ELSEVIER

Burns 31 (2005) 459–464

BURNS

www.elsevier.com/locate/burns

Endothelial thrombomodulin (CD 141) in a rabbit burn model

Silvester von Bülow *, Th. Hartmann, P.C. Fuchs, C. Schrimpf, N. Pallua

Klinik fuer Plastische Chirurgie, Hand- und Verbrennungschirurgie, Universitaetsklinikum Aachen, Pauwelsstrasse 30, 52074 Aachen, Germany

Accepted 16 November 2004

Abstract

Thrombomodulin (CD 141) is an endothelial surface transmembrane glycoprotein. It is involved in the activation of protein C in the inactivation of thrombin. In severe sepsis CD 141 is shed from the endothelial surface. This leads to disseminated intravascular coagulation (DIC), disturbed organ functions and multi organ failure (MOF). In this study, we investigated if endothelial bound thrombomodulin is shed in thermal injuries.

Material and methods: In 10 New Zealand white rabbits full thickness and superficial partial thickness burns were produced. Dermal blood flow was analyzed by measuring the fluorescence of intravenously injected indocyanine green. Skin-biopsies were taken from the burn wounds from the zones of stasis between full thickness burns and from unburned skin 72 h post burn. Specimens were processed for immunoperoxidase staining using a specific monoclonal antibody against CD 141.

Results: Dermal blood flow was reduced in burned skin areas and in the zones of stasis. Thrombomodulin was only detectable on the surface of capillary endothelial cells in specimens taken from unburned skin areas. No thrombomodulin was detectable in specimens taken from burn wounds or from the zones of stasis. Thus, shedding of thrombomodulin was detectable in areas with reduced dermal blood flow.

Conclusion: Thermal injuries affect the dermal endothelial surfaces resulting in a shedding of thrombomodulin. This mechanism might be involved in the development of progressive skin damage in the zone of stasis. Disseminated intravascular coagulation following inactivation of thrombomodulin might lead to multiple organ dysfunctions in severe burn injuries.

Keywords: Thermobomodulin; Activated protein C; CD 141; Dermal blood flow; Burn wound

1. Introduction

Thrombomodulin is an endothelial surface transmembrane glycoprotein. It plays a central role in the activation of protein C and initiation of the activated protein C (APC) anticoagulant pathway. At the same time thrombomodulin binds thrombin, directly inhibiting its clotting and cell activation potential. Thrombomodulin also has direct anti-inflammatory activity, minimizing cytokine formation in the endothelium and decreasing leukocyte–endothelial cell adhesion [1]. Vascular endothelial damage alters these functions of thrombomodulin. The state of generalized inflammatory host response in sepsis leads to shedding of thrombomodulin from the endothelial surface. The activity of thrombomodulin is reduced resulting in a complex imbalance of pro- and anticoagulant pathways followed by disseminated intravascular coagulation. Multi organ failure (MOF) in sepsis was shown to be caused by the inactivation of thrombomodulin.

Symptoms similar to those in severe sepsis can be observed in major burn injuries. In this experimental study, we investigated if endothelial bound thrombomodulin is affected by thermal injuries.

2. Material and methods

Ten New Zealand white rabbits (female, 2.5–3 kg) were used for the experiments. Anaesthesia was started with

* Corresponding author. Tel.: +49 241 800.
E-mail address: svonbuelow@ukaachen.de (S. von Bülow).

0305-4179/$30.00
doi:10.1016/j.burns.2004.11.021

subcutaneous administration of Ketamin® 10% (0.3 ml/kg) and Meditomidin® (0.1 ml/kg) and maintained by intravenous doses administered through a 24-gauge catheter inserted into a marginal ear vein. Burn injuries were produced at the shaved dorsum. Full thickness burns were produced by application of heated brass probes at 100 °C to the backs of the animals over 30 s. Six areas of 10 mm × 30 mm were burned with a distance of 5 mm between the areas [2,3]. Additionally, two areas of superficial partial thickness burns were produced by applying the probe for 7 s. Postoperative analgesia was ensured by 0.025 mg/kg Buprenorphine® s.c. and 15 mg/kg Paracetamol® i.v. every 12 h.

Cutaneous blood flow was visualized with the IC-View-System (Pulsion Medical Systems, Munich). Measurement of the blood flow was performed at 1, 2, 3, 4, 24, 48 and 72 h post burn in burned skin areas, zone of stasis and in normal skin (reference-area). Doses of 0.5 mg indocyanine green kg^{-1} were injected into the ear vein. Fluorescence to laser-stimulation (with a wavelength of 780 nm) was recorded with a digital video camera for 1 min after injection. Blood flow was calculated by analysis of pixel-intensity using the IC-Calc-software (Pulsion Medical Systems, Munich) [4–6]. The blood flow (fluorescence signal intensity) in burned skin areas was compared to the blood flow in unburned areas of the same animal. Results were recorded as the relative amount in percent of the blood flow at the unburned area. The calculation was done for zones of stasis, defined as the gap between two full thickness burn sites, and for burned zones separately for each animal.

The animals were sacrificed 72 h after trauma. Skin-biopsies were taken from burned skin areas, from the zone of stasis between full thickness burns and from unburned skin. Samples were fixed in neutral buffered formalin and embedded in paraffin. Micron sections were deparaffinized and processed for immunoperoxidase staining. A monoclonal mouse anti-rabbit antibody specifically binding to thrombomodulin (Acris, Hiddenhausen, Germany Clone: QBEND/40 Mouse IgG2a) was used at a 1:10 dilution according to the manufacturers datasheet. The DAKO LSAB+ System HRP (DAKO Corporation, Carpinteria, CA, USA) was used for immunoperoxidase reaction. In brief, endogenous peroxidase activity was quenched by peroxide block for 5 min. After washing, non-specific binding sites were blocked with protein block (serum free) for 5 min, followed by incubation with primary antibody (1:10 dilution) for 45 min. Slides were rinsed and a biotinylated secondary antibody was added for 20 min at RT. Following a washing step, cells were incubated in streptavidin–horseradish peroxidase conjugate for 5 min. After rinsing again AEC substrate was added for 10 min at RT. For counterstaining Mayer's Hematoxylin was used for 2 min. Histological analysis was done by two independent examiners. The slices were judged for dermal capillaries, showing positive staining for thrombomodulin at 100 times enlargement. Histological results are descriptive.

Table 1
Percentage of relative blood flow (mean ± S.D.) in full thickness burns

Time post burn (h)	Percentage of relative blood flow
1	54.1 ± 6.7
2	30.2 ± 13.0
3	22.2 ± 11.7
4	27.1 ± 13.1
24	29.5 ± 12.7
48	42.5 ± 9.6
72	57.5 ± 15.3

3. Results

3.1. Analysis of cuntaneous blood flow

In full thickness burns blood flow was decreased to 40–50% 1 h post burn in all animals. The blood flow further decreased to a minimum 3 h post burn, being reduced to approximately 20–30% compared to normal skin. Afterwards the blood flow slightly increased up to 72 h post burn. In full thickness burns it never exceeded 60% compared to unburned skin (Table 1).

In the zone of stasis slight hyperaemia with a mean blood flow of 105% of the flow in normal skin was detected 1 h post burn (Table 2). Afterwards the perfusion in the zone of stasis decreased down to 23.6 ± 9.0% compared to unburned skin at 4 h post burn. In the further time course blood flow increased again up to 33.7 ± 17.0% after 24 h and up to 82.2 ± 34.2 at 72 h post burn.

3.2. Histological findings

Positive immunoperoxidase staining specific for thrombomodulin was detectable in all specimens taken from unburned skin samples (Fig. 1). Dermal capillary vessels in these samples show a red colour of the cytoplasm of endothelial cells. Samples taken from the zone of stasis between full thickness burn injuries showed no positive peroxidase staining for thrombomodulin (Fig. 2). Despite blocking of unspecific peroxidase activity in these

Table 2
Percentage of relative blood flow (mean ± S.D.) in the zones of stasis

Time post burn (h)	Percentage of relative blood flow
1	105.4 ± 26.4
2	33.2 ± 12.5
3	26.4 ± 14.0
4	23.6 ± 9.0
24	33.7 ± 17.0
48	60.5 ± 21.8
72	82.2 ± 34.2