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Cellular and Molecular Mechanisms in Hypertrophic Scar Formation

van der Veer, W.M.

2011

document version

Publisher's PDF, also known as Version of record

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citation for published version (APA)

van der Veer, W. M. (2011). *Cellular and Molecular Mechanisms in Hypertrophic Scar Formation: Potential Targets for Therapy*.

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SUMMARY

Research into the biological nature of scars has led to an increased understanding of mechanisms in hypertrophic scar formation, which are described in **chapter 2**. This resulted in the development of more specific therapeutic options. Despite this, hypertrophic scars remain difficult to treat. In **chapter 3** multiple therapies and studies are discussed. The aim of this thesis research was to identify factors, both at a clinical and molecular level, that are involved in human hypertrophic scar formation, to provide potential targets for new therapies. For this we employed several studies investigating patient characteristics and patterns of gene and protein expression of various cytokines and chemokines in scar tissue samples that are associated with hypertrophic scar formation.

In **chapter 4**, we described the incidence of hypertrophic scar formation and its association with several patient characteristics in standardized wound healing models. In our series 60% of patients developed a hypertrophic scar after surgery, typically during the first three months after surgery. Most hypertrophic scars that were present three months after surgery were still hypertrophic after 12 months. Young, non-smoking patients were most susceptible for hypertrophic scar formation. These data contribute to the determination of a population that is at high-risk for developing a hypertrophic scar, and underline the importance of stratifying for age and smoking when analyzing experimental studies on the treatment of hypertrophic scars. Furthermore, in the future these data can contribute to the development of an accurate prediction model, by which clinicians can predict the scar outcome of patients.

Besides an increased incidence in predisposed individuals and susceptible regions of the body, hypertrophic scar formation occurs more often after delayed reepithelialization. Although it is mainly described as a disorder of the dermis, histologic evaluation of hypertrophic scar tissue has also revealed epidermal abnormalities. These abnormalities strongly resemble epidermal alterations observed in the skin disorder psoriasis. Psoriasis can be effectively treated with topical application of calcipotriol, a synthetic derivative of vitamin D. We hypothesized that topical application of calcipotriol could prevent hypertrophic scar formation by altering the biochemical properties of the epidermis. Therefore, a randomized, double-blind, placebo-controlled trial was performed to investigate the preventive effect of topical calcipotriol use on hypertrophic scar formation, and to investigate the biochemical properties of the epidermis during hypertrophic scar formation (**chapter 5**). We established that topical application of calcipotriol during the first 3 months of wound healing does not affect the incidence of hypertrophic scar formation. Furthermore, we observed a strong association between keratinocyte activation and hypertrophic scar formation. Because the activated phenotype is generally present only during the inflammatory phase of wound healing, it seems that a prolonged inflammatory phase is an important prerequisite for, or sign of, hypertrophic scar formation.

Evidence is increasing that immunologic responses manifested shortly after wounding play an important role in hypertrophic scar formation. Corticosteroids are widely used as treatment for excessive scarring by intralesional injection. The use

of corticosteroids to prevent hypertrophic scar formation seems rational, and it is conceivable that systemically administered corticosteroids affect a wider range of inflammatory processes that influence wound healing and may therefore be more successful in preventing hypertrophic scar formation. To study this assumption we conducted a prospective cohort study in patients undergoing cardiothoracic surgery through a median sternotomy incision (**chapter 6**). Dexamethasone was administered systemically at high dose before and after cardiac surgery that required the use of cardiopulmonary bypass. The presternal scars were clinically evaluated, measured and scored as normotrophic or hypertrophic up to 52 weeks postoperatively. We observed no statistically significant differences in incidence of hypertrophy when comparing patients who did and who did not receive dexamethasone perioperatively. However, scars became significantly wider and thicker in the dexamethasone group compared with the control group. These differences in scar width and thickness were mainly present in patients that developed hypertrophic scars. These findings contribute to the concept of the involvement of perioperative immunologic responses in the etiology of hypertrophic scar formation. An explanation for our findings could lie in the effect of the cardiopulmonary bypass, which activates the inflammatory response, or in the effect that corticosteroids have on macrophage activation and differentiation, as corticosteroids are known to stimulate the alternative activation of macrophages.

Macrophages are considered key players in wound healing. They can display different functional phenotypes. The currently most widely used classification scheme for the activation of macrophages defines classically activated macrophages as M1, and the group of non-classically activated (or alternatively activated) macrophages as M2. M1 macrophages have an increased expression of CD40 and exhibit antimicrobial properties releasing inflammatory mediators including tumor necrosis factor- α , nitric oxide and interleukin (IL)-6 and IL-12. M2 macrophages produce transforming growth factor- β and IL-10 and express both mannose receptor (CD206) and haemoglobin scavenger receptor (CD163). They are involved in fibrosis. Whether the proposed phenotypes actually represent macrophage populations in the human host, and how these phenotypes are represented in the human wound during normotrophic and hypertrophic scar formation is currently unknown. Therefore, we investigated the time course of the cellular influx of inflammatory cells, and especially the macrophage phenotype, associated with normotrophic and hypertrophic scar formation (**chapter 7**). The number and distribution of CD45⁺, CD68⁺, CD40⁺, CD163⁺ and CD206⁺ cells were evaluated, and expression levels of the genes associated with classically- and alternatively-activated macrophages were investigated. In scar tissue samples developing to hypertrophic scars an increased number of macrophages was present 4 and 6 weeks postoperatively. These macrophages showed an increased expression of CD163 and CD40, which are markers for M1 and M2 macrophages, respectively. However, an association between hypertrophic scar formation and a macrophage phenotype that fits the current dogmas on macrophage activation and differentiation could not be established.

Compared with normal skin, hypertrophic scars have an increased number of blood vessels with a more dilated phenotype. Furthermore, in hypertrophic scars

the blood flow is increased as measured by laser-doppler. This implies the presence of an increased vascular density in hypertrophic scars compared with normotrophic scars. To provide further insight in, and to identify new molecular details about the angiogenic profile during normotrophic and hypertrophic scar formation, we examined the time course of the angiogenic response during hypertrophic scar formation in humans in comparison with normotrophic scar formation (**chapter 8**). In our study microvessel densities were significantly higher in the hypertrophic compared with the normotrophic scars 12 weeks and 52 weeks postoperatively. Moreover, we established that differential expression of angiopoietin-1, angiopoietin-2 and urokinase-type plasminogen activator in time is associated with an increased vascular density in hypertrophic scars compared with normotrophic scars.

In **chapter 9**, the findings described in this thesis are summarized and discussed in the context of the current developments in wound healing research. Conclusions are presented and future directions for research of wound healing and hypertrophic scar formation are proposed.