THE ROLE OF DIMETHYLARGININES IN CUTANEOUS WOUND HEALING

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Summary

Wound healing is an essential process for whole body recovery. The factors that support an adequate intercellular communication in all wound healing phases (inflammatory, proliferative, tissue remodelling) are not fully known. Experimental evidence from research in both animals and humans suggest that dimethylarginines may have a key role in cutaneous wound healing. Knowledge of molecular mechanisms that initiate and promote chronic wounds may represent a way of manipulating the metabolism into fast restoration of skin integrity.

Key words: *cutaneous wounds, tissue regeneration, dimethylarginines.*

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Introduction

Recent progress in cutaneous wound healing and treatment showed that an acute wound heals rapidly, its healing well controlled by the organism and with minimal functional loss, while a chronic wound does not heal, remaining stuck in the inflammatory phase [1]. Identification of factors that could hinder or foster cutaneous wound healing is a major objective of the medical community worldwide. Numerous experimental studies over the past two decades showed that the process of cutaneous wound healing involves three phases, interconnected over time and space:

a) inflammatory phase;
b) proliferative phase;
c) tissue remodelling phase [1].

Inflammatory response is an immediate defence reaction of the body against the action of harmful factors (lesions, infections). The inflammatory phase (removal of pathogens and wound cleansing) features:

a) cutaneous neurogenic inflammation;
b) haemostasis.

These early events include cellular involvement, vascular changes, extracellular matrix alterations, synthesis of many biochemical factors acting as pro- and antioxidants, by modifying or promoting the inflammatory focus.

Monocytes in the peripheral blood and tissue-resident macro-phages are the dominant inflammatory cells that orchestrate the initiation, evolution and resolution of the inflammatory phase in the wound healing process [1–10].

The proliferative (granulation) phase is identified through:

a) fibroplasia (fibroblast proliferation and differentiation into myofibroblasts, extracellular matrix deposition, wound contraction);
b) reepithelialisation and epithelial mesenchymal interactions between keratinocytes and fibroblasts;
c) angiogenesis (proliferation and formation of new blood vessels);

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d) peripheral nerve repair. Macrophages are the dominant inflammatory cells that orchestrate the proliferative phase in the wound healing process [1–10].

The remodelling phase (skin integrity restoration) consists of:

a) gradual reduction of granulation tissue;
b) remodelling of the epidermis, dermal vascularity, nerve endings and myofibrils, with formation of functional tissue [1–10].

Changes in the normal skin healing process leads to complications, such as:

a) fibrosis;
b) cutaneous chronic wounds [1–10].

Fibrosis is described as excessive production of extracellular matrix. On the skin, fibrosis is recognized as keloid, atrophic and hypertrophic scar. Atrophic scars are depressed and take the form of a sunken recess in the skin. Hypertrophic scars are prominent and remit with time. Keloid scars are non-malignant tumours consisting of fibrous tissue that cross the limits of the initial lesions, are elevated, expansive and continue growing. Most of the skin lesions can heal through keloid development. Among these, we mention acne, burns, chickenpox, ear piercing, surgical incisions, vaccination.

Chronic wounds with tissue loss, that do not heal spontaneously, are identified as venous and arterial ulcers, decubitus ulcers, diabetic foot ulcers [1–10]. Their occurrence is promoted by circulation disorders, diabetes, inflammatory skin diseases, age [1]. Knowledge of metabolic processes that initiate and promote chronic wounds may represent a way of manipulating the metabolism into solving these afflictions.

Nitric oxide status in cutaneous wound healing

Experimental proof for both animal and human studies indicate that nitric oxide (NO) plays a key part in the healing of uncomplicated skin wounds. NO is involved in cellular and molecular events concerning wound healing, such as vasodilation, angiogenesis, inflammation, cell proliferation, matrix deposition, tissue fibrosis, immune responses and remodelling [11,12].

The importance of NO was demonstrated through delayed wound healing in animals with genetic impairment of NO synthesis. In addition, NO therapy is efficient in ischemic and diabetic ulcer healing in experimental animals, by inducing reepithelialisation, angiogenesis and collagen synthesis [1,11–15]. Numerous factors that modulate NO level and bioavailability in the cutaneous wound healing process were described. Among them: enzyme substrates, enzyme inhibitors, inflammatory and immune mediators [1,11].

Nitric oxide and the skin

In mammals, NO synthesis is catalysed by NO synthase (NOS, E.C.1.14.13.39). NOS catalyses the oxidation of non-proteinogenic amino acid L-arginine to citrulline and NO. Molecular cloning highlighted the presence of three NOS isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). All three NOS are homodimer proteins, their activity depending on essential cofactors (NADPH, reduced flavins, haemoproteins, 6 (R) 5,6,7,8-tetrahydrobiopterin). Constitutive NOS isoforms expressed in the skin are activated by an increased level of Ca2+/calmodulin. nNOS expression was observed in keratinocytes and melanocytes, eNOS expression was detected in keratinocytes of the basal layer of the epidermis, in dermal fibroblasts, endothelial capillaries and eccrine glands, while iNOS can be induced in keratinocytes, fibroblasts, Langerhans and endothelial cells. Thus, NO may regulate melanogenesis mediated by ultraviolet B radiation, and maintenance of the skin barrier.

Generally, iNOS expression was described in skin inflammatory diseases (psoriasis, lupus erythematosus, atopic dermatitis, contact dermatitis, scleroderma, pemphigus vulgaris, Sjogren syndrome, Stevens-Johnson syndrome) [1,11–13].

Nitric oxide as an antimicrobial agent

NO shows bimodal antimicrobial activity, depending on concentration [11–15]. At low concentrations, it stimulates the immune system

by improving proliferation, differentiation and apoptosis of the immune cells, cytokine synthesis and synthesis of extracellular matrix. On the other hand, as a component of the innate immune system, iNOS produces large quantities of NO when activated by polysaccharides and bacterial endotoxins, as well as proinflammatory cytokines. At concentrations larger than 1 μM, the utility of NO against pathogen agents derives largely from its capacity of reacting with reactive oxygen intermediates in order to generate reactive nitrogen oxide species (RNOS). Peroxynitrite (ONOO-), the most reactive and cytotoxic RNOS, is formed when NO reacts with superoxide. RNOS have many antimicrobial properties, through inducing nitrosative and oxidative stress, inactivating essential enzymes, depleting intracellular iron deposits, damaging microbial DNA (by generating alkylating agents and hydrogen peroxide, and by inhibiting DNA repair), disrupting microbial cellular membrane by peroxidation of lipids. iNOS knockout mice show increased susceptibility to herpes simplex virus infection, as well as decreased elimination of latent virus. Mice treated with an iNOS inhibitor proved more vulnerable to intracellular bacterial infections [14,15].

ADMA / SDMA and cutaneous wounds

The activity of NOS enzymes is regulated by methylated arginine derivatives, of which asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are of enzyme activity regulators. (16-23). Despite being stereoisomers, ADMA and SDMA have different biological behaviour and clinical significance (Table 1). Firstly, ADMA is a strong competitive inhibitor of NOS, while SDMA is a weak competitive inhibitor of cationic amino acid transporters (CAT). Secondly, ADMA and SDMA compete with L-arginine for its transporters, thus their accumulation decreases NO production by decreasing L-arginine availability for NOS. ADMA and SDMA syntheses are regulated by different protein arginine methyltransferases (PRMTs). ADMA is mainly catabolised to L-citrulline and dimethylamine (DMA), by dimethylarginine dimethylaminohydrolase (DDAHs). SDMA is excreted through urine by choice.

Recent results [16,23] showed an increase in both ADMA and SDMA concentrations, as compared with healthy people and cardiometabolic patients without chronic wounds. The increase in ADMA is associated with cardiovascular risk factors. The explanation of this phenomenon is the following: ADMA interferes with NO synthesis by inhibiting NOS enzymes and by reducing arginine availability, competing for its membrane transporters. In addition, it affects NO signalling by inhibiting eNOS phosphorylation. Furthermore, ADMA increases in patients with limb ulcerations significantly more than in diabetic patients without neuropathy. SDMA negatively affects arginine availability by inhibiting, but does not spark the same interest as ADMA because it is only a weak inhibitor of NOS. There is little information about SDMA in chronic wounds. In addition, data obtained from metanalyses linking SDMA with mortality risk in cardiovascular disease are conflicting. Moreover, functional studies showed that SDMA eliminates anti-inflammatory and antiatherogenic properties of HDL. Consequently, SDMA was claimed to be a marker of HDL dysfunction [23]. Other evaluations show that ADMA and SDMA levels were elevated, and Arg/ADMA and Arg/SDMA ratios were significantly reduced in chronic wound patients, which indicated low NO and, respectively, arginine availability. Wound type seems to have an impact on NO availability, considering that Arg/ADMA ratio was significantly reduced in patients with ulcers. In turn, wound etiology affected arginine availability, as venostasis patients had high levels of SDMA and low levels of Arg/SDMA. ADMA and SDMA levels are mainly regulated through their rates of synthesis (type 1 PRMTs for ADMA and type 2 PRMTs for SDMA) and degradation by DDAH enzymes (for ADMA), and renal excretion (for SDMA). In patients with chronic wounds, PRMTs and DDAH could be modified more, because they are positively (PRMTs) and negatively (DDAH) affected by inflammatory mediators [16,23].

Inflammatory cytokines are among the initiators of endothelial disfunction and key players in supporting the inflammation in chronic wounds. They induce iNOS expression but inhibit eNOS expression and contribute to

Table 1 - ADMA and SDMA - metabolism and pathophysiology implications [16–23]

Origin
A. Intranuclear posttranslational synthesis mediated by PRMTs (E.C.2.1.1.125) ADMA is obtained by NG-asymmetric dimethylation of L-Arg residues in proteins, under the action of type 1 PRMT (PRMT-1, -3,-4, -6 , -8), followed by proteolysis; it is released and taken up by target organs via CAT. ; SDMA is obtained by NG-symmetric dimethylation of L-Arg residues in proteins, reaction mediated by type 2 PRMT (PRMT-5, -9), followed by proteolysis; it is transported outside the cell B. Exogen absorption from lipid and cholesterol-rich foods.
Elimination
A. Renal excretion is the secondary excretion route for ADMA (less than 10%) and the main route for SDMA (over 90%); B. Intracellular degradation of ADMA, but not SDMA, by conversion to citrulline and dimethylamine (over 90%), by action of DDAH (E.C. 3.5.3.18), enzyme expressed in kidneys, brain, pancreas, liver, immune cells; C. Transamination of ADMA and SDMA to DMGV, by action of AGTX2 (E.C.2.6.1.44), enzyme expressed by choice in the kidneys; D. Conversion of ADMA and SDMA by butylation and methylation.
Biological activity
A. ADMA and SDMA generate NFkB activation, through induction of proinflammatory cytokine expression; B. ADMA and SDMA impact NO production: • NG-methylated compounds inhibit NOS activity (E.C. 1.14.13.39) MMA>ADMA>SDMA; • ADMA and SDMA are inhibitors of cell absorption of L-Arg via CAT. C. ADMA and SDMA are activators of oxidative stress and inflammation: • Mutual stimulation ADMA - ROS by increasing PRMT1 activity, reducing DDAH, decreasing CAT; • SDMA increases ROS production in fMLP-stimulated monocytes, by modulating calcium influx via SOC; • SDMA induces the increase in endothelial production of ROS, associated with a decrease in Arg absorption and inhibition of NO production; • ADMA accelerates ROS synthesis. D. ADMA and SDMA have a toxic effect on the immune system. E. Effect on lipoproteins: • SDMA causes adverse changes in HDL with TLR activation; • ADMA impairs oxidation of LDL-cholesterol. F. ADMA and SDMA are non-proteinogenic amino acids, hydro soluble uremic toxins, with low molecular weight (less than 0.5KDa), easy to remove through dialysis, with incompletely demonstrated effects in the body. G. ADMA is a mediator of endothelial dysfunction, SDMA is a sensitive indicator of renal function. H. ADMA and SDMA modulate NO bioavailability and have an impact on the healing of chronic cutaneous wounds.

ADMA accumulation. Recently, it has been documented that chronic wounds are associated with systemic increase of IL-1β, IL-4, IL-6, IL-8, FGF-2, MIP-1α, PDGF-BB and VEGF-A. Also, significantly increased CRP and low HDL in chronic wound patients, as compared to those with similar cardiometabolic burden, indicates a higher level of inflammation. ADMA concentrations were independently and inversely associated with VEGF-A levels, which indicates negative impact of ADMA accumulation on angiogenesis in chronic wound patients. In addition, the tight relationship between inflammatory response, NO availability, and Arg/ADMA and Arg/SDMA ratios may suggest the complexity of events involved in chronic wound repair [23]

Conclusions

Reported data show that patients with chronical wounds present alterations in NO and arginine homeostasis, resulting from ADMA and SDMA accumulation. ADMA and SDMA levels are inversely related with NO and arginine bioavailability. Thus, recent discoveries do not support arginine or citrulline supplementations in chronic wound patients, and rather suggest the necessity of treatment aiming to lower the concentrations of ADMA and SDMA.

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Conflict of interest
NONE DECLARED

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