NITRIC OXIDE METABOLISM IN WOUNDS.

Schäffer MR, **Tantry U**, **van Wesep RA**, **Barbul A**.

Department of Surgery, Chirurgische Klinik, Eberhard-Karls-Universität, Tübingen, Germany.

Arginine can be metabolized in wounds to nitric oxide and citrulline by nitric oxide synthase or to urea and ornithine by arginase. We investigated the expression of these arginine metabolic pathways over a 3-week period. Groups of 8-10 male Balb/C mice underwent a dorsal skin incision and subcutaneous polyvinyl alcohol sponge implantation. The animals were sacrificed at various times, and sponges were harvested to obtain wound fluid and wound cells. Cells or whole sponges were incubated with L-[2,3-(3)H]arginine, with or without N(G)-L-monomethyl-arginine (NMMA, a competitive inhibitor of nitric oxide synthase). Nitrite and nitrate (both stable end products of nitric oxide metabolism) and amino acids were measured in wound fluid and wound cell culture supernatants. Increasing concentrations of nitrite and nitrate were noted in wound fluid and in whole sponge cultures until the second week postwounding, indicating sustained wound nitric oxide synthesis. In wound fluid arginine levels were undetectable at all times, suggesting sustained utilization. Wound fluid citrulline levels showed an early peak and then a gradual decrease, suggesting that recycling for continued nitric oxide production may occur. Wound fluid ornithine levels increased until Day 10 and remained elevated, indicative of continued arginase activity. In vitro production of nitrite/nitrate and citrulline by cells and whole sponges was inhibitable by NMMA. Inducible nitric oxide synthase expression was confirmed by immunoblotting, while immunohistochemistry demonstrated that macrophages are a major source of wound nitric oxide. The data show that nitric oxide synthesis occurs for prolonged periods after injury and macrophages appear to be a major cellular source.

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NITRIC OXIDE IN THE HEALING WOUND: A TIME-COURSE STUDY.

Lee RH, **Efron D**, **Tantry U**, **Barbul A**.

Division of Plastic & Reconstructive Surgery, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21287, USA.

INTRODUCTION: We studied the time course of nitric oxide expression in the healing wound and the cell populations responsible for its synthesis. METHODS: Twenty four Lewis rats underwent subcutaneous implantation of polyvinyl alcohol sponges. Rats were sacrificed in groups of three on days 1, 3, 5, 7, 10, 14, and 35 after wounding. The conversion of 3H-labeled arginine to 3H-labeled citrulline, with or without N(G)-L-monomethyl-arginine (L-NMMA) in harvested sponges, was measured. Nitrate/nitrite (NOx) in plasma and wound fluid was quantified by Greiss reaction. Inducible nitric oxide synthase (iNOS) gene expression was determined by Northern analysis and reverse transcriptase-polymerase chain reaction (RT-PCR). Inducible NOS was identified in specific wound cell populations by dual-label flow cytometry. RESULTS: Nitric oxide synthase (NOS) activity peaked at 24 h after wounding (37.7 +/- 0.9 micromol citrulline per milligram sponge), with a steady decline thereafter. Percentage inhibition of NOS activity by l-NMMA was highest on days 1-7 (70-80%). This declined to 50% by day 10 and to 25% by days 14-35. The iNOS gene expression paralleled NOS biochemical activity. RT-PCR confirmed low-level expression up to 10 days after wounding. Plasma NOx levels remained within a narrow range of 22.6 +/- 1.3 to 29.3 +/- 1.5 microM throughout the postwounding period, while corresponding levels in wound fluid (microM) increased steadily from 27 +/- 3.8 on day 1 to 107.2 +/- 10.0 on day 14. Inducible NOS expression was detectable by fluorescence-activated cell sorting in wound macrophages on days 1 and 3 after wounding. CONCLUSIONS: Our findings suggest maximal NOS activity early in cutaneous wound healing, with sustained production up to 10 days after wounding. NOS biochemical activity was paralleled by iNOS gene expression. Plasma NOx remained constant, while wound fluid NOx increased steadily to peak at day 14. Wound macrophages appear to be a source of nitric oxide production in the early phase of wound healing. Copyright 2001 Academic Press.

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ROLE OF NITRIC OXIDE IN WOUND REPAIR.

Witte MB, **Barbul A**.

Department of Surgery, the Sinai Hospital of Baltimore, and the Johns Hopkins Medical Institutions, Baltimore, MD 21215, USA.

After injury, wound healing is essential for recovery of the integrity of the body. It is a complex, sequential cascade of events. Nitric oxide (NO) is a small radical, formed from the amino acid L-arginine by three distinct isoforms of nitric oxide synthase. The inducible isoform (iNOS) is synthesized in the early phase of wound healing by inflammatory cells, mainly macrophages. However many cells participate in NO synthesis during the proliferative phase after wounding. NO released through iNOS regulates collagen formation, cell proliferation and wound contraction in distinct ways in animal models of wound healing. Although iNOS gene deletion delays, and arginine and NO administration improve healing, the exact mechanisms of action of NO on wound healing parameters are still unknown. The current review summarizes what is known about the role of NO in wound healing and points out path for further research.

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NITRIC OXIDE DRIVES SKIN REPAIR: NOVEL FUNCTIONS OF AN ESTABLISHED MEDIATOR.

Frank S, **Kämpfer H**, **Wetzler C**, **Pfeilschifter J**.

Pharmazentrum Frankfurt, Klinikum der Johann Wolfgang Goethe-Universität, Frankfurt/Main, Theodor-Stern-Kai, D-60590 Frankfurt am Main, Germany. S.Frank@em.uni-frankfurt.de

Wound healing of the skin represents a highly ordered process of important tissue movements that aims for a rapid closure of the wound site and a subsequent regeneration of the injured tissue. The factors ensuring the intercellular communication during repair are only known in part. However, although protein-type mediators are well-established players in this process, it has become evident that the diffusible, gaseous molecule nitric oxide (NO) participates in the orchestration of wound healing. The role of wound-derived NO that critically influences macrophage, fibroblast, and keratinocyte behaviour within the intercellular communication network during repair is subject of this review. Thus, cutaneous wound healing prototypically reflects processes that generally occur also in kidney injury and regeneration.

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BIOLOGICAL FATE AND CLINICAL IMPLICATIONS OF ARGININE METABOLISM IN TISSUE HEALING.

Curran JN, **Winter DC**, **Bouchier-Hayes D**.

Department of Surgery, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland. curranjn@eircom.net

Since its discovery in 1987, many biological roles (including wound healing) have been identified for nitric oxide (NO). The gas is produced by NO synthase using the dibasic amino acid L-arginine as a substrate. It has been established that a lack of dietary L-arginine delays experimental wound healing. Arginine can also be metabolized to urea and ornithine by arginase-1, a pathway that generates L-proline, a substrate for collagen synthesis, and polyamines, which stimulate cellular proliferation. Herein, we review subjects of interest in arginine metabolism, with emphasis on the biochemistry of wound NO production, relative NO synthase isoform activity in healing wounds, cellular contributions to NO production, and NO effects and mechanisms of action in wound healing.

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ARGININE METABOLISM IN WOUNDS

J. E. Albina, C. D. Mills, A. Barbul, C. E. Thirkill, W. L. Henry Jr, B. Mastrofrancesco and M. D. Caldwell

Department of Surgery, Rhode Island Hospital, Providence 02902.

Arginine metabolism in wounds was investigated in the rat in 1) lambda-carrageenan-wounded skeletal muscle, 2) Schilling chambers, and 3) subcutaneous polyvinyl alcohol sponges. All showed decreased arginine and elevated ornithine contents and high arginase activity. Arginase could be brought to the wound by macrophages, which were found to contain arginase activity. However, arginase was expressed by macrophages only after cell lysis and no arginase was released by viable macrophages in vitro. Thus the extracellular arginase of wounds may derive from dead macrophages within the injured tissue. Wound and peritoneal macrophages exhibited arginase deiminase activity as demonstrated by the conversion of [guanido-14C]arginine to radiolabeled citrulline during culture, the inhibition of this reaction by formamidinium acetate, and the lack of prokaryotic contamination of the cultures. These findings and the known metabolic fates of the products of arginase and arginine deiminase in the cellular populations of the wound suggest the possibility of cooperativity among cells for the production of substrates for collagen synthesis.

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