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Poloxamer-188 Improves Capillary Blood Flow and Tissue Viability in a Cutaneous Burn Wound

Harihara Baskaran, Ph.D., Mehmet Toner, Ph.D., Martin L. Yarmush, M.D., Ph.D., and François Berthiaume, Ph.D.[1]

Center for Engineering in Medicine, Massachusetts General Hospital, Harvard Medical School, and the Shriners Hospitals for Children, Boston, Massachusetts 02114

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After cutaneous burn injury, an area of tissue 1–2 mm thick surrounding the wound is the site of a pronounced inflammatory response where blood flow is reduced. This "zone of stasis" undergoes progressive necrosis within 24–48 h, resulting in an expansion of the burn wound. Poloxamer-188 (P-188) is a surfactant that has been shown to prevent cell death due to electrical injury *in vivo* and heat shock *in vitro*. In this study, we investigated the effect of P-188 on blood flow within and around a burn wound and on the expansion of the wound area within 24 h after administration of a full-thickness burn injury. Results show that immediately (0–2 h) after the burn, red blood cell speed decreased to zero in a zone extending up to 1 mm from the center of the burn in both P-188 (200 mg/kg)- and saline (0.9%)-treated animals. Between 1 and 3 mm from the center of the burn, red blood cell speed decreased to 50% of preburn levels in saline controls ($n = 5$), while no decrease occurred in P-188-treated animals ($n = 5$). Beyond 3 mm from the center of the burn, red blood speed was equal to the preburn levels in saline controls, while it increased by about 10% in P-188 animals. Twenty-four hours after administration of burn, the "zero red blood cell speed zone," termed as the zone of coagulation, became smaller in P-188-treated animals, with an area of 2.4 ± 0.5 mm^2 ($n = 5$) compared to 3.5 ± 0.5 mm^2 ($n = 4$) in saline controls ($P < 0.01$). These results suggest that P-188 prevented the formation of a zone of stasis within 2 h after the burn injury and reduced the area of coagulation observed 24 h after cutaneous burn injury.

INTRODUCTION

Over forty years ago, Jackson [1] described the three functional zones of burn injury: (1) the peripheral zone of hyperemia, (2) the intervening zone of stasis, and (3) the central zone of coagulation. The latter consists of tissue irreversibly damaged via protein denaturation and breakdown of biological membranes at elevated temperatures. The outer zone of hyperemia contains healing tissue that exhibits high metabolic activity. The intervening zone of stasis consists of a layer of tissue 1–2 mm thick characterized by reduced blood flow and progressive tissue necrosis, which can result in additional tissue death within 24–48 h after the occurrence of the injury. In addition to causing an extension of the initial burn wound area, this process can result in the conversion of a partial-thickness burn into a full-thickness burn, which takes longer to heal and increases the probability of forming hypertrophic scars [2].

Prior studies have indicated that various events could contribute to blood flow reduction, including blood coagulation, increased vascular permeability, neutrophil recruitment and plugging of capillaries, and damage to the endothelium mediated by locally released oxygen radicals. In rats subjected to a deep second-degree skin burn, plasma levels of xanthine oxidase (XO), a source of reactive oxygen species that can cause cell and tissue damage, increased within 15 min after the injury, followed by a reduction of blood flow in the burned area; however, treatment with XO inhibitors did not prevent the blood flow reduction [3]. Stronger evidence exists for a role of neutrophils in blood flow reduction. Antibodies which block adhesion of neutrophils to endothelial cells administered 30 min after the burn injury improved blood flow in a "comb-

[1] To whom correspondence and reprint requests should be addressed at 51 Blossom Street, Boston, MA 02114.

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burn" model of burn injury consisting of a series of burns separated by narrow lanes of unwounded skin [4, 5]. Bucky *et al.* [6] showed that treatment with a monoclonal antibody to the CD18 neutrophil surface adhesion molecule in a rabbit burn model resulted in improved hair follicle retention and re-epithelialization at 8 days following the burn. Intravascular coagulation has also been implicated as a potential reason for the reduction in blood flow, since the administration of a recombinant tissue-type plasminogen activator 2 h following burn injury increased blood flow in the unwounded interspaces of a comb burn 7 days after the burn [7]. Aggarwal *et al.* [8] studied burn-induced vasoactive changes in peripheral cutaneous microcirculation using a hamster model and concluded that burns cause vasodilatation and increased vascular permeability. Increased vascular permeability can potentially lead to protein leakage from the vasculature into extravascular space. Decreases in protein concentrations in the blood can affect pressure equilibrium between the interstitial and vascular spaces, leading to a reduction in the blood flow [9].

Strategies aimed at blocking neutrophil adhesion and enhancing fibrinolysis have been successful in reducing experimental burn-induced progressive necrosis in animal models [4–7]. Poloxamer-188 (P-188) is a nonionic block copolymer surfactant of polyoxyethylene and polyoxypropylene commonly found in artificial blood substitutes as an additive. P-188 increases whole blood clot permeability and fibrinolysis *in vitro* [10]. In addition to these potentially beneficial hemorheological effects, P-188 also inhibits leukocyte chemotaxis, adhesion, and migration [11–13]. Other evidence suggests that P-188 can directly protect cells by sealing their plasma membrane, as shown in electropermeabilized skeletal muscle preparations *in vivo* [14] and heat-shocked fibroblasts *in vitro* [15].

Most researchers who studied the zone of stasis have used the comb-burn model and laser Doppler to monitor skin blood flow [4, 5, 7, 16]. The main disadvantage of the above burn model is that it is not possible to visualize dynamic events that occur after a burn injury. In this study, we developed a small-burn injury model which allows microscopic visualization of red blood cell flow in the zone of stasis. We demonstrated that intravenously injected P-188 prevented the formation of the zone of stasis and reduced the burn wound area after 24 h.

MATERIALS AND METHODS

Reagents. *N*-[2-Hydroxyethyl] piperazine-*N'*-[2-ethanesulfonic acid] (Hepes) balanced salt solution (HEBSS) was prepared according to the following formula: 0.137 M NaCl, 0.54 mM KCl, 5.55 mM glucose, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, and 4.2 mM Hepes. Two HEBSS solutions, one at pH 7.4 and the other at pH 8.2, were obtained by the addition of 10 M NaOH. A stock solution of fluorescein isothiocyanate (FITC; Molecular Probes, Eugene, OR) was made by dissolving FITC in dimethylsulfoxide at a concentration of 75 mg/ml. Dextran/fluorescein (500,000 Da, Molecular Probes) was diluted in phosphate-buffered saline to 10 mg/ml. P-188 (F-68; Sigma Chemical Company, St. Louis, MO) was diluted to 60 mg/ml in 0.9% saline. Ketamine (Ketalar) was from Parke-Davis (Morris Plains, NJ), xylazine (Xyla-ject) was from Phoenix pharmaceutical, Inc. (St. Joseph, MO), buffered 10% formalin was from Fischer Scientific (Pittsburgh, PA), and the Masson trichrome staining kit was from Sigma.

Implantation of intravital microscopy chamber. Male CD1 mice (~30 g body weight) were obtained from Charles River Laboratories (Wilmington, MA). The animals were maintained in accordance with National Research Council Guidelines, and experimental protocols were approved by the Subcommittee on Animal Care, Committee on Research, at the Massachusetts General Hospital, Boston, MA. Anesthesia was induced using 80 mg/kg of ketamine and 12 mg/kg of xylazine via intraperitoneal injection. All hair from the dorsal skin area was removed using electric clippers and a hair depilatory cream (Carter-Wallace, New York, NY).

A dorsal skin fold chamber with a design similar to that of Gourgouliatos *et al.* [17] was implanted on the back of mice. Briefly, a skin flap on the back of the mouse was pulled perpendicularly and sutured to a titanium plate with a 12-mm circular opening allowing access to the skin. A complete layer of skin over a circular area of 12 mm in diameter was removed on the other side of the skin flap. The wounded area was wetted with saline, and another titanium plate with a window was sutured to the skin flap. Special care was taken to avoid damaging exposed blood vessels during implantation. The window in the second plate consisted of a glass coverslip (Fischer Scientific), which was held inside the chamber with the help of a snap ring and two Teflon rings, one on each side of the coverslip, to form a seal.

The stability of FITC/red blood cells stored at 4°C over a period of 4 days was measured using a flow cytometer (Becton-Dickinson, San Jose, CA). The peak intensity of labeling, in relative fluorescent units, decreased as a function of storage time (data not shown), which was verified by microscopic examination. The reduction in intensity over 24 h was so great that monitoring the flow of the labeled red blood cells *in vivo* became difficult. For this reason, we used red blood cells labeled within 12 h prior to the flow measurement.

Burn injury. With the animal under anesthesia, a brass rod with a circular tip of 2-mm diameter heated to 100°C was applied to the skin for 5 s. The temperature at the tip was regulated by a rectangular strip resistance heater (Omega Engineering, Inc., Stamford, CT) wrapped around the rod, a mini thermocouple (T type, Omega Engineering, Inc.) located at the burn tip, and a temperature controller (Omega Engineering, Inc.). During the process of injury the temperature at the tip varied by less than 2°C. A full-thickness burn injury (i.e., through the epidermis and dermis) resulted from this procedure.

Capillary blood flow measurements. Approximately 1 ml of blood from a donor mouse was collected in 20 ml of heparinized (500 U) saline. The solution was centrifuged at 230 *g* and the pellet washed with HEBSS (pH 7.4). This procedure was repeated once at 230 *g* and three times at 920 *g*. The pellet, containing primarily red blood cells, was resuspended in 10 ml HEBSS (pH 8.2) containing FITC at a concentration of 1.5 mg/ml. The suspension was gently shaken at 4°C for 1 h. After incubation, unreacted FITC was removed by centrifuging at 920 *g* and washing the pellet with HEBSS (pH 7.4) four or five times until the supernatant was clear. In the final step, labeled red blood cells were suspended in HEBSS (pH 7.4) at a concentration of 5×10^9 cells per milliliter. Flow cytometry analysis (FACS Calibur, Becton Dickinson) was carried out to determine the labeling intensity and its stability over time.

About 100 μl of red blood cell suspension (i.e., 500 million labeled

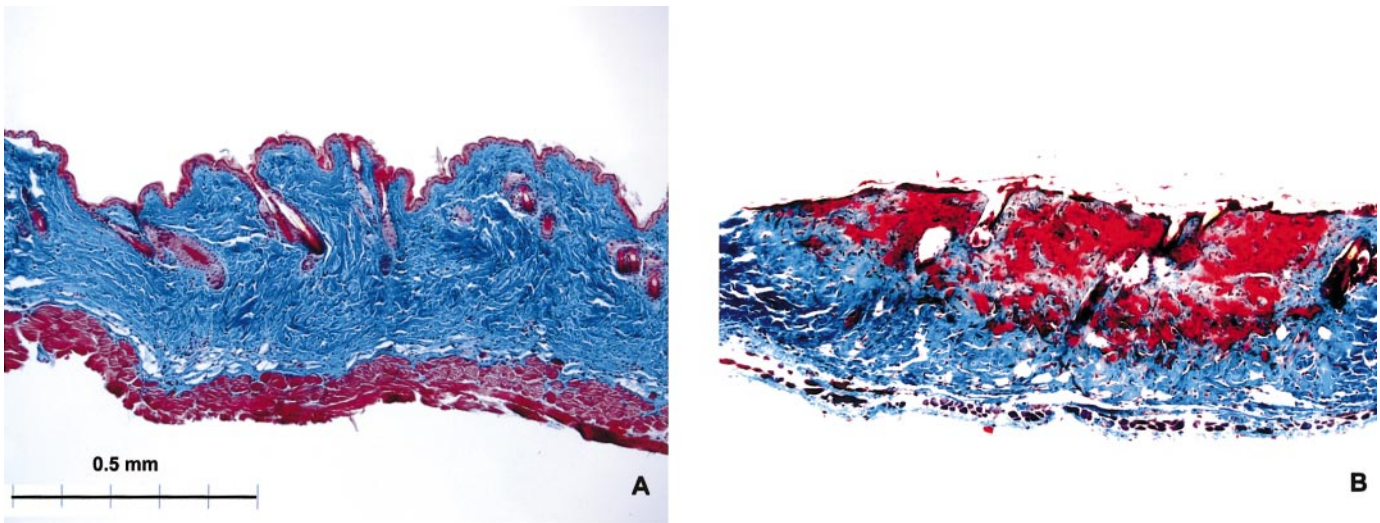

FIG. 1. Masson trichrome staining of burned skin sections. A brass rod with a tip diameter of 2 mm was used to burn mouse skin. Skin was formalin fixed, paraffin embedded, serially sectioned, and stained. (A) and (B) denote sections obtained respectively at 1.3 and 0.6 mm from the center of the burn.

red blood cells) were injected via the tail vein. While under microscopic examination, the chamber was held steady in a Plexiglas® holder, which allowed the animal to breathe normally and move its limbs. The skin vasculature was viewed through a 20× objective (0.4 DN 160/1.2 ELWD, Nikon, Tokyo, Japan) on an inverted fluorescence microscope (Diaphot, Nikon). Real-time fluorescent images of red blood cell flow at various distances from the center of the wound were recorded on videotape (Panasonic, Japan). Capillaries were identified as the vessels where the arterial bifurcations terminate and whose diameters are similar to that of a red blood cell. Red blood cell speed in capillaries was determined based on the time taken by the fluorescently labeled red blood cells to cross a fixed capillary length. Capillary red blood cell flow was measured at several locations, each separated by 500 μm from the center of the wound, before and after administration of the burn. Red blood cell speed was normalized and averaged in the following manner: four orthogonal locations equidistant from the burn center were chosen and three to four capillary red blood cell speeds per location were obtained. At each location, postburn red blood cell speed was normalized to preburn red blood cell speed. This procedure was repeated in each animal and the normalized red blood cell speeds were averaged.

Coagulation area measurement. Mice fitted with the dorsal skin fold chamber were subjected to the burn injury. 24 h after the burn injury and subsequent treatment with 0.9% saline or P-188, 10 mg/kg of 500,000-Da dextran/fluorescein was injected. Fluorescent images of the burn area were taken using a 4× planar objective (Nikon). The coagulation area was identified as the unstained area and was quantified using image analysis software (Metamorph, Universal Imaging Corp., West Chester, PA)

Histology of burn wounds. Skin samples were obtained from the burn locations immediately after administration of the burn injury. The samples were immediately fixed in buffered 10% formalin, dehydrated, paraffin-embedded, and serially sectioned at 6-μm intervals. Tissue sections were stained with the Masson trichrome procedure.

Statistics. Statistical analysis of the red blood cell speed data was carried out using analysis of variance. Tukey's test was used whenever multiple comparisons were made. An α value of 0.05 was considered significant.

RESULTS

Histology

In order to determine the initial burn characteristics, we obtained serial cross-sections of the burned mouse skin obtained at different locations relative to the center of the burn and stained them with the Masson trichrome stain. Figure 1A shows a control unburned skin section obtained at 1.3 mm from the center of the burn and Fig. 1B shows a burned skin section obtained at 0.6 mm from the center of the burn. It is well known that Masson trichrome stains normal collagen green or blue-green and collagen heated above its denaturation temperature (60–65°C) red [18, 19]. Also, burn injury to the skin is known to change the organization of collagen fibers. It is clearly seen that in the burned skin section, the dermis was stained red, whereas in the unburned section, it was stained blue. In addition, the collagen fibers were disorganized in the burned skin. In contrast, Masson trichrome stained the underlying muscle (panniculus carnosus) blue in the burned skin section, whereas it stained the muscle red in the unburned skin section (Fig. 1A). Epidermal damage is also clearly visible in the burned skin, as the basal keratinocytes and the lamina propria were coagulated and indistinguishable. We calculated the extent of damage by measuring the width of the damaged epidermis and the muscle layer that was stained blue in the images of serial sections obtained from the center of the burn using image analysis software (Metamorph). Based on the epidermal damage and the muscle damage, the average diameters of the burn were obtained to be 1.84 ± 0.24 and 1.78 ± 0.28 mm, respectively. The diameters of the damaged epidermis and

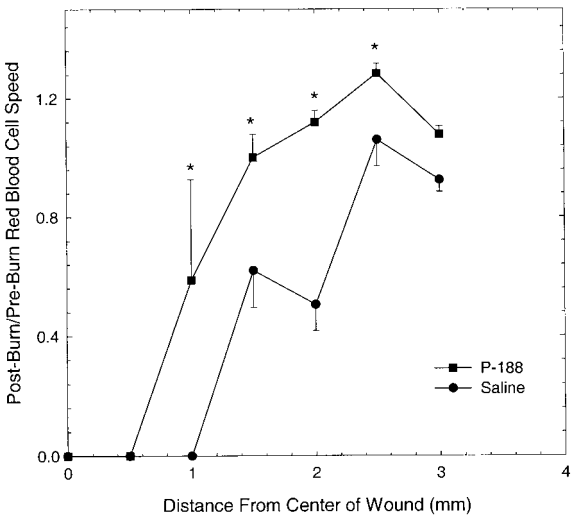

FIG. 2. Effect of P-188 on burn-induced capillary blood flow in the wound. Postburn (0–2 h) to preburn ratio of red cell speed in capillaries is plotted against the distance from the center of the injury. P-188 (200 mg/kg) or saline (0.9%) was injected immediately before the burn injury. Data shown are the average ± SEM of five animals. *Significantly different from saline control.

muscle layer are within 10% of the diameter of the tip of the brass rod used to inflict the injury on the skin.

Effect of P-188 on Capillary Blood Flow

To determine whether P-188 alters the blood flow distribution within and around the burn wound, we measured the average red blood cell speed in capillaries at various distances from the center of the burn wound (Fig. 2). In the following description of the results, we denote the areas where the red blood cell speed is zero as the zone of coagulation, those below preburn levels as the zone of stasis, and those equal to or above preburn levels as the zone of hyperemia. In the control group injected with saline immediately before the burn, the zone of coagulation extended to 1 mm from the center of the burn, the zone of stasis was located between 1 and 3 mm from the center of the burn, and the zone of hyperemia was absent. In mice receiving P-188 immediately before the burn, the extent of the zone of coagulation was similar to that in group A, but the zone of stasis was absent, and the zone of hyperemia appeared from 1 mm onward.

Effect of P-188 on Coagulation Area

To assess the effect of P-188 in reducing tissue necrosis, mice were injected with dextran/fluorescein 24 h after the burn, after which unperfused areas corresponding to the coagulation zone could be easily identified. Figure 3 shows typical wound areas of mice that were treated with 0.9% saline and P-188, with a clear demarcation between perfused and unperfused areas. Quantification of the area of coagulation by image analysis revealed that treatment of mice with P-188 (200 mg/kg) 20 min after the burn reduced the coagulation area by 40% compared to saline-injected controls (Fig. 4).

DISCUSSION

Capillary blood flow is a key indicator of nutrient supply, especially oxygen, to tissue. Thus, dynamic studies of blood flow measurements after burns can be used to unravel mechanisms of progressive ischemia in the zone of stasis. In this study, we used intravital microscopy to visualize dynamic changes in blood flow following a small-size burn injury. We also tested the efficacy of P-188 on the secondary tissue death after burn injury. We found that burn injury causes a reduction in blood flow to occur surrounding the burn wound within 2 h after the injury. Our results also show that P-188 is beneficial in preventing the immediate reduction in blood flow and in reducing the necrotic area measured 24 h after the injury.

Most prior research in this area utilized relatively larger burns separated by areas of unburned tissue and used laser Doppler imaging to monitor blood flow in the interspaces [4, 5, 7, 16]. Following injury, blood flow in these interspaces was reduced and the tissue became necrotic after 24–48 h. In our study, we used a small 2-mm circular burn to determine the effect of P-188 on these zones. Intravital microscopy was used to directly measure capillary blood flow before and after the burn injury and to characterize the zones based on zero flow (coagulation), reduced flow (stasis), and normal or enhanced flow (hyperemia). The current model has the advantage of direct investigation of dynamic events with observation periods which could be extended over several days.

Following a burn injury to the skin, dynamic alterations to blood flow take place near the site of injury. Arteriole constriction and dilatation and venule dilatation, aggregation of red blood cells and platelets, and leukocyte adhesion to postcapillary endothelium occur. Prior studies suggest that the most likely events responsible for the formation of zone of stasis are blood coagulation and neutrophil recruitment, since administration of a recombinant tissue-type plasminogen activator, anti-Mac-1, and intercellular adhesion molecule-1 antibodies improved blood flow in the interspaces of a comb-burn model [4, 5, 7]. The formation of the zone of stasis is a dynamic phenomenon; however, the dynamics of the events that cause blood flow stasis are still unknown.

Edema formation is one of the events that occurs within minutes following the injury. Intracellular swelling occurs when endothelial cells cannot maintain transmembrane potential and swell by taking up sodium and water. Swollen endothelial cells open pores in capillary walls, aiding in interstitial edema formation, and obstruct blood flow in the capillaries [9].

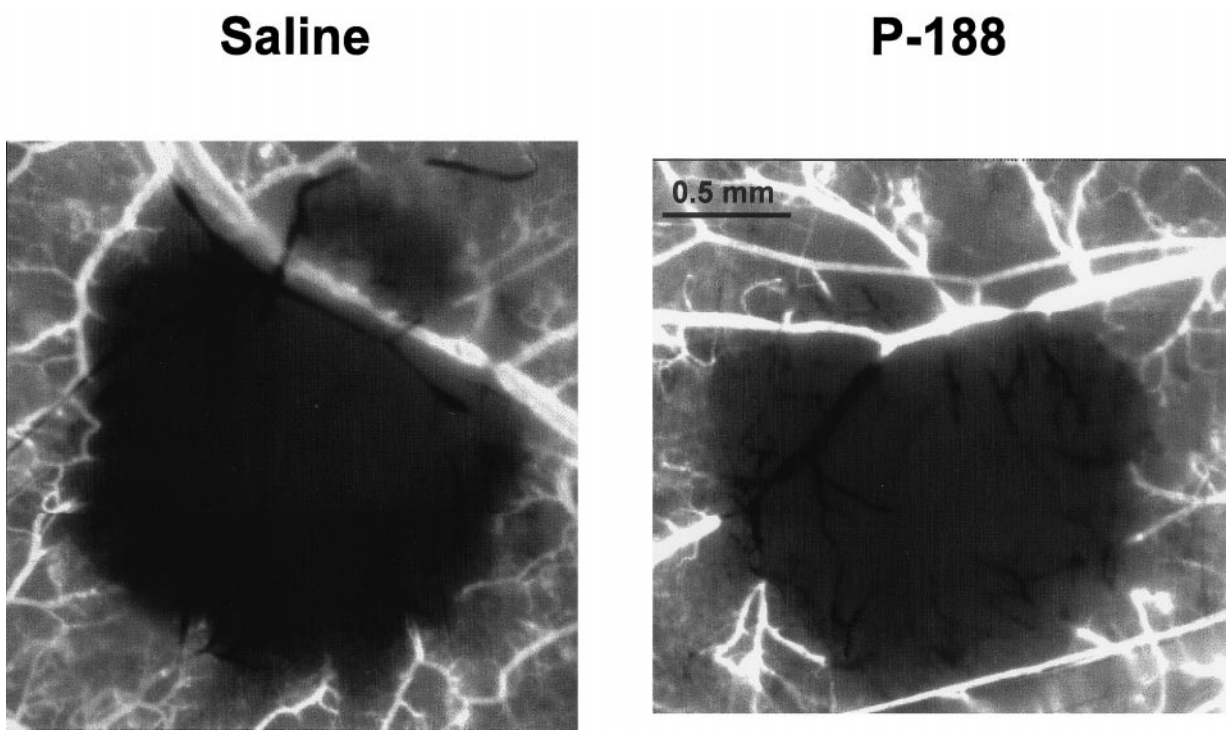

FIG. 3. Effect of P-188 on coagulation area 24 h after the burn. Mice were treated with 0.9% saline and P-188 (200 mg/kg) 20 min after the burn. Fluorescein/dextran (500,000 MW) was injected 24 h after the burn, and fluorescent images were obtained.

P-188 is known to preserve the structure of endothelial cells after a reperfusion injury and to reduce capillary obstruction [20]. It has also been shown to act as a membrane sealant and, therefore, can prevent swelling of endothelial cells and aid in the restoration of the capillary blood flow [14, 15]. This may explain the absence of the zone of stasis within 0–2 h after the burn in animals that received the P-188 injection prior to the burn (Fig. 2).

Reasons for progressive blood flow reduction in the zone of stasis after a burn injury are not well understood. Instead of restoration of blood flow to normal levels, progressive necrosis occurs within 24–48 h after the injury. It is possible that fibrinolysis is delayed at the wound edge because treatment with recombinant tissue-type plasminogen activator has been shown to be effective in preventing progressive necrosis in the zone of stasis [7]. Thrombogenic activity near the wound can also trigger an inflammatory response of endothelium [21] leading to the sequestration and aggregation of neutrophils, which, when activated by local mediators such as platelet-activating factor, platelet-derived growth factor, and XO, can undergo adhesion, degranulation, and subsequent respiratory burst that are deleterious to the vessel endothelium. Since P-188 is known to enhance fibrin network permeability [10, 22] and is an inhibitor of neutrophil chemotaxis *in vitro* [11], it can keep capillaries patent and maintain blood flow within them. More recently, P-188 has been shown to bind to blood platelets [23]. Although P-188 does not interfere with the formation of the primary platelet plug, it prevents the formation of occlusive thrombus. This raises the concern that P-188 can potentially exacerbate hemorrhage complications that mainly occur in burned patients when they undergo wound excision and grafting. The half-life of P-188 in humans is about 4.5 h [24]. This will result in a faster clearance of the compound and reduce the impact of P-188 by the time the patient undergoes burn wound excision and grafting.

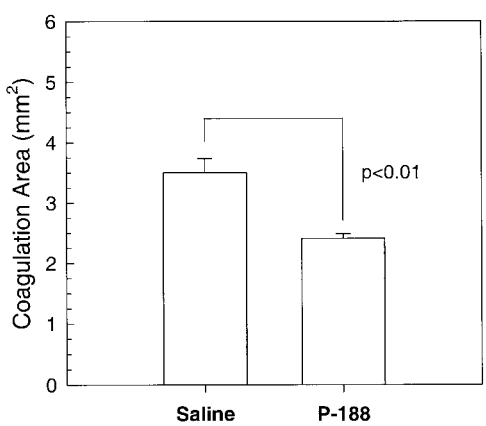

FIG. 4. Effect of P-188 on coagulation area 24 h after the burn. Coagulation areas shown in Fig. 3 were quantified by image analysis. Data shown are the average ± SEM of five saline controls and four P-188-treated animals.

In summary, we used intravital microscopy to characterize capillary blood flow changes in the vicinity of a burn wound. Our results show that within 2 h after the burn, capillary blood flow reduction occurs in an area near the wound. This zone of stasis becomes part of the zone of coagulation within 24 h. P-188 given prior to or early after the burn prevented the formation of the zone of stasis. Furthermore, administration of P-188 after the burn reduced the area of the zone of coagulation 24 h after the burn, suggesting that P-188 may alleviate the progressive ischemic injury. Intravital microscopy used in conjunction with the dorsal skin-flap model of burn injury is a useful approach to study the events leading to the extension of a burn wound. Future studies to assess the efficacy of P-188 will involve the use of pigs as these animals have skin characteristics that are much closer to humans than do mice.

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REFERENCES

1. Jackson, D. M. The diagnosis of the depth of burning. *Br. J. Surg.* **40:** 588, 1953.
2. Deitch, E. A., Wheelahan, T. M., Rose, M. P., Clothier, J., and Cotter, J. Hypertrophic burn scars: Analysis of variables. *J. Trauma* **23**(10): 895, 1983.
3. Till, G. O., Guilds, L. S., Mahrougui, M., Friedl, H. P., Trentz, O., and Ward, P. A. Role of xanthine oxidase in thermal injury of skin. *Am. J. Pathol.* **135**(1): 195, 1989.
4. Choi, M., Rabb, H., Arnaout, M. A., and Ehrlich, H. P. Preventing the infiltration of leukocytes by monoclonal antibody blocks the development of progressive ischemia in rat burns. *Plast. Reconstr. Surg.* **96**(5): 1177, 1995.
5. Mileski, W., Borgstrom, D., Lightfoot, E., Rothlein, R., Faanes, R., Lipsky, P., and Baxter, C. Inhibition of leukocyte-endothelial adherence following thermal injury. *J. Surg. Res.* **52**(4): 334, 1992.
6. Bucky, L. P., Vedder, N. B., Hong, H. Z., Ehrlich, H. P., Winn, R. K., Harlan, J. M., and May, J. W., Jr. Reduction of burn injury by inhibiting CD18-mediated leukocyte adherence in rabbits. *Plast. Reconstr. Surg.* **93**(7): 1473, 1994.
7. Isik, S., Sahin, U., Ilgan, S., Guler, M., Gunalp, B., and Selmanpakoglu, N. Saving the zone of stasis in burns with recombinant tissue-type plasminogen activator (r-tPA): An experimental study in rats. *Burns* **24:** 217, 1998.
8. Aggarwal, S. J., Da Costa, R., Diller, K. R., and Hinich, M. J. The effects of burn injury on vasoactivity in hamster peripheral microcirculation. *Microvasc. Res.* **40**(1): 73, 1990.
9. Arturson, G. Local effects. In J. A. D. Settle (Ed.), *Principles and Practice of Burns Management*. New York: Churchill Livingstone, 83, 1996.
10. van Gelder, J. M., Nair, C. H., and Dhall, D. P. The permeability of fibrin network developed in human plasma. *Blood Coagul. Fibrinolysis* **6**(4): 293, 1995.
11. Schaer, G. L., Hursey, T. L., Abrahams, S. L., Buddemeier, K., Ennis, B., Rodriguez, E. R., Hubbell, J. P., Moy, J., and Parrillo, J. E. Reduction in reperfusion-induced myocardial necrosis in dogs by RheothRx injection (poloxamer 188 N.F.), a hemorheological agent that alters neutrophil function. *Circulation* **90**(6): 2964, 1994.
12. Forman, M. B., Ingram, D. A., and Murray, J. J. Role of perfluorochemical emulsions in the treatment of myocardial reperfusion injury. *Am. Heart J.* **124**(5): 1347, 1992.
13. Lane, T. A., and Lamkin, G. E. Paralysis of phagocyte migration due to an artificial blood substitute. *Blood* **64**(2): 400, 1984.
14. Lee, R. C., River, L. P., Pan, F. S., Ji, L., and Wollmann, R. L. Surfactant-induced sealing of electropermeabilized skeletal muscle membranes in vivo. *Proc. Natl. Acad. Sci. USA* **89**(10): 4524, 1992.
15. Merchant, F. A., Holmes, W. H., Capelli-Schellpfeffer, M., Lee, R. C., and Toner, M. Poloxamer 188 enhances functional recovery of lethally heat-shocked fibroblasts. *J. Surg. Res.* **74**(2): 131, 1998.
16. Regas, F. C., and Ehrlich, H. P. Elucidating the vascular response to burns with a new rat model. *J. Trauma* **32**(5): 557, 1992.
17. Gourgouliatos, Z. F., Welch, A. J., and Diller, K. R. Microscopic instrumentation and analysis of laser-tissue interaction in a skin flap model. *J. Biomech. Eng.* **113**(3): 301, 1991.
18. Flint, M. H., and Lyons, M. F. The effect of heating and denaturation on the staining of collagen by the Masson trichrome procedure. *Histochem. J.* **7:** 547, 1975.
19. Chvapil, M., Speer, D. P., Owen, J. A., and Chvapil, T. Identification of the depth of burn injury by collagen stainability. *Plast. Reconstr. Surg.* **73**(3): 438, 1983.
20. Forman, M. B., Puett, D. W., Bingham, S. E., Virmani, R., Tantengco, M. V., Light, R. T., Bajaj, A., Price, R., and Friesinger, G. Preservation of endothelial cell structure and function by intracoronary perfluorochemical in a canine preparation of reperfusion. *Circulation* **76**(2): 469, 1987.
21. Becker, B. F., Heindl, B., Kupatt, C., and Zahler, S. Endothelial function and hemostasis. *Z. Kardiol.* **89**(3): 160, 2000.
22. van Gelder, J. M., Nair, C. H., and Dhall, D. P. Effects of Poloxamer 188 on fibrin network structure, whole blood clot permeability and fibrinolysis. *Thromb. Res.* **71**(5): 361, 1993.
23. Ahmed, F., Alexandridis, P., and Neelamegam, S. Synthesis and application of fluorescein-labeled pluronic block copolymers to the study of polymer-surface interactions. *Langmuir* **17:** 537, 2001.
24. Jewell, R. C., Khor, S. P., Kisor, D. F., LaCroix, K. A., and Wargin, W. A. Pharmacokinetics of RheothRx injection in healthy male volunteers. *J. Pharm. Sci.* **86**(7): 808, 1997.