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Deferoxamine: Potential Novel Topical Therapeutic For Chronic Wounds

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Deferoxamine and Chronic Wound Healing.

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Abbreviations: PU: pressure ulcer; VLU: venous leg ulcer; DFU: diabetic foot ulcer; DFO: Deferoxamine; hypoxia inducible factor-1 (HIF-1); PHD: prolyl 4-hydroxylases; VEGF: vascular endothelial factor; ROS: reactive oxygen species; CBP: CREB binding protein; DO: distraction osteogenesis;

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Although chronic wounds have multifactorial etiologies, they share a common characteristic of compromise of the local vasculature leading to diminished oxygen tension.[1] In pressure ulcers (PU), the obstruction of capillary blood flow and lymphatics from the local shear pressure results in ischemia. The subsequent reperfusion causes hyperemia and edema further reducing the regional oxygen level.[1]Similarly, in venous leg ulcers (VLU), vascular permeability and increase in venous hypertension generate local edema. In diabetic foot ulcers (DFU), hypoxia results from local pressure and high metabolic stress on the wound bed. During the initial phase of healing for all three wound types, the local and acute hypoxic climate results in the release of numerous

growth factors by endothelial cells, fibroblasts, and macrophages.[1] One of those essential factors is the hypoxia inducible factor-1 (HIF-1). The regulation of the HIF-1 activity resides in its oxygen-dependent degradation, by prolyl 4-hydroxylase (PHD) enzymes that require iron (Fe^{2+}) as a co-factor. Under hypoxic conditions, HIF-1α is stabilized against PHD enzymatic degradation and moves to the nucleus, where it dimerizes with HIF-1β and binds to a hypoxia response element. For its transcriptional activity, HIF-1α also needs to bind to the co-activators p300/CREB binding protein (CBP).[1] This results in the up-regulation and the activation of more than 60 HIF-1α target genes for tissue repair, cell growth and proliferation, and important angiogenic factors such as the vascular endothelial factor (VEGF, Figure 1).[1] Although VEGF induces angiogenesis, studies have shown that prolonged hypoxia results in increased acidosis, decrease ATP production and inhibition of neovascularization that cannot be reversed by VEGF.[1] This explains the need for a therapeutic agent that would maintain HIF-1α's multi target activity within the chronic wound environment (Figure 1).

Another characteristic of chronic wounds is the local deposition of free iron. In VLU, leakage of erythrocytes into the interstitium and their degradation by macrophages leads to the release of the iron bound to ferritin. In diabetic patients, insulin resistance results in the increase in ferritin synthesis and iron stores, which concurrently reduce the hepatic extraction and metabolism of insulin, leading to hyperinsulinemia.[2] We speculate that the local tissue ischemia with vessel occlusion encountered in PU also leads to increased iron deposition (Figure 1).

Iron is a transition metal that has the ability to donate or accept single electrons. This allows iron to neutralize free radicals and thus to serve as a critical antioxidant defense.[2] In order to maintain the physiologic redox status of the cells, iron is sequestered in a bound with either proteins or co-factors.[2] If released from its bound form, free iron serves as a chemo-attractant for neutrophils and macrophages. The combination of iron and hydrogen peroxide (from activated neutrophils) generates ferric ion (Fe^{3+}) and reactive oxygen species (ROS), whose prolonged production results in a chronically inflamed environment.[1] A vicious cycle is created by the reduction of ferric

to ferrous ion (Fe^{2+}), generating more ROS, and the subsequent ROS enhancement of release of iron from carriers (Figure 1).[1] Iron also induces an unrestrained pro-inflammatory M1 macrophage phenotype, which contributes to perpetual tissue damage and compromised tissue regeneration as the cells fail to switch to the pro-reparative M2 phenotype.[3] All of the above sustain the chronic inflammatory state encountered in chronic wounds (Figure 1).

We postulate that the topical application of Deferoxamine (DFO), which simultaneously acts as an iron-chelator and effective HIF-1α inducer and stabilizer, is a potential therapeutic approach to improve healing of chronic wounds. DFO could be topically administered to the impaired wound bed at concentrations 100-fold lower than the therapeutic parenteral infusion warranted in the treatment of thalassemia or iron poisoning, and with a higher safety profile.

Pre-clinical studies have demonstrated that DFO topically applied[1] or injected[4] improved wound healing in db/db mice with increased granulation tissue and neovascularization. DFO modulated the expression of several cytokines and growth factors (including HIF-1α and VEGF).[4] As a result, DFO led to decreased inflammation, enhanced angiogenesis and wound maturation. The authors proposed that DFO ointment could potentially heal cutaneous wounds in diabetic patients.[4] The addition of DFO to fibroblasts derived from diabetic db/db mice cultured in hyperglycemic and hypoxic conditions resulted in increased expression of HIF-1α target genes responsible for motility and angiogenesis.[1] DFO enhanced wound healing rates in the db/db mouse even in the presence of continuous hyperglycemia.[1]

Human aortic endothelial cells cultured in high glucose and hypoxia with DFO, resulted in a 2-fold elevation in HIF-1α binding to p300 co-activator, approaching levels found in cells cultivated in low glucose.[1] DFO prevented the methylglyoxal modification of p300, which has been shown to negatively impact the heterodimerization of the two HIF-1 subunits.[1] Additionally, the local injection of DFO into ischemic flaps of diabetic mice resulted in wound healing enhancement and prevention of flap necrosis by

increasing HIF-1α and VEGF expression endothelial cell recruitment and neovascularization.[4]

A very different approach by Duscher et al demonstrated the transdermal drug delivery of DFO in reverse micelles that penetrate the intact stratum corneum. This transdermal delivery system improved PU healing as well as prophylactically prevented ulcer formation in diabetic mice. The authors suggested that this DFO delivery system could prevent diabetic ulcers and improve wound healing in humans.[6]

The authors' (CTF and SRB) translational experiments in radiation-induced delayed bone healing have led us[7] and others to investigate DFO as a therapeutic option to optimize tissue repair and regeneration. We used a murine mandibular model of distraction osteogenesis (DO) and pathologic fracture in the setting of radiation. DO is a reconstructive technique that leads to endogeneous bone regeneration after the separation of two osteogenic fronts. In these models DFO increased the vascular metrics compared to radiated untreated controls.[7] All of our aforementioned studies demonstrate that DFO improves bony tissue repair even in the absence of hyperglycemia-induced deficits. Although both skin and bone are dynamic organs with different repair mechanisms, our findings corroborate with that of others in the context of delayed wound healing.

Clinical studies[3,8] have correlated the severity of VLU with the abundance of iron in the wound dermis. They also revealed sizable amounts of iron in chronic wound exudates of VLU compared to acute wound exudates.[3,8]

In vivo studies of diabetic patients[2] have shown that hyperglycemia impairs transferrin binding ability and augments ferritin synthesis, thus increasing the availability of free iron and promoting ROS formation. In addition, hyperglycemia interferes in a dose-dependent manner with HIF-1α protein stabilization,[1] resulting in low levels found in diabetic wounds, with concomitant decrease in angiogenesis contributing to wound chronicity.

One of the earliest human studies linking DFO and glycemic control in diabetic patients was done in 1989.[2] Following DFO intravenous administration, diabetic patients with high serum ferritin no longer required hypoglycemic therapy.[2] Thus, studying DFO therapy in clinical trials may decrease diabetic complications, such as DFU.[6] Most other human clinical studies have focused on DFO protective effect on the myocardium against reperfusion-induced free radical formation in cardiac patients or in the prevention of iron-induced heart failure and arrhythmia in thalassemia.[2,6] In addition, DFO has been examined as a preventative measure for intracerebral hemorrhage and therefore ischemic stroke as it counteracts iron-mediated neurotoxicity by decreasing cerebral edema and neuronal death.[6] However, to date there are no human studies that have investigated the clinical application of DFO for the treatment of any type of chronic wound.

Given the hypoxia-driven environment of chronic wounds and the toxic effect of local iron deposition, it is fair to postulate that a topical delivery of DFO could reverse those deleterious effects and make significant contribution to wound healing. Pharmacokinetic studies have shown that DFO is highly hydrophilic[6] and rapidly metabolized by plasma enzymes,[6,9] which leads to a short half-life of 12 min.[6] Therefore, there is decreased absorption by the gastrointestinal tract and limited efficacy. Consequently, DFO has preferentially been administered subcutaneously for the treatment of iron overload.

DFO is usually well tolerated, and adverse reactions to DFO have insufficiently been reported to quantify their frequency. At high doses (exceeding 40mg/kg/day) subcutaneous administration of DFO may produce pain and swelling at the injection site, ocular, auditory, neurologic disturbances, and renal insufficiency, with the later shown to reverse upon discontinuation of DFO therapy.[9] Hypotension, growth retardation in children, and opportunistic infections, (in particular mucormycosis in dialysis patients) have also been reported.[9] Severe pulmonary effects have been observed at intravenous infusion doses of 10-20mg/kg/hr.[9] As far as the skin, a very rare generalized rash has been described.[6,9] Several randomized clinical trials on the use of iron chelators for the

treatment of thalassemia have shown that DFO has a good safety profile compared to other chelators. There have not been any reactions reported from the topical use of DFO.

Here we propose that DFO be administered topically at concentrations 100-fold lower than the therapeutic dosage used parenterally in thalassemia or iron poisoning, thus with a better safety profile. A major potential barrier to penetration of topically administered DFO is the stratum corneum, the outermost layer of the skin. However, chronic wounds lack the intact stratum corneum. In addition, numerous studies have now documented that DFO uptake does not occur via permeation across cellular membrane but rather via fluid-phase endocytosis, leading to an exclusive intracellular location within the endosome-lysosome complex.[10] Since the stratum corneum is absent in chronic wounds and DFO absorption occurs through an endosomal pathway, we anticipate easy delivery of DFO and a direct absorption at the local wound site, including weight-bearing areas such as plantar DFU."

We envision that DFO can be administered topically as a solution, cream or medication delivery patch directly on the wound bed. DFO will be readily available at the local wound site since it will avoid the first pass by the liver.

The topical use of DFO as an iron chelator seems to have multiple potential advantages. DFO can prevent iron-release in the tissue of diabetic, venous or pressure ulcers and therefore indirectly inhibit the deleterious cascade of iron-induced oxygen free radicals that overload the wound's antioxidant capacities and perpetuate the inflammatory damage cycle. Furthermore, DFO-induced HIF-1α stabilization would circumvent the chronic hypoxic milieu of PU, VLU and DFU, and stimulate new vessels, potentiate cell motility and recruitment of endothelial precursors; all of which are essential to the healing phase of cutaneous wounds. Altogether, DFO has the potential to provide a novel therapeutic tool to improve healing in chronic wounds.

Authors' contributions:

Dr. Tchanque-Fossuo drafted the article. Drs. Dahle, Buchman and Isseroff critically revised its content, including references and figures and approved the final version to be submitted for publication.

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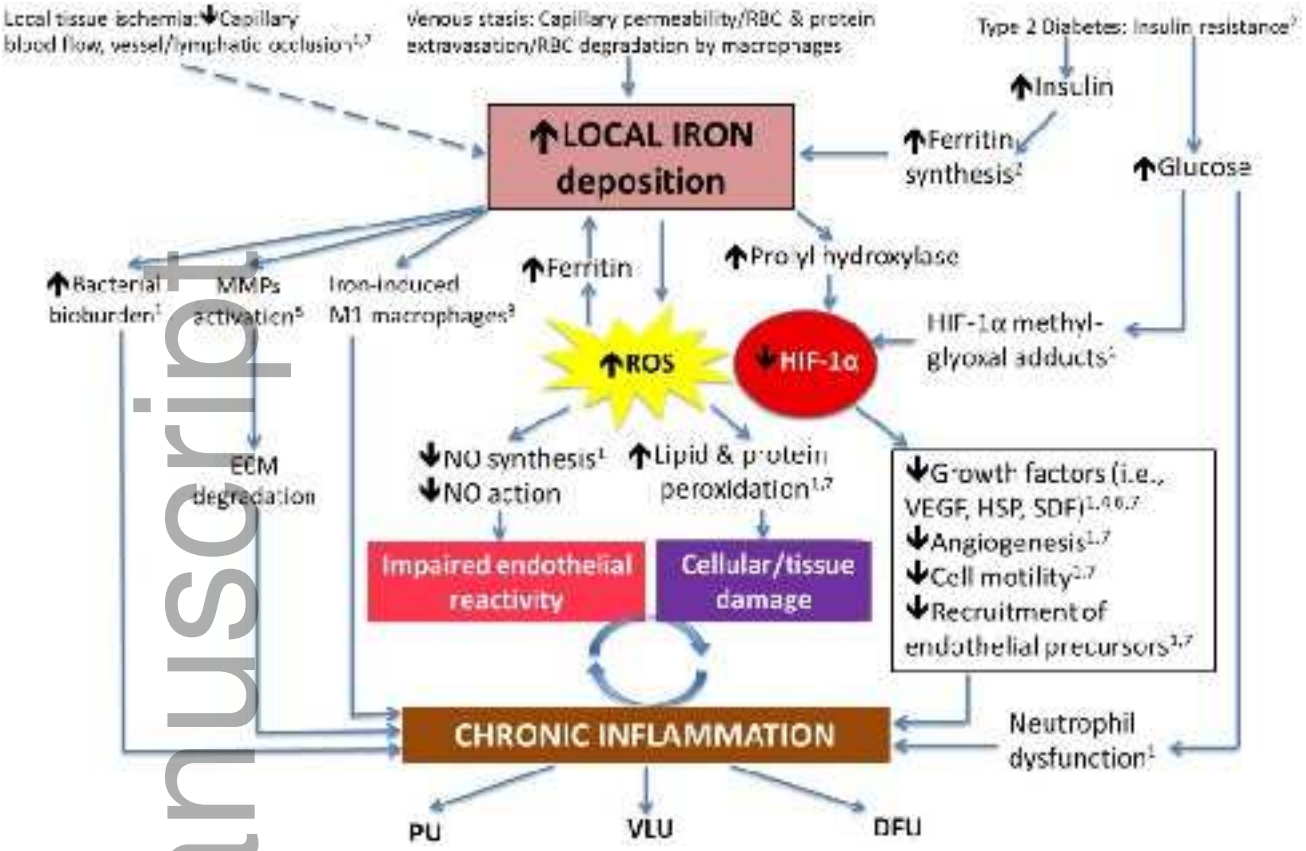
Figure legends:

Figure 1. Schematic role of iron in the pathogenesis of chronic wound formation. DFU, diabetic foot ulcer; ECM, extra-cellular matrix; HIF-1α, hypoxia-inducible factor-1 alpha; HSP, heat shock protein; MMPs, matrix metalloproteases; NO, nitric oxide; PU, Pressure ulcer; RBC, red blood cell; ROS, reactive oxygen species; SDF, stromal derived factor; VEGF, vascular endothelial growth factor; VLU, venous leg ulcer. Note: the dashed line refers to a hypothetical concept, not yet evidenced in the literature.



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