Influence of Comorbidities: Neuropathy, Vasculopathy, and Diabetes on Healing Response Quality

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Submitted for publication January 28, 2013.

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Abbreviations and Acronyms

AGE = advanced glycation end
products

CGRP = calcitonin gene-related
peptide

GK = diabetic Goto-Kakizaki rats

GAL = galanin

GAP-43 = growth associated
protein 43

IPC = intermittent pneumatic
compression

MMP-3 = matrix metallo-
proteinase 3

MMP-13 = matrix metallo-
proteinase 3

MCL = medial collateral
ligament

NPWT = negative pressure
wound therapy

(continued)

Significance: Prolonged and nonhealing connective tissue injuries are often seen associated with common diseases, such as metabolic disorders, obesity, hypertension, arteriosclerosis, neuropathy, and diabetes mellitus and these influences result in considerable burden on society via the health care system, the economy, and quality of life for patients.

Recent Advances: Emerging findings have established important new links in our understanding of effective connective tissue healing. Thereby, the function of the nervous system, vascular supply, and metabolic state of the patient can be directly linked to the quality of the connective tissue healing process.

Critical Issues: As some of these conditions are also more common in individuals as they age, and aging can also impact healing effectiveness, such complications will have an emerging significant impact as the demographics of many societies change with expanding percentages of the populations >60–65 years of age.

Future Directions: Comorbidities have to be early identified in patients with acute wounds or planned surgery. Necessary interactions between physicians with different subspecialties have to be initiated to optimize wound healing potentials.

SCOPE AND SIGNIFICANCE

THE PURPOSE OF THIS REVIEW is to describe relatively new knowledge in how common comorbidities influence wound healing and its outcomes. Novel approaches which target the specific underlying deficits and optimization of current wound healing strategies are discussed. Wound healing complications will have an emerging significant impact on our society due to lifestyle diseases and due to the aging population.

TRANSLATIONAL RELEVANCE

This review will mainly focus on the impact on acute injury/healing

responses, and while the risk of developing chronic wounds is increased in such environments, chronic wounds will be dealt in more detail elsewhere in this issue. The novel approaches discussed in this review are also intended to inspire translational research, which is very much needed in this area.

CLINICAL RELEVANCE

The clinician reader of this review should get new understanding in how different comorbidities affect the healing process and how new targeted treatments may be integrated into the current therapy to optimize the healing process.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Neuropathy

Nearly all connective tissues are innervated to some extent (articular cartilage being an exception). Evidence accumulated over the past decades has indicated that neuropeptides in these connective tissues (*e.g.*, skin, ligaments, tendons, *etc.*) can participate in the regulation of the tissues in a dynamic manner. Thus, neuropeptides, such as Substance P (SP) and calcitonin gene-related peptide (CGRP) and others can influence the activity of normal cells in these tissues.^{1–3} Furthermore, additional studies have indicated gender differences in neuropeptide responsiveness, and in females, responsiveness can be transiently abrogated during pregnancy.^{4,5} Thus, neural elements appear to play a role in normal tissue homeostasis in a dynamic manner.

Given a role in normal tissue regulation, it is likely that such innervation/neuropeptides also play a role in connective tissue repair after injury. Thus, experimental evidence and clinical observations suggest that functional disturbance of the peripheral nervous system, in particular neuropathy, can lead to impaired connective tissue healing. Interestingly, all phases of the healing process are hindered in denervated tissue, and the mechanisms seem to be unrelated to those involved in vasculopathy or metabolic disorders like diabetes mellitus.⁶ Thus, neuropathy may affect each or a combination of the sensory, autonomic, and motor nervous systems.

Denervation. Denervated skin, ligament, and tendons have all been shown to exhibit delayed connective tissue healing.^{7,8} Wound contraction is delayed in rats, when spinal nerves are divided.⁷ One theory has postulated that denervation would only ex-

ert its effects on vascular responses. However, denervated and revascularized rat skin flaps still have a greatly reduced rate of wound healing.⁹

Another possibility would be that denervation purely affects the motor system by decreased muscular or smooth muscular mechanical stimulation of connective tissue. However, denervation specifically of the sensory system can be applied via injections of capsaicin (Spanish pepper), which reduces sensory nerve fiber density by 30%–40% in wounded tissue (Fig. 1).^{6,10} Specific sensory denervation results in delayed reepithelialization, as well as hindered tendon wound healing.^{6,10,11} Treatment with capsaicin also delays healing of gastric mucosal injuries.¹²

Denervation affects wound reinnervation. Neuropathy has been demonstrated to affect the healing process in multiple ways. One major pathway in connective tissue repair involves reinnervation after injury. These observations of early nerve regeneration are in line with observations on skin, tendon, ligament, and bone healing indicating that nerve ingrowth is a fundamental aspect of tissue repair.^{13–18} Reinnervation, that is, peripheral nerve ingrowth and sprouting, is especially obvious after tendon injury, since tendon midsubstance in healthy conditions is more or less aneuronal (Fig. 2).^{13,19} Nerve sprouting and growth within the injury site is followed by a time dependent expression of neuropeptides during the healing process (Figs. 3–5).¹⁹ After the healing process is finished, sprouting nerve fibers within the tendon proper retract to the surrounding structures, that is, the paratenon and associated loose connective tissue.¹³ Presumably, wound site reinnervation provides a “highway” for delivery of essential neuronal substances during the healing process (Fig. 3).

Abbreviations and Acronyms (*continued*)

NGF = nerve growth factor

NPY = neuropeptide Y

NO = nitric oxide

PGP 9.5 = protein gene product 9.5

SP = Substance P

VEGF = vascular endothelial growth factor

VIP = vasoactive intestinal peptide

Figure 1. (A, B) Immunofluorescence micrographs of sections of tendon tissue after capsaicin denervation (B) and controls (A) after incubation with antisera to Substance P (SP). Arrows denotes varicosities and nerve terminals. A marked reduction of SP immunoreactivity is seen in the denervated group (A, B). Reprinted with permission from Bring *et al.*¹⁰ v, blood vessel; t, proper tendon tissue. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Denervation affects neuropeptide availability. Notably, sensory denervation with capsaicin mainly reduces the levels of two essential sensory neuropeptides, that is, SP by $\sim 60\%$ and CGRP by $\sim 40\%$ from small sensory fibers (Fig. 1).¹⁰ Thus, capsaicin-mediated denervation is believed to exert its effects mainly via SP- and secondly by CGRP-inhibition. Higher residual SP levels in rats treated with capsaicin correlated with better resultant biomechanical tissue properties of the scar tissue, that is, transverse area, ultimate tensile strength and stress at failure.¹⁰

Effects of neuropeptides on healing processes. During the inflammatory phase of healing, increased levels of SP- and CGRP-positive nerve fibers locate in blood vessel walls surrounded by inflammatory blood derived cells (*e.g.*, platelets and growth factors) in the associated loose connective tissue (Fig. 5).^{16,18,19} Such observations are consistent with a proinflammatory role for sensory neuropeptides. Thus, SP release enhances vasopermeability and stimulates recruitment of leukocytes and cytokine production.²⁰ Therefore, a decreased level of SP in denervated tissue would be

Figure 2. (A–C) Overview micrographs of longitudinal sections through the Achilles tendon. Incubation with antisera to general nerve marker PGP 9.5. Micrographs depict the proximal half of the Achilles tendon at increasing magnification. Arrows denote varicosities and nerve terminals. The typical vascular localization of autonomic neuropeptides is depicted in lower left (B), whereas the free nerve endings are typical localization of sensory neuropeptides (C). The immunoreactivity is seen in the paratenon and surrounding loose connective tissue, whereas the proper tendinous tissue, notably, is almost devoid of nerve fibers. Reprinted with permission from Ackermann *et al.*¹³ m, muscle; pt, paratenon; PGP 9.5, protein gene product 9.5.

Figure 3. (A, B) Overview micrographs of longitudinal sections through the Achilles tendon at 2 weeks postinjury (rupture). Incubation with antisera to a nerve growth marker, GAP-43. Micrographs depict the proximal half of the Achilles tendon at increasing magnification. Arrows denote varicosities and nerve terminals. The GAP-positive fibers, indicating wound reinnervation, are abundantly observed in the healing proper tendon tissue. Reprinted with permission from Ackermann *et al.*¹³ GAP-43, growth associated protein 43.

expected to lead to delayed wound healing, as denervation results in delayed protein extravasation and cell migration.^{7,21} In animals exposed to capsaicin, vasodilation, and plasma protein extravasation are absent with injury leading to significantly reduced monocyte, macrophage, and T-lymphocyte cell numbers.^{20,22} Interestingly, endogenous neuropeptides with known anti-inflammatory actions have been demonstrated to be present at low levels during the inflammatory healing phase (Fig. 4).¹⁹

Figure 4. Area occupied by nerve fibers (%) immunoreactive to SP, CGRP, and GAL in relation to total healing area, over 16 weeks posttendon injury (mean $\pm$ SEM). Reprinted with permission from Ackermann *et al.*¹⁹ CGRP, calcitonin gene-related peptide; GAL, galanin.

During the proliferative healing phase, there is a peak expression of SP and CGRP (Fig. 4). Notably, this peak occurs in concert with reinnervation at the injury site. There is a conspicuous occurrence of SP and CGRP in free sprouting nerve endings among fibroblasts and also around newly formed blood vessels in the healing connective tissue (Fig. 5). Sensory neuropeptides are also well known to stimulate proliferation of fibroblasts and endothelial cells.^{23,24} Recently, SP has also been demonstrated to stimulate recruitment of stem cells to the healing area,²⁵ which would comply with the localization of SP in free nerve endings unrelated to vessels within the healing area.

During the remodeling healing phase, nerve fiber retraction from the injury site represents an important aspect.¹³ At this stage, neuropeptides with anti-inflammatory effects seem to emerge (Fig. 4).¹⁹ Thus, vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), and galanin have been demonstrated to mitigate the proinflammatory and nociceptive effects of SP.^{26,27} This seems to occur simultaneously or coordinately with apoptosis, which is important in downregulating inflammatory cell infiltration, fibroblasts, and collagen deposition.²⁸ Denervation results in enlarged wound tissue because of increased granulation cell division without a proportionate increase in apoptosis.⁶ Neuropeptide depletion, as a result of denervation, appears to delay epithelial cell migration and apoptosis signaling.²⁹ Without sufficient apoptosis,

Figure 5. Immunofluorescence micrograph of longitudinal sections through healing Achilles tendon at 1 (**A**) and 2 (**B**) weeks postrupture after incubation with antisera to CGRP. Nerve fibers immunoreactive to CGRP at week 1 are seen as vascular and free nerve endings in the loose connective tissue (**A**). At week 2, CGRP- immunoreactivity occurs mainly in the healing tendinous tissue as sprouting free nerve fibers (**B**). Scale bar = 50 μ m. Reprinted with permission from Ackermann *et al.*¹⁹ lct, loose connective tissue. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

hypertrophic scarring and keloids may form.²⁸ This delay in apoptosis is independent of the delay in cellular infiltration and can be reversed by topical growth factors.²⁸

Effects of nerve growth factor. One essential growth factor upregulated in response to injury and important for the regulation of apoptosis is nerve growth factor (NGF).³⁰ After injury, NGF is taken up by sympathetic and small sensory nerves, where it undergoes retrograde transport and then stimulates the production of several nerve substances in the dorsal root ganglia, for example, Substance P.³⁰ NGF displays a wide spectrum of biological functions, for example, acceleration of wound contraction and increased leukocyte chemotaxis.^{30,31} Patients with diabetic neuropathy have lower serum NGF than controls, and the decreases in NGF are proportional to decreases in the patients' nerve conduction velocity.³²

Supplementation of injury sites with NGF. Addition of NGF promotes the healing process.³⁰ Thus, topical 2.5S NGF increases nerve density, accelerates time to wound closure and augments epithelialization, but does not affect wound contraction.³³ High molecular-weight 7S NGF, however, accelerates wound contraction.³⁴ During ligament healing in rats, NGF was continuously administered to the injury site for 7 days via an implanted mini-osmotic pump.³⁵ At 7 days after injury, enhanced angiogenesis was noted and at 14 days, increased nerve fiber density. At 42 days after injury, nerve fiber, and blood vessel densities were progressively increased, and scar mechanical properties were also significantly improved in the NGF group.³⁵ Hence, early exposure of injured

tissues to exogenous NGF can lead to improved biomechanical tissue strength, a critical factor for connective tissue healing. However, it remains to be determined whether this enhanced scar tissue quality is due to improved organization of the scar matrix, the composition of the matrix, or a combination of both variables.

Supplementation of injury sites with SP. Exogenous administration of SP promoted wound and tendon healing.^{10,36–39} Moreover, inhibition of neutral endopeptidase, which degrades SP, augments epithelialization.⁴⁰ Thus, an exogenous SP supply after tendon injury resulted in increased fibroblast number, diameter of the organized collagen, and the amount of collagen III after injury (Fig. 6).³⁷ Histological parameters of collagen fiber orientation and angiogenesis were also improved by SP treatment. Treatment with SP also led to increased stress at maximal load and work to maximal load in the healing Achilles tendon.³⁹ One important observation denoted was that the sensory nerve in growth in the healing tendons appeared to withdraw at a quicker pace in the SP supplemented tendons.³⁷

One study assessed the effect of exogenous neuropeptide administration on the healing of denervated ligaments in rats.⁴¹ The aim was to restore the neuronal supply to improve the healing responses of the medial collateral ligament (MCL) in joints partially denervated by femoral nerve transection or surgical sympathectomy. In this study, exogenous SP, VIP, and NPY all led to improved healing of denervated MCLs. Interestingly, SP and VIP treatment resulted in enhanced mechanical properties, including failure load and failure stress, that were actually higher than those

Figure 6. (A–C) Sirius red-polarized light micrographs of longitudinal sections through healing Achilles tendon at one postinjury of (A) substance P + captopril/thiorphan-, (B) captopril/thiorphan- and (C) saline-treated rats. Short green fibers denote collagen III-like structures. Scale bar = 200 μ m. Reprinted with permission from Carlsson *et al.*³⁷ To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

of intact, uninjured MCLs.⁴¹ The supply of SP, VIP, and NPY also significantly improved the histological appearance of the healing MCLs. Treated ligaments showed more organized scars and the appearance of increased matrix production. Interestingly, CGRP did not improve any of the measured histological or mechanical parameters of MCL healing in this model.

Vasculopathy

Clinical and experimental observations provide evidence that blood vessel disorders also can lead to impaired connective tissue healing. This is most obviously seen in patients with peripheral vascular diseases, such as arteriosclerosis or diabetes mellitus, which in severe cases often result in limb amputations. Clinically, smoking, hypertension, obesity, excess blood cholesterol levels, and low degree of physical activity are intimately associated with suboptimal connective tissue healing. Venous insufficiency, another blood vessel disorder, likewise leads to increased risk for nonhealing wounds.⁴² Patients with peripheral vascular disease should be referred to a vascular surgeon in order to optimize vascular supply.

Macroangiopathy. This form of atherosclerosis of the large blood vessels, macroangiopathy, has a multifactorial genesis and largely affects connective tissue healing. Advanced glycation end products (AGE), defective signal transduction, and an imbalance of matrix metalloproteinases (MMPs) can all lead to the progression of atherosclerosis in major arteries.⁴³ Increased levels of prothrombotic factors and restricted formation of collateral arteries in diabetic patients with postischemic lesions are important pathophysiological pathways in impaired healing.⁴³

Microangiopathy. Interestingly, microangiopathy has been suggested to be the major underlying factor in the poor healing process of both ischemic and venous ulcers.⁴² Microangiopathy also plays a significant role in the pathogenesis of tissue breakdown in the diabetic foot. Microvasculature in the skin is comprised of nutritive capillaries and thermoregulatory arteriovenous shunt flow, which is regulated through the complex interaction of neurogenic and neurovascular control. Therefore, the close association between a properly functioning nervous system and a functional microvascular system becomes obvious. The interplay among impaired nerve axon reflex activities, endothelial dysfunction, and microvascular dysregulation are crucial factors in the poor healing of wounds.⁴⁴

Angiogenesis. Angiogenesis, new blood vessel formation, is an essential process in connective tissue healing. As early as the third day after wounding new vessels appear,⁴⁵ capillary growth into the wound subsequently provides a conduit for nutrients and other mediators of the healing response as well as removal of metabolites. Inhibition of angiogenesis impairs wound healing.⁴⁵

The opposite dysregulation of angiogenesis, that is, excessive neoangiogenesis, may also lead to dysfunctional healing, such as in degenerative tendon diseases, that is, tendinopathy (Fig. 7).⁴⁶ Notably, two nerve-based factors, SP and NGF, stimulate angiogenesis, thereby demonstrating the close connection between the neuro- and vascular systems (Fig. 7). SP promotes angiogenesis by stimulating endothelial cell proliferation.⁴⁷ NGF has been demonstrated to enhance angiogenesis partly via stimulation of the vascular endothelial growth factor (VEGF).⁴⁸

Vascular endothelial growth factor. VEGF induces endothelial cell migration in wound healing mainly by two mechanisms, chemotaxis and vasodilatation. During the inflammatory and in the initial phase of angiogenesis, endothelial cells migrate before mitotic division.⁴⁹ VEGF induces production of integrins, which are both chemotactic for endothelial cells and increase the vascular permeability.⁴⁹ VEGF further stimulates endothelial cell proliferation and prevents apoptosis.⁴⁹

During the proliferative phase of repair, capillary growth and differentiation are at a maximum. During this period, approximately 3 to 7 days after skin injury, VEGF is upregulated likely to promote the early stages of angiogenesis (*i.e.*, vascular dilation and permeability, cell migration, and proliferation).⁴⁹ Once the wound is granulated, blood

vessel density declines as endothelial cells undergo apoptosis. The reduction in VEGF appears to contribute to this transition from a hypercellular granulation tissue to a more normocellular scar. Prolonged VEGF upregulation may lead to a non-resolving fibrogenic scar as can be observed in some tissues, for example, in tendinopathy.⁴⁶

Diabetes

Patients with diabetes often with time develop the above mentioned disorders implying neuropathy and vasculopathy are intimately involved in their development, and thereby, some of the underlying mechanisms of failed healing are fairly understandable. However, healing of wounds is intrinsically different in diabetes. Diabetic wounds tend to heal by cellular infiltration, deposition of granulation tissue, and reepithelialization, not by contraction, as occurs in normal wounds. This pattern of wound closure is common to the tissue of chronic ulcers in patients with both diabetic decubitus ulcers and venous stasis.⁴⁹ Recent knowledge about patients with diabetic wounds, mostly foot ulcers, have demonstrated that multidisciplinary care in specialized centers with standardized conservative therapeutic approach, will improve outcome and allow limb salvage in a high percentage of cases.⁵⁰ Essential aspects of the multidisciplinary therapy are the treatment of infection, relief of ischemia as well as operative and medical promotion of wound healing.⁵⁰

Metabolic disturbance. In diabetes there also exist pathogenic components, such as disturbed metabolism (*e.g.*, AGE and others) which directly affect the healing response.⁵¹ Thus, diabetic rats have been found to exhibit a decrease in collagen matrix formation, and therefore, a resultant reduced wound

Figure 7. (A, B) Immunofluorescence micrographs of longitudinal sections from biopsies of healthy Achilles tendon (A) and tendinosis tissue (B) after immunostaining for SP. Arrows denote varicosities and nerve terminals. The micrograph illustrates SP-positive nerve fibres in close vicinity to neoangiogenesis in the tendon proper (B). Scale bar = 50 μ m. Reprinted by permission from Lian *et al.*⁷⁸ To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

tensile strength (Fig. 8).⁵² The expression and assembly of collagen has been shown to be related to glucose utilization.⁵³ Diabetic rats treated with insulin exhibited more normal wound healing.⁵²

Even non-insulin dependent diabetic rats exhibited down-regulated collagen I and III production after tendon injury, and these changes correlated with impaired biomechanical properties (Fig. 8).⁵⁴ In this study, a concomitant decreased expression of MMP-3 indicated that decreased degradation of matrix proteins may be a part of the impaired tissue remodeling process.⁵⁴

Topical treatments. Interestingly, even locally applied insulin can promote wound healing. In diabetic patients, topical insulin cream markedly improved wound healing, possibly by increasing the expression of other proteins, such as endothelial nitric oxide (NO) synthase and VEGF in the wounded skin.⁵⁵

Topical treatment with insulin could represent an attractive and a relatively inexpensive method of promoting tissue healing in diabetes patients. The metabolic disturbance in diabetes may be re-

sponsible for endothelial dysfunction, including oxidative stress, hyperglycemic pseudohypoxia, nonenzymatic glycation, and activation of the coagulation cascade.⁴⁹ Several different factors are being explored as topical treatments to improve diabetic wound healing, such as growth factors, extracellular matrix protein surfaces, *etc.*⁵⁶ Moreover, topical ozone, as gaseous or liquid mixture has been demonstrated effective as anti-infective local treatment, especially in countries where there is lack of antibiotics.⁵⁶

Negative pressure wound therapy. Negative pressure wound therapy (NPWT) has emerged as an adjuvant therapy in treatment of difficult healing wounds. NPWT augments the wound bed for closure by promoting granulation tissue formation and decreasing the wound size.⁵⁷ The benefits of NPWT include rapid wound granulation, epithelialization, and contraction reduction of dressing changes, reduced infection risk and treatment costs, control of exudates and concurrent rehabilitation.⁵⁷ Several studies have demonstrated faster healing times with NPWT when compared to moisturized saline

Figure 8. Relative expression of collagen I (A), collagen III (B), biglycan (C), versican (D), MMP-3 (E), and MMP-13 (F) mRNA in the intact and ruptured tendon of control (Wistar) and diabetic (GK) rats at day 14 postinjury. Results are expressed as means $\pm$ SE with $n=4$ in Wistar groups and $n=5$ in diabetic GK groups. * $p=0.05$, ** $p=0.005$ compared with intact Wistar and # $p=0.05$, ## $p=0.005$ compared with intact GK group (single-factor analysis of variance). Reprinted by permission from Ahmed *et al.*⁵⁴ GK, diabetic Goto-Kakizaki rats; MMP-3, matrix metalloproteinase 3.

gauze.⁵⁷ Development of NPWT from large devices to smaller, more portable devices, has also allowed a smoother transition from hospital to community setting treatments.

Intermittent pneumatic compression treatment. Intermittent pneumatic compression (IPC) therapy is also an emerging method for prophylaxis and treatment of patients with impaired venous,⁵⁸ arterial,⁵⁹ and interstitial circulation.^{60,61} IPC treatment passively increases blood flow by cycling external pressure on lower or upper limbs by means of inflating or deflating a pneumatic cuff. By applying lower limb compression cuffs in one of five different positions (on foot, foot and calf, calf, calf and thigh, or whole limb) venous stasis can be reduced, arterial blood flow enhanced and interstitial circulation increased.⁶² IPC has been suggested to be incorporated into a multidisciplinary therapeutic approach in patients with wound healing problems and is evidence based also for the reduction of edema, which can hinder diabetic wound healing.^{61,63,64}

A randomized, double-blinded, placebo-controlled trial on diabetic patients demonstrated better and more rapid wound healing in the IPC group as compared to the placebo-controlled pump group. Moreover, in the compression pump group, healing was achieved in more of the therapy-compliant patients than in those who were noncompliant.⁶⁵ The Mayo Clinic experience demonstrates similarly good results when using intermittent compression treatment in wound healing in patients with diabetes and limb ischemia.⁶⁶

Compression therapy is considered to exert three distinctive mechanisms of action: (1) mechanical, (2) chemical, and (3) cell-mediated/molecular. Mechanical actions include increased venous, arterial and interstitial circulation improving the supply of oxygen. Moreover, these mechanical actions on for example blood vessel walls, can induce release of chemical factors, such as antithrombotic substances (tissue factor pathway inhibitor), and profibrinolytic substances (tissue plasminogen activator),⁶² and substances that enhance tissue repair, including NO,⁶⁷ sensory neuropeptide SP and CGRP.⁶⁸ Prolonged treatment has demonstrated alterations in cellular gene expression of important regulators of vasodilatation (NO)⁶² and angiogenesis (*e.g.*, VEGF).⁶⁹ The gene expression alterations have been verified by subsequent improvements in tissue structure, for example, neurovascular ingrowth, callus production, increased bone mineral density, and bone mineral content following IPC treatment of experimental fractures.^{70–72}

TAKE-HOME MESSAGES

- Assess the neuronal, vascular, and metabolic state of your wound healing patient or if the patient is planned for surgical intervention.
- Initiation of prophylactic interventions which suspend the development of metabolic disorders, obesity, hypertension, arteriosclerosis, neuropathy, and diabetes mellitus are of uttermost importance.
- In patients with such established comorbidities new means to promote systemic and local neurovascular and metabolic status will prove to accelerate and improve the connective tissue repair process.

Neuropathy. Notably, in chronic ulcers of diabetes, venous stasis, and inflammatory disorders, the peripheral nervous system has been identified as common causes of dysregulation of connective tissue healing and homeostasis.⁷³ Thus, diabetic humans with early neuropathy have a reduction in NGF and SP, but not CGRP in the skin. Patients with diabetic neuropathy have lower serum NGF than controls, and the decreases in NGF are proportional to decreases in the patients' nerve conduction velocity.³² Diabetic neuropathy mainly affects the targets of NGF, namely sensory and sympathetic neurons.³² It is also noted that since NGF is structurally similar to insulin, it may be ancestrally related to insulin and therefore also affected in diabetes.⁷⁴

Supplementation of injury sites with neuronal factors. In line with the role of NGF in neurovascular pathology, it has been demonstrated that exogenous NGF can accelerate wound closure in diabetic animals by facilitating vascular regeneration, and suppressing endothelial apoptosis via a VEGF dependent mechanism.⁷⁵ NGF moreover up-regulates the production of SP. Thus, exogenous SP promotes wound healing in diabetic mice, partly by increased epithelialization.³⁸ Moreover, serum levels of substance P are lower in diabetes patients and may also have a role in the pathogenesis of coronary artery disease.⁷⁶ SP has further been speculated to have a role in the regulation of glucose metabolism via insulin signaling-associated pathways.⁷⁷

ACKNOWLEDGEMENTS AND FUNDING SOURCES

Studies from the author's laboratories were supported by the Regional Agreement on Medical Training and Clinical Research (ALF) between Stockholm County Council and Karolinska Institutet (project no. SLL 20110177), and the

Swedish National Center for Research in Sports, as well as the Swedish Medical Research Council (2012–3510) (P.W.A.); and The Arthritis Society, The CIHR Institute for Gender and Health, and the Canadian Arthritis Network (D.A.H).

AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

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