The Effect of Air Pressure on Edema and Healing of Scalded Tissue of Rats

Zheng Shi, MD,* Chunying Li, MD,* Charles Li, MD†

To study the effectiveness of using high-pressure air on edema and healing of second-degree scald burns in rats. A self-designed high pressure airtight box in which the air pressure can be controlled is used to observe the edema and healing time of second degree burned tissues in rat under different air pressures. With the air pressure increased by 30 cm H_2O, there was a significant reduction of edema, exudation and healing time of the scalded tissue. Increasing air pressure can reduce edema, exudation and healing time of scalded tissue. (J Burn Care Res 2007;28:286–290)

At the early stage of burn injury, the most important pathological changes are the increase of capillary permeability and large amount of plasma-like fluid exuding out, which causes local edema and local reduction of effective blood volume[1] in the organs. Severe edema of limbs may cause ischemia, leading to limb loss, whereas the severe reduction of effective blood volume may lead to scalding shock, which causes scalded tissue ischemia, and hypoxemia, which plays an important role in the early period of burn. Currently, the pathologic and physiologic mechanisms for the increase of vessel permeability and the exuding of plasma-like fluid are not yet clearly understood. These kinds of reactions, which are not limited to the injured area, have led researchers to think that the releasing of vessel-active mediator systematically or locally may be the cause of the body fluid loss.[2–4] Thus, the critical strategy in managing the early stages of burns is to reduce capillary permeability and plasma-like exudation. In recent years, many researchers have performed studies using anti-inflammatory substances, lipotropic peroxide, and Chinese herbs to find effective methods to control these reactions. These substances can reduce the capillary permeability to some degree but have to be used before the trauma occurs, which is not practical in the clinical use. So far, there is no single effective method to stop or reduce plasma-like exudation from capillaries after scalding.[5,6] Once a burn takes place, exudation and edema are unavoidable. In this study, we designed an air pressure-controlled device and used the mesenteric capillary model and scalded tissue model in rats to observe the exuding of capillary, edema, and the healing of the scalded tissue under different air pressures to investigate the relationship among these factors and to provide a basis for clinical treatment.

*From the *Yangzhou People's Hospital, Yangzhou, China and †Georgia Regional Hospital, Savannah, Georgia.*
Address correspondence to Charles Li, MD, Georgia Regional Hospital, 1915 Eisenhower Drive, Savannah, Georgia 31406.
Copyright © 2007 by the American Burn Association.
1559-047X/2007

DOI: 10.1097/BCR.0B013E318031A195

MATERIALS AND METHODS

Experimental Device

We used a self-designed airtight glass case (500 × 500 × 700 cm) with a hinged sealing door, air-in and air-out pipe with valves, and a barometer inside. The other end of the air-in pipe was connected to an air pump (power 6.5 W, airflow ≥8 liters/min), by which the air pressure within the case was controlled and new air and airflow could be maintained.

Experimental Animals

Wistar male rats weighing 220 to 250 g, provided by the Animal Experimental Center of Yangzhou University, were used in the study. The rats lived in the experimental environment at least 1 week before the experiment.

Rat Mesenteric Capillary Model

The rats were anesthetized via abdominal pentobarbital injection and fixed supinely on board. The abdomen was cut up along the middle line. The mesen-

Copyright © American Burn Association. Unauthorized reproduction of this article is prohibited.
Downloaded from https://academic.oup.com/jbcr/article-abstract/28/2/286/4605514
by Ed 'DeepDyve' Gillespie user
on 19 April 2018

tery was pulled out for observation. Fluorescence illuminant was injected through caudal vein. An Olympus BH-2 (Olympus, Center Valley, PA) fluorescence microscope with ocular magnification of 10×, target objective magnification of 4×, and violet wavelength set to 540 nm was used to observe the fluorescence illuminant in the capillary going into the surrounding tissues (Figure 1).

Rat Scalding Model

The rats were anesthetized via an abdominal pentobarbital injection 24 hours after the back hair was shed by sodium sulfide. The scalding was obtained by submerging the rats in hot water (80°C for 6 seconds), which caused a deep 4 × 40-cm second-degree burn (verified by the pathology department) on the back of the rat. Resuscitation was initiated, followed immediately by injecting 5 ml of Ringer's solution into the rat's abdomen.

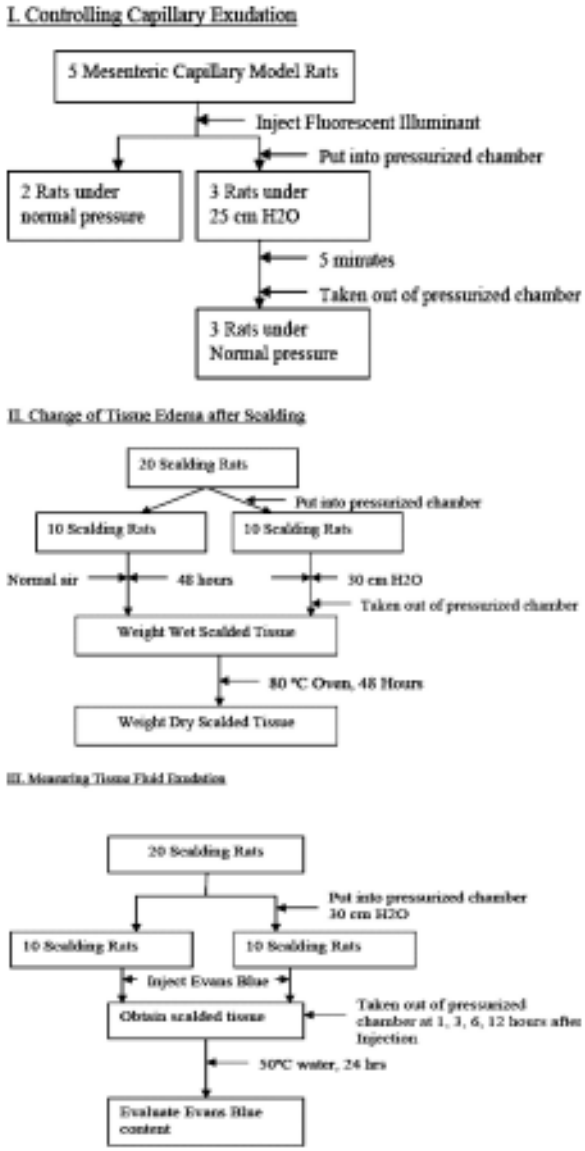

Figure 1. Experiment procedures.

Measuring of Tissue Water Content

The burned rat was put into the air pressure-controlled case immediately and fed for 48 hours before being sacrificed to obtain tissue, which was performed outside the case. The scalded tissue from the rat was weighed, and then the tissue was put into an oven set at 60°C to dry for 48 hours before being weighed again. The water content was measured by using the following formula:

$$\text{Water content } (\%) \text{ per gram of tissue} = \text{gross weight} - \text{dry weight}/\text{gross weight} \times 100\,\%$$

Measuring of Tissue Fluid Exudation

We adopted Lu's method[7] by using a spectrophotometer (Beckman Coulter, Fullerton, CA). The standard curve was obtained by setting the wavelength to 620 nm and by measuring the photoabsorption degree of Evans blue (0.005–50 mg/l). Evans blue was injected into the rat's caudal vein (2 mg/kg) 30 minutes before each measuring time point, at which the rat's chest was opened and 300 ml of 0.9% sodium chloride was injected into the left cardiac ventricle to wash out the circulating blood, leaving only the left Evans blue. Immediately after the aforementioned procedures, the scalded tissue was obtained, weighed, put into 2 ml of dimethyformamide, and kept in a 50°C water-bathing container for 24 hours. The Evans blue content (μg/g) in the extract fluid was evaluated by measuring the photoabsorption degree at the 620 nm wavelength and by checking the standard curve. Then, the ratio between the content and the tissue weight was calculated as the Evans blue content in tissue (μg/g). The change of vessel permeability of the wound was expressed as the percentage of Evans blue compared with that in the control group.

Healing and Constriction Percentage

The healing percentage, healing time, and constriction percentage were calculated by weighing method and wound area copying method,[8] which are described here:

1. Weighing method: the weight of a thin transparent film was weighed by analytical balance with division value of 0.1 mg. The area of the film can be calculated by the weight.
2. Area copying: a thin transparent film was used to cover the wound area and the wound bound-

Copyright © American Burn Association. Unauthorized reproduction of this article is prohibited.
Downloaded from https://academic.oup.com/jbcr/article-abstract/28/2/286/4605514
by Ed 'DeepDyve' Gillespie user
on 19 April 2018

Table 1. The influence of air pressure on the water content of scalding tissue

Group	Number	Water Content (%)	Wet/Dry (g)
Burn + HAP	10	73.5 ± 0.53*	3.76 ± 0.093*
Burn	10	77.5 ± 0.51	4.43 ± 0.063
Control	10	67.3 ± 0.50	3.08 ± 0.047

*$P < .01$ vs. "Burn" group.

ary was copied on the film. The copied area of the film was cut down and weighed so the area size could be calculated with the weighing method.

The healing percentage was calculated by the following formula:

$$\text{Healing percentage}(\%) = \text{original wound area} - \text{not healing area/original wound area} \times 100\%$$

Statistics

SPSS statistic software (SPSS Institute, Chicago, IL) was used (analysis of variance) to analyze the deviations. The results were shown as $\bar{\chi} \pm s$.

RESULTS

Controlling Capillary Exudation

Five mesenteric capillary model rats were put into the airtight glass case one by one. Two were injected with fluorescence illuminant under normal air pressure. The fluorescence illuminant was observed as flowing through capillary into the surrounding tissues quickly. The other three rats were injected with fluorescence illuminant and placed into the case with an air pressure that was increased by 25 cm H_2O. The fluorescence illuminant remained inside the capillary without infiltrating the capillary. Five minutes later, the air pressure was reduced to normal, and then the same result was observed as that of the first two models.

Change of Tissue Edema After Scalding

After scalding, 10 rats were put into the airtight glass case immediately under high air pressure (HAP; pressure increased by 30 cm H_2O) and were noted as the "Burn + HAP" group. Another 10 scalded rats were designed as the pure "Burn" group, that is, under normal air pressure. Ten more regular rats were used as the "Control" group, that is, also under normal air pressure. All rates were sacrificed at 48 hours after the scalding. The water content of scalding tissue was measured: the "Burn + HAP" was 73.5 ± 0.53%, and the "Burn" group 77.5 ± 0.51%. There was a significant difference between the two groups ($P < .01$), which indicated that there was a significant reduction of edema in the group under HAP (Table 1).

Tissue Fluid Exudation Measurement.

Healing and Constriction Percentage of Scalding Tissue and Healing Time

Ten of the 20 scalded rats were kept in HAP (by increasing the air pressure to 30 cm H_2O) for 48 hours and then under normal air pressure as (ie, Group A). The remained 10 rats were kept under normal air pressure (ie, Group B). In each group, the burn area was treated with silver sulfadiazine and wrapped. The procedure was repeated twice a week until the area was healed. At day 14 and 18 after the scalding, the healing percentage of Group A was 90.86% and 99.62%, respectively, whereas Group B was 77.40% and 90.01%. The difference was significant ($P < 0.05$; Table 3). The constriction percentage of Group A was 36.26% and 53.1%, respectively, whereas Group B 34.87% and 51.95%, or no significant difference ($P > .05$; Table 3). The average healing time was 18.02 days for Group A and 23.16 days for Group B. Group A had an average healing time of 5.1 days earlier than Group B, which was significant ($P < .01$; Table 2).

DISCUSSION

Preventing exudates from leaking out of the capillary is an important strategy in burn treatment. Stopping exudation at the early stages of burn can prevent edema, facilitate burn area treatment, and prevent shock caused by the reduction of effective blood volume, which will further reduce the risk of damage to major organs. The reduction and stopping of exudate and edema can significantly improve burn treatment.

Table 2. Measurement of burn tissue exudation (Evans Blue $\mu g/g$, $\bar{\chi} \pm$ SD, n = 7)

	1 Hour	3 Hours	6 Hours	12 Hours
Burn + HAP	0.60 ± 0.15*	1.00 ± 0.30*	0.65 ± 0.18*	0.50 ± 0.12*
Burn	0.80 ± 0.22	1.45 ± 0.35	0.85 ± 0.25	0.70 ± 0.19

*$P < 0.01$ vs "Burn" group.

Copyright © American Burn Association. Unauthorized reproduction of this article is prohibited.
Downloaded from https://academic.oup.com/jbcr/article-abstract/28/2/286/4605514
by Ed 'DeepDyve' Gillespie user
on 19 April 2018

Table 3. Healing, time, and constriction percentage ($\bar{\chi} \pm s$)

		Constriction Percentage		Healing Percentage		
Group	Number	14 Days	18 Days	14 Days	18 Days	Healing Time
A	10	36.26 ± 1.92*	53.11 ± 1.74*	90.86 ± 2.41†	99.62 ± 1.62†	18.02 ± 1.76†
B	10	34.87 ± 2.10	51.95 ± 1.73	77.40 ± 1.98	90.01 ± 1.62	23.16 ± 1.80

†$P < .01$ vs. Group B; *$P > .05$ vs. Group B.

After thermal injury, the major factors affecting fluid exudation out of the vessels are increased intravascular hydrostatic pressure, decreased interstitial hydrostatic pressure, and increased permeability of capillary.[9] There are three zones in the burned tissue: coagulation, stasis, and hyperemia. The exudation happens in the last two zones. Many studies suggested that the increased permeability of capillary is the major reason for exudation.

Many vasoactive mediators and inflammatory mediators cause the increased capillary permeability after thermal injury, and studies focused on the inhibitors of these mediators became very popular in burn research in the past.[10] However, because of the complexity of the mediators and the processes, blocking one mediator or one connection would not have any significant effect. Plus, the treatment has to be performed before the injury, which is not practicable. Besides the aforementioned inhibitors, there are very few other treatments or measures reported.

The result from our study indicates that increased air pressure can significantly reduce the exudation of the scalded tissue and further reduce the edema. During the experiment, we observed that when the air pressure is increased by 30 cm H_2O, there was a significant reduction of exudation and edema, and the healing time is shortened. The reason that we increased pressure 30 cm H_2O was based on the fact that 1) a significant change was observed with this increased pressure and 2) that this increased pressure does not affect heart rate, breathing, eating, and other life activities of the rats. This increased pressure roughly equals the water pressure that a human feels when submerged in a bathtub; it will not cause any discomfort.

We also observed in the study on rat mesenteric capillary model that when the air pressure increased by 25 cm H_2O, the exudation of fluorescence illuminant stopped, which means that the exudation from the end of capillary artery was "0." It indicates that a pressure is evenly exerted on the mesenteric and causes the increased interstitial hydrostatic pressure. When this pressure is greater than the filtration pressure of the artery end, the exudation rate becomes "0." Even when the capillary permeability increases as the result of some reasons, such as thermal injury, the exudation would not happen because of the increased interstitial hydrostatic pressure.

The change of interstitial hydrostatic pressure affects the exudation and absorption of the capillaries. From the viewpoint of liquid kinetics, for every 1-mm H_2O interstitial hydrostatic pressure increase, there is a 1-mm H_2O reduction of filtration pressure at the capillary artery end. Plus, it will increase 1 mm H_2O absorption pressure at the capillary vein end. Therefore, the high pressure has dual actions on reducing the edema. Hart[11] reported unexpectedly that, during the first several days after thermal damage, there would be a significant reduction of fluid infusion needed when using hyperbaric therapy. That was significant to the developing of edema, the complicated respiratory tunnel blockage and to the operation of eschar resection.

Davis et al[12] studied the early burn treatment on dogs with hyperbaric oxygen and under high air pressure. The result indicated that hyperbaric oxygen therapy has some statistics significance, which was resulted from the observation of plasma volume change and hematocrit change combined with heart output volume and peripheral resistance. At the same time, they also pointed out that the reason for reducing plasma loss is increasing the tensile force, not increasing air pressure. Although there are more authors reporting about the effects of hyperbaric oxygen on burn wound healing, very few researches studied the cause of this effect and implication for future clinical treatment.

Currently, most researchers agree that hyperbaric oxygen causes the contraction of capillaries[13] and limits tissue perfusion that increases capillaries permeability; thus, it reduces fluid loss on animals that is treated by hyperbaric oxygen therapy. But this mechanism is still not supported by all experiments.[12]

Our idea is that, under a hyperbaric oxygen environment, the wound area is placed under an increased pressure and that the interstitial hydrostatic pressure increases, so the pathologic dilation of capillaries is reduced and pure oxygen may only stimulate the con-

Copyright © American Burn Association. Unauthorized reproduction of this article is prohibited.
Downloaded from https://academic.oup.com/jbcr/article-abstract/28/2/286/4605514
by Ed 'DeepDyve' Gillespie user
on 19 April 2018

traction of normal capillaries, not the pathologic dilated capillaries.

From the clinical point of view, the earlier the treatment under HAP after scalding, the better the result. The exudation period of wound is the first 48 hours; therefore, it is most effective to give HAP treatment immediately after the burn and stop the treatment after 48 hours. The earlier the treatment exerts, the less the exudate emerges. It is obviously unsuitable to administer HAP when the exudate stops.

Typical burn treatment during the exudation period is to give intravenous fluid to compensate the amount of fluid lost as the result of exudation. Often, the large amount of fluid infusion causes an electrolyte imbalance, a large amount of protein loss that makes the edema more severe, and increases the chance of infection and other complications.

The increased air pressure treatment within 48 hours after burn can obviously reduce fluid loss from the wound and alleviate the trauma to patient's body. Correspondingly, the infusion of fluid and protein as well as the electrolyte could be reduced. This method provides a good basis for the treatment of postexudation and at the same time reduces the cost of therapy.

This study manifests that increasing the air pressure can reduce the infiltration and edema of the scald burn and accelerate the healing speed. It provides a base for further study on using hyperbaric air therapy to treat burns in humans.

REFERENCES

1. Arturson G. Microvascular permeability to macromolecules in thermal injury. Acta Physiol Scand Suppl 1979;463: 111–22.
2. Kaley G, Weiner R. Prostaglandin E-1: a potential mediator of the inflammatory response. Ann N Y Acad Sci 1971;180: 338–50.
3. Royal Society of London meeting. Summary of discussion. Eur Sci Notes 1975;29:2, 76.
4. Wilhem DL, Mason B. Vascular permeability changes in inflammation: the role of endogenous permeability factors in mild thermal injury. Br J Exp Pathol 1960;41:487–506.
5. Li Auo. Burn treatment. 2nd ed. Beijing: People's Public Health Publications; 1995. p. 1183.
6. Jiang DJ, Tao JY, Xu SY. Inhibitory effects of clonidine on edema formation after thermal injury in mice and rats. Acta Pharmacol Sin 1989;10:540–2.
7. Lu W, Chen YL, Xia ZF, et al. Modified Evans blue fluorimetry for determination of pulmonary vascular permeability in rats sustaining burns and delayed fluid resuscitation of burn shock. Burns 1997;23:490–2.
8. Zheng J, Huang WY, Wei X. The effects of epidermal growth factor on the wound healing of deep partial thickness burn in rats. Chin J Burns 2003:19:289–92.
9. Davies JWL. Physiological responses to burning injury. London: Academic Press; 1982. p. 195–208.
10. Hilton JG. Effects of alteration of polyunsaturated fatty acid metabolism upon plasma volume loss induced by thermal trauma. J Trauma 1980;20:663–9.
11. Hart GB, O'Reilly RR, Broussard ND, Cave RH, Goodman DB, Yanda RL. Treatment of burns with hyperbaric oxygen. Surg Gynecol Obstet 1974;139:693–6.
12. Davis JC, Hunt TK.. Hyperbaric oxygen therapy (Chinese Version). Beijing: People's Army Health Press, Beijing;1989.
13. Bergofsky EH, Wang MCH, Yamaki T, et al. . Tissue oxygen and carbon dioxide tensions during hyperbaric oxygenation. JAMA 1964;189:841–4.

Copyright © American Burn Association. Unauthorized reproduction of this article is prohibited.
Downloaded from https://academic.oup.com/jbcr/article-abstract/28/2/286/4605514
by Ed 'DeepDyve' Gillespie user
on 19 April 2018