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# Overview of the Basic Research on Vascular Surgery in China

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*This paper sums up the fundamental research projects on Vascular Surgery supported by National Natural Science Foundation of China from 1996 to 2000, and presents the experimental results and advances in the aspects of ischemia disease, the formation mechanism and prevention of restenosis, the development of abdominal aorta aneurysm and portal hypertention.*

**Key words** vascular surgery, experimental research

This paper generalizes and analyses the progress of the fundamental research projects on Vascular Surgery supported by National Natural Science Foundation of China from 1996 to 2000.

During the 5 years, 1.41 millions RMB were invested into the vascular surgical research and seven institutions obtained the financial assistance in all. The first five institutions on the list who obtained the financial assistance are: Shanghai Second Medical University, Second Military Medical University, Sun Yat-Sen University of Medical Sciences, Chongqing Medical University, and Beijing Medical University. The achievements are mainly focused on the treatment of ischemic disease, the formation mechanisms and prevention of vascular restenosis, the formation mechanism of abdominal aortic aneurysm, the development of domestic apparatus for the treatment of abdominal aortic aneurysm by the method of intravascular graft exclusion and portal hypertension.

## 1 Research on the Treatment of Ischemic Disease

With the alternation of dietary construction and many Chinese people stepping into geratic period, the ischemic disease represented by arteriosclerotic occlusion has become one of the main diseases to threaten Chinese health. Because of the poor effect of the available therapeutic strategies on the treatment of severe ischemic disease, nowadays cytobiological and molecular biological technique is studied to aid in the treatment. Recombinant plasmid pSV-VEGF165 was constructed in vitro, and then was directly injected into the ischemic hindlimb muscles in rabbit models. Thirty days after gene transfer, the capillary density and the capillary to muscle fiber ratio increased significantly. This experiment indicates that the capillary formation in ischemic limbs can be significantly augmented by VEGF165 gene. Another study is on angiopoietin. After limb ischemica model was developed in rabbits, various doses of recombinant human angiopoietin (rhANG) were injected intraarterialy via catheters into the bifurcation of iliac artery. Revascularization and perfusion of ischemic limbs were compared by the method of calf blood ratio, 99TC-MAA perfusion scan, serial angiography and thigh muscle biopsy. Results showed that recombinant human angiopoietin could effectively promote revascularization and recovery of blood perfusion in the experimental ischemic hindlimb model after administration at the dose of 20-40 ug, and the effect become more obvious with the dose increasing.

## 2 Research on Formation Mechanisms of Restenosis After Vascular Anastomosis

### 2.1 Formation mechanisms

The main cause of long-term dystherapeutic after vascular surgery is vascular restenosis. Vascular intimal hyperplasia is the leading cause of stenosis and occlusion developing within 2-24 months after reconstruction of artery. After vascular lesion, the proliferation of smooth muscle cells is tightly associated with the expression of proto-oncongene. It has been proved by ex-

periment that the expression of early response gene c-myc in vein graft increases unusually, and the abnormal expression of c-myc gene may contribute to the proliferation of smooth muscle cells, thus induce the intimal hyperplasia in vein graft. C-myc proteins and c-myc mRNA in smooth muscle cells of veins graft were detected at different times by the method of immunohistochemical staining and in situ hybridization. Results showed that c-myc mRNA increased significantly at 2 h, 6h after operation, c-myc protein production and staining for proliferative cellular nuclear antigen (PCNA) peaked at 1 week. Therefore, it can be concluded that the changes of c-myc protein parallel with PCNA, c-myc gene is one of the initial gene that can regulate the proliferation of smooth muscle cells in vein graft, and it can induce smooth muscle cells steping from stationary phase to proliferating cycle. Tissue type plasminogen activator (t-PA) is a kind of serine proteinase, synthesized and secreted by vascular endothelial cell. It has the effect to promote both profibrinolysin transforming into fibrinolysin and procollagenase transforming into collagenase. At the same time, it can induce the releasing of extracellular matrix. The role that t-PA plays in the formation and development of restenosis after vascular transplantation is still in controversy. The variation of t-PA activity in human organ-culture medium of saphenous vein was detected. It was found that low t-PA activity may facilitate thrombosis at early stage of bypass grafting and the increased t-PA activity may relate to the migration of vascular smooth muscle cells and intimal proliferation at late stage. Different concentration of fibrinogen plays different role in intimal hyperplasia. Human saphenous vein was harvested during infrainguinal vein bypass surgery. The culture medium was supplemented with fibrinogen at different concentration. The experiment showed that high concentration fibrinogen at local preianastimotic area could induce vein graft restenosis or occlusion but low concentration fibrinogen had no effect on vascular intimal hyperplasia. This experiment indicates that decreasing fibrinogen might be a possible method to prevent restenosis. Further study on organ-culture medium of human saphenous vein showed that high concentration of fibrinogen significantly reduced t-PA activity at early stage of culture but had no effect on t-PA activity at late stage. Proliferation of vascular smooth muscle cells is the central part of intimal hyperplasia. The right external jugular vein of rabbit was interposed between ipsilateral common carotid artery and the vein graft was harvested at different times after operation, the ultrastructure of medial smooth muscle cells was observed under electronic microscope. This experiment showed that after autogenous grafting, the medial smooth muscle cells began to proliferate and migrate to intima, and join in the formation of neointima on the 7th day, the proliferation peak around 14 days hints an optimal opportunity for inhibition of intimal hyperplasia[1].

Vascular anastomosis can lead to endothelium stripping and injury of media, the velocity and rang of reendothelialization can affect intimal hyperplasia through the regulation of proliferation and migration of smooth muscle cells and the regulation of synthesis of extracellular matrix. Present study shows that transforming growth factor beta 1 (TGFβ1) can induce accumulation of extracellular matrix, while VEGF has the function to promote proliferation of endothelial cell and to intensify permeability of blood vessel. Protein expression of TGFβ1 emerges 24 hours after grafting, within the first week it mainly locate in smooth muscle cells of media, after 2 weeks, both in media and in endangium the expression of TGFβ1 can be found, the ratio of positive cells are at their peak. At the same time, VEGF in smooth muscle cells begins to increase 24 hours after grafting and peaks 2 weeks later. This indicates that the expression peak of TGFβ1 and VEGF are in accordance with intimal hyperplasia in smooth muscle cells, they may affect each other and cooperate in the formation of intimal hyperplasia[2].

### 2.2 Research on prevention of restenosis after vascular anatomosis

Medicine has some therapeutic effectiveness on prevention intimal hyperplasia. Vascular intimal hyperplasia can be inhibited through restraining smooth mucsle cell from proliferation and migration by utilizing angiotensinase inhibitor and calcium antagonist. The lysine, which is the substrate of nitric oxide, can produce nitric oxide and inhibit intimal by promoting cure of endothelium. Because proliferation of smooth muscle cells are tightly related to the expression of proto-oncogene after vascular lesion, some scholars utilize medicine which can inhibit proto-oncogene to prevent intimal hyperplasia. After establishing vein graft model in rats, different doses of actinomycin D were given just before and after operation. Results showed that the expression of c-myc mRNA and the intimal thickness

were both significantly reduced in large dose group of actinomycin compared with control group. This experiment indicates that large dose of actinomycin D can inhibit expression of c-myc mRNA and intimal hyperplasia in graft. Main photodynamic therapies include traditional in vitro laser radiate therapy and endovascular laser radiate therapy, the later is more suitable for the development of endovascular surgery. Researches of photodynamic therapies are mainly focused on non-toxic photosensitizer, well-distributedly radiated laser fiber and proper local radiated channel. Carotid arteries were sectioned and anastomosed. During the operation, photosensitizer PSD-007 was injected into vessels and laser light illumination was given intravascular. Results showed that the cross section areas abated significantly. Scanning electronic microscope showed that the intima at cross section was continuative, ranging tidily and possessing less blood cells and other components. It can be concluded from this experiment that intravascular photodynamic therapy can be effective on inhibiting intimal hyperplasia following arterial anastomosis. Inspired by the fact that β-rays has the ability to kill tumor cells and inhibit cicatricial tissue, some scholars utilize β-rays to prevent blood vessel from restenosis by illuminating intravascularly. The advantages of this method are not only better biological value, high effect in short time illumination, but also pale penetrating power and no radiated injury. Autogenous vein graft model was established by transplanting the internal branch of jugular vein to the carotid artery by end to end anastomosis. The veins were irradiated by 32P solution before anastomosis in vitro. Results showed that in radiation group, the average intimal thickness was reduced significantly and the proliferation of smooth muscle cells was also inhibited significantly. Another experiment revealed that the soft extravascular model with 32P had obvious ability to inhibit the proliferation of smooth muscle cells and could notably restrain intimal hyperplasia at early to middle stage of graft (1-4weeks)[3].

The most popular research on prevention of restenosis is gene therapy. Therapeutic effectiveness of gene therapy is mainly determined by target gene, vector system and the way which target gene is guided to. Various antisense gene therapies can be divided into three types. The first is antisense proto-oncongene such as antisense c-myc, c-myb and c-ras. A fibrin tissue gel carring antisense oligonucleotides of c-myc was directly painted on the tunica adventitia vasorum. Results showed that this method could prevent intima from proliferation significantly and inhibit stenosis efficiently[4]. The second is antisense cell cycle regulator gene, including antisense cdc2, cdk2 and proliferative cellular nuclear antigen (PCNA). Discontinuing expression of PCNA can inhibit proliferation and migration of smooth muscle cells but cannot induce cellular death. PCNA can be successfully discontinued by utilizing antisense technique. The third is a variety of antisense cytokines gene. At present, monocyte chemotactic protein 1 (MCP-1) is generally accepted as a main material which can regulate monocytes/macrophages adherence and migration into blood vessel. Continually high expression of MCP-1 plays an important role in infiltration of inflammantory cell and proliferation of neointima at early stage of vein graft. Chinese scholars transferred antisense MCP-1 gene into graft vein by the method of cationic liposome, and discovered that the MCP-1 mRNA expression was significantly inhibited and intimal hyperplasia was markedly reduced. Expression of antisense MCP-1 successfully inhibited the expression of MCP-1 in vein grafts, which provided a new strategy for prevention of restenosis after vascular anastomosis[5]. Guiding target gene into blood vessel by using ideal vector, making target gene express safely, faithfully and continually are the key technique of gene therapy. Gene can be transferred by the method of virus or non-virus. Expressional adeno-associated virus plasmid vector carring wild type p53 gene was constructed and transfected into cultured smooth muscle cells mediated by cationic liposome. The mRNA and p53 protein expressed by transfected smooth muscle cells was confirmed by polymerase chain reaction technique and immunohistochemical technique. This experiment proved that the constructed expressional p53 adeno-associated virus plasmidcan be used as a vector of gene therapy and the smooth muscle cells might be served as the target cell of gene therapy for cardiovascular disease. Gene can be transferred in vivo and in vitro. In vivo as a method of transference is developing fast. Antisense medicine was dissolved into fibrin tissue gel, and then the gel was directly painted onto the surface of blood vessel. By this method, ideal effect on inhibiting intimal hyperplasia can be obtained[6]. Gene thread is a newly developed technique in recent years. Coated vicryl thread marked with EB was soaked in antisense oligonueleotide, and then the common carotid artery of rabbit was cut and anastomosed with coated vicryl

thread. This experiment revealed that coated vicryl thread can carry considerably large quantity of antisense oligonueleotide and directly deliver it to anastomosis by slowly releasing.

## 3 Formation Mechanism of Abdominal Aorta Aneurysm

Formation of abdominal aorta aneurysm (AAA) is related to atherosclerosis. Mechanisms of it are mainly on the deficiency of nutritional blood vessel, large amount of collagen degrading and lengthening duration of cirrhosisgenic factor in blood contacting with vessel wall stimulated by naked smooth muscle cells activating collagenase. AAV inclines to familial clustering. Losing some genes on number 16 autosome and X-chromosome can result in AAV through excessively degrading of elastin and collagen in arterial. In 1994, Newman and his colleagues discovered that both activity of matrix metalloproteinase 1 (MMP-1) and matrix metalloproteinase 9 (MMP-9) increased in the patients of AAV[7]. Chinese scholars performed in situ hybridization in the tissue sections of 20 cases of AAV to detect mRNA expressions of MMP-1 and MMP-9 and discovered that the expression of MMP-1 and MMP-9 could be found in macrophages, smooth muscle cells and lymphocytes, and the highest expression was found in macrophages. It indicated that MMPs play important role in development and expansion of AAV, and inflammantory cells mainly contribute to the production of MMPs and influence the expression of MMPs in interstitial cells[8].

## 4 Domestic Apparatus for the Treatment of AAV by Endovascular Graft Exclusion

Compared with traditional reconstruction of abdominal aortic, endovascular graft exclusion (EVGE) combines the advantages of the significant decrease in hemorrhage during operation, remarkable decrease in duration of supporting respiration and monitoring in ICU and in hospitalization after operation, as well as the obvious decrease in morbidity on severe complication. The safety and microtrauma of EVGE have been wildly acknowledged. The development of apparatus and improvement of technique for EVGE are all in stage of researching and exploring around the world. Chinese experts are also developing domestic technical device actively. Complex of stent-grafts can be divided into three types: bifurcation-shaped, tuber-shaped and monoarm-shaped[9]. A Ni-Ti alloy wire was knitted in Z-shaped stents, and every connected point was ligated by 6-0 no-injury brocade thread to form stent frame, and then the stent was wrapped with domestic super-thin silk-dacron graft to form integrated graft. EVGE was performed a month after successfully building canine AAA model. Results showed that of the 10 cases of EVGE, 9 cases successed and no mismatch or endoleak was found. Another experiment reported that domestic Ni-Ti alloy was knitted in tuber mesh stents, wrapped with domestic silk-dacron woven graft matched with stent diameter. Either tips of stent were fixed by 6-0 no-injury brocade thread and were left 0.5 mm naked area to form bugle-shaped stent-graft, and then graft was introduced with endovascular technique into lumen of sac and anchored at the proximal and distal aorta of aneurysm under x-ray transillumination. Results showed that the success ratio of EVGE was about 95%. Six months later, 79% of stents remained unobstructed, and neither migration nor endoleak was found[10].

## 5 Research on Portal Hypertension

Portal hypertension is characterized by hyperhemodynamics. Present study mainly focuses on mechanisms of hyperhemodynamics. Previous study had confirmed that $\alpha_1$ adrenoceptor proteins significantly decreased in hepatic tissues of cirrhotic portal hypertension patients. Chinese scholars performed semi-quantitative reverse transcription and polymerase chain reactions to investigate the expression of $\alpha_1$ adrenoceptor subtypes mRNA in the hepatic tissues of cirrhotic patients with portal hypertension. Results revealed that the mRNA expression of both $\alpha 1_a$ and $\alpha 1_b$ adrenoceptor subtypes are significantly decreased in cirrhotic portal hypertension, which may be a key reason for the decrease of the volume of $\alpha_1$ adrenoceptor proteins in cirrhotic hepatic tissues[11]. The decrease of $\alpha_1$ adrenoceptor proteins volume may affect the metabolism and action of epinephrine and norepinephrine, resulting in the elevated levels of cirrhotic portal hypertension and contributing to the formation and maintenance of portal hypertension. Nitric oxide is also the important mediator of hyperhemodynamical circulation. Nitric oxide synthase inhibitor L-N-monomethyl-arginc (L-NMMA) was injected into portal hypertension model in rats and the hemodynamical phase was detected. Results

showed that L-NMMA could reverse hyperhemodynamical phase of portal hypertension rats. This experiment indicates that nitric oxide is an important mediator of hyperdynamic circulation in portal hypertension. Reduced arterial responsiveness to endogenous vasoconstrictor substance such as norepinephrine plays an important role on hyperdynamical circulation in portal hypertension. Development of reduced arterial responsiveness maybe related to excessive production of endogenous vasodilator substance, but the relationship between potassium channels and reduced responsiveness is not confirmed. Some scholars built pre-hepatic portal hypertension model in rats by partial portal vein stenosis, then investigated the isolated intestinal mesenteric arterial responsiveness to norepinephrine and the effects of potassium channel blockers on responsiveness to norepinephrine. Results showed that arterial sensitivity to norepinephrine in pre-hepatic portal hypertensive rats was reduced. After inhibiting voltage-gated potassium channels by potassium channel blockers, the arterial response to norepinephrine remained significantly attenuated. It indicated that opening of a single type of potassium channels is not the major cause of reduced vascular norepinephrine responsiveness in portral hypertension.

How to reduce the occurrence of esophageal varicose and the reformation of esophageal varicose after devascularization is one of the focuses on therapeutic territory of portal hypertension. Basic fibroblast growth factor (bFGF) is recognized as a more important role in neovascularization. The expression of bFGF in esophagus was detected by the method of immunohistochemistry. Results showed that bFGF increased significantly with portal hypertension and might take part in the formation of esophageal varicose before and after devascularization[12].

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