Arginine metabolism and wound healing

Abstract

Wound healing is a complicated process needed to restore homeostasis in the body. The two arginine utilising pathways involved in wound healing are the inducible nitric oxide synthase (iNOS) to produce NO, and the arginase pathway producing ornithine, proline, and the polyamines. iNOS is highly active in the inflammatory phase with NO affecting the cyclooxygenase enzymes and the release of inflammatory mediators. In the proliferative phase, NO affects arginase and therefore collagen production and cell proliferation. Both supplemental arginine and ornithine appear to be beneficial in wound healing. The roles and regulation of these pathways are discussed in the different stages of wound healing. Interpretation of data from wound healing research is complicated by the extrapolation of animal model data to the human situation and a lack of human studies.

Introduction

Wound healing, following trauma or other surgical procedures, is an important process to quickly and effectively restore homeostasis in the body. Wound healing occurs in four phases: homeostasis, inflammation, proliferation, and remodelling and for proper healing to occur these phases need to be well controlled.

The dibasic amino acid arginine is of fundamental importance in wound healing as its metabolites nitric oxide (NO), proline, and polyamines affect all phases of the wound healing process. In the wound milieu, the concentration of arginine may decrease to undetectable levels and in this context arginine shifts from being a non-essential to a semi-essential amino acid as these low concentrations of arginine may limit NO and the whole wound healing process. Infusion of arginine into a wound produces sustained NO production and improves the overall state of the wound.

The major arginine utilising metabolic pathways active during the wound healing process are the inducible nitric oxide synthase (iNOS) which produces NO, and the arginase pathway which produces ornithine and subsequently proline, a major component of collagen. (See Figure 1.)

This review summarises recent research focusing on arginine utilising pathways and the regulatory role of the products of these pathways, particularly NO, have on wound healing.

Nitric oxide synthase and arginase pathways

NO plays a major role in many other physiological and pathophysiological processes, including wound healing. The effects of NO on wound repair are diverse involving angiogenesis, inflammation, cell proliferation, matrix deposition, and remodelling as well as mediating apoptosis.
The arginase pathway (See Figure 1) results in the production of L-ornithine and urea. This pathway favours proliferation as the metabolites proline and polyamines are directly involved in collagen synthesis and cell proliferation respectively.

The iNOS and arginase pathways act at separate times during the wound healing process. Wound fluid collected from polyvinyl alcohol sponges indicated that iNOS was predominant in the first 3 days post-wounding with arginase dominating thereafter. In addition, regulatory effector molecules and metabolites may activate the one enzyme pathway and have the opposite effect on another pathway. Shearer et al showed that interferon-γ (IFN-γ) and bacterial lipopolysaccharide (LPS) increase the activity of the iNOS whereas the products of the iNOS production (NO and citrulline) decrease the arginase activity. These data are supported by studies showing IFN-γ and LPS increase NO production by increasing the expression of iNOS. At the same time transforming growth factor-β1 decreases the effects of iNOS, leading to an increase in urea, ornithine and arginase metabolites. Urea from the arginase pathway decreases the activity of iNOS by acting post transcriptionally. Both hydroxy-L-arginine, the intermediate metabolite in the NO production and nitrite/nitrate, the stable products of NO decomposition decrease arginase activity. Other molecules, IL-4, IL-10, and PGE2, decrease the activity of iNOS and promote the activity of arginase.

Table I: Molecules active at different stages in the wound healing process

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Phase</th>
<th>Homeostasis</th>
<th>Inflammation</th>
<th>Proliferation</th>
<th>Remodelling</th>
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<tr>
<td>NO</td>
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<td>Arginase</td>
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<tr>
<td>Urea</td>
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<td>COX-1</td>
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<td>PGE2</td>
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**Supplementary feeding on wound healing**

Several researchers have demonstrated the beneficial effects of supplemental arginine feeding on wound healing. Arginine supplementation in elderly people improves wound healing and immune function and enhances wound healing in acute situations such as trauma. Shi et al and Witte et al demonstrated improved wound healing in diabetic patients. Their results suggest that arginine reverses impaired healing conditions present in these patients and at the same time the arginase pathway is maintained. Evans et al however, noted that supplemental arginine dose-response may be highly patient dependent and supplementation would have to be tailored to different individuals. Continuous infusion of arginine into a wound produced sustained NO production, reduced VEGF content, wound blood vessel number and vascular surface area as well as granulation thickness, so improving the overall state of the wound.

The effect of supplemental ornithine in diets increased collagen production and increased scar breaking strength in both iNOS knockout and wild type mice. NO production between the strains was similar as indicated by nitrate and nitrite concentrations. Thus ornithine supplementation appears to bypass the arginase and iNOS pathways and produces beneficial results similar to supplemental arginine feeding. However the data supporting these conclusions is limited and experiments to determine differences in NO production with respect to arginine and ornithine supplemental feeding are needed.

**Inflammatory phase: link between COX and NO**

The release of prostaglandin which enhances the inflammatory responses during the inflammatory phase of wound healing, has been reported. Prostaglandins are synthesised from arachidonic acid and its subsequent metabolites, released from the cell membranes. They are converted by the cyclo-oxygenase (COX) enzymes to form the cyclic endoperoxides prostaglandin G2 (PGG2) and prostaglandin H2 (PGH2). PGH2 is the precursor of prostaglandins E2 (PGE2) and F2a (PGF2a), both of which are necessary in regulating cell integrity and producing a non-specific inflammatory response. COX-1 has been consistently associated with general inflammatory processes whereas COX-2 appears important in acute inflammatory processes. The latter is induced following trauma to the skin and has pro-angiogenic properties.

Recent research reports have investigated the relationship between NO and COX enzymes during the inflammatory response as both enzymes, iNOS and COX-2 are induced during this phase. Salvemini et al showed the involvement of NO in the inflammatory process, increasing the activity of the COX enzymes and so increasing the inflammatory mediators. Elevated iNOS activity in the first 24 hours of injury increases NO in subcutaneous wounds. COX inhibitors conversely inhibit NOS and decrease production of thromboxane A2. The study suggests a close relationship between thromboxane A2 and NO production during the inflammatory phase.

Perkins and Kniss further characterised the relationship between NOS and COX-2 in murine macrophages showing that inhibition of NO formation with aminoguanidine decreases PGE synthesis by reducing COX-2 mRNA expression but, de novo COX-2 expression was not prevented. Therefore, it appears that NO is not active in de novo induction of COX-2 but is active in regulating sustained COX-2 activity and prostaglandin formation. Stimuli and products from this inflammatory phase lead onto the activation of the proliferative phase of wound healing.
Proliferative phase: changes in arginine metabolism

It would seem logical, as proline is involved in collagen synthesis, that the arginase pathway would be more important in the proliferative phase of wound healing than the iNOS pathway. However, investigations have shown that decreasing the activity of the iNOS pathway leads to a decrease in collagen synthesis and impaired wound healing. This implies that the two pathways are not independent of each other, with both being essential for efficient collagen and polyamine synthesis. Shaffer et al. showed that iNOS inhibition in mice decreased collagen concentration in the wound fluid, whereas supplemental arginine or ornithine feeding increased appropriate collagen production in wounds. S-nitrosoglutathione (GSNO), a nitric oxide donor, applied during both the inflammatory and proliferative phases increased the rate of wound healing. However GSNO applied alone, or during the other healing phases, did not show the beneficial effect as when it was applied to the two phases consecutively. Other studies have shown excessive NO synthesis decreases the rate of wound healing and collagen production, similar to the effect of NO depletion and may even cause cytotoxic effects. It appears that only appropriate NO production during the correct phase is beneficial in wound healing.

Conclusions

There are two arginine utilising pathways involved in wound healing a) the inducible nitric oxide synthase (iNOS) to produce NO, and b) the arginase pathway forming ornithine, a major constituent of collagen, proline, and polyamines. iNOS is highly active in the inflammatory phase, with NO affecting the cyclooxygenase enzymes to release inflammatory mediators. In the proliferative phase, NO affects arginase to enhance collagen production and cell proliferation. Both supplemental arginine and ornithine appear to be beneficial in wound healing. The products of these pathways facilitate to regulate both the pathways, affecting activity at the different stages of wound healing. However, data should be regarded with caution as data from animal studies cannot always be extrapolated to the human situation and there is a lack of comparable studies.

References