ISSN 1864-1121 74528 Vol. 6, No. 5 | 2012

Wound Management

Reprint

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Hypoxia prevents healing!

(Wound Management 2012; 5: 212–217)

REVIEW ARTICLE

Official bulletin of the

Initiative Chronische Wunden e. V. (ICW e. V.)

Österreichische Gesellschaft für Wundbehandlung

(AWA)

Schweizer Gesellschaft für Wundbehandlung (SAfW)

Wundnetz Kiel e. V.

Wundverbund Nord e. V.

Wundverbund Südwest e. V.

Wundzentrum Hamburg e. V.

Wundzentrum Nord e. V.

mhp

Verlag GmbH

Wound Management 05/2012, pp. 212–217

REVIEW ARTICLE

Chronic wounds:

Hypoxia prevents healing!

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ABSTRACT

In many chronic wounds, there is a prolonged undersupply of oxygen to the tissue (hypoxia). This rapidly leads to a slowing or stagnation of wound healing processes, since numerous molecular

processes are dependent on a sufficient supply of oxygen or are induced by reactive oxygen species (ROS). The most common chronic wounds are venous crural ulcers, foot lesions in peripheral arterial occlusive disease (PAOD) and/or diabetes mellitus and decubitus. What they all have in common is a vascular component that either promotes their development or prevents their healing through local hypoxia. If no other wound-healing inhibitory factors such as infections, hypothermia, macerations or pressure are present, local hypoxia is the pathophysiological correlate of impaired wound healing. Besides causal treatment of the primary disease to improve the supply of oxygen to the affected tissue, phase-adapted wound treatment is the basis for a positive healing prognosis. Beyond this, innovative therapeutic options are now available for wound treatment by means of an additional local improvement of the oxygen supply in the wound.

KEYWORDS

Chronic wound, hypoxia, oxygen, wound healing, reactive oxygen species, crural ulcers, PAOD, diabetes mellitus

Hypoxia and chronic wounds

The most common chronic wounds are venous crural ulcers, foot lesions in peripheral arterial occlusive disease (PAOD) and/or diabetes mellitus and decubitus [19, 68]. What they all have in common is a vascular component that either promotes their development or prevents their healing through local hypoxia. If no other wound healing inhibitory factors such as infections, hypothermia, macerations or pressure are present, local hypoxia is the pathophysiological correlate of impaired wound healing.

Hypoxia in venous crural ulcers is caused by venous hypertension. The chronically elevated ambulatory pressure in the veins and the upstream venules causes structural changes in the blood vessels. This leads to the demise of capillaries, which becomes manifest in a reduced capillary density [44, 45], and to a retardation of the diffusion pathways for oxygen from the capillaries to the tissue cells [25, 46, 60, 84]. These pathophysiological changes are also described in the S-3 guideline "Diagnosis and therapy of venous crural ulcers" of the German Society of Phlebology [17]. Here, it is stated:

• *"Measurements of transcutaneous oxygen partial pressure above the medial ankle in patients with chronic venous insufficiency showed a reduced transcutaneous oxygen partial pressure ($tcpO_2$) compared with healthy subjects [55, 64].",*

• *"The capillary thrombosis and reduced capillary density are probably also responsible for the reduction in the oxygen partial pressure (PO_2) in the nutritive capillaries and consequently also in the $tcpO_2$ [63, 83]."*

Chronic wounds in the area of the feet are often the result of PAOD, often caused by polyneuropathy in diabetes mellitus or, in the most unfavourable case, a combination of polyneuropathy with diabetic foot syndrome and PAOD. In PAOD, the connection between oxygen deficiency and reduced arterial perfusion is obvious [11]. Depending on the degree of the reduced arterial perfusion, the hypoxia reaches a critical threshold below which wound healing is not to be expected. This stage of PAOD is termed chronic critical ischaemia in the current literature and is easy to detect with the appropriate apparatus. The current "Guidelines for the diagnosis and therapy of (PAOD)" of the German Society of Angiology then give the following threshold values below which chronic critical ischaemia is present [16].

Chronic critical ischaemia is present if any of the following criteria are fulfilled: Patient with pain at rest or a poorly healing wound and

• an absolute ankle artery pressure < 50–70 mmHg or

• an absolute toe artery pressure < 30–50 mmHg or

• a transcutaneous oxygen partial pressure < 30–50 mmHg.

In the case of pure neuropathic diabetic foot lesions, the traumatic, repetitive pressure load is at the forefront as the obvious trigger, i.e. ischaemia caused externally, whereby the patient does not sense the pressure or the ischaemia, due to anaesthesia. Apart from this, the mechanisms of reactive recovery are impaired after pressure loading. In addition, the patient's entire anthropological matrix is altered: the feet become components of the surroundings, because subjectivity is detached by the neural disturbance [70, 71].

The evidence-based guideline of the German Diabetes Society 2008 [62] states: Occluding microangiopathy as a cause of an ulceration is, however, unlikely [58, 87], even if functional microangiopathy [30, 42] with a thickening of the basal membrane and endothelial capillary swelling is detectable [87]. In addition, as a consequence of the neuropathy, there is a dysfunction in neuronal regulation of the precapillary arterioles [74]. The endothelium-dependent regulation of the vascular lumen is also affected by NO (nitric oxide). As a result, an adequate reaction in the foot, e.g. to stress or injury, via an increase in blood flow cannot be achieved [34, 66, 88]. Thus, an additional oxygen requirement for wound healing after an injury can only be met inadequately or not at all.

Oxygen and wound healing

The healing of a wound is accompanied by an increased energy metabolism of the skin and therefore requires considerably more oxygen than the normal metabolism of intact skin. In the different phases of wound healing, numerous biochemical and cellular processes are highly dependent on a sufficient supply of oxygen [8, 9, 29, 39, 73, 75, 79]. Thus, in the immune response to pathogens, reactive oxygen species (ROS), which are produced by neutrophils (granulocytes, microphages) and macrophages (histiocytes) from molecular oxygen, are released in considerable amounts. This release of ROS leads to "oxidative stress" [20]. In addition, oxygen is substantially involved, directly or indirectly, in the chemotaxis of these cells via the release of messenger substances [89]. Apart from this, cells located in the tissue, such as fibroblasts, endothelial cells and keratinocytes (epithelial cells), require a continuous and sufficient supply of oxygen to build up new functioning tissue via granulation, angiogenesis and epithelialisation. The sufficient supply of oxygen also plays a decisive role in the synthesis and especially in the maturation of functioning collagen.

Therefore, it makes sense that the status of the oxygen supply to a wound and the surrounding tissue represents an important criterion for the course of its healing.

Cleaning or inflammatory phase

In the first phase of wound healing, after an activation of the complementary system and the coagulation cascade in injured blood vessels, the immune response to infection and the removal of dead tissue by micro- and macrophages is at the forefront [58]. Histiocytes and neutrophils mobilised by chemotaxis migrate into the area of the wound, where they produce and release large amounts of ROS or use them intracellularly within the context of the "respiratory/oxidative burst".

The ROS play a central role in the immune response to infection (oxidative eradication of bacteria [5, 27, 78]) and are continuously required in order to prevent wound infection. A central enzyme in the "respiratory burst" for ROS production is NADPH oxidase (phox) [4]. This enzyme converts oxygen into hydrogen peroxide H_2O_2 and superoxide anions O_2-, whereby the oxygen requirement increases enormously (50- to 100-fold). The semi-maximal conversion rate for this reaction is at a tissue oxygen partial pressure of approx. 75 mmHg and its maximum is over 300 mmHg. But already at a partial pressure of 50 mmHg, the reaction is markedly slower and can virtually come to a stop at an oxygen partial pressure of less than 20 mmHg [1, 73, 85].

Therefore, it is not surprising that the effectiveness of the immune response to bacterial infection in wounds is inversely correlated with the oxygen partial pressure measured subcutaneously in the tissue [38]. Thus, the use of perioperative oxygen inhalations with a respective increase in oxygen partial pressure in the subcutis very effectively reduced the incidence of an infection [31]. This also means that a normoxic tissue oxygenation above around 60 mmHg counteracts the infection of wounds.

It could also be shown that die NADPH oxidase has a decisive regulatory influence on chemotaxis of neutrophils through the production of ROS [33, 48].

In addition, at a reduced concentration, ROS also function as messenger molecules for other processes. In this connection, it could be shown that, in the case of acute and transient hypoxia, the transcription factor HIF-1a (hypoxia inducible transcription factor 1a) is induced and thus operates as an oxygen sensor [14, 56]. Subsequently, interacting with other transcription factors, HIF-1a binds to the "hypoxia response elements" (HRE) in the promoter area of genes that are required in particular in the proliferation phase subsequent to the cleaning phase (e.g. genes in glucose metabolism, angiogenesis or cell proliferation) [12, 57, 59, 76, 92].

In the case of severe and persistent hypoxia, on the other hand, the HIF-independent processes are dominant. It is of paramount importance to simply keep the available cells alive, since insufficient energy (in the form of ATP) can be made available for further cellular functions, including wound healing processes [32]. As a result, the protein synthesis and cell proliferation required for wound healing is limited or stopped completely [50, 51].

Proliferation and epithelialisation phase

The cleaning and inflammation phase is followed by the proliferation and epithelialisation phase. Depending on the size of the wound, this can last for days and weeks.

For wound closure and the complete healing of the wound, processes such as the development of granulation tissue, a neo-vascularisation, the formation of an extracellular matrix (ECM) and a re-epithelialisation interact to produce a new functional tissue.

Angiogenesis:

Stimulated by cytokines and chemokines such as VEGF and TGF-ß that are released by macrophages, fibroblasts initially begin with cell proliferation, differentiation and migration in the wound [54, 89]. An important growth factor for the production of new blood vessels (angiogenesis) is VEGF [77]. Its release by macrophages, fibroblasts, endothelial cells and keratinocytes is stimulated by acute hypoxia and the ROS produced in this connection [12, 24, 49, 77, 78, 80]. Both the inward migration of the above-mentioned cells and the budding of the blood vessels is orientated on the oxygen concentration present in a wound, which declines from the wound margin (high oxygen partial pressure) to the centre of the wound (hypoxia, low oxygen partial pressure) [28, 65]. However, if a chronic undersupply of oxygen occurs as a whole, angiogenesis is impaired [26, 37]. The European Committee for Hyperbaric Medicine (ECHM) and the European Tissue Repair Society (ETRS) confirmed after a joint consensus conference [21]: "Angiogenesis is reduced to zero at tissue tensions of 10 mmHg." and: "In clinically oxygen-deficient situations, granulation tissue is not formed."

Build-up of the ECM:

In parallel to the production of granulation tissue through cell proliferation and angiogenesis, fibroblasts form a preliminary fibrin-rich provisional extracellular matrix, which is predominantly composed of fibrin, fibronectin, hyaluronic acid, proteoglycans and immature collagen (type III) [13, 82]. Over the course of further wound healing, this matrix is restructured into a strong collagen-containing ECM.

Oxygen again plays a decisive role in this process. Acute hypoxia initiates the production and release of the growth factor TGF-1ß, which stimulates collagen synthesis by influencing gene expression, and in-vitro experiments have shown that prolonged hypoxia inhibits precisely this process [23, 36, 81].

Besides having an influence on collagen synthesis at a transcriptional level, oxygen is also required as a co-factor in the posttranslational collagen modification of proline and lysine by prolyl and lysyl hydroxylases [40, 67]. In the event of a disturbance of proline modification, a triple helix cannot be formed and thus a functional (pro-) collagen cannot be secreted. The synthesis of collagen is proportional to the oxygen partial pressure; in wound areas with an oxygen partial pressure below around 20 mmHg the collagen synthesis in fibroblasts comes almost entirely to a stop, while only at an oxygen partial pressure of above 30–40 mmHg does one find a sufficient collagen incorporation into the extracellular matrix [81].

In the further collagen maturation process, a cross-linking of the collagen fibrils (maturation of the collagen) takes place in the extracellular matrix. The tensile strength and elasticity of the newly formed tissue depends on the degree of cross-linking of the collagen molecules. The enzymes that are important for this process, lysyl hydroxylase and lysyl oxidase, require oxygen as a co-factor [37, 67, 72]. Therefore, in the event of chronic hypoxia, the cross-linking of the newly formed collagen is much poorer, with negative effects on the new scar tissue.

Without the formation of an extracellular matrix, blood vessels cannot grow into the new ECM, in order to supply other cells, such as the fibroblasts necessary for collagen synthesis, with oxygen and nutrients. These cells can only develop the ECM further if they receive an optimal supply, which in turn affects angiogenesis. Because there is a very complex direct connection here between the different processes in the granulation phase, in contrast to the positive stimulus of granulation through acute hypoxia, a complete stagnation of healing can occur as a result of chronic hypoxia.

Re-epithelialisation:

In the case of re-epithelialisation as a further component of the proliferation phase [13, 82], wound closure is achieved by cell division and migration of epidermal Keratinocytes [43, 47]. In this process, numerous cytokines and chemokines, such as TNF, TGF-ß1, KGF or PDGF, are again released in order to stimulate epidermal cells at the wound margin to proliferate and migrate [22, 61, 91]. Re-epithelialisation is also accompanied by high metabolic activity, whereby the different steps such as differentiation, division and migration of epidermal keratinocytes are dependent on oxygen and ROS [18, 47, 89, 90, 91].

The proliferation and epithelialisation phase ends with wound closure after granulation and epithelialisation and wound contraction through fibroblasts that have differentiated into myofibroblasts [86, 93].

Tissue restructuring phase

With the restructuring of the new tissue and wound closure, a reduction in the proliferation activity then occurs. This is accompanied by the further restructuring of the extracellular matrix, whereby the less stable, preliminary type-III collagen is replaced by the more stable and more elastic type-I collagen. As already mentioned above, the synthesis by fibroblasts and the incorporation and especially the maturation of the collagen into the ECM is a highly oxygen-dependent process, which only takes place adequately at an oxygen partial pressure of greater than 30 mmHg.

Treatment of chronic wounds

Causal therapy is the central element in the treatment of chronic wounds. Thus, compression therapy should be applied to treat all venous crural ulcers, in order to reduce the effects of elevated venous pressure. Insufficient arterial perfusion must be alleviated by effective recanalisation of the obstructed vessels. Interventional or surgical procedures are available for this purpose. If they are not feasible, an attempt should be made to improve perfusion by administering prostaglandin, as, apart from its different local inflammatory properties, it also has vasodilatory effects and thus can have a positive influence on the healing of ulcers via an improvement of microcirculation [53]. Neuropathy-related foot lesions in diabetes mellitus occur as a result of abnormally elevated, repetitive tissue pressures on the basis of anaesthesia. Here, healing without consistent pressure relief is not possible.

The second pillar of the therapy of chronic wounds is local therapy. In general, phase-adapted, moist wound treatment is considered to be the "gold standard" for the treatment of the majority of all patients with chronic wounds [68].

Now the question arises as to how one can additionally specifically treat the local hypoxia, in order to promote the oxygen-dependent processes of wound healing in a targeted manner.

Treatment of the hypoxia of chronic wounds

In addition to causal and local standard therapy, a range of adjuvant therapies are available today, with the aim of improving the local oxygen supply. Beside the technically very complicated hyperbaric oxygen therapy, which requires a pressure chamber that can be entered or walked through and can therefore only be offered at appropriate medical centres, equipment for topical

oxygen therapy (discontinuously in chambers [10] or bags or continuously through aeration of the base of the wound [35]) are also used. For adjuvant hyperbaric oxygen therapy (HBO) of chronic ulcers of the lower limb [52], a Cochrane Review that evaluated the benefit and risk showed a reduction of the risk of major amputations if additional hyperbaric oxygen therapy was administered, compared with a standard therapy (relative risk 0.31; 95 % confidence interval 0.13 to 0.71). However, these study results are very controversial in the discussion about clinical relevance.

Besides the technical therapeutic options, preparations such as oxygen-generating wound dressings [15, 41] or a haemoglobin spray for improving the transportation of atmospheric oxygen through the barrier of the wound fluid [6, 7] are available. The results on this published thus far, e.g. concerning the haemoglobin spray [2, 3], also point to a markedly improved wound healing.

All of these therapies are aimed at improving the oxygen supply in the area of the wound, so that a more rapid and better healing and skin regeneration can take place. The promising results achieved with these methods confirm the importance of the local supply of oxygen for wound healing described above.