

Growing blood vessels to treat limb ischemia : studie in mice and man  ${\sf Weel}, {\sf V}. {\sf van}$ 

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**General Introduction** 

## **General Introduction**

Symptomatic peripheral arterial occlusive disease (PAOD) can be treated by interventions such as stenting, angioplasty or bypass surgery. However these interventions, especially those using prosthesis material, are often complicated by restenosis. In a substantial number of patients there is no possibility for revascularization by interventions as indicated above due to e.g. the extent of the disease, leading to limb amputation as the only therapeutic option. Collateral artery growth forms the corner stone of the often mild natural history of PAOD and stimulation of the development of collateral circulation is an important first option of treating ischemic disease. In patients with intermittent claudication, exercise training is a first step in the treatment regime according to the ACC/AHA 2005 guidelines for the management of patients with peripheral arterial disease.<sup>1</sup> Although the exact mechanism of action is not completely understood, it is suggested that exercise exerts its beneficial effect by stimulation of collateral artery growth, but also by induction of changes in muscle metabolism and by enhancement of endothelial function.<sup>2</sup> Interestingly, from clinical practice it is known that patients with ischemic disease show either good or poor collateral formation, the latter often leading to incapacitating claudication or even critical limb ischemia. For these patients, stimulation of collateral growth using vascular growth factors or (stem) cell therapy seems a promising treatment that has been studied for the last couple of years. This dissertation covers a number of aspects related to this topic.

**Chapter 1** provides an overview of molecular and cellular mechanisms of vascular growth, as known to date, and a review of clinical trials to treat PAOD by angiogenic approaches. Unfortunately, the initial therapeutic angiogenesis "hype" has now been tempered due to disappointing results from randomized placebo-controlled trials, calling for more mechanistic insights.

The concept of collateral circulation has been established for many centuries. As early as 1669, anatomist Richard Lower found that collateral anastomoses exist in the human heart.<sup>3</sup> Subsequently, in the 18th century, the famous British physiologist and surgeon John Hunter (1728-1793) found the functional meaning of a collateral circulation in a marvelous experiment. He studied the effects of unilateral ligation of the carotid artery of a deer captured in Richmond Park, London. He observed that shortly after ligation the antler (which was only partially developed and consequently very vascular) on the side of the obliterated artery became cold, whereas a few days later, to his surprise, the antler had become as warm as its fellow, and was apparently increasing in size. On examination, he found that smaller arteries had become enlarged so as to supply the antler with blood by a different route. Hunter's knowledge of collaterals led, among other things, to his successful operation upon popliteal aneurysms by ligation of the femoral artery in the subsartorial (Hunter's)

canal proximal to the aneurysm, first performed in 1785. Subsequently, his disciple Ashley Cooper (1768-1841) performed numerous studies on the collateral circulation in dogs, by ligation of the femoral, brachial, carotid and vertebral artery, and even the abdominal aorta. The Italian Luigi Porta (1800-1875) performed around 600 experiments in 270 animals of various species (goats, donkeys, sheep, horses), in which he demonstrated the presence of collateral vessels after complete obliteration of the aorta that he illustrated marvellously.<sup>4</sup> Similar experiments followed by many others, such as Kast<sup>5</sup>, Halsted<sup>6;7</sup>, Reichert<sup>8</sup> and Leriche<sup>9</sup>. In 1965, Fulton showed that the presence of collateral arteries in the human heart depended on a history of prior coronary artery disease.<sup>10</sup> He observed that patients with slowly progressing atherosclerosis had better-developed collateral arteries than patients with a more acute clinical history. Nevertheless, it was obvious that this collateral network in many cases is insufficient to fully protect against myocardial ischemia or infarction. The idea came to life that stimulation of collateral circulation may be used to treat ischemic disease. With this aim in mind, Wolfgang Schaper commenced studies on cellular and molecular mechanisms of collateral artery growth in the 1960s, which continue to date, now chaperoned by many other research groups. In 1996, Schaper et al. introduced arteriogenesis as a term for development of collateral arteries from a pre-existing arteriolar network as opposed to angiogenesis, which is the sprouting of new capillaries.<sup>11</sup> They postulated that arteriogenesis is more important for restoration of blood flow towards ischemic tissues than angiogenesis.

Vascular growth factors play a crucial role in angiogenesis and arteriogenesis, and their therapeutic implications were first identified by Judah Folkman.<sup>12</sup> To date, a broad spectrum of angiogenic and arteriogenic factors have been shown to be able to successfully stimulate vascular growth in ischemic hind limb models in animals (mainly mice and rabbits). Nevertheless, a large variety of surgical techniques and end-point measurements complicate the interpretation of these models. For instance, surgery ranged from excision of the whole femoral artery including all its side-branches<sup>13</sup> to arterial occlusion over a small segment<sup>14</sup> to excision of femoral artery and vein.<sup>15</sup> In 2004, a consensus meeting on ischemia models was organized by the European Vascular Genomics Network (EVGN) in Porto Conte, Sardinia, to discuss these issues, which provided guiding principles for the use of these models, as described in **Chapter 2**. In this thesis, a short proximal occlusion of the femoral artery was performed in mice, leaving the pre-existing collateral side-branches intact, thereby enabling collateral growth to be studied.

Using this model we encountered, to our surprise, large differences in collateral formation between two mouse strains, similar to the "good" and "poor" collateral formation as observed in patients. Interestingly, these two strains are known to extensively differ in their lymphocyte-mediated immune system. The immune system, mainly involving monocytes, was already shown to play a crucial role in arteriogenesis.<sup>11</sup> Together, this let us to the hypothesis that lymphocytes modulate collateral formation as well. Until then, only one study<sup>16</sup> described a possible role of

CD4+ cells in collateral formation, a surface antigen that, however, not only is expressed on T-cells, but also on a large variety of other inflammatory cells. In **Chapter 3**, we study the role of different types of lymphocytes on collateral formation in mice.

Apart from inflammatory cells, bone marrow-derived cells (BMCs) also gain interest in arteriogenesis research. Promising results are observed from initial clinical studies using autologeous bone marrow transplantation<sup>17</sup>, although the mechanism of action is only partly understood. Recent studies point in the direction of a paracrine role for BMCs in collateral formation, merely excreting angiogenic factors in a perivascular location, rather than incorporating into the vasculature as endothelial progenitor cells.<sup>18-21</sup> Furthermore, it remains to be elucidated how BMCs are attracted to sites where neovascularization is required. Stromal cell-derived factor-1 (SDF-1, a chemokine) has been proposed as a key regulator bridging hypoxia with BMC recruitment in animal studies.<sup>22</sup> In **Chapter 4**, we study for the first time expression patterns of SDF-1, and its receptor CXCR4, which is expressed on BMCs, in ischemic amputated limbs of patients with PAOD, together with other crucial angiogenic factors.

Obviously, patients included in this study suffered from chronic ischemic disease with insufficient collateral compensation, ultimately leading to amputation. One major challenge is to understand how collateral formation is impaired in these patients. In mouse models to primarily study angiogenesis, it was shown that deregulation of either lipid metabolism<sup>23-25</sup> or glucose metabolism<sup>26-28</sup> results in impaired neovascularization. In clinical practice, poor collateral formation is most evidently observed in diabetics. Interestingly, a disturbed lipid metabolism has recently been proposed to play a role in both the pathogenesis<sup>29-34</sup> and complications<sup>35;36</sup> of diabetes. This warranted a study of the relative contributions of either a disturbed lipid- or a disturbed glucose metabolism on impairment of collateral formation, as described in **Chapter 5**.

Vascular endothelial growth factor is the most extensively studied angiogenic growth factor. Although VEGF administered to humans with severe lower limb ischemia showed salutary effects in early trials, evidence for ameliorated perfusion is weak. In pilot experiments applying intramuscular VEGF gene therapy in our mouse model, we noted that ischemic muscles expressing VEGF became deeply red in color, in the absence of changes in angiographic scores of collateral vessels. We hypothesized that this is due to increased myoglobin expression, a protein involved in muscle oxygenation. VEGF may prove additionally beneficial by changing properties of skeletal muscle fibers to function better during limited perfusion. In **Chapter 6**, the mechanism of VEGF-induced myoglobin up-regulation is studied in mice and human muscle samples. In an editorial published in Circulation Research<sup>37</sup> our findings are put in a broader perspective, holding promise for the treatment of not only ischemic disease, but also heart failure, renal failure, pulmonary disease, advanced age and diabetes.

Parallel to our basic research, we brought VEGF gene therapy from bench to bedside, as described in **Chapter 7**. In a multi-center randomized trial, treatment with intramuscular VEGF-containing plasmid was compared with placebo for 54 patients with diabetes mellitus and critical limb ischemia. Although, since the start of this trial in 2000, placebo-controlled trials using VEGF had been published with disappointing results<sup>38;39</sup>, and VEGF had been shown to merely promote angiogenesis, not arteriogenesis in pre-clinical models<sup>40;41</sup>, patient inclusion was finished in 2004 and it appeared that VEGF treatment had significant beneficial effects on hemodynamics and ulcer healing. Hopefully, this randomized study could serve to regenerate interest in the angiogenic approach to treat ischemic disease.

## References

- 1. Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, Hiratzka LF, Murphy WR, Olin JW, Puschett JB, Rosenfield KA, Sacks D, Stanley JC, Taylor LM, Jr., White CJ, White J, White RA, Antman EM, Smith SC, Jr., Adams CD, Anderson JL, Faxon DP, Fuster V, Gibbons RJ, Hunt SA, Jacobs AK, Nishimura R, Ornato JP, Page RL, Riegel B. ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. *Circulation*. 2006;113:e463-e654.
- 2. Stewart KJ, Hiatt WR, Regensteiner JG, Hirsch AT. Exercise training for claudication. *N Engl J Med*. 2002;347:1941-1951.
- 3. Lower R. Tractus de Corde. Amsterdam: Elsevier. 1669.
- 4. Porta L. Delle Alterazioni Patologiche delle Arterie per la legatura e la torsione. Milano:Tipografia di Giuseppe Bernardoni di Gio. 1845.
- 5. Kast A. Die Unterbindung der Bauchaorta. Ztschr. f. Chir, XII, 405. 1880.
- 6. Halsted WS. Partial, progressive and complete occlusion of the aorta and other large arteries in the dog by means of the metal band. *J Exp Med.* 1909;XI:381.
- 7. Halsted WS. The effect of ligation of the common iliac artery on the circulation and function of the lower extremity. Johns Hopkins Hospital Bulletin, XXIII, 65. 1912.
- 8. Reichert F. An experimental study of the anastomotic circulation in the dog. Johns Hopkins Hospital Bulletin, XXXV, 385-390. 1924.
- 9. Leriche R. Physiologie Pathologique et Chirurgie des Artères, Masson et Cie. 1943.
- 10. Fulton WFM. The Coronary Arteries. Springfield, Illinois: Charles C Thomas. 1965.
- 11. Scholz D, Cai WJ, Schaper W. Arteriogenesis, a new concept of vascular adaptation in occlusive disease. *Angiogenesis*. 2001;4:247-257.
- 12. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med*. 1971;285:1182-1186.
- 13. Couffinhal T, Silver M, Zheng LP, Kearney M, Witzenbichler B, Isner JM. Mouse model of angiogenesis. *Am J Pathol*. 1998;152:1667-1679.
- 14. Van Weel V, Deckers MM, Grimbergen JM, van Leuven KJ, Lardenoye JH, Schlingemann RO, Nieuw Amerongen GP, van Bockel JH, van Hinsbergh VW, Quax PH. Vascular endothelial growth factor overexpression in ischemic skeletal muscle enhances myoglobin expression in vivo. *Circ Res.* 2004;95:58-66.
- 15. Masaki I, Yonemitsu Y, Yamashita A, Sata S, Tanii M, Komori K, Nakagawa K, Hou X, Nagai Y, Hasegawa M, Sugimachi K, Sueishi K. Angiogenic gene therapy for experimental critical limb ischemia: acceleration of limb loss by overexpression of

vascular endothelial growth factor 165 but not of fibroblast growth factor-2. *Circ Res*. 2002;90:966-973.

- 16. Stabile E, Burnett MS, Watkins C, Kinnaird T, Bachis A, la Sala A, Miller JM, Shou M, Epstein SE, Fuchs S. Impaired arteriogenic response to acute hindlimb ischemia in CD4-knockout mice. *Circulation*. 2003;108:205-210.
- 17. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, Shimada K, Iwasaka T, Imaizumi T. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet*. 2002;360:427-435.
- Peters BA, Diaz LA, Polyak K, Meszler L, Romans K, Guinan EC, Antin JH, Myerson D, Hamilton SR, Vogelstein B, Kinzler KW, Lengauer C. Contribution of bone marrowderived endothelial cells to human tumor vasculature. *Nat Med*. 2005;11:261-262.
- 19. Rajantie I, Ilmonen M, Alminaite A, Ozerdem U, Alitalo K, Salven P. Adult bone marrowderived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. *Blood*. 2004;104:2084-2086.
- Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science*. 2002;297:2256-2259.
- 21. Ziegelhoeffer T, Fernandez B, Kostin S, Heil M, Voswinckel R, Helisch A, Schaper W. Bone marrow-derived cells do not incorporate into the adult growing vasculature. *Circ Res*. 2004;94:230-238.
- 22. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med*. 2004;10:858-864.
- 23. Couffinhal T, Silver M, Kearney M, Sullivan A, Witzenbichler B, Magner M, Annex B, Peters K, Isner JM. Impaired collateral vessel development associated with reduced expression of vascular endothelial growth factor in ApoE-/- mice. *Circulation*. 1999;99:3188-3198.
- Tirziu D, Moodie KL, Zhuang ZW, Singer K, Helisch A, Dunn JF, Li W, Singh J, Simons M. Delayed arteriogenesis in hypercholesterolemic mice. *Circulation*. 2005;112:2501-2509.
- 25. Van Belle E, Rivard A, Chen D, Silver M, Bunting S, Ferrara N, Symes JF, Bauters C, Isner JM. Hypercholesterolemia attenuates angiogenesis but does not preclude augmentation by angiogenic cytokines. *Circulation*. 1997;96:2667-2674.
- 26. Abaci A, Oguzhan A, Kahraman S, Eryol NK, Unal S, Arinc H, Ergin A. Effect of diabetes mellitus on formation of coronary collateral vessels. *Circulation*. 1999;99:2239-2242.
- 27. Rivard A, Silver M, Chen D, Kearney M, Magner M, Annex B, Peters K, Isner JM. Rescue of diabetes-related impairment of angiogenesis by intramuscular gene therapy with adeno-VEGF. *Am J Pathol.* 1999;154:355-363.
- Tamarat R, Silvestre JS, Huijberts M, Benessiano J, Ebrahimian TG, Duriez M, Wautier MP, Wautier JL, Levy BI. Blockade of advanced glycation end-product formation restores ischemia-induced angiogenesis in diabetic mice. *Proc Natl Acad Sci U S A*. 2003;100:8555-8560.
- 29. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes.* 1997;46:3-10.

- 30. Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *Eur J Clin Invest*. 2002;32 Suppl 3:14-23.
- 31. Lam TK, Carpentier A, Lewis GF, van de WG, Fantus IG, Giacca A. Mechanisms of the free fatty acid-induced increase in hepatic glucose production. *Am J Physiol Endocrinol Metab.* 2003;284:E863-E873.
- 32. Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev.* 2002;23:201-229.
- 33. Petersen KF, Shulman GI. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *Am J Cardiol.* 2002;90:11G-18G.
- 34. Shulman GI. Cellular mechanisms of insulin resistance in humans. *Am J Cardiol*. 1999;84:3J-10J.
- 35. Festa A, Kopp HP, Schernthaner G, Menzel EJ. Autoantibodies to oxidised low density lipoproteins in IDDM are inversely related to metabolic control and microvascular complications. *Diabetologia*. 1998;41:350-356.
- 36. Lopes-Virella MF, Virella G. Cytokines, modified lipoproteins, and arteriosclerosis in diabetes. *Diabetes*. 1996;45 Suppl 3:S40-S44.
- 37. Williams RS, Annex BH. Plasticity of myocytes and capillaries: a possible coordinating role for VEGF. *Circ Res.* 2004;95:7-8.
- Henry TD, Annex BH, McKendall GR, Azrin MA, Lopez JJ, Giordano FJ, Shah PK, Willerson JT, Benza RL, Berman DS, Gibson CM, Bajamonde A, Rundle AC, Fine J, McCluskey ER. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. *Circulation*. 2003;107:1359-1365.
- 39. Rajagopalan S, Mohler ER, III, Lederman RJ, Mendelsohn FO, Saucedo JF, Goldman CK, Blebea J, Macko J, Kessler PD, Rasmussen HS, Annex BH. Regional angiogenesis with vascular endothelial growth factor in peripheral arterial disease: a phase II randomized, double-blind, controlled study of adenoviral delivery of vascular endothelial growth factor 121 in patients with disabling intermittent claudication. *Circulation.* 2003;108:1933-1938.
- Deindl E, Buschmann I, Hoefer IE, Podzuweit T, Boengler K, Vogel S, van Royen N, Fernandez B, Schaper W. Role of ischemia and of hypoxia-inducible genes in arteriogenesis after femoral artery occlusion in the rabbit. *Circ Res.* 2001;89:779-786.
- 41. Hershey JC, Baskin EP, Glass JD, Hartman HA, Gilberto DB, Rogers IT, Cook JJ. Revascularization in the rabbit hindlimb: dissociation between capillary sprouting and arteriogenesis. *Cardiovasc Res.* 2001;49:618-625.