General introduction
INTRODUCTION

Burns are a significant problem worldwide. According to a report of the World Health Organisation (September 2016) accounting for an estimated 265 000 burn-related deaths annually. Burns can result in fatal complications, including shock and infection as well as severe emotional and psychological distress because of long-term hospitalization, scarring and deformity [1]. Especially severe burns often induce a massive and long-term inflammatory response and hypercoagulability both systemic and locally in the burn wound that not only can negatively affect the wound healing but also may result in systemic multiple organ dysfunction [2].

This thesis describes the crucial role of acute inflammation, coagulation and oxidative stress in the pathophysiology of burns.

I: The role of complement in the acute phase response after burns

Severe burn injuries induce a complex systemic inflammatory response characterized by a typical prolonged acute phase response (APR) that persists for months up to a year after the initial burn trauma. Although the inflammatory response initially is indispensable for proper wound healing, in burn victims it can be over-activated. As such, it can contribute to secondary wound expansion, excessive scarring and can exert systemic effects, including inflammation in the heart, that can result in secondary organ failure and hence be life threatening [3]. As part of the acute phase response (APR), the complement system has been shown to negatively affect the local pathophysiology of the burn wound, but also to have negative systemic effects in burned patients [2, 4, 5]. It is therefore of utmost importance to obtain better insight into the role of the APR and the complement system after burns. In Chapter 2 we discuss the current knowledge of this post-burn APR, including its local and systemic pathophysiological effects, in more detail, with a specific focus on complement.

Systemically, complement activation was shown to relate to the severity of burn trauma, clinical outcome [6-8], post-burn sepsis and organ failure [2]. Therefore, complement might be a promising target for therapy in burn wound patients. Indeed previous pre-clinical studies in rats and pigs showed that complement inhibition by the natural (endogenous) inhibitor C1 esterase inhibitor (C1inh) resulted in reduced capillary leakage, wound depth and scarring [9-11], supporting the pathogenic involvement of complement in burn wounds as well as the therapeutic potential of C1inh in burns. However, the pathophysiology of the burn wound is still not fully understood, including the role of inflammatory mediators herein [6-8,12]. In order to optimize therapeutic strategies targeting the complement system, a more detailed knowledge regarding complement blood levels and their relation with complement levels and the cellular inflammatory response locally in the burn wound as well as systemically in other
organs is warranted in a model close to humans. Therefore, in Chapter 3 we studied the effects of burn wounds on complement levels and inflammatory cell infiltration in the burn wound (local effects), as well as in blood and several organs, including the heart, lungs, liver, and kidney (systemic effects) in time after burn wound induction in pigs.

Burn wounds also induce long-term C-reactive protein (CRP) blood levels that persist for up to months [13,14]. In children it was shown that post-burn CRP blood levels positively correlated with burn size [14]. Previous studies in non-burn patients have shown that changes in CRP levels can differ between adults and children [15]. Therefore, in Chapter 4a we studied whether in adults there is a correlation between burn-induced CRP blood levels and burn wound size in time up to 30 days post-burn.

In the clinic, C1inh has already been used in patients with hereditary angioedema (HAE) [16-22], acute myocardial infarction (AMI) [23-25] and sepsis [26-29]). However, in patients with burn wounds, C1inh has never been used therapeutically before. In order to determine the most optimal application scheme of C1inh in time, in relation with patients with burn wounds, more detailed knowledge about the post-burn levels of natural (endogenous) C1inh in patients with burn wounds is warranted. For this, post-burn C1inh protein levels and C1inh activity were determined in time in patients with burn wounds as described in Chapter 4b.

Previous studies determining the therapeutic effects of C1inh administration in animals with burn wounds focused mostly on the short-term (i.e. up to the first 96 hours post-burn) effects of complement inhibition [10,11]. However, in animals and in humans, the post-burn complement blood or tissue levels were shown to be elevated up to months after burn injury [8-11]. We have found in a rat burn wound model that daily administration of C1inh for a longer time (i.e. until 14 days post-burn), resulted in reduced granulation tissue formation and reduced infiltration of macrophages in the burn wound and showed beneficial effects on wound healing parameters (e.g. reepithelialization and tissue destruction) [9]. This indicates that C1inh can have positive long-term effects on burn wound healing. Subsequently, in Chapter 5 the effects of long-term treatment with complement inhibitor C1inh on post-burn inflammation and wound healing parameters were analyzed in a pig burn wound model in time up to 60 days post-burn.

II: The role of coagulation and oxidative stress in the pathophysiology of burns

Patients suffering from burn injuries endure lifelong consequences, due to loss of vital tissue and subsequent defective healing resulting in extensive scarring. The severity of these consequences is determined by multiple factors, including the depth of the wound and the percentage of body surface affected [30]. A significant proportion of the tissue loss can be caused by secondary expansion of necrosis into the surrounding vital dermis neighboring the initial burn injury, leading to an increased
burn depth and area [28]. As such, partial thickness burns often convert into deeper or full thickness burns, a process called “burn wound conversion” [31]. A potentially important contributing factor is the severe loss of perfusion in the center and the border zone (i.e. zone of stasis) of the burn wound [32]. This loss of perfusion may delay clearance of dead tissue and leads to ischemia, which in turn result in delayed wound healing, wound expansion and excessive scarring. This damage is probably instigated by a combination of both the intense local inflammatory responses and the burn-induced hypercoagulation [31,32]. However, the exact mechanisms underlying this burn-induced microangiopathy and intravascular thrombosis are not fully understood yet.

An important feature of local complement activation is its chemotactic recruitment of inflammatory cells, such as neutrophilic granulocytes, to sites of injury [33,34]. Neutrophilic granulocytes are thought to be essential mediators of microvascular damage in the zone of stasis [35] and are the first inflammatory cells known to infiltrate the wound after burn trauma and persist for weeks post-burn [36]. In 2004, a novel strategy of neutrophils was introduced, whereby so-called Neutrophilic Extracellular Traps (NETs) are formed. NETs are assembled through an active, generally suicidal process called NETosis, whereby neutrophils eject net-like structures consisting of DNA and histones, and a number of different anti-microbial and pro-inflammatory granular proteins [37,38]. NETs are essential in the innate immune response against microbial infection as they trap and neutralize microbial pathogens [37]. However NETosis also occur independent of infection and was recently identified as a possible link between sterile inflammation and thrombosis [39]. In Chapter 6 post-burn intravascular thrombosis, NETs formation and presence of pro-coagulant tissue factor (TF) in the microvasculature of burned skin in time after burn trauma is described in animal models (rat and pig) and patients.

Thrombosis not only is related to NETs, but is also induced via reactive oxygen species (ROS). Previously we have shown that high levels of homocysteine (Hcy) not only promote oxidative stress in the vasculature [51-55], but also thrombosis [56,57]. Hcy namely can induce tissue factor (TF) expression, initiating a pro-coagulant state [58,59]. Several studies have suggested a role for NADPH oxidase (NOX) as an important source of ROS, in Hcy-induced endothelial injury and/or dysfunction [61-63]. In addition, we showed in ischemic endothelial cells in the heart that loss of serine protease dipeptidyl peptidase IV (DPP4) expression was correlated to induction of tissue factor (TF) expression. Since Hcy can initiate thrombosis through the induction of TF expression, we evaluated in Chapter 7 whether the inversely relation of TF and DPP4 is also Hcy dependent and whether NOX-mediated ROS is possibly playing a role herein.

In addition to intravascular thrombosis, extravascular thrombosis also occur in burn wounds. In non-burn wound skin injuries extravasation of erythrocytes occurs after damaging blood vessels that is followed by extravascular coagulation [40]. We hypothesized that activation of the coagulation cascade
proteins Fibronectin, P-selectin (CD62p) and Factor VIII [41-50] play an important role herein, albeit exact knowledge of its activation in time is lacking. For this in Chapter 8 we have analyzed the time frame of extravascular coagulation activation in non-burn skin injury.

Successful wound healing of burn injuries needs coordination of multiple cellular processes including control of cell apoptosis and activation of keratinocytes [64]. In response to epidermal injuries, keratinocytes undergo proliferation, migration, differentiation and apoptosis [65]. Keratinocytes are a major cellular component of the epidermis, and are responsible for restoring the epidermis after injury, through a process termed reepithelialization. As such, reepithelialization is a fundamental part of wound healing also in burn wounds [66]. The NOX enzyme complexes are known to play important roles in host defence, inflammation, signal transduction, gene expression, cell death, cell growth, and wound healing [67]. However, knowledge concerning the role of NOX2-mediated signalling in keratinocytes, especially in burn wound repair, is limited and relies mainly on studies conducted in other skin diseases. Therefore, in Chapter 9 we have studied the possible role of NOX2 in keratinocytes in the skin after burns.

REFERENCES


