Original article

Erythropoietin in the prevention of experimental burn progression

M. Tobalem[1,2], Y. Harder[1,2,4], T. Schuster[5], F. Rezaeian[1,4] and R. Wettstein[2,3]

[1]Faculty of Medicine, University of Geneva, and [2]Division of Plastic, Reconstructive and Aesthetic Surgery, Geneva University Hospitals, Geneva, and [3]Department of Plastic, Reconstructive, Aesthetic and Hand Surgery, University Hospital Basle, Basle, Switzerland, and [4]Department of Plastic Surgery and Hand Surgery and [5]Institute of Medical Statistics and Epidemiology, Klinikum rechts der Isar, Technische Universtät München, Munich, Germany
Correspondence to: Dr R. Wettstein, Department of Plastic, Reconstructive, Aesthetic and Hand Surgery, University Hospital Basle, Spitalstrasse 21, CH-4031 Basle, Switzerland (e-mail: drwette@yahoo.com)

Background: Damage control is essential in first aid of burn lesions. The aim of the present study was to investigate whether systemic erythropoietin (EPO) administration could prevent secondary burn progression in an experimental model.

Methods: The burn comb model creates four rectangular burn surfaces intercalated by three unburned zones prone to progression. Twenty-one Wistar rats were randomized to a control group or to receive intraperitoneal EPO (500 units per kg) once a day for 5 days starting 45 min (EPO45min) or 6 h (EPO6h) after burn injury. Histological analyses assessing burn depth, inflammation and neoangiogenesis, planimetric evaluation of burn progression, and laser Doppler flowmetry to assess perfusion were performed after 1, 4 and 7 days. Final scarring time and contracture rate were assessed once a week.

Results: Burn progression was decreased significantly with EPO45min but not EPO6h; progression of burn depth stopped in the intermediate dermis (mean(s.e.m.) burn depth score 3·3(0·6) for EPO45min *versus* 4·7(0·3) and 5·0(0·0) for EPO6h and control respectively on day 7; $P = 0·026$) and the surface extension was significantly reduced (45(8), 65(4) and 78(4) respectively on day 7; $P = 0·017$). This was paralleled by faster re-establishment of perfusion with EPO45min (114(5) per cent on day 4 *versus* 85(6) and 91(3) per cent for EPO6h and control respectively; $P = 0·096$). The reduction in progression resulted in a decreased healing time (7·3(0·7) weeks for EPO45min *versus* 11·5(1·0) and 10·8(0·5) weeks for EPO6h and control; $P = 0·020$) and contracture rate ($P = 0·024$).

Conclusion: Early EPO prevented burn progression, mainly by improved vascular perfusion.

Surgical relevance

Although the benefit of rapid cooling of burn injuries is well known, there is little understanding of the process of burn progression, that is, secondary deepening of the burn wound during the first 2–3 days after the injury, which can dramatically influence the final outcome.

The present study of experimental burn injury showed that the intraperitoneal administration of erythropoietin at 45 min, but not 6 h, after injury reduced burn severity. The effect appeared to be due mainly to improved microcirculatory perfusion.

The use of erythropoietin, or other substances that improve the microcirculation, could become an important early treatment for patients with burns.

Presented in part to the 97th Congress of the Swiss Society of Surgery–Association for Research in Surgery, Interlaken, Switzerland, September 2010; published in abstract form as *Swiss Knife* 2010; **7**: 45

Paper accepted 18 May 2012
Published online in Wiley Online Library (www.bjs.co.uk). **DOI:** 10.1002/bjs.8847

Introduction

Burns are dynamic injuries that may progress over the first 48–72 h. The worst development of secondary burn progression is the conversion of an initially superficial lesion that would heal with conservative measures to a full-thickness lesion that necessitates surgical treatment, and leads to debilitating scarring and contracture[1].

Three distinct concentric zones characterize burn lesions: a central zone of necrosis damaged beyond repair; a surrounding zone of stasis with decreased, stagnant microcirculatory perfusion that can progress to necrosis if untreated; and a marginal zone of hyperaemia with high metabolic activity that shows rapid restitution[2]. Secondary burn progression is the development of necrosis of the

marginally perfused zone that represents a potential target for therapeutic intervention.

Even though the outcome after major burn injuries has improved considerably over recent decades owing to the regionalization of treatment, advances in standard of care and ongoing research[3], there exists no therapeutic strategy to prevent secondary burn progression of superficially burned areas. In burns with a large surface area this progression can be life-threatening. In smaller burns the deepening of the wound can significantly change the treatment and require surgery with inferior outcome. It significantly affects subsequent quality of life and can also increase the cost of burn treatment.

Erythropoietin (EPO) is a pluripotent molecule that is expressed in the adult kidney in response to hypoxia in order to regulate erythropoiesis[4]. However, more recently, additional tissue-protective effects have been attributed to EPO, including nitric oxide (NO)-mediated relaxation of arterial vessels[5,6], induction of angiogenesis[7], prevention of ischaemia-induced apoptotic cell death and inflammation[8], as well as tissue regeneration[9]. The aim of the present study was to investigate the effectiveness of EPO treatment at 45 min or 6 h after burn injury in preventing burn progression in the rat comb burn model.

Methods

All experiments were carried out in accordance with the Swiss guidelines for animal experimentation and followed approval by the Geneva Cantonal Veterinary authority. A total of 21 male Wistar rats weighing mean(s.e.m.) 456(7) g (Charles River Laboratories, L'Arbresle, France) were housed in single cages at a room temperature of 22–24°C and relative humidity of 60–65 per cent, with a 12-h light–dark cycle. Chow and water were freely available. To ensure freedom from disease, the animals were observed for 1 week before experimentation.

Animal preparation

The animals were prepared 24 h before the experiments, including shaving and chemical depilation of the dorsal skin with a depilatory cream (Veet®; Reckitt Benckiser, Hull, UK). The areas to be burned were outlined with a marker pen and the corners marked with intradermal ink. The rats were anaesthetized for preparation and all measurements using inhalational anaesthesia with a 2 per cent isoflurane–air mixture. Animals received subcutaneous buprenorphine at a dose of 0·05 mg/kg 15 min before burn induction and thereafter every 12 h for the first 3 days.

Burn induction

The comb burn model was used to create the burn wounds[10,11]. A chromium–nickel steel (V2A) template (Ornaplast Kunststofftechnik, Dagmersellen, Switzerland) weighing 136 g was immersed in boiling water for 15 min until equilibration of the temperature. With the animal prone, the template was applied to the previously shaved and marked area on the back, perpendicular to the skin surface and parallel to the spine at a distance of approximately 5 mm. No pressure was applied while inducing the burn and the template was removed after 60 s. Application of the template resulted in four 20 × 10-mm burn areas separated by three 20 × 5-mm unburned interspaces as defined by the notches of the template. The back was burned bilaterally to the spine. No dressing or topical treatment was applied.

Experimental groups and protocol

Animals were assigned randomly to one of three experimental groups, each of seven rats. At the end of all measurements at each of the following time points (except baseline), one animal was killed in order to harvest skin for histological examination. Necrosis (depth and surface) and perfusion were analysed at five time points (baseline, 1 h, day 1, day 4 and day 7). This resulted in four animals per group at the end of the experiments on day 7. Skin for baseline analyses was harvested from separate animals.

A control group was treated with local cooling alone for 20 min. Two treatment groups each received cooling for 20 min, followed by intraperitoneal administration of 500 units/kg EPO either 45 min (EPO45min) or 6 h (EPO6h) after burn injury, and daily for 5 days. Cooling was initiated immediately after burn induction by applying gauzes (10 × 10 cm) that had been soaked in cold water (17°C) directly to the burn area; these were changed every minute for 20 min. Animal preparation and baseline measurements were performed 24 h before burn induction. The parameters were then assessed after 24 h, 4 and 7 days. The time to wound healing (complete epithelialization without presence of eschar) and wound contracture were assessed once a week. After complete wound healing, the animals were killed with an intraperitoneal overdose of pentobarbital under anaesthesia.

Erythropoietin

Epoetin (recombinant human EPO, Neo-Recormon®; Roche Pharma, Basle, Switzerland) was admixed with 1 ml 0·9 per cent sodium chloride to achieve a final

concentration of 500 units/kg EPO. The freshly prepared solution was stored at a maximum of 8°C for 45–60 min until use.

Blood samples

Blood samples of 250 μl were taken from the tail vein and analysed for haematocrit 1 day before burn induction, and 24 h, 4 and 7 days, and 2 weeks after burn injury using an analyser designed for rat blood (OSM3; Radiometer, Copenhagen, Denmark).

Microcirculatory perfusion

Microcirculatory perfusion within the interspaces was measured using a multichannel laser Doppler imager (PIM II Laser Doppler Perfusion Imager, LDPIwin 2.0.6 software; Lisca, Linköping, Sweden). The surface probe measured microcirculatory blood flow to a depth of approximately 1 mm. The laser Doppler unit was calibrated according to the guidelines of the manufacturer. Because of the high intersite variability, data are presented as a percentage of baseline value.

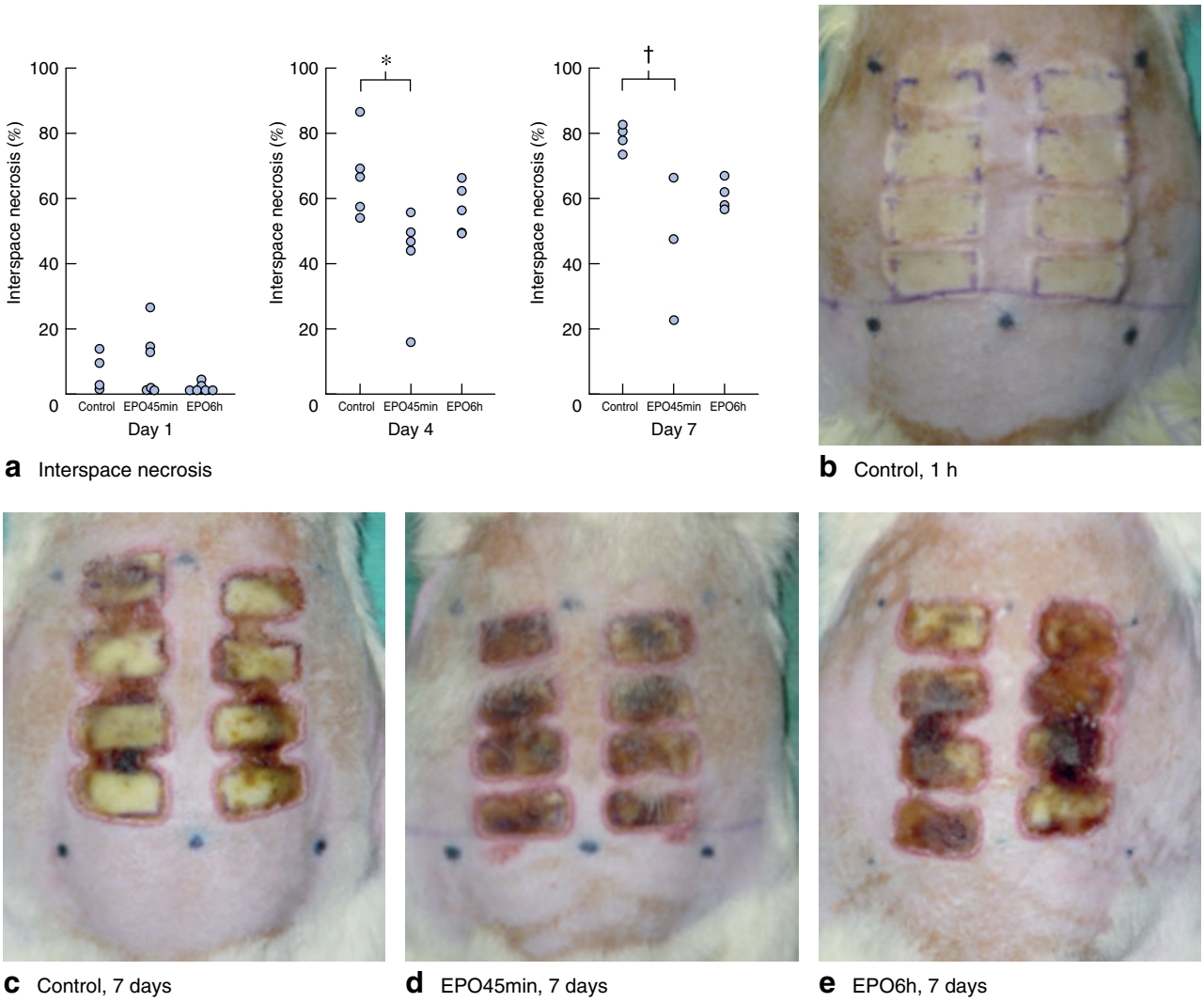

Fig. 1 **a** Scatter plot showing changes in interspace necrosis between treatment groups over time ($P = 0{\cdot}007$), a significant effect of time ($P < 0{\cdot}001$) and differences in time slopes ($P = 0{\cdot}001$) between the control group and groups that received erythropoietin (EPO) at 45 min (EPO45min) and 6 h (EPO6h) after injury (two-way ANOVA). *$P = 0{\cdot}006$, †$P = 0{\cdot}017$ (Bonferroni *post hoc* test). **b–e** Tissue morphology **b** 1 h after burn injury in a control animal, and after 7 days in **c** control, **d** EPO45min and **e** EPO6h groups. Note the increased width of interspace and overall decreased rate of necrosis in animals administered EPO at 45 min

Observation and planimetry

Observations were performed daily for the first week and then weekly until the wounds had healed. Photographs were taken in a standard manner with the camera (Panasonic DMC-TZ1; John Lay Electronics, Lucerne, Switzerland) fixed on a tripod. A computer-assisted image analysis system (Cap Image®; Zeintl Software, Heidelberg, Germany) was used to determine the total amount of interspace necrosis (percentage of the interspace surface) and the contracture rate. The latter was determined as the percentage decrease in total surface area within the corner points marked with ink before burn induction.

Histology and immunohistochemistry

The entire burn area (8 burns and 6 interspaces per animal) was harvested at 1 h, 1, 4 and 7 days after the burn. This tissue was fixed immediately in 10 per cent formalin, stored in 70 per cent alcohol for 24 h, and embedded in paraffin. Thereafter 4-μm sections were obtained for haematoxylin and eosin, and immunohistochemical staining.

Inducible nitric oxide synthase immunostaining

Antigen retrieval was performed for 30 s in citrate buffer at pH 6. Peroxidase blocking was carried out using Dako real peroxidase blocking solution (Dako Schweiz,

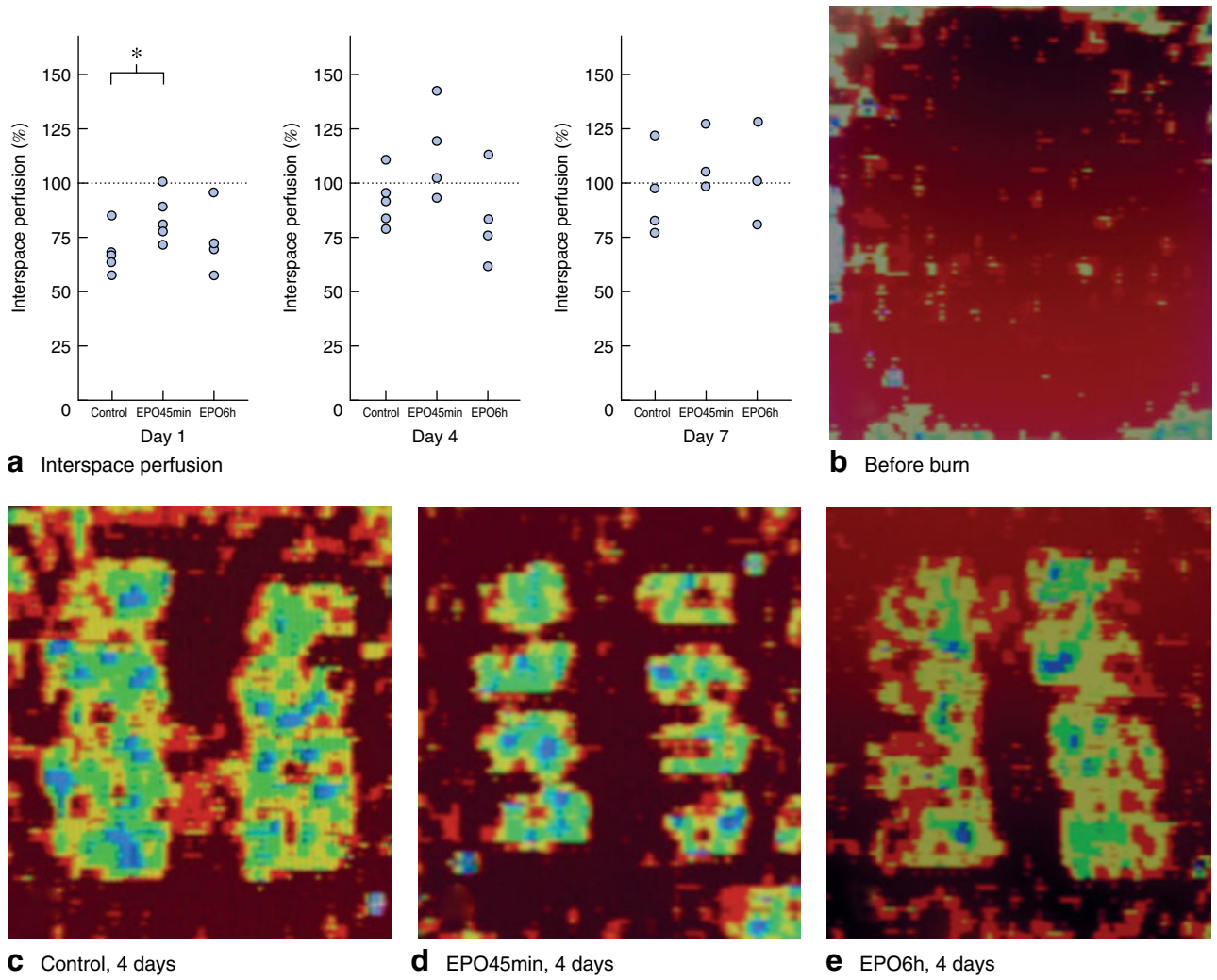

Fig. 2 **a** Scatter plot showing changes in interspace perfusion between treatment groups over time ($P = 0{\cdot}044$), a significant effect of time ($P < 0{\cdot}001$) and differences in time slopes ($P = 0{\cdot}011$) between the control group and groups that received erythropoietin (EPO) at 45 min (EPO45min) and 6 h (EPO6h) after injury (two-way ANOVA). *$P = 0{\cdot}048$ (Bonferroni *post hoc* test). **b–e** Flow pattern **b** before burn injury, and 7 days after in a control animal, and 4 days after the burn injury in **c** control, **d** EPO45min and **e** EPO6h groups. Note that interspace perfusion was maintained in animals administered EPO at 45 min, indicated by yellow and red pixels

Baar, Switzerland) for 10 min. This was followed by incubation for 60 min with primary antibody (1 : 800 rabbit polyclonal antirat/antimouse inducible NO synthase (iNOS) antibody; BD Biosciences, Heidelberg, Germany) and for 30 min with secondary antibody (rabbit EnVision™ HRP antibody; Dako). The peroxidase activity was detected by incubation with 3-amino-9-ethyl carbazole (BioGenex, Basle, Switzerland) for 10 min. Slides were counterstained with Mayer's haemalum and mounted in Aquatex® (Merck, Geneva, Switzerland).

CD31 immunostaining

Antigen retrieval was performed for 30 s in EDTA buffer at Ph 7 within a pressurized heating chamber (Dako, Glostrup, Denmark). This was followed by incubation for 60 min with primary antibody (1 : 100 goat polyclonal antimouse CD31 platelet endothelial cell adhesion molecule 1 antibody (M-20); Dako) and for 30 min with secondary antibody (1 : 150 rabbit antigoat biotin; Dako). CD31-stained sections were incubated for 5 min with Dako real peroxidase blocking solution and for 15 min with streptavidin peroxidase (Dako). The peroxidase activity was detected with diaminobenzidine for 10 min. Slides were counterstained with Mayer's haemalum and mounted in Ultrakitt™ (J. T. Baker, Deventer, the Netherlands).

Evaluation

All sections were recorded with a Zeiss® Axiophot microscope (Carl Zeiss, Jena, Germany). To assess burn depth, a pathologist blinded to the treatment group examined haematoxylin and eosin-stained sections (6 per group), and rated burn depth based on a validated scale from 1 to 5: 1, epidermis; 2, superficial dermis; 3, intermediate dermis; 4, deep dermis; and 5, muscle[12]. To assess inflammation, dilatory response and angiogenesis, three visual fields per haematoxylin and eosin-, iNOS- and CD31-stained section were selected randomly, and recorded with a charged coupled-device camera (Zeiss® Axiocam) using Axiovision software. Thereafter, leucocytes as well as iNOS- and CD31-positive vessels were quantified.

Statistical analysis

All values are expressed as mean(s.e.m.). To control for inflation of type I error probability in the course of multiple data analysis, hierarchical modelling and hypothesis testing was performed. Linear mixed regression models comprising the main-effect terms time and treatment group and the interaction term time by treatment group were used in order to perform a two-way ANOVA. If the global test of the main-effect treatment group was significant at a level of 0·05, one-way ANOVA group comparison for each time point was carried out (followed by the appropriate *post hoc* test, which considered α-error correction according to the Bonferroni method). In case of right skewed data distribution, log transformation was applied to normalize data before applying the respective ANOVA model, and back-transformed marginal means and confidence intervals were provided as effect measures. To adjust for baseline group differences in

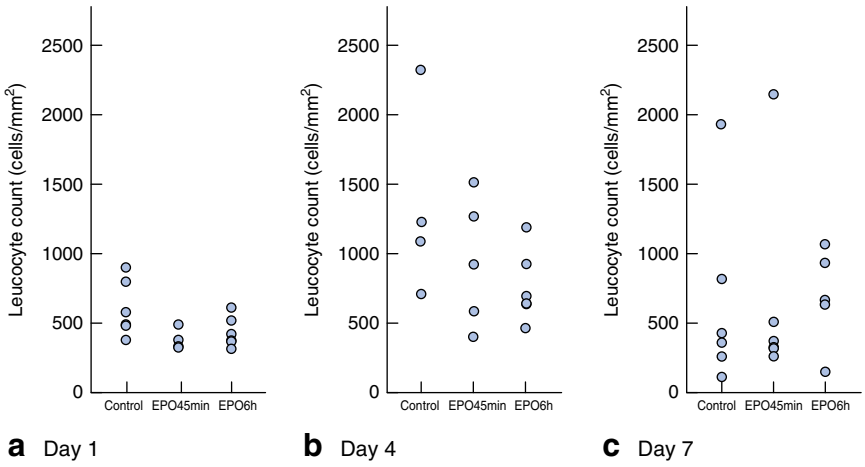

Fig. 3 Scatter plot showing distribution of leucocyte counts on **a** day 1, **b** day 4 and **c** day 7 in the control group and groups that received erythropoietin (EPO) at 45 min (EPO45min) and 6 h (EPO6h) after injury. There was a significant change in leucocyte count over time ($P = 0{\cdot}005$) indicating an anti-inflammatory effect, but no difference in leucocyte levels between groups ($P = 0{\cdot}499$) (two-way ANOVA)

the statistical analysis, ANOVA models were extended with the respective baseline co-variable. Group differences in ordinal data at a single time point were assessed by means of the non-parametric Kruskal–Wallis test followed by Bonferroni-adjusted pairwise comparisons using the Mann–Whitney *U* test. Statistical testing was done with SPSS® (IBM, Armonk, New York, USA).

Results

Burn depth and interspace necrosis

Histological evaluation of burn depth 1 h after burn induction showed consistent and uniform damage to the epidermis and superficial dermis in all animals (score 2·0(0·0) for control, EPO45min and EPO6h). By day 7, progression to full-thickness burn was observed both in controls and in animals administered EPO 6 h after thermal injury, whereas administration of EPO 45 min after the burn limited the damage to the intermediate dermis (burn depth score 5·0(0·0), 3·3(0·6) and 4·7(0·3) for control, EPO45min and EPO6h respectively; $P = 0·026$). Interspace necrosis developed during the first 4 days in all animals (*Fig. 1a*). Administration of EPO resulted in a significantly reduced surface extension of tissue necrosis that was time-dependent: 78(4), 45(8) and 65(4) per cent on day 7; $P = 0·017$) (*Fig. 1*).

Interspace perfusion

Perfusion within the interspace (the ischaemic zone surrounding the thermally injured tissue) decreased substantially in all animals 24 h after burn induction (day 1: 67(2), 86(3) and 73(4) per cent for control, EPO45min and EPO6h respectively; $P = 0·043$) (*Fig 2a*). Thereafter, interspace perfusion increased steadily to reach baseline values by day 4 in the EPO45min group (114(5) per cent *versus* 85(6) and 91(3) per cent for EPO6h and control respectively; $P = 0·096$). Preburn perfusion was re-established by day 7 in the EPO6h group but never completely achieved in controls (day 7: 94(4) per cent for control *versus* 110(4) per cent for EPO45min and 105(7) per cent for EPO6h) (*Fig. 2*).

Haematocrit

The haematocrit in EPO-treated animals significantly increased compared with baseline; however, it was not significantly different from that in untreated controls up to 7 days following the burn, after five consecutive administrations of EPO (*Table 1*).

Table 1 Quantitative analysis of haematocrit

	Baseline	Day 1	Day 4	Day 7	Week 2
Control	38(1)	38(1)	42(2)	40(0)	36(1)
EPO45min	37(1)	37(2)	45(2)*	49(2)*†	47(3)*†
EPO6h	40(1)	40(1)	43(1)*	53(0)*†	45(1)*†

Values are mean(s.e.m.) haematocrit values in the control group and groups that received erythropoietin at 45 min (EPO45min) and 6 h (EPO6h) after burn injury ($n = 7$ per group). *$P < 0·050$ *versus* baseline, †$P < 0·050$ *versus* control (Bonferroni *post hoc* test).

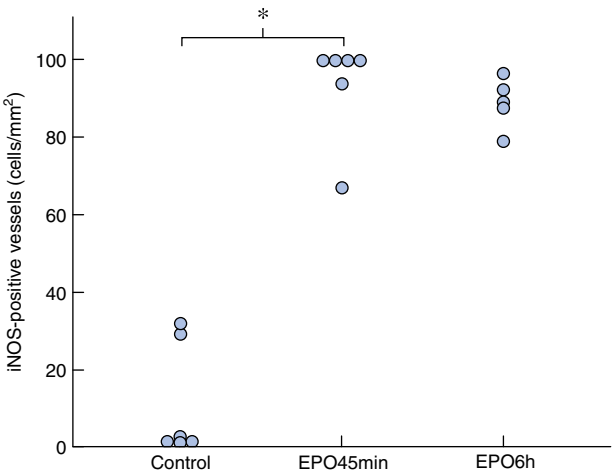

Fig. 4 Scatter plot showing inducible nitric oxide synthase (iNOS)-positive vessels on day 1 in the control group and groups that received erythropoietin (EPO) at 45 min (EPO45min) and 6 h (EPO6h) after injury. *$P = 0·001$ (Bonferroni *post hoc* test)

Leucocyte count

The tissue of control animals developed an inflammatory response to thermal injury and ischaemia with leucocyte extravasation into the interstitial tissue. This response was already present on day 1 and peaked on day 4. EPO administration decreased this response (day 1: 635(97) cells/mm^2 for control *versus* 380(32) cells/mm^2 for EPO45min and 403(34) cells/mm^2 for EPO6h; $P = 0·499$) (*Fig. 3*).

Inducible nitric oxide synthase

EPO significantly increased iNOS expression following administration (day 1: 10(6) stained vessels/mm^2 for control *versus* 93(6) and 87(3) stained vessels/mm^2 for EPO45min and EPO6h respectively; $P = 0·001$) (*Fig. 4*).

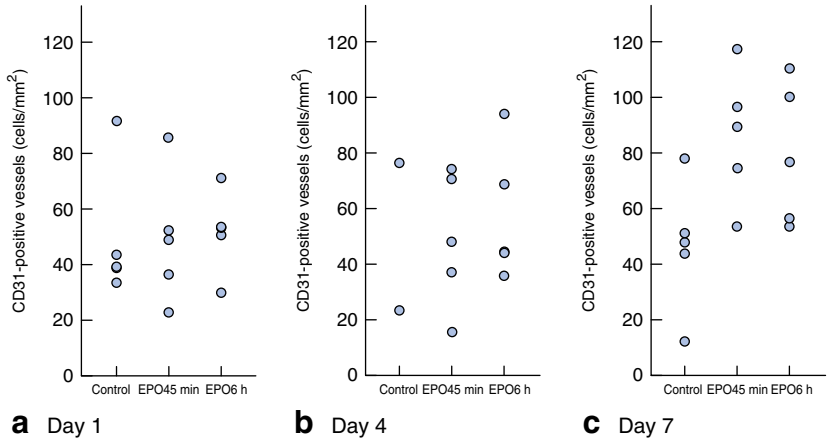

Fig. 5 Scatter plot showing the distribution of CD31-positive vessels on **a** day 1, **b** day 4 and **c** day 7 in the control group and groups that received erythropoietin (EPO) at 45 min (EPO45min) and 6 h (EPO6h) after injury. There was a significant increase in vessels over time indicating a late angiogenic effect ($P = 0{\cdot}049$, two-way ANOVA), but no statistically significant group heterogeneity ($P = 0{\cdot}301$, Bonferroni *post hoc* test)

Angiogenesis

A significant increase in CD31 protein expression that correlated with an angiogenic response was observed from day 7 in EPO-treated animals, irrespective of administration time (47(10) stained vessels/mm^2 for control *versus* 87(11) and 80(11) stained vessels/mm^2 for EPO45min and EPO6h respectively; $P = 0{\cdot}014$) (*Fig. 5*).

Healing time and wound contracture

Burn wounds of animals treated with EPO 45 min after the burn, but not after 6 h, healed significantly faster than those in control animals (7·3(0·7) weeks for EPO45min *versus* 10·8(0·5) weeks for control and 11·5(1·0) weeks for EPO6h; $P = 0{\cdot}020$). The contracture rate was also significantly reduced with EPO45min (23(4) per cent *versus* 33(2) per cent for control and 27(3) per cent for EPO6h; $P = 0{\cdot}024$).

Discussion

The main finding of the present study was that secondary burn progression in the rat burn comb model was reduced by the administration of EPO in a time-dependent manner. Administration of EPO 6 h after burn injury was too late to prevent progression to a deep lesion. In contrast, EPO treatment 45 min after burn injury significantly attenuated burn progression and eventual tissue necrosis. This was attributed to early restoration of blood flow in the critically perfused zone of stasis. The observed anti-inflammatory and angiogenic effects of EPO did not seem to be involved in the containment of burn-induced tissue damage.

Depending on the level of energy absorption, irreversible tissue damage results immediately after burn injury. Within the first few days, the limit between this coagulated, necrotic tissue and the underlying, still viable dermis is fluent and the damage can extend. Basically, tissue coagulation leads to interruption of microcirculatory blood flow, and neighbouring vessels are partly damaged and thrombosed, resulting in an ischaemic zone (zone of stasis) surrounding the necrotic core. Within this zone of stasis, mechanical and rheological disturbances facilitate leucocyte–endothelial interaction, and trigger complement and coagulation cascades. Vascular permeability is increased, causing oedema[13]. The supply of oxygen and other nutrients becomes critical, and metabolic, potentially toxic byproducts accumulate. This highly complex, chaotic interplay of different pathophysiological pathways can lead to an extension of the damage within the zone of stasis, which is eventually recruited into the necrotic core.

Rapid interruption of ischaemia, inflammation and coagulation to control damage and limit progression of tissue destruction should be the goal in emergency management of burn lesions. Early EPO treatment 45 min after a burn injury significantly reduced secondary progression in this experimental model and thus limited the expansion of burn depth to the level of the intermediate dermis. Early EPO treatment reduced the development of necrosis on the skin surface, suggesting that these tissue-protective effects are the result of an early re-establishment of interspace perfusion. As necrosis demarcates within

4 days following burn trauma, perfusion has to be re-established within this window.

The concept of the zone of stasis in burns can be compared with the penumbra in acute ischaemic stroke[14,15], defined as functionally impaired, yet still viable tissue surrounding the ischaemic core. This condition is potentially salvageable by intravenous administration of tissue plasminogen activator, but only if treatment is initiated within the first 3–6 h[16,17], although lesions continue to evolve for 90 days[18]. A meta-analysis described the suitability of EPO as a novel candidate drug for stroke to reduce infarct size and improve functional recovery; EPO administration within the first 6 h was more effective than later initiation of treatment[19].

In the present study, independently of the timing of administration, two well known EPO-mediated effects were observed the day after burn induction within the critical perfused zone: upregulated iNOS expression and a decreased leucocyte count. iNOS is the enzyme that synthesizes NO, one of the most important signalling molecules in the cardiovascular system that induces vasodilatation through smooth muscle cell relaxation, among other functions[20]. Inhibition of neutrophil adherence resulting in a decreased leucocyte count has also been demonstrated to improve microvascular perfusion in the zone of stasis following burn injury[21,22]. In contrast to administration at 45 min after burn injury, giving EPO after 6 h did not re-establish blood flow within the critically perfused zone. There may be a threshold duration of critical perfusion that cannot be reversed by EPO despite its vasodilatory and anti-inflammatory effects. Similar time-dependent effects of EPO have been observed previously in critically perfused skin flap tissue where administration before flap preparation (induction of ischaemia) significantly reduced necrosis owing to NO-mediated, improved microvascular perfusion[23]. In contrast, EPO administration after induction of ischaemia did not improve tissue survival despite a significant reduction in inflammatory response. A low dose of EPO was chosen for the present study to prevent effects on haematocrit, as shown before[23], thus obviating any rheological effect from increased blood viscosity that could counteract the beneficial effects of EPO on microcirculatory perfusion.

Angiogenesis, another well known EPO-mediated effect, was observed in the present study by detection of CD31-positive vessels after 7 days. This was after definite demarcation of the necrosis, which occurred within 4 days, and consequently did not influence burn progression. Therefore, EPO-induced angiogenesis did not play a role in containment of secondary burn progression in this rat burn comb model. This is in contrast to previous work of Galeano and colleagues[24] that showed a tissue protective effect of 400 units/kg EPO for 14 days starting 3 h following injury in a murine burn wound model. They observed increased microvascular density secondary to a vascular endothelial growth factor-associated angiogenic effect, which increased the re-epithelialized burn area and reduced the time to wound closure. It was postulated that angiogenesis, detected as an increased number of CD31-positive cells, might play an important role in EPO treatment of thermal injuries of the skin.

Other positive effects of EPO on wound healing have been shown previously in ischaemic wounds in rats[25] and diabetic mice[26], mainly as a result of neovascularization and improved dermal regeneration. As beneficial results in the present study were seen only in the EPO45min group, and were probably unrelated to angiogenesis, additional experiments at the molecular level are needed to understand the wound healing process in burn injuries in more detail.

Although burns evolve during the first few days following thermal injury, there appears to be a critical window during which systemically applied EPO could ameliorate progressive ischaemia and consequently prevent burn deepening. Although EPO is expensive, its early use could reduce the high cost of managing patients with serious burn injury.

Acknowledgements

M.T. and Y.H. contributed equally to this article. The authors thank Professor Brigitte Pittet-Cuénod (Division of Plastic, Reconstructive and Aesthetic Surgery, Geneva University Hospitals, Geneva, Switzerland) for her support; Joan Stalder, Carole Verdan, Patricia Gindre, Corinne Zwicki and Dr Elisabeth Tschanz (Department of Pathology, Geneva University Hospitals) for their pathology work; and Anton Haselbeck (Roche Diagnostics, Penzberg, Germany) and Pierre-Alain Delley (Roche Pharma Schweiz, Reinach, Switzerland) for drug supply. This study was supported by the Swiss National Science Foundation (grant no. 32003B-108408).
Disclosure: The authors declare no conflict of interest.

References

1 Singer AJ, Dagum . Current management of acute cutaneous wounds. *N Engl J Med* 2008; **359**: 1037–1046.
2 Jackson DM. The diagnosis of the depth of burning. *Br J Surg* 1953; **40**: 588–596.

3 Cioffi WG. What's new in burns and metabolism. *J Am Coll Surg* 2001; **192**: 241–254.
4 Erslev A. Humoral regulation of red cell production. *Blood* 1953; **8**: 349–357.
5 Haroon ZA, Amin K, Jiang X, Arcasoy MO. A novel role for erythropoietin during fibrin-induced wound-healing response. *Am J Pathol* 2003; **163**: 993–1000.
6 Rezaeian F, Wettstein R, Egger JF, Sandmann F, Rucker M, Tobalem M *et al*. Erythropoietin-induced upregulation of endothelial nitric oxide synthase but not vascular endothelial growth factor prevents musculocutaneous tissue from ischemic damage. *Lab Invest* 2010; **90**: 40–51.
7 Ribatti D, Presta M, Vacca A, Ria R, Giuliani R, Dell'Era P *et al*. Human erythropoietin induces a pro-angiogenic phenotype in cultured endothelial cells and stimulates neovascularization *in vivo*. *Blood* 1999; **93**: 2627–2636.
8 Yatsiv I, Grigoriadis N, Simeonidou C, Stahel PF, Schmidt OI, Alexandrovitch AG *et al*. Erythropoietin is neuroprotective, improves functional recovery, and reduces neuronal apoptosis and inflammation in a rodent model of experimental closed head injury. *FASEB J* 2005; **19**: 1701–1703.
9 Calvillo L, Latini R, Kajstura J, Leri A, Anversa P, Ghezzi P *et al*. Recombinant human erythropoietin protects the myocardium from ischemia–reperfusion injury and promotes beneficial remodeling. *Proc Natl Acad Sci U S A* 2003; **100**: 4802–4806.
10 Regas FC, Ehrlich HP. Elucidating the vascular response to burns with a new rat model. *J Trauma* 1992; **32**: 557–563.
11 Mahajan AL, Tenorio X, Sean Pepper M, Baetens D, Montandon D, Schlaudraff KU *et al*. Progressive tissue injury in burns is reduced by rNAPc2. *Burns* 2006; **32**: 957–963.
12 Yuan J, Wu C, Holland AJ, Harvey JG, Martin HC, La Hei ER *et al*. Assessment of cooling on an acute scald burn injury in a porcine model. *J Burn Care Res* 2007; **28**: 514–520.
13 Hettich R. Interactions between local and generalised burn oedema. *Resuscitation* 1986; **14**: 105–112.
14 Astrup J, Siesjö BK, Symon L. Thresholds in cerebral ischemia – the ischemic penumbra. *Stroke* 1981; **34**: 723–725.
15 Bandera E, Botteri M, Minelli C, Sutton A, Abrams KR, Latronico N. Cerebral blood flow threshold of ischemic penumbra and infarct core in acute ischemic stroke: a systematic review. *Stroke* 2006; **37**: 1334–1339.
16 Selvaratnam R, Srirangalingam U, Ahmad S, Goulden P. Thrombolysis beyond 3 hours for acute ischaemic stroke. *Emerg Med J* 2009; **26**: 756–757.
17 Ringleb PA, Schellinger PD, Schranz C, Hacke W. Thrombolytic therapy within 3 to 6 hours after onset of ischemic stroke: useful or harmful? *Stroke* 2002; **33**: 1437–1441.
18 Gaudinski MR, Henning EC, Miracle A, Luby M, Warach S, Latour LL. Establishing final infarct volume: stroke lesion evolution past 30 days is insignificant. *Stroke* 2008; **39**: 2765–2768.
19 Minnerup J, Heidrich J, Rogalewsky A, Schäbitz WR, Wellmann J. The efficacy of erythropoietin and its analogues in animal stroke models: a meta-analysis. *Stroke* 2009; **40**: 3113–3120.
20 Strijdom H, Chamane N, Lochner A. Nitric oxide in the cardiovascular system: a simple molecule with complex actions. *Cardiovasc J Afr* 2009; **20**: 303–310.
21 Mileski W, Borgstrom D, Lightfoot E, Rothlein R, Faanes R, Lipsky P *et al*. Inhibition of leukocyte–endothelium adherence following thermal injury. *J Surg Res* 1992; **52**: 334–339.
22 Choi M, Rabb H, Arnaout MA, Ehrlich HP. Preventing the infiltration of leucocytes by monoclonal antibody blocks the development of progressive ischemia in rat burns. *Plast Reconstr Surg* 1995; **96**: 1177–1185.
23 Rezaeian F, Wettstein R, Amon M, Scheuer C, Schramm R, Menger MD *et al*. Erythropoietin protects critically perfused flap tissue. *Ann Surg* 2008; **248**: 919–929.
24 Galeano M, Altavilla D, Bitto A, Minutoli L, Calo M, Lo Cascio P *et al*. Recombinant human erythropoietin improves angiogenesis and wound healing in experimental burn wounds. *Crit Care Med* 2006; **34**: 1139–1146.
25 Buemi M, Galeano M, Sturiale A, Lentile R, Crisafulli C, Parisi A *et al*. Recombinant human erythropoietin stimulates angiogenesis and wound healing of ischemic skin wounds. *Shock* 2004; **22**: 169–173.
26 Galeano M, Altavilla D, Cucinotta D, Russo GT, Calò M, Bitto A *et al*. Recombinant human erythropoietin stimulates angiogenesis and wound healing in the genetically diabetic mouse. *Diabetes* 2004; **53**: 2509–2517.