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The Role of Nitric Oxide in the Pathogenesis of Venous Ulcers

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SUMMARY Chronic venous insufficiency frequently leads to ulceration. The exact pathophysiological mechanisms underlying the development of venous ulceration remain to be elucidated. One major etiological factor of the trophic changes is the phenomenon of leukocyte trapping. The aim of the study was to review the pathophysiological events culminating in venous ulceration, focusing primarily on the role of alterations in nitric oxide (NO) production. We establish the hypothesis that venous stasis in the microcirculation reduces the rate of shear stress on the endothelial cells, effectively resulting in a decrease in cellular levels of NO, a key event of enhanced adhesion molecule expression and subsequent massive neutrophil activation. A similar series of events is proposed to explain the ischemic-reperfusion tissue injury. Inducible NO (iNO) produced by the inflammatory cells causes free radical injury seen as a venous ulceration.

KEY WORDS venous ulceration; pathophysiology of venous ulcers; nitric oxide

INTRODUCTION

Venous ulcers are chronic wounds due to chronic venous insufficiency (CVI) in the lower limbs. This pathophysiological state is the consequence of sustained elevation of venous pressure in the macrocirculation of the affected leg due to valve incompetence. Chronic venous hypertension leads to secondary disorders of the microcirculation and results in interstitial and skin damage (lipodermatosclerosis and venous ulceration). However, the mechanisms underlying the development of venous ulceration remain to be elucidated.

Many theories have been advanced in the past to explain the sequence of events that give rise to venous ulceration. The simple concept that tissue hypoxia leads to venous ulceration has been largely abandoned (1). In recent years attention has focused on the inflammatory events and the deleterious effects of white cells “trapped” in the microcirculation, which attend venous disease and development of venous ulceration (2).

The chronic inflammation in venous ulcers is similar to cellular events seen in ischemia-reperfusion injury, mainly increases in adhesion molecule expression, NO production and oxidant production (3).

By analogy with the sequence of events that occur during repeated ischemia-reperfusion injury in patients with venous hypertension, it is tempting to speculate a pathogenetic role of disturbed NO production in the development of venous ulceration.

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PATHOPHYSIOLOGY OF VENOUS ULCERS

Congenital or acquired incompetence of valves in the superficial or communicating veins or post-thrombotic damage of valves in deep veins permits venous reflux. Furthermore, an obstruction of the venous outflow may lead to an increased resistance and increased intravascular pressure. Manifestation of these disorders is the development of chronic hypertension in the venous macrocirculation as well as in the capillaries and postcapillary venules. Venous stasis leads to a decrease in the level of shear stress and subsequent endothelial dysfunction, which is manifested in disturbed releases of agents that regulate vasomotor function, trigger inflammatory processes, and affect hemostasis (4,5).

Histopathologic features of the periulcer skin include perivascular infiltration of the capillaries of the papillary plexus with monocytes, macrophages and connective tissue proteins including fibrin (6). These findings have focused attention on the chronic inflammation associated with venous ulcers. The phenomenon of leukocyte trapping in the microcirculation and leukocyte sequestration is similar to the series of events following reperfusion of ischemic tissue that causes tissue damage. In venous ulcers, the ischemic event occurs when the lower extremity is dependent and the normal arterial-venous pressure gradient is no longer present. There is blood stasis and effective loss of circulation. When the leg is elevated, circulation is restored, and the inflammatory changes that occur with reperfusion worsen the injury.

Ischemia-reperfusion: cellular events

During the reperfusion process after an ischemic event secondary to lower extremity dependency in the setting of venous insufficiency, the endothelial surface expresses adhesion molecules. In the initial stages of the adhesion process, P- or E-selectin expressed on the endothelial cells binds to carbohydrates on the leukocyte surface (7). This interaction slows white blood cells and causes them to roll along the endothelial surface. At the same time activated leukocytes release reactive substances such as leukotrienes, interleukins and platelet activating factor (8). Other, subsequently expressed cell adhesion molecules including ICAM-1 and VCAM-1 then latch onto the integrin receptors and completely arrest white cells (7). In the second phase of leukocyte recruitment, activation of leukocytes as well as *de novo* expression of endothelial adhesion proteins and chemokines (e.g. monocyte chemoattractant protein-1, MCP-1) promote leukocyte migration out of the blood vessel and into the interstitium (8,9). Activated leukocytes release toxic metabolites, proteolytic enzymes and free radicals, which cause tissue injury.

Repeated ischemia perfusion events potentiate the cycle of inflammatory cytokines, leukocyte migration, and protease and oxidant injury with loss of tissue perfusion and resultant tissue necrosis.

Nitric oxide

Nitric oxide (NO) is a structurally simple compound that participates in a wide range of biochemical reactions and evokes a variety of biologically relevant responses. It was first discovered in 1980 by Furchgott and Zawadzki as an endothelium-derived relaxing factor (EDRF) (10). In 1987, NO was identified as a biologically active agent in EDRF (11). This finding was awarded Nobel Prize for Medicine and Physiology five years later.

NO is produced from the precursor amino acid L-arginine by the enzyme NO synthase (NOS). NOS is known to be present in at least two isoforms. The first, calcium-dependent isoform, is normally expressed constitutively in the tissues. It includes endothelial NOS (eNOS) in the vascular endothelial cells and neuronal NOS (nNOS) in peripheral nerves (12). The second isoform is calcium-independent, inducible isoform (iNOS) which is produced mainly in pathologic conditions (13). Constitutive NOS rapidly synthesizes small amounts of NO in response to increases of intracellular calcium in pulsative manner. In contrast, iNOS is under transcriptional control whose expression can be induced in a wide range of cells and tissues by cytokines and other agents such as endotoxin (14,15).

Shear forces exerted on the endothelial surface appear to modulate the release of NO (16). NO produced by endothelial cells is important in the regulation of the local blood flow. It is now recognized as a key determinant of vascular health by regulating several physiologic effects, including vascular tone, vascular permeability, endothelial-leukocyte interaction, cell proliferation, and the antithrombotic properties of the endothelium (17). Many studies have shown that NO plays a protective role in several models of inflammation including ischemia-reperfusion by reducing leukocyte-endothelial cell adhesion (18). These functions converge to maintain normal endothelial phenotype and an antithrombotic intravascular milieu.

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NO produced by iNOS has been described to have beneficial microbicidal, antiviral, antiparasitic, and antitumoral effects (14) However, aberrant iNOS induction seems to be involved in the pathophysiology of human diseases such as asthma, arthritis, multiple sclerosis, colitis, psoriasis, chronic wounds, tumor development, transplant rejection, or septic shock (19).

High concentrations of NO are toxic and act as free radicals. Large amounts of NO produced by the induction of iNOS interact with oxygen free radicals derived from polymorphonuclear cells (PMNs) and macrophages to form peroxynitrite (20). NO, either alone or when combined with other oxygen or nitrous free radicals, results in tissue damage.

In addition, sufficient amounts of NO are able to promote apoptotic cell death due to its damaging effects on DNA and the subsequent expression of the tumor suppressor gene, p53 (21,22).

Nitric oxide and wound healing

NO is known to be synthesized in the wound, but its role in the healing process is only beginning to be defined (23). The cellular sources of NO during healing process are probably multiple and are not fully established although inflammatory cells such as macrophages and polymorphonuclear neutrophils have been shown to synthesize large amounts of NO (24).

In acute wound, iNOS predominates in the early stage where the wound environment is cytotoxic. Expression of iNOS at this time is consistent with the known effects of NO, such as vasodilatation (25), antimicrobial activity (26), and anti-platelet aggregation activity (27).

Thus, the expression of NO is important in the normal process of wound healing. We hypothesize that impaired production of NO is associated with disturbed wound healing in chronic wounds including chronic venous ulcers.

The role of nitric oxide in venous ulceration

The increased capillary and venular diameter associated with CVI leads to a decrease in the level of shear stress in the affected vessels and to a diminished synthesis and release of NO from the vascular endothelium. Low endothelial NO bioavailability can upregulate VCAM-1 in the endothelial cell layer (28). The expression of VCAM-1, ICAM-1, and E-selectin plays a role in the initiation of the inflammatory process. VCAM-1 binds monocytes and T lymphocytes, the first step of invasion of the vessel wall by inflammatory cells (29). A reduction in NO results in the induction of MCP-1 expression that recruits mononuclear phagocytes (20).

In the second phase, large amounts of NO produced by the induction of iNOS will interact with oxygen free radical derived from PMNs and macrophages to form peroxynitrite. Peroxynitrite is a potent oxidant that can attack many types of biological molecules and has strong oxidizing and cytotoxic properties causing tissue damage (30). Increased iNOS activity has been demonstrated in the skin of venous ulcer patients (31). NO, either alone or when combined with other oxygen or nitrous free radicals, could result in tissue damage associated with chronic venous ulcers.

In the presence of superoxide (O_2), however, NO reacts extremely rapidly to produce the very reactive and toxic peroxynitrite ($ONOO^-$), which subsequently decomposes into additional reactive intermediates (32).

In macrophages, activation of iNOS generates sufficient amounts of NO to promote the apoptotic cell death (33). Induction of apoptosis could be another mechanism through which NO produces its damaging effect in CVI tissues.

FUTURE DIRECTIONS

The impaired endothelial function and impaired NO production in chronic venous ulcers are thought to be one of the possible mechanisms responsible for developing and delayed healing of venous ulcers. NO may represent a novel target molecule to circumvent many difficulties that occur throughout the healing process of chronic wounds. Because NO is a short-lived gas molecule, maintaining an effective level of NO at the wound site is an obvious problem for clinical therapy. In recent studies, Luo *et al.* have demonstrated that gene therapy of NOS or SOD is effective in restoring cutaneous NO levels and accelerating wound healing in diabetic mice (34).

CONCLUSION

The hemodynamic and hemorheologic changes, together with the pathologic changes in vessel wall physiology, lead to venous ulceration. Impaired NO production manifested by a decrease in the levels of eNO and an increase in the levels of iNO have an important role in the development of

venous ulcers. Gene therapy strategies aimed at increasing NO or reducing superoxide levels may represent an effective means of reversing cutaneous NO deficiency at the wound site for refractory wound healing. Future preclinical studies are warranted to optimize the designs and regimens before clinical trails can be conducted and ultimate translation of basic science to clinical settings for human gene therapy.

REFERENCES

1. Browse NL, Burnard KG. The cause of venous ulceration. Lancet 1982;ii:243-5.
2. Coleridge-Smith PD, Thomas P, Scurr JH, Dormandy JA. Causes of venous ulceration: a new hypothesis. Br Med J 1988;296:1726-7.
3. Kvietys PR, Granger DN. Endothelial cell monolayers as a tool for studying microvascular pathophysiology. Am J Physiol 1997;273: G1-G10.
4. Endemann DH, Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol 2004;15:1983-92.
5. Verma S, Anderson TJ. Fundamentals of endothelial function for the clinical cardiologist. Circulation 2002;105:546-9.
6. Hahn J, Junger M, Friedrich B, Zuder D, Steins A, Hahn M, *et al.* Cutaneous inflammation limited to the region of the ulcer in chronic venous insufficiency. Vasa 1997;26:277-81.
7. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. Blood 1994;84:2068-101.
8. Saharay M, Shields DA, Porter JB, Scurr JH, Coleridge-Smith PD. Leukocyte activity in the microcirculation of the leg in patients with chronic venous disease. J Vasc Surg 1997;26:265-73.
9. Powell CC, Rohrer MJ, Barnard MR, Peyton BD, Furman MI, Michelson AD. Chronic venous insufficiency is associated with increased platelet and monocyte activation and aggregation. J Vasc Surg 1999;30:844-51.
10. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373-6.
11. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelial-derived relaxing factor. Nature 1987;327:524-6.
12. Nathan C, Xie QW. Nitric oxide synthase roles, tolls, and controls. Cell 1994;78:915-8.
13. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991;43:109-42.
14. MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. Annu Rev Immunol 1997;15:323-50.
15. Nathan CF, Hibbs JB Jr. Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr Opin Immunol 1991;3:65-70.
16. Kurose I, Wolf R, Grisham MB, Granger DN. Modulation of ischemia/reperfusion-induced microcirculatory dysfunction by nitric oxide. Circ Res 1999;74:376-82.
17. Kubes P. The role of shear forces in ischemia/reperfusion-induced neutrophil rolling and adhesion. J Leukoc Biol 1997;62;458-64.
18. Bogdan C. Nitric oxide and the immune response. Nat Immunol 2001;2:907-16.
19. Kröncke KD, Fehsel K, Kolb-Bachofen V. Inducible nitric oxide synthase in human diseases. Clin Exp Immunol 1998;113:147-56.
20. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implication for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 1990;87:1620-4.
21. Wang BY, Ho HK, Lin PS, Schwarzacher SP, Pollman MJ, Gibbons GH, *et al.* Regression of atherosclerosis: role of nitric oxide and apoptosis. Circulation 1999;99:1236-41.
22. Sulowska Z, Majewska E, Klink M, Banasik M, Tchorzewski H. Flow cytometric evaluation of human neutrophil apoptosis during nitric oxide generation in vitro: the role of exogenous antioxidants. Mediators Inflamm 2005;2005(2):81-7.
23. Frank S, Kampfer H, Wetzler C, Pfeilschifter J. Nitric oxide drives skin repair: novel functions of an established mediator. Kidney Int 2002;61:882-8.
24. Lyons CR, Orloff GJ, Cunningham JM. Molecular cloning and functional expression of an inducible nitric oxide synthase from murine cell line. J Biol Chem 1992;267:6370-4.
25. Stuehr DJ, Gross SS, Sakuma I, Levi R, Nathan CF. Activated murine macrophages secrete a metabolite of arginine with the bioactivity of endothelium-derived relaxing factor and the chemical reactivity of nitric oxide. J Exp Med 1989;169:1011-20

26. Granger DL, Hibbs JB, Perfect JR, Durack DT. Specific amino acid (L-arginine) requirement for the microbiostatic activity of murine macrophages. J Clin Invest 1988;81:1129-33.
27. Salvemini D, Denucci G, Gryglewski RJ, Vane JR. Human neutrophils and mononuclear cells inhibit platelet aggregation releasing nitric oxide like factor. Proc Natl Acad Sci USA 1989;86:6328-32.
28. Khan BV, Harrison DG, Olbrych MT, Alexander RW, Medford RM. Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. Proc Natl Acad Sci USA 1996;93:9114-9.
29. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 1991;88:4651-5.
30. Zeiher AM, Fisslthaler B, Schray-Utz B, Busse R. Nitric oxide modulates the expression of monocyte chemoattractant protein 1 in cultured human endothelial cells. Circ Res 1995;76:980-6.
31. Abd-El-Aleem SA, Ferguson MW, Appleton I, Kairsingh S, Jude EB, Jones K, *et al.* Expression of nitric oxide synthase isoforms and arginase in normal human skin and chronic venous ulcers. J Pathol 2000;191:434-42.
32. Wink DA, Hanbauer I, Grisham MB, Laval J, Cook J, Pacelli R, *et al.* Chemical biology of nitric oxide: regulation and protective and toxic mechanisms. Curr Top Cell Regul 1996;34:159-87.
33. Albina JE, Cui S, Matheo RB, Reichner JS. Nitric oxide mediated apoptosis in 52 murine peritoneal macrophages. J Immunol 1993;150:5080-5.
34. Luo JD, Wang YY, Fu W, Wu J, Chen AF. Gene therapy of eNOS and MnSOD restores delayed wound healing in type 1 diabetic mice. Circulation 2004;110:2484-93.

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