's solution at a rate of 2 mL/kg/h. Urine output was monitored every 4 hours and the infusion rate was changed if necessary to maintain an output of 0.5 to 2.0 mL/kg/h. Water balance was calculated by subtracting urine output from fluid intake every 4 hours.

Hemodynamic and Pulmonary Measurements

Cardiopulmonary variables and blood gases were measured before smoke and at 2, 4, 8, 12, 24, 36, and 48 hours after smoke. Pulmonary artery pressure, pulmonary capillary wedge pressure, and systemic arterial pressure were measured using a pressure monitor (Model 78354A, Hewlett-Packard Company, Waltham, Mass) and a 1290A quartz transducer (Hewlett-Packard Company). Cardiac output was measured by the thermodilution technique (Cardiac Output Computer Model 9520A, American Edwards Laboratories, Santa Ana, Calif). Blood gas analyses were performed using an IL1303 pH/blood gas analyzer (Instrumentation Laboratories, Inc., Lexington, Mass) and an IL482 CO-oximeter (Instrumentation Laboratories, Inc.).

At the end of 48 hours, the animals were anesthetized with sodium pentobarbital (25 mg/kg IV), orally intubated, paralyzed with pancuronium bromide (0.03–0.04 mg/kg, Pavulon, Organon Pharmaceuticals, West Orange, NJ), and mechanically ventilated. During mechanical ventilation, the tidal volume was set at 15 mL/kg and the respiratory rate at 12 breaths/min. Positive end-expiratory pressure was 5 cm H_2O and F_{IO_2} was maintained at 0.21 throughout the remainder of the study period.

Measurement of ventilation perfusion (V_A/Q) distribution on a 50-compartment scale was performed using the multiple inert gas elimination technique (MIGET).[7] Lactated Ringer's solution containing six inert gases (sulfur hexafluoride, ethane, cyclopropane, halothane, diethyl ether, and acetone) was infused at a rate of 0.1 mL/kg/min. After 40 minutes when equilibrium of gas exchange occurs, arterial and mixed-venous blood (10 mL each) was drawn anaerobically into preweighed, heparinized syringes (30-mL matched glass, Becton Dickinson and Company, Franklin Lakes, NJ) simultaneously. Mixed-expired gas was collected from a temperature-controlled (40°C) copper coil (outer diameter, 3.5 cm; length, 550 cm) about 1 minute after blood sampling, compensating for the delay of the mixing chamber. The gas concentration contained in these samples was immediately analyzed by gas chromatography. From these data the V_A/Q distribution was determined using a specially designed computer program.

After the MIGET study, all animals were killed. The right lung was excised for the determination of wet to dry lung weight ratio (W/D) and lung MDA, and the left lung was excised for histologic evaluation. W/D was determined by the method described previously.[8] Briefly, the lung was homogenized with an identical weight of distilled water. Some of the homogenized lung was stored at −80°C until MDA levels were measured. Samples of the homogenate and blood were weighed and dried at 80°C for 48 hours. Dry weights were measured and the wet to dry ratios of the homogenate and blood were calculated. A sample of the homogenate was centrifuged at 12,500 rpm for 1 hour, and blood samples were diluted with the same volume of distilled water. To determine the hemoglobin levels in the homogenate and blood, 20 μL of the homogenate supernatant or the diluted blood were added to 2.5 mL of Drabkin's solution. The absorbance of both solutions was measured spectrophotometrically at 540 nm. Then, the weight of the blood in the wet lung was calculated. From these data, blood-free W/D was determined.

The MDA level in the lung was measured by the thiobarbituric acid (TBA) method.[9] Stored lung was homogenized in a ratio of 1 g of sample to 2 mL of 20% of acetic buffer (Ac-buffer; pH 3.5) by using a Polytron homogenizer. Three milliliters of Ac-buffer and 1 mL of TBA reagent (0.67% TBA aqueous solution plus glacial acetic acid, 1:1, v/v) were added to 50 μL of homogenized sample. The reaction mixture was heated at 98°C for 60 minutes in an oil bath. After cooling with tap water, 3 mL of *n*-butanol were added and the mixture was shaken vigorously. After centrifugation at 3,000 rpm for 15 minutes, the *n*-butanol layer was taken for fluorometric measurement at 515 nm excitation and 553 nm emission. The fluorescence intensity of a standard solution was also obtained by reacting 5 nmol of tetraethoxypropane with TBA by the same steps.

The light microscopic histologic grading of the tracheobronchial injury of each animal was performed using the following criteria. Parenchymal damage was evaluated only in an apical lobe because bronchoalveolar lavage was done in other lobes. Thr tracheobronchoepithelial damage score is as follows: 0 = normal; 1 = some loss of cilia, loss of apical epithelium; 2 = marked attenuation of epithelium, single layer of epithelium; 3 = <50% segmental/focal ulceration of epithelium; 4 = >50% ulceration of epithelium. The lung parenchyma damage score is as follows: 0 = normal; 1 = minimal to mildly thickened alveolar septa, a few inflammatory cells or a small, single focus of inflammatory cells; 2 = multifocal areas with increased inflammatory cells in alveolar septa and in alveoli; 3 = diffuse inflammation and/or edema that affects less than half of the section; 4 = diffuse inflammation and/or edema that affects more than half the section.

Statistical Analysis

Statistical analysis was performed using repeated measures analysis of variance with post hoc Scheffe's test for compar-

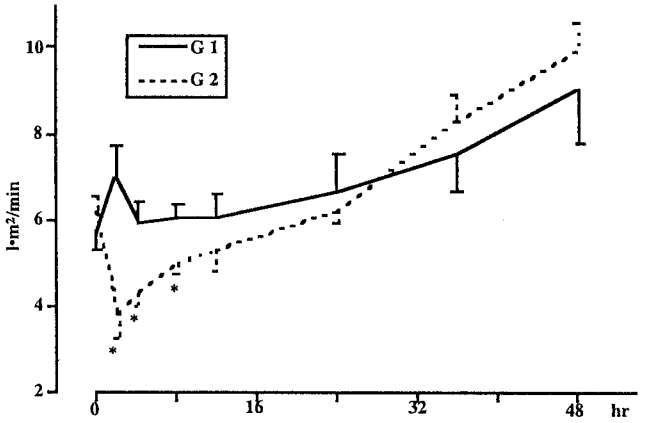

FIG 1. Serial cardiac index (CI). The CI in G2 dropped immediately after burn and increased gradually after resuscitation. The CI in G1 did not fall after smoke injury. During the second 24 hours, the CI in both groups increased gradually as hypoxemia worsened. *$p < 0.05$ versus G1.

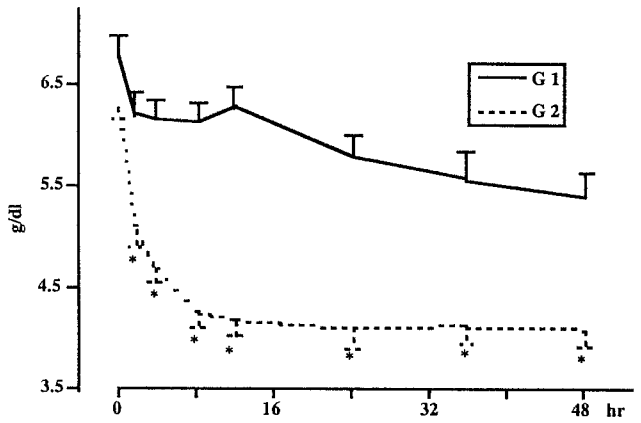

FIG 3. Serum total protein concentration. Although protein levels fell significantly after injury in both groups, the decrease in concentration was much greater in G2 than G1. *$p < 0.05$ versus G1.

ison between groups. An unpaired t test was used for non-repeated measures. Mann-Whitney U test was used for histologic evaluation. Data are shown as mean ± SEM. Significance was assigned at $p < 0.05$.

RESULTS

The arterial carboxy-hemoglobin levels immediately after smoke exposure were not different between groups (G1: 91.7 ± 1.3 vs. G2: 90.3 ± 2.3%). All animals survived the 48-hour observation period.

Figure 1 depicts the serial cardiac index (CI) for the two groups. The CI in G2 dropped immediately after burn and increased gradually after resuscitation. The CI in G1 did not fall after smoke injury. During the second 24 hours, the CI in both groups increased gradually. The differences between two groups were significant until 8 hour after injury but not during the later period.

Figure 2 depicts the serial water balance. Water retention was significantly greater in G2 than G1 during the first 24 hours due to resuscitation and burn edema, but there was no significant difference during the second 24 hours. Total water balance during 48 hours was significantly higher in G2 (122 ± 12.2 mL/kg) than in G1 (58.2 ± 18.3 mL/kg).

Figure 3 shows the serum total protein concentration. Although protein levels fell significantly after injury in both groups, the decrease in concentration was much greater in G2 than G1.

Lung MDA content, an index of lipid peroxidation, was significantly higher in G2 than G1 (G1: 1.06 ± 0.10 vs. G2: 1.38 ± 0.08 nmol/mg protein; $p < 0.05$) suggesting that combined injury results in increased cellular damage. Despite these changes, the degree of lung dysfunction was not different between the two groups. Figure 4 shows the serial Pao_2. Although progressive hypoxemia was noted in both groups, there was no difference between two groups.

The results of the MIGET study demonstrated that the degree of V_A/Q mismatching in G2 was not different from that in G1 (Table 1). Although mean V_A/Q of Q was decreased and the Q dispersion on log V_A/Q axis (Log SDQ) and the percentage of Q distribution with V_A/Q less than 0.1 ($V_A/Q < 0.1$) area much greater than normal in both groups, there was no difference between two groups.

Table 2 depicts other cardiopulmonary variables. In G2, pulmonary vascular resistance index increased immediately after injury, peaking at 2 hours and then decreasing gradually. Pulmonary vascular resistance index in G1 increased gradually coincident with the progression of hypoxemia. Although there is a statistically significant difference between the two

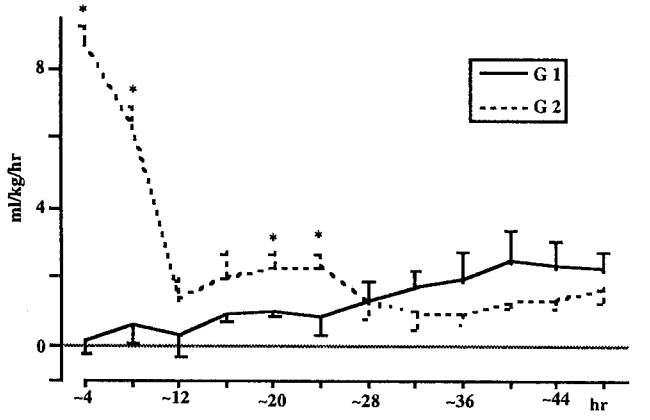

FIG 2. Serial water balance. Water retention was significantly greater in G2 than G1 during the first 24 hours due to resuscitation and burn edema, but there was no difference during the second 24 hours. *$p < 0.05$ versus G1.

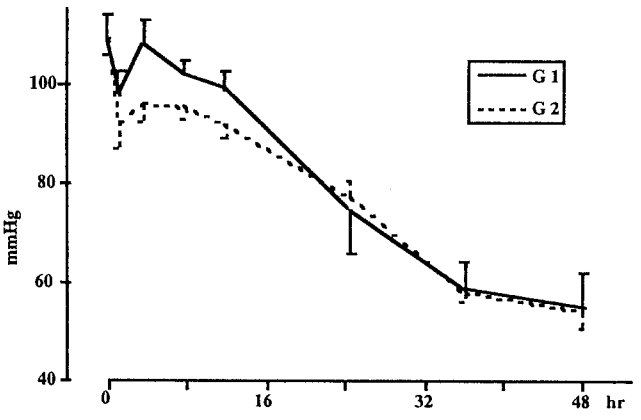

FIG 4. Serial Pao_2. Although progressive hypoxemia was recorded in both groups, there was no difference between two groups.

TABLE 1. V_A/Q distribution by MIGET analysis

	Group 1	Group 2
Mean V_A/Q of Q	0.27 ± 0.02	0.27 ± 0.04
Log SDQ	2.17 ± 0.16	2.21 ± 0.19
$V_A/Q < 0.1$ (%Q)	38.3 ± 5.47	34.7 ± 3.58

Q, pulmonary blood flow; V_A, ventilation; mean V_A/Q, mean value of Q distribution; log SDQ, Q dispersion on log V_A/Q axis; $V_A/Q < 0.1$ (%Q), percentage of Q distribution with V_A/Q less than 0.1.

groups, it was dependent on the difference during the first 12 hours. There was no difference during the second 24 hours. Total peripheral resistance index was significantly higher in G2 at 2, 4, and 8 hours after injury but significantly lower at 36 and 48 hours. Pulmonary capillary wedge pressure and $Paco_2$ did not differ between two groups. Lung W/D ratios were not different (G1: 5.94 ± 0.38 vs. G2: 5.63 ± 0.43) although both values are greater than normal.

Table 3 contains the histologic evaluation. There was no difference between groups. Bronchoepithelial damage was extensive in both groups, whereas lung parenchymal damage was very mild.

DISCUSSION

It is well known that the addition of smoke injury to burn injury results in greater fluid requirements during initial resuscitation.[10–12] However, it is undefined whether the lung dysfunction that occurs after smoke inhalation is accentuated by the addition of thermal injury. In accordance with clinical experience, the addition of a 40% total body surface burn in this study was associated with significant changes in selected hemodynamic indices, serum protein levels, and amount of lipid peroxidation.

CI was significantly decreased in G2 as compared with G1 only during the first 8 hours after injury. Subsequent to that time, CI was comparable in both groups. An adverse effect of the decrease in CI on pulmonary function was not seen during the first 48 hours after injury.

TABLE 3. Lung damage score

	Group 1	Group 2
Midtrachea	3.4 ± 0.4	3.1 ± 0.6
Distal trachea	2.7 ± 0.6	2.7 ± 0.6
Proximal bronchus	3.6 ± 0.4	2.6 ± 0.5
Distal bronchus	3.4 ± 0.4	2.6 ± 1.4
Apical lobe	1.1 ± 0.1	0.7 ± 0.4

Hypoproteinemia occurred in all animals and was significantly more severe in G2 as a reflection of increased vascular permeability in burned tissue and the greater resuscitation volumes given those animals. The rate of accumulation of fluid in the lung (R) must be the difference between the filtration rate (Q) and the lymph flow rate (L): $R = Q - L = K((Pmv - Ppmv) - \sigma(\Pi mv - \Pi pmv)) - L$, where K is the filtration coefficient, Pmv is the microvascular pressure, Ppmv is the perimicrovascular (interstitial) fluid hydrostatic pressure, Πmv is the plasma protein osmotic pressure, Πpmv is the perimicrovascular protein osmotic pressure, and σ is the microvascular barrier reflection coefficient to protein. Hypoproteinemia decreases Πmv, which would be expected to increase pulmonary edema. However, the oncotic gradient (Πmv-Πpmv) in the lung is known to be rapidly restored after hypoproteinemia induced by burn or plasmapheresis,[13,14] suggesting that the increase in Q is of brief duration. In this study, lung edema in G2, indexed as W/D ratio, was not accentuated at all compared with G1. This finding confirms that hypoproteinemia is not the sole cause of pulmonary edema after inhalation injury. Demling et al. measured L with lung lymph fistulas in an ovine model of smoke inhalation with or without burn and reported that with combined injury, L was still elevated at 24 hours after injury, whereas with inhalation injury alone, L had returned to baseline values by that time. Nonetheless there was no difference in lung water

TABLE 2. Cardiopulmonary data after smoke inhalation injury

		Time (hours)							
		pre	2	4	8	12	24	36	48
PVRI	G1	140 ± 14	173 ± 20[a]	166 ± 17[a]	167 ± 16[a]	184 ± 21	195 ± 17[b]	202 ± 26	181 ± 17[b]
	G2	103 ± 11	368 ± 33[b]	325 ± 39[b]	251 ± 29[b]	231 ± 38[b]	170 ± 15[b]	169 ± 12[b]	150 ± 18[b]
MPAP	G1	15.4 ± 0.2	18.7 ± 0.8[b]	18.3 ± 0.8	16.3 ± 0.9	16.9 ± 1.0	20.3 ± 1.3[b]	23.2 ± 1.6[b]	23.2 ± 2.5[b]
(mm Hg)	G2	15.5 ± 0.3	22.8 ± 1.7[b]	23.6 ± 0.9[b]	21.6 ± 1.3[b]	20.4 ± 1.4[b]	21.9 ± 1.4[b]	22.6 ± 1.6[b]	24.7 ± 2.1[b]
TPRI	G1	1572 ± 127	1561 ± 152[a]	1518 ± 123[a]	1478 ± 88[a]	1563 ± 78	1390 ± 116	1299 ± 130[a,b]	1096 ± 110[a,b]
	G2	1373 ± 62	2816 ± 291[b]	2285 ± 215[b]	1831 ± 89[b]	1751 ± 160	1093 ± 116[b]	901 ± 67[b]	764 ± 39[b]
MAP	G1	91 ± 2	110 ± 4[b]	97 ± 3	89 ± 3	93 ± 3	92 ± 3	99 ± 5[a]	97 ± 14
(mm Hg)	G2	92 ± 3	102 ± 5[b]	100 ± 5[b]	95 ± 4	91 ± 5	82 ± 6	86 ± 3[b]	88 ± 3
$Paco_2$	G1	29.5 ± 0.7	31.6 ± 1.6	30.1 ± 1.2	29.8 ± 0.6	29.1 ± 0.8	29.2 ± 1.6	30.4 ± 2.3	35.4 ± 4.6
(mm Hg)	G2	31.0 ± 0.9	30.8 ± 0.5	31.8 ± 0.7	31.6 ± 0.6	31.6 ± 0.7	30.5 ± 0.9	32.3 ± 2.8	36.1 ± 4.2
PCWP	G1	7.4 ± 0.5	6.7 ± 0.8	7.9 ± 0.7	6.4 ± 0.4	6.6 ± 0.6	7.9 ± 0.4	8.8 ± 1.1	7.8 ± 1.9
(mm Hg)	G2	8.9 ± 0.5	9.6 ± 1.3	10.0 ± 1.0	9.5 ± 1.1	9.5 ± 1.1	10.3 ± 1.1	7.9 ± 1.0	8.6 ± 0.7

PVRI (dyne.sec/cm^5/m^2), pulmonary vascular resistance index; MPAP, mean pulmonary artery pressure; TPRI (dyne.sec/cm^5/m^2), total peripheral resistance index; MAP, mean arterial pressure; PCWP, pulmonary capillary wedge pressure; G1, group 1; G2, group 2; pre, presmoke measurement. Values are means ± SEM.

[a] $p < 0.05$ vs. G2;

[b] $p < 0.05$ vs. pre.

between the groups.[3,4] These findings are consistent with our results indicating that even if Q was increased with combined injury, a proportional increase in L prevented greater fluid accumulation from occurring in the lung.

Oxygen radicals released by activated polymorphonuclear leukocytes are considered to be significant effectors of the progressive airway inflammation, which occurs after smoke injury. Polymorphonuclear leukocytes are reported to be activated after burn injury.[15] Therefore, our hypothesis was that the addition of a burn injury would further activate polymorphonuclear leukocytes leading to a greater degree of lung dysfunction. The tissue content of MDA is often used as an index of free radical involvement in tissue damage by disease or toxins. Indeed, in traumatic injury to the brain and spinal cord, good evidence exists that iron ion release into the surrounding area, and consequent iron-related free radical reactions, worsen the injury.[16] However, Gutteridge and Halliwel reported that in some other diseases, it is likely that the increase in free radical activity induced as a result of tissue injury makes no significant contribution to the disease pathology.[17] In the present study, although lung MDA was elevated in the animals with the combined injury (G2) suggesting greater pulmonary damage compared with animals with only inhalation injury (G1), no increase in lung dysfunction was noted. It is possible that the lung tissue in the animals with both burn and smoke injuries had cellular damage that was not functionally significant. Another possibility is that the more severe cellular damage in the combined injury group will exert deleterious functional effects at a later time beyond the duration of the current study. The early lipid peroxidation process might sensitize the lung to a second insult such as infection in the postresuscitation period. Tranbaugh et al. reported that they could not identify an early increase in pulmonary capillary permeability in burn patients even if inhalation injury was present. In their study, the occurrence of sepsis after resuscitation resulted in rapid accumulation of lung water.[18,19] Because pneumonia, a septic process, is a common complication of inhalation injury in burn patients, the morphologic and functional implications of the combined injuries may become fully evident only with the second insult of infection occurring after resuscitation.

In summary, the addition of a 40% burn to smoke injury significantly exaggerated the early postinjury hemodynamic changes as well as the hypoproteinemia and the lung content of MDA noted in animals with only inhalation injury. Despite these changes, hypoxemia, pulmonary hypertension, lung edema and V_A/Q mismatching were not accentuated during the first 48 hours after injury by the addition of cutaneous burn. A longer observation period may be necessary to identify morphologic and functional changes responsible for the deleterious effects of combined cutaneous burn and smoke inhalation injury.

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