Review paper

The cardiologists' dream may come true – vascular therapy

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Abstract

Recently there has been a great evolution of new diagnostic and therapeutic methods of cardiovascular diseases. However, they still remain the most common death cause in the Western countries. Despite the fact that new methods of dealing with cardiovascular diseases like for example percutaneous coronary interventions or coronary artery bypass grafting are constantly being improved, there is still a group of patients that can not undergo the routine treatment. It is a good stimulus for searching different ways of dealing with cardiovascular diseases. New vascular methods based on interfering in processes of vessels' creation and regression, seem to be an interesting direction of current studies. This article reviews the basis of vasculogenesis, angiogenesis and arteriogenesis and angiogenic therapies, learned from the latest trials. Vascular therapies offer a promise as a novel treatment for ischemic heart disease, particularly for patients who are not candidates for current methods of revascularization.

Key words: vasculogenesis, angiogenesis, arteriogenesis, vascular therapy, angiogenic therapy.

Introduction

Vascular network's creation in the embryo is one of the most important stages of the prenatal life without which further structural and functional changes in the organism would not be possible. From the first weeks of the intrauterine life till the last moments of human existence the circulatory system pumps around the body great amounts of blood, linking tissues and affirming homeostasis.

A dysregulated formation of new blood vessels has serious consequences on the body's function and can be a cause of many diseases. An excessive blood vasculature contributes to neoplasms, psoriasis, arthritis, diabetic retinopathy, obesity, asthma, atherosclerosis and infectious disease.

On the other hand, an insufficient vessel growth or abnormal vessel regression can lead to the heart and brain ischemia, neurodegeneration, arterial hypertension, respiratory distress syndrome of adults and osteoporosis. It seems that keeping a balance between creation and degradation of vessels is crucial for a normal structure and function of the organism.

One could suspect that issues concerning formation and function of the cardiovascular system are well known but in fact the most important discoveries in the vascular biology took place during the last 10 years.

Medicine has great expectations for studies on vessels physiology and large amounts of money are invested in this field of science. Results and conclusions from those trials may be extremely important for developing an effective treatment for diseases with abnormal, insufficient or excessive

vascular pathogenesis. Those issues are especially important for the cardiologist dealing every day with ischemic heart disease which still remains the most frequent death cause in Western countries.

According to the American Heart Association, 571 000 of percutaneous transluminal coronary angioplasties (PTCA), 516 000 coronary artery bypass surgeries (CABG) and 128 000 endartectomies were performed in 2001 in the USA. Although treatment methods in cardiology have become very subtle and effective, there is still a big group of patients with severe or multivessel type of ischemic heart disease who can not undergo the routine treatment because of a very low efficacy and many contraindications. Another serious problem is restenosis observed among about 30% of patients after PTCA [1].

All these problems are a very good stimulus for scientists to search and improve new therapeutic options for patients with cardiovascular diseases.

Structure and function of blood vessel

From the early stages of the prenatal life a great anatomical and physiological variety can be noticed in different parts of the cardiovascular system. Blood vessels vary from large strong conduits to tiny lace-like networks. It is due to a wide range of tasks to be fulfilled by all cells, tissues and organs. Also it is a reason why there are so many problems for scientists dealing with the interruption in the processes of blood vessels formation and regression.

Cells that form walls of the arteries, capillaries and veins have specific receptors for proper agonists. Moreover, cells present different expression and activity levels of molecules such as growth factors, chemokins, cytokins, hormones or neuropeptides.

According to many studies angiogenic factors have the influence on blood vessels' permeability, development and reconstruction [2]. With the help of those factors scientists may try to stimulate or inhibit vessels formation in vitro and in vivo. The amount of factors with proved angiogenic properties constantly increases with upcoming new trials and studies.

The complicated laminar structure provides high vessels' resistance to blood flow mechanical stress and also links the endothelium with the extravascular space. Interactions between the endothelium, myocytes and pericytes have a major influence not only on endothelial cells proliferation, migration and differentiation, but also on vessels sprouting, permeability and blood flow.

When a structure of the vascular wall is disrupted because of abnormalities or an insufficient amount of cells, the vessels become more susceptible to pathogens. It creates conditions for easier bleeding, abnormal perfusion, hypoxia and excessive permeability.

According to all those facts mentioned above, it is a great challenge for the vascular biologist to create the functional blood vessels network. Moreover, it is a very attractive chance for finding some new treatment for a certain group of diseases.

Blood vessels creation

Vessels can be formed due to a few processes such as vasculogenesis, angiogenesis and arteriogenesis. Each plays an important role under certain conditions.

Vasculogenesis is a complex process of in situ formation of blood vessels from the endothelial precursors cells and angioblasts (Figure 1). Initially, mesenchymal cells distinguish into early hemangioblasts that form cellular aggregates (vascular islands). The inner cell population differentiates into haematopoietic precursors while the outer cell population gives rise to the vascular endothelium. Finally, the primitive vascular plexus develops into a complex network of mature blood vessels. Until recent years, vasculogenesis was thought to be restricted to embryonic development. Trials demonstrated that bone-marrow derived endothelial precursors cells circulate in the peripheral blood and can be incorporated into areas of neovascularization in adults [3].

Angiogenesis is a complicated and still not very well known multi-step process involving extensive interplay between cells, soluble factors and extracellular matrix components. It refers to the sprouting and growth of small vessels, branching and extension of existing capillaries by the assembly of endothelial cells from preexisting vessels (Figure 2). Crucial steps of the process are included in Figure 3 [4]. Angiogenesis is important in physiological conditions such as development of the vascular network in a maturing organism, wound healing and endometrial changes during the menstrual cycle. However, angiogenesis has been implicated in numerous pathological processes like for example tumor growth as well.

The major physiological stimuli for angiogenesis are: tissue ischemia and hypoxia, inflammation, shear stress. A number of specific factors are known to stimulate or inhibit angiogenesis, including vascular growth factors, inflammatory cytokines, adhesion molecules, and nitric oxide. Many recent trials have revealed many of the factors stimulating and inhibiting angiogenesis (Table I), so it is merely probable that a single factor could play the crucial role in blood vessels' formation and regression. It may be more probable that cytokines form a complicated 'cocktail' in which a prevalence of some factors over the others decides about a direction of vascular processes [5]. In these conditions it is not surprising that some attempts of monotherapy do not come up to scientists' expectations. A dynamic balance between angiogenesis and vascular regression is a necessary condition for keeping haemostasis in the body.

Recently 5 isoforms of VEGF have been described: VEGF 121, VEGF 145, VEGF 165, VEGF 189 and VEGF 206 [6]. VEGF is expressed by many lines of cells and has an influence mainly on endothelial cells by binding to VEGF's receptor (VEGFR-1 and VEGFR-2). This specificity of VEGF for endothelial cells is a great advantage because endothelial cells represent the most critical structures on a cellular level for the formation of new vessels. VEGF can be crucial for therapeutic strategies of forming and destroying blood vessels [7].

It was observed that angiogenesis and inflammation have many common features and very often coexist in some diseases like in atherosclerosis. Neutrophils, macrophages, mastocytes, dendritic cells, monocytes have an indirect influence on new vessels' creation due to releasing numerous angiogenic factors [8]. On the other hand, angiogenic factors have chemotactic features and can intensify inflammation by attracting inflammatory cells from the blood stream. Angiogenesis is also tightly connected with processes of haemostasis which is observed in vessels sprouting in the wound's clot [9].

Endothelial cells can create new vessel branches only if a wall is destroyed by proteases such as urokinase-type plasminogen activator (uPA), plasminogen activator inhibitor-1 (PAI-1), matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [10]. Factors mentioned above release other activators of angiogenesis and chemokins which are necessary for the next steps of vascular network's formation.

Also crucial for angiogenesis are some glycoproteins from the family of angiopoietins (Ang-1 and Ang-2) [11]. Ang-1 is a potent growth factor that induces vessel formation in the skin, ischemic limbs, gastric and duodenal ulcers, some tumors [12]. On the other hand, Ang-1 has an antiangiogenic action, inhibiting angiogenesis in many tumors and the heart [13]. It is suggested that Ang-2 by inducing growth of immature blood vessels in tumors has some antagonistic action to Ang-1. In the cardiac muscle, Ang-2 with a company of VEGF stimulates angiogenesis, but alone leads to the death of endothelial cells [14].

On the one hand, vascular regression plays an important role in pathogenesis of such diseases as stroke, atherosclerosis, arterial hypertension, diabetes, Alzheimer's disease, neuropathies and osteoporosis. However, it is also a very interesting approach towards treatment of diseases with excessive vessels growth.

It was proven that the removal of proangiogenic factors leads to vessels' regression not only in tumors but also in the heart. It was observed clearly especially with immature vessels [15].

Angiogenic factors that bind to their receptors begin a multi-step process which ends in the formation of a vascular network. Using antibodies against VEGF's receptors (Flt-1) we can inhibit blood vessels creation in tumors, ischemic retina or inflamed articulations [16]. It is a new therapeutic solution for diseases with excessive vessels creation.

Table I. Stimulators and inhibitors of angiogenesis. The quantity of stimulators and inhibitors alludes cooperation of factors in vessels' formation

Angiogenesis	
Stimulators	**Inhibitors**
Ang-1	2-metoxyestradiol
Angiogenin	Angioarrestin
EGF	Angiostatin
FGF	Canstatin
Follistatin	CD59
G-CSF	CDI
HGF	Endostatin
Leptin	hCG
LPA	Heparinases
PDGFB	IFN alfa/beta/gamma
PGF	Il-12
Progranulin	Kringle 5
Proliferin	PAI
PTN	Prolactin 16kD
TGF alfa	Retinoids
TGF beta	TIMPs
VEGF	TSP-1
	Tumstatin
	Vasculostatin
	Vasostatin

Arteriogenesis is the transformation of small arterioles into much larger well-muscularized, conductance arteries (Figure 4). It has been recognized that patients with ischemic vascular disease tend to develop natural bypass vessels and a degree of their development decides about clinical symptoms. Occasionally, patients with complete occlusion of a major coronary artery, have no or only minimal symptoms and normal myocardial function associated with well-deformed collateral vessels. In clinical trials, the presence of angiographically visible collateral vessels has been associated with fewer ischemic electrocardiographic changes and less anginal pain on occlusion of a coronary artery by balloon inflation. Collateral vessels also have been associated with a lower chance of developing left ventricular aneurysms and with higher survival rates after myocardial infarction [17].

The existence of the collateral flow is crucial for tissues' survival in the case of the narrowing or the total occlusion of the blood vessel. Capillaries supply a few cells with blood but arteries provide the flow

Table II. Summary of the most important clinical studies with the use of angiogenic factors and gene and cell therapies. n – number of patients; ↓ – less, ↑ – more; EF – Ejection Fraction; FIRST – FGF-2 Initiating Revascularization Support Trial; FGF – Fibroblast Growth Factor; VIVA – VEGF in Ischemia for Vascular Angiogenesis; AGENT – Angiogenic GENe Therapy; BMC – bone marrow-derived mononuclear cells; TOPCARE-AMI – Transplantation of Progenitor Cells and Regeneration Enhancement in acute Myocardial Infarction); EPC – Endothelial Progenitor Cells; AMI – Acute Myocardial Infarction; CAD – Coronary Arteries Disease

Reference	Angiogenic factor/ Cells' type	Administration	Patients	Effect
FIRST [24]	FGF-2	Intracoronary	n=337 aggravation of CAD and drug-resistant angina	↓ angina symptoms
VIVA [25]	VEGF	Intracoronary	n=178 stable angina on exertion	↓ angina symptoms, fewer angina pains, better results on exertion test
AGENT [26]	Ad5-FGF4	Intracoronary	n=79 II/III CCS with chronic stable angina	better results in the exertion test
Strauer [30]	BMC	Intracoronary	n=10 up to 9 day after AMI	↑ transcutaneous oxygen pressure, ↓ pain at rest, no pain on exertion
TOPCARE-AMI [14]	BMC vs EPC	Intracoronary	n=20 4 days after AMI	↑ global and regional EF, ↓ End-systolic volume, ↑ viability, ↑ coronary flow reserve
Tse [32]	BMC	Intramyocardial	n=8 CAD	↑ wall motion and thickening
Fuchs [33]	BMC	Intramyocardial	n=10 CAD	angina score and stress-induced ischemia improved
Perin [31]	BMC	Intramyocardial	n=14 chronic CAD	↑ EF, ↓ end-systolic volume

in bigger areas and consequences of their occlusion are much more severe.

There are a few theories about collateral vessels creation. One of them presupposed the existence of precollaterals, vessels in which the flow is minimal in normal conditions. When the narrowing or the critical occlusion appears the pressure in the pre-occluded part of the vessel increases, the laminar flow is disturbed and the blood can be directed to precollaterals. It is stated that the main factor which influences formation of collaterals is a bigger blood flow.

According to another theory, collateral arteries are created de novo. The proof comes from angiography where vessels that were not observed before, appear after the occlusion of the artery. Also it has to be underlined that angiography has a specified resolution. It is possible that in normal conditions we do not see any collaterals because a very little amount of contrast enters collaterals [18]. There are observations that after the occlusion of the coronary artery some cells (like for example monocytes) accumulate near the endothelium and give the origin to the collateral vessels network [19]. The basal membrane is degradated by proteases. Myocytes lose their contractile properties, start to proliferate and create neointime. After some time they regain the previous phenotype and function. Around blood vessels leucocytes can accumulate and create inflammation features. It was proven that administration of glycocorticosteroids inhibits collateral growth [20]. In comparison with normal vessels in collaterals there is higher blood pressure and excessive mechanical shear stress becomes a factor that activates endothelial cells and monocytes, and higher expression of growth factors and proteases is started.

Studies proved that arteriogenesis is a local process. Bone marrow cells are not needed for development of collaterals. In contrast to angiogenesis, arteriogenesis does not depend on the presence of hypoxia or ischemia [21]. After the first stage of arteriogenesis where inflammation plays a very important role, a stage of slow rebuilt and mature vessels network creation comes [22].

Vascular therapies

For the treatment of patients with ischemic vascular diseases, the augmentation of vascular growth is a very attractive therapeutic strategy. The type of disease may determinate which kind of vascular growth (arteries, veins or capillaries) is likely to be the most beneficial.

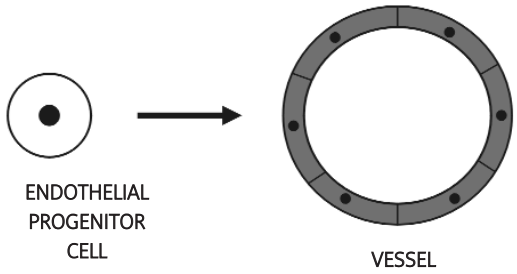

Figure 1. Vasculogenesis. Transformation of progenitor endothelial cells into mature cells capable of forming a new vessel

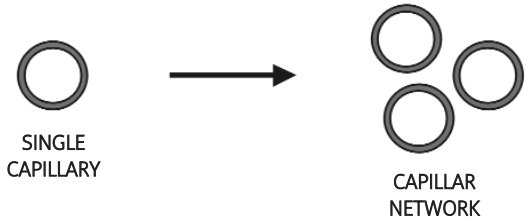

Figure 2. Angiogenesis. Extension of vessels' network by forming new ramifications of already existing vessels and creating new anastomoses

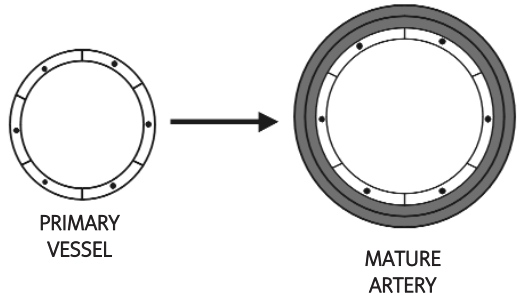

Figure 4. Arteriogenesis. Transformation of a primary vessels into a mature artery. Walls of the artery consist of many layers formed by differentiated cells

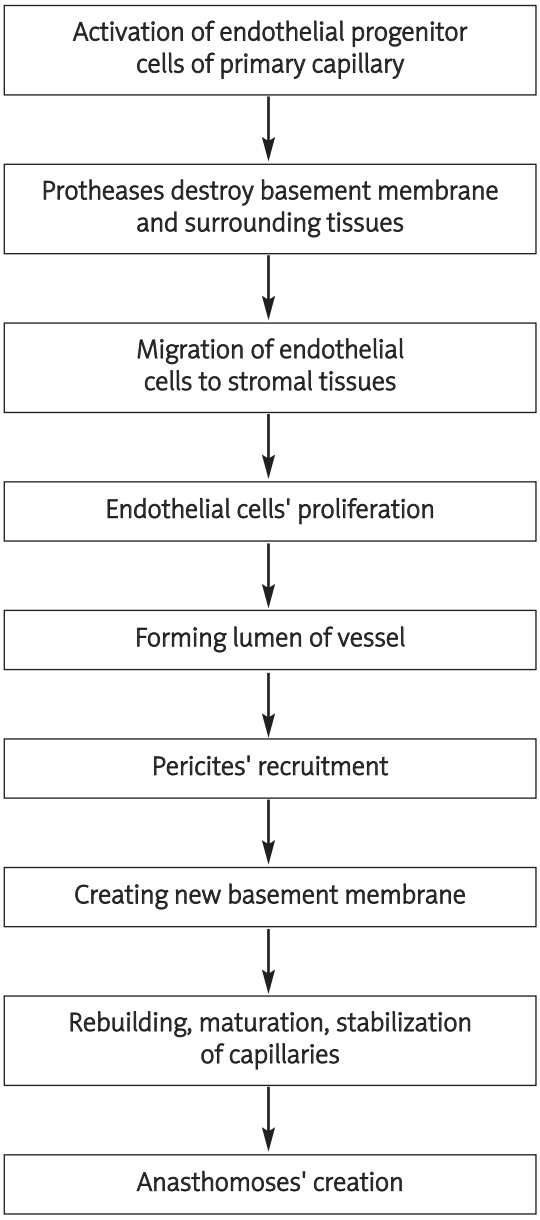

Figure 3. Stages of angiogenesis. Formation of vessels' network from a single capillery

Among patients with Burger's disease and thromboangiitis obliterans, where microcirculation (arterioles, capillaries or venules) is mainly affected, therapeutic angiogenesis may be very profitable. Arteriogenesis would be useful in the case of patients with atherosclerotic changes located in the arteries [23]. Also it could be very beneficial to stimulate transformation of precollaterals into mature collaterals in the ischemic tissues.

The creation of functional blood vessels network in ischemic areas could be possible due to protein, gene and cell therapy. Those approaches base on the fact that growth factors stimulate vessels growth in areas where regression or degradation of vessels appears.

The first published results from the studies over angiogenesis concerned on various animal models with the use of such factors as FGF-1, FGF-2, VEGF. Very attractive results from experiments performed in vitro encouraged scientists to start some pilot clinical studies.

The study groups for trials over vascular therapies were chosen from patients who were not candidates for conventional revascularization techniques of balloon angioplasty and stenting, or coronary artery bypass grafting. The patients had not only a chronic but also acute type of ischemic heart disease. Angiogenic factors were administered with the use of proteins or vectors with genetic information (gene

therapy) to the coronary arteries or to the epicardium, or directly to the myocardium. Also endothelial pregenitor cells (EPC) potent in forming new blood vessels were used (cell therapy). Below there are presented results of the most important trials performed in recent years (Table II).

The FGF Initiating Revascularization Trial **(FIRST)** is a multicenter, randomized, double blind, placebo-controlled trial of a single intracoronary infusion of rFGF2 in 337 patients which evaluated the efficacy and safety of recombinant fibroblast factor 2 (rFGF2) [24]. A single intracoronary infusion of rFGF2 did not improve exercise tolerance, myocardial perfusion or angina symptoms at 180 days significantly, due to continued improvement also seen in the placebo group. Adverse events were similar across all groups.

The **VIVA** trial (Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis) was the study of 178 patients with stable exertion angina unsuitable for standard revascularization. Intracoronary infusions of rhVEGF were followed by intravenous infusions on days 3, 6, and 9. The study was double-blinded, placebo-controlled, designed to evaluate the safety and efficacy of intracoronary and intravenous infusions of the recombinant vascular endothelial growth factor (rhVEGF). The RhVEGF seemed to be safe and well tolerated. By day 120, only high-dose rhVEGF resulted in a significant improvement in angina and favorable trends in exercise tolerance time and angina frequency [25].

The **AGENT** (Angiogenic GENe Therapy), randomized double-blind study was among the first published viral gene therapy studies. One of the objectives was to evaluate the safety and anti-ischemic effects of 5 ascending doses of intra-coronary pro-angiogenic FGF4 delivered in an adenoviral vector (Ad5-FGF4) to 79 patients with stable mild to moderate angina and no clinically significant heart failure. Another aim was to select potentially safe and effective doses for the subsequent study. The Ad5-FGF4 was administered by a percutaneous intracoronary infusion. The limitation of this trial is, that only one dose group showed positive results. Thus, there was overall no significant difference in the clinical efficacy between the treated and placebo patients at the time of onset of angina, although a post-hoc analysis suggested that patients who received active treatment improved more than the placebo patients in treadmill walking times [26].

There were also continuations of the AGENT study, but they did not meet their endpoints.

The Kuopio Angiogenesis Trial **(KAT)** was the randomized, placebo-controlled, double-blind phase II study with 103 patients of CCS class II to III coronary heart disease after performed PTCA combined with stent implantations in 90% of patients, followed by gene transfer with a perfusion-infusion catheter. VEGF adenovirus was compared to VEGF plasmid liposomes, and controls. After 6 months, myocardial perfusion showed a significant improvement in the VEGF-Adv-treated patients. Some inflammatory responses were transiently present in the VEGF-Adv group, but no increases were detected in the incidences of serious adverse events in any of the study groups [27].

In the **REVASC** study direct intramyocardial adenoviral delivery of a VEGF-A isoform was performed. The study group consisted of no-option patients with severe angina that was poorly controlled on standard medical therapy. The trial was randomized but not blinded. Injections were made directly to the target ischemic zone of the left ventricle via mini-thoracotomy, not by intra-arterial infusions (like in the AGENT). There was a difference in the time to the 1 mm ST segment depression at 26 weeks. Secondary endpoints were positive, including the onset of angina, total exercise duration, and improvement of the CCS angina class [28].

Investigators of the **TACT** study (Therapeutic Angiogenesis by Cell Transplantation) performed a randomized controlled trial in 22 patients with peripheral artery disease. After the intramuscular injection of the bone marrow-derived mononuclear cells (BMCs) they reported a significant increase in transcutaneous oxygen pressure, rest pain and pain-free walking time [29]. The study supported the concept that cell therapy may augment neovascularization, leading to oxygen supply to the tissue.

Strauer infused bone marrow-derived mononuclear cells 5 to 9 days after the acute myocardial infarction [30]. In comparison to nonrandomized control patients, who did not undergo cell therapy or additional catheterization, bone marrow-derived mononuclear cell infusion enhanced regional infarct region perfusion as assessed by thallium scintigraphy. Moreover, stroke volume, end-systolic volume, and contractility indices were improved after cell therapy.

In the **TOPCARE-AMI** trial (Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction), patients were randomized to receive either bone marrow-derived mononuclear cells or endothelial progenitor cells. Bone marrow cells or endothelial progenitor cells were infused 4 days after myocardial infarction. Bone marrow cells and endothelial progenitor cells significantly improved the global ejection fraction assessed by left ventricular angiography, compared with a nonrandomized control patient group. Functional improvement was confirmed by MRI in a patient subgroup [29].

Also four other investigations should be mentioned in which bone marrow-derived stem cells were injected to the myocardium during CABG or

using the NOGA catheter. Perin et al. showed a significant improvement of the ejection fraction and end-systolic volume in 14 patients with chronic ischemic heart failure [31]. Tse et al. treated 8 patients with ischemic heart disease and reported improvement of the wall motion and wall thickening as well as decreased hyperperfusion after cell therapy [32]. Fuchs et al. tested the effect of total unfractionated total bone marrow in 10 no-option patients with advanced coronary artery disease. The CCS angina score and stress-induced ischemia were significantly improved after a 3-month follow up [33]. Results of those studies were promising because of better coronary flow, significant improvement in CCS angina, higher ejection fraction.

All those studies are limited by small patients and by the design of pilot safety and feasibility studies, which precludes a randomized, placebo-controlled, double-blind design.

The recently performed EUROINJECT ONE Trial is a double-blind randomized study in which 80 no-option patients with severe stable ischemic heart disease, CCS class 3 to 4 were observed. An analysis of changes in the myocardial perfusion in the NOGA-defined regions with intramyocardial injections of plasmid encoding phVEGF-A165 was performed. It revealed that injections of phVEGF-A165 plasmid improve, but do not normalize, the stress-induced perfusion abnormalities [34].

Potential complications of vascular therapy

Although current efforts are focused on targeting angiogenesis to ischemic myocardium, there exists the theoretical risk of unwanted blood vessels growth in adjacent or distant tissue sites. In addition, VEGF is known to increase vascular permeability and tissue edema and to cause hypotension, whereas the FGF therapy is associated with proteinuria. Broader safety concerns include the possibility of accelerating occult tumor growth, diabetic retinopathy, or atherosclerosis. Up till now, about 1000 patients took part in different trials with the use of gene or cell angiogenic therapies and no increased risk of aberrant neovascularization has been reported, although a long-term clinical follow-up is needed [35, 36].

Summary

Nowadays trials performed on animal models concentrate mainly on major mechanisms of angiogenesis and on determining the exact role of singles factors. Results of in vivo studies and early phase clinical trials suggest that a mixture of factors is more effective than an administration of a single factor. Stem and progenitor cells can be a source of all the components needed for new myocardium and functional vascular bed.

Much remains to be learned about different steps and mediators involved in the formation of collateral arteries. More systematic studies of the gene and protein expression profiles may help to improve our understanding about various events that are important for generating new vessels. What we should focus on is the way of administration of the therapeutic material, choice of appropriate end points and a well selected group of patients.

Ischemic vascular diseases have remained the most common cause of death in the Western world and are the cause of major morbidity. Despite the positive impact of primary and secondary prevention efforts, the aging of populations in many countries is not likely to change in the foreseeable future. All this situation is a reason for the intensive search for new therapeutic methods with the use of angiogenic factors and development of vascular therapy.

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