Venous and arterial coronary artery bypass grafts in a pharmacological perspective
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CHAPTER 1

Introduction
History of surgical myocardial revascularisation

The development of procedures for myocardial revascularisation runs parallel to that of selective coronary angiography. Four years after the introduction of selective coronary angiography by Sones and collaborators in 1958, Effler and his team repaired a severe obstruction in the left main trunk coronary artery, by using the patch graft technique developed by Senning. At the same time Sones demonstrated angiographically the successful application of the Vineberg’s procedure, which is the direct implantation of the left internal mammary artery (IMA) into the myocardium.\textsuperscript{1,2} Until 1967 the surgical therapy of coronary artery disease consisted of these two techniques.

During the nineteen sixties two groups reported the use of saphenous vein (SV) in coronary artery surgery. The group of Garrett performed a successful coronary artery bypass graft (CABG) in 1964, using SV and in 1967 Favaloro and colleagues reconstructed a coronary artery by the interposition of a segment of SV.\textsuperscript{1,3} Soon thereafter the same group started placing the proximal anastomosis of coronary artery bypass grafts in the wall of the ascending aorta. During the following years the technique developed to procedures where CABG was combined with ventricular aneurysmectomy or valvular replacement, for emergency revascularisations, and for multiple bypass. By June 1970 more than 1000 CABG with SV were performed.\textsuperscript{1}

In 1965 Green demonstrated the successful anastomosis of the IMA on the coronary system of dogs.\textsuperscript{4} It was not until 1970 that Favaloro and Loop used the IMA for direct coronary anastomosis in humans. In 1986 Loop demonstrated in a 10-year follow up study a better patency for IMA compared to SV grafts.\textsuperscript{5} Since then other sources for arterial conduits were exploited as well. Bailey used already in 1967 the right gastroepiploic artery for the Vineberg type operation. Twenty years later several groups reported the application of this artery for coronary anastomosis. The use of the inferior epigastric artery was introduced in 1990. The early application of the radial artery in 1971 by Carpentier showed poor angiographic results. In 1989 spasm of the conduit was recognised as the cause for early graft failure and the use of the radial artery revived owing to the use of calcium channel blockers.\textsuperscript{1}

At present different options are available for multiple graft coronary revascularisation. Saphenous vein and internal mammary, radial, inferior epigastric, and gastroepiploic arteries can be used with or without sequential anastomosis, and with or without graft-to-graft anastomosis. CABG continues to be the treatment of choice in multivessel-, or left main trunk disease. More and more patients are accepted for CABG, despite co-morbidity and old age.\textsuperscript{6,7} However, hospital mortality is about 1% and stable and the risk adjusted combination of mortality and morbidity rate is declining.\textsuperscript{6}
Pharmacology and coronary artery bypass grafting

In the early days of CABG the SV was seen as a passive conduit. Spasm of the vein occurring during its removal was overcome by high-pressure distension using a syringe. With the introduction of IMA as a bypass graft, spasm became a relevant clinical problem, due to the ischemic complications of the myocardium supplied by this conduit. Since then it was recommended to counteract graft spasm by the application of papaverine.\textsuperscript{4-8} Thereby, the pharmacological treatment of vascular grafts during CABG was established. However, several questions remained to be solved concerning the most effective vasodilator agent and the applied concentration. Since then pharmacologists started to study these questions using the isolated vessel rings in an organ bath.\textsuperscript{9} Surgeons, measuring the effect of pharmacological agents on blood flow of IMA and SV in vivo, extended the study of graft pharmacology.\textsuperscript{10-12}

In addition to the application for the understanding of graft spasm, pharmacological studies can be used to determine the effect of factors influencing the quality of the vessel wall. Especially since it became obvious that the patency rates of venous grafts are worse compared to arterial grafts, surgeons are interested in factors influencing the integrity of the vessel wall. Usually these factors are tested by histological and morphological studies. However, the value of these studies is limited by the fact that the presence of certain structures and/or their organisation in the vessel wall does not guarantee a normal function.\textsuperscript{13}

Characteristics of saphenous vein and internal mammary artery

Morphological properties

The wall of SV is different from that of arterial grafts (Table 1). The intima is lined by endothelium that lies on a fenestrated basement membrane. Cell junctions in venous endothelium are predominantly of the long occluding type and more permeable than those of arterial endothelium. SV endothelial cells are large but flat when compared to those of the IMA. Focally, intimal cells lie immediately beneath the endothelium. A rudimentary internal elastic membrane separates the intima from the media. The tunica media is thin when compared to that in arteries, and it is composed of smooth muscle cells (SMC), arranged in circular layers. Circumferential collagen is often adjacent to longitudinal bundles of smooth muscle and collagen. The IMA, structured like an elastic artery, is dominated by a media composed of discrete lamellae of wavy collagen and SMC, aligned nearly circumferentially. The relative thick adventitia of SV contains the
Table 1: Morphology

<table>
<thead>
<tr>
<th></th>
<th>Vein</th>
<th>Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelial cells</strong></td>
<td>Larger, thinner, less anchored to subendothelium</td>
<td>Smaller, thicker, anchored to subendothelium</td>
</tr>
<tr>
<td><strong>Tunica intima</strong></td>
<td>More permeable</td>
<td>Less permeable</td>
</tr>
<tr>
<td><strong>Internal elastic membrane</strong></td>
<td>Poorly defined</td>
<td>Well defined</td>
</tr>
<tr>
<td><strong>Media</strong></td>
<td>Thin</td>
<td>Thick</td>
</tr>
<tr>
<td><strong>Elastic lamellae</strong></td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Medial SMC</strong></td>
<td>Few, circular and longitudinal arrangement, widely separated by collagen</td>
<td>Circular, orderly array with collagen, elastic fibres, and matrix</td>
</tr>
<tr>
<td><strong>Vasa vasorum</strong></td>
<td>More anastomoses</td>
<td>Fewer anastomoses</td>
</tr>
<tr>
<td><strong>Valves</strong></td>
<td>Present</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Overview of the morphological characteristics of arteries and veins with respect to coronary artery bypass grafting. SMC = smooth muscle cells. (adapted from Cox 1991)

Biophysical and biochemical properties

Physiological and biochemical control of basal vascular tone differs among arteries and veins and type of artery/vein. (Table 2) The degree of contraction of vascular SMC depends on the myogenic activity and the responses to endogenous vasoactive substances. Differences in the control of basal vascular tone also determine the responses to pharmacological compounds (see Pharmacological properties). The predominant mechanism of action of vasoconstriction in arteries appears to be the phasic depolarisation and entry of calcium through voltage-dependent calcium channels, while the major dilator influence appears to be endothelium-derived nitric oxide (NO). The predominant mechanism of vasoconstriction in veins is tonic contraction in response to noradrenaline released from sympathetic nerves present in the vessel wall. Veins are especially sensitive to nitrates (NO-donors). In response to NO the concentration of guanosine 3',5'-cyclic monophosphate (cGMP) increases substantially. Furthermore, the tonic phase of vasoconstriction, seen predominantly in veins, is highly sensitive to cGMP-dependent mechanisms, and veins have a low production of NO.
that is thought to mediate the metabolism of organic nitrates in vivo\textsuperscript{17}. The endothelium of veins releases predominantly vasoconstrictor substances, whereas the release of vasodilatory agents is more pronounced in arteries.\textsuperscript{14,16,18,19}

The elastic properties of the vessel wall vary among veins and arteries as well. Veins are highly compliant over the normal range of venous pressure, but relatively inelastic at arterial pressures. The high fraction of longitudinal muscle, in addition to the circumferential muscle cells in the SV determine the high mechanical stiffness when exposed to an arterial pressure. Additionally, elastic fibres are more important determinants of their rheological behaviour than the collagen fibres, in contrast to arteries.\textsuperscript{20}

Veins possess relatively weak intrinsic antithrombotic properties when compared to arteries. Heparan sulphate, a proteoglycan molecule with anticoagulant properties, is less prominent in the media and the poorly developed internal elastic lamina of veins.\textsuperscript{14,21} In addition, the production of the inhibitors of platelet activation by the endothelium (NO, prostacyclin (PG\textsubscript{12})) is low in veins, when compared to arteries.\textsuperscript{21} The response of SV to substances released from aggregating platelets is contractile as demonstrated for thromboxane A\textsubscript{2} and serotonin. In IMA these substances could not induce a contraction and ADP (adenosine-diphosphate) mediated endothelial NO-release and subsequent SMC relaxation.\textsuperscript{22} Furthermore, thrombin, released from aggregating platelets during their activation causes constriction of SV and dilation in IMA.\textsuperscript{23}

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
 & Veins & Arteries \\
\hline
\textbf{Vasoconstrictors} & More sensitive & Less sensitive \\
\textbf{Vasodilators} & Less sensitive & More sensitive \\
\textbf{Endothelial-derived agents} & SMC contraction & SMC relaxation \\
\textbf{Elasticity} & Relatively inelastic at arterial pressures & Elastic at arterial pressures \\
\textbf{Role of collagen} & Inconsequential & Important \\
\textbf{Lipolysis} & Slower & More rapid \\
\textbf{Lipid uptake} & Rapid & Slow \\
\textbf{Lipid synthesis} & More active & Less active \\
\textbf{PG\textsubscript{12} production} & Less & More \\
\hline
\end{tabular}
\caption{Physiology}
\end{table}

Overview of the biophysical and biochemical properties of veins and arteries with respect to aortocoronary bypass grafting. PG\textsubscript{12} = prostacyclin, SMC = smooth muscle cells. (adapted from Cox 1991)
Thrombus formation is also different between the two types of blood vessels. In the SV extensive microthrombi are formed, resulting in occlusion, whereas in arteries a “platelet carpet” is deposited, and not an occlusive thrombosis. This might be due to the lower blood velocity and hence lower shear stress in the venous circulation resulting in the less rapid removal of thrombogenic substances.\textsuperscript{14,20}

The metabolism of lipids in veins is proatherogenic, when compared to arteries. The combination of slow lipolysis and rapid lipid uptake and synthesis in veins is responsible for this observation.\textsuperscript{14,21,24}

**Pharmacological properties**

I. Pharmacology of the saphenous vein and internal mammary artery

*Adrenergic responses.* In vascular SMC, two types of postjunctional $\alpha$-adrenoceptors are identified, the $\alpha_1$, and the $\alpha_2$-adrenoceptor, respectively. The predominance of subtypes of adrenoceptors varies in between blood vessels. In SV the $\alpha_{1a}$ and $\alpha_{1b}$-subtypes of the $\alpha_1$-adrenoceptor are present and the postjunctional $\alpha_2$-adrenoceptor consists of the $\alpha_{2c}$-subtype.\textsuperscript{25,26} The predominant $\alpha$-adrenergic receptor present on IMA vascular SMC is the $\alpha_1$-adrenoceptor. Different subtypes have been specified with the following rank order of density: $\alpha_{1a} > \alpha_{1b} > \alpha_{1d}$.\textsuperscript{26-28} Stimulation of the $\alpha$-adrenoceptor results in the activation of membrane-bound phospholipase C (PLC) and the subsequent hydrolysis of phosphatidylinositol 4,5-biphosphate to produce diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP$_3$). DAG activates protein kinase C leading to the phosphorylation of some intracellular proteins. IP$_3$ causes the release of calcium from intracellular stores, followed by an increase in intracellular calcium and/or an increase in sensitivity to calcium of the contractile apparatus, and subsequent contraction (Figure 1A).\textsuperscript{29} As described previously, veins are highly responsive to $\alpha$-adrenoceptor stimulation, because the effect of noradrenaline released from nerve endings in the vessel wall is the main mechanism of their basal contractile tone. (Table 3) Compared to other vasoconstrictor stimuli the combined $\alpha_1$-, and $\alpha_2$-adrenoceptor agonist noradrenaline causes nearly twofold contractions in isolated SV preparations.\textsuperscript{9,30} In the IMA, the exposure to noradrenaline and the selective $\alpha_1$-adrenoceptor agonists methoxamine and phenylephrine causes contraction (this thesis).\textsuperscript{31} In SV, phenylephrine or methoxamine, and the $\alpha_2$-adrenoceptor by B-HT 933 provoke contractile responses with the following rank order of efficacy: noradrenaline $>$ phenylephrine $>$ methoxamine $>$ B-HT 933 (this thesis).\textsuperscript{9}

The $\beta$-adrenoceptors involved in vascular smooth muscle and endothelial cell responses are mainly of the $\beta_1$-subtype. Via a G-protein-mediated mechanism adenylate cyclase becomes activated after $\beta$-adrenoceptor stimulation (Figure 1B). Subsequently the
Figure 1: Mechanism of contraction (A) and relaxation by β-adrenergic agonists (B), nitrovasodilators (C), calcium channel blockers (D), or potassium channel activators (E), of vascular smooth muscle cells. Acyclase = adenylyl cyclase, cAMP = adenosine 3,5-monophosphate, cGMP = guanosine 3,5-monophosphate, CM = calmodulin, DAG = diacylglycerol, GTP = guanosine triphosphate, IP₃ = inositol 1,4,5-triphosphate, MLCK = myosin light-chain kinase, PDE = phosphodiesterase, PIP₂ = phosphatidylinositol 4,5-bisphosphate, PLC = phospholipase C. (adapted from Brody 1998)
concentration of adenosine 3,5-monophosphate (cAMP) increases, resulting in the decrease of the intracellular calcium concentration and SMC relaxation, or NO release by endothelial cells. In SV and IMA β-agonists such as isoproterenol, are moderate vasodilators (this thesis). Furthermore, the relaxation to this agent is dependent on the vasoconstrictor stimulus applied. A possible reason for the weak dilatory action in SV, is the presence of prejunctional β-adrenoceptors that augment rather than depress the contraction in response to activation of adrenergic nerve endings. In IMA, dobutamine or another β-adrenoceptor agonist can relax precontracted rings completely, also in the range of therapeutic plasma concentrations.

Angiotensin II-mediated responses. Activation by angiotensin II of its specific AT-receptors leads via G-proteins to the activation of PLC, a signalling mechanism as described for α-adrenoceptor activation (see above) (Figure 1A). Responses to angiotensin II are mediated via the AT₁-receptors in human SV and IMA. In human SV angiotensin II provoked a maximal contractile response that amounted to ~60% of the depolarisation-induced contraction, whereas in rabbit SV only small contractions could be evoked (this thesis). In human IMA responses to angiotensin II are less when compared to human SV (Table 3)

Other vasoconstrictor agents. Endothelin, dopamine, prostaglandin F₂α, serotonin, and the thromboxane A₂ analogue U46619, all provoke vasoconstriction of SMC in SV and IMA (Table 3). The second messenger system of the endothelin-receptor is complex. Both serotonin-receptors and thromboxane A₂ (TXA₂) receptors induced contractions

<table>
<thead>
<tr>
<th>Vasoconstrictor stimulus</th>
<th>Arterial (A) or venous (V) contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Adrenergic agonists</td>
<td>V &gt; A</td>
</tr>
<tr>
<td>Depolarisation</td>
<td>V &gt; A</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>V ≥ A</td>
</tr>
<tr>
<td>Others:</td>
<td></td>
</tr>
<tr>
<td>- endothelin-1</td>
<td>V &gt; A</td>
</tr>
<tr>
<td>- thromboxane A₂</td>
<td>V &gt; A</td>
</tr>
<tr>
<td>- serotonin</td>
<td>V &gt; A</td>
</tr>
</tbody>
</table>

Overview of the effect of the vasoconstrictor agents in internal mammary artery (A) and saphenous vein (V), blood vessels used for coronary artery bypass grafting.
are mediated via a PLC-dependent signalling mechanism. In SV endothelin is as effective as noradrenaline.\textsuperscript{30} Endothelin is a more potent vasoconstrictor in veins than in arteries\textsuperscript{16,36}, and in both vessels the response is mediated mainly by ETA receptors\textsuperscript{36}. Serotonin induces maximal contractile responses that are \(\sim 50\%\) of KCl-induced responses in SV.\textsuperscript{37} U46619, is a potent vasoconstrictor in human SV and in IMA.\textsuperscript{12,22,27,31} Endothelin and thromboxane are the most potent vasoconstrictors in IMA.\textsuperscript{12}

**Papaverine.** This non-specific vasodilator substance, relaxes blood vessels through multiple mechanisms. It is a phosphodiesterase-inhibitor, raising cGMP levels in SMC (Figure 1B). Papaverine-induced relaxation is also caused by other actions such as a decreased calcium influx or inhibition of the release of calcium from intracellular stores.\textsuperscript{12} Papaverine is a potent vasodilator in most vessel preparations and independent of the precontraction applied. In SV and IMA papaverine indeed inhibits precontractions mediated by depolarisation, \(\alpha\)-adrenoceptor stimulation or U46619 (this thesis).\textsuperscript{9,12} Also after incubation in a papaverine-containing solution, SV and IMA preparations the responses to depolarisation and \(\alpha_1\)-adrenoceptor stimulation are almost completely abolished. This inhibitory effect is characterised by a quick onset, and a relatively short duration of action (this thesis).\textsuperscript{30}

**Nitrates.** The mechanism of action of these vasodilators (such as glyceryltrinitrate and sodium nitroprusside) involves the donation of NO, a powerful stimulant of the enzyme guanylate cyclase, that increases the formation of cGMP in the SMC (Figure 1C). The cGMP subsequently reduces intracellular calcium concentrations and leads to the relaxation of the SMC (Figure 1).\textsuperscript{12,38} Because of the high responsiveness of veins to NO (see Biophysical and biochemical properties), nitrates are effective dilators of veins, independent of the mechanism of precontraction.\textsuperscript{9,30,39} In IMA NO-donors were more effective in reversing receptor-mediated, than depolarisation-induced precontraction.\textsuperscript{9,30}

**Other vasodilatory agents.** \(\alpha\)-Adrenoceptor antagonists bind, hence their nomenclature, at the \(\alpha\)-adrenoceptor, which especially in veins results in a highly effective dilation of the vessel.\textsuperscript{16} PDE-inhibitors exhibit their action by inhibition of phosphodiesterase, the enzyme responsible for the metabolism of cAMP and cGMP. By increasing the intracellular concentrations of these substances vasorelaxation is achieved (Figure 1B). PDE-inhibitors are weak vasodilators in human SV, whereas a complete dilation of precontracted IMA preparations can be achieved independent of the preconstricting stimulus.\textsuperscript{33,40} The effect of ATP-dependent potassium channel openers (Figure 1E) is
dependent on the vasoconstrictor stimulus applied. Glibenclamide was able to inhibit responses induced by U46619, but not noradrenaline-, or endothelin-1-mediated vasoconstriction. In arteries potassium channel openers are more potent dilators when compared to veins.

**Endothelium-dependent relaxation.** Stimulation of several receptors on the endothelial cell can provoke NO-release by the endothelium and secondary relaxation of the SMC. The signalling mechanism activated after the stimulation of these receptors involves the activation of NO-synthase (NOS) and cyclooxygenase (COX) that catalyse the production of NO and PGI\textsubscript{2} from arachidonic acid, respectively. Relaxation to endothelium-dependent vasodilatory agents (acetylcholine, thrombin, ADP) is weak in SV. However, the calcium ionophore A23187, which releases endothelium-derived relaxing factor by non-receptor-operated mechanisms, induces relaxation in SV more prominently. IMA relaxation is most pronounced in response to the muscarinic receptor agonist acetylcholine, followed by thrombin and ADP. Receptor-independent stimulation of the endothelium produces similar relaxations as acetylcholine. The activation of venous endothelium provokes SMC contraction in vitro, and to a lesser extent relaxation. This indicates a more prominent genesis of vasoconstrictor compounds compared to vasodilator substances in veins, whereas, in response to stimulation the IMA endothelium produces predominantly vasodilator substances.

**II Calcium antagonists in saphenous vein and internal mammary artery**

The common mode of action of CA is the inhibition of calcium entry through voltage dependent calcium channels (VDCC) into cells (Figure 1). From the characterized VDCC, only long-lasting (L-type) and transient (T-type) calcium channels have been identified in vascular SMC. N(euronal)-type calcium channels are present at the nerve endings in the vessel wall. The CA currently available can be divided into three groups: the phenylalkylamines, the 1,4-dihydropyridines, and the benzothiazepines. In recent years new CA have been developed to reduce negative side effects associated with the clinical use of these compounds (negative inotropic action, reflex tachycardia) and have been claimed to exert a higher vasoselectivity. One of these new CA, mibefradil (Ro 40-5967), blocks T-type Ca\textsuperscript{2+}-channel currents in VSMC at lower concentrations than those necessary for L-type current blockade. Furthermore, mibefradil exerted an inhibitory effect on sympathetic neurotransmission, and a K<sub>ATP</sub>-channels-dependent infarct-size limiting effect in the myocardium. Some newer 1,4-dihydropyridines CA, such as lacidipine,
Table 4: Arterial and venous effects of vasodilator agents

<table>
<thead>
<tr>
<th>Vasodilator type</th>
<th>Arterial (A) or venous (V) dilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic nitrates</td>
<td>V &gt; A</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>A » V</td>
</tr>
<tr>
<td>Papaverine (this thesis)</td>
<td>A = V</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>V ≥ A</td>
</tr>
<tr>
<td>Others:</td>
<td></td>
</tr>
<tr>
<td>- α-Adrenoceptor antagonists</td>
<td>V &gt; A</td>
</tr>
<tr>
<td>- PDE-inhibitors</td>
<td>A &gt; V</td>
</tr>
<tr>
<td>- Potassium channel openers</td>
<td>A &gt; V</td>
</tr>
</tbody>
</table>

Overview of the effect of the vasodilator agents in internal mammary artery (A) or saphenous vein (V), blood vessels used for coronary artery bypass grafting. ACE = angiotensin I converting enzyme, NO = nitric oxide, PDE = phosphodiesterase. (adapted from Van Zwieten 1997)

posses a high lipophilicity. From lipid-rich depots (like the cell membrane) in which they easily dissolve, such compounds are slowly released to reach the L-type calcium channel. This explains their slow onset, and long duration of action which results in their beneficial kinetic profile.52-55

In CABG calcium antagonists are successfully applied in the treatment of graft spasm. Verapamil, a predominantly L-type calcium channel blocker, has been shown to prevent spasm of the SV when locally applied during its dissection.56 Furthermore, after coronary revascularisation with arterial grafts, patients are treated systemically with nitroglycerine or CA (nifedipine, diltiazem) to prevent spasm of the grafts.30,57 Another possible beneficial effect associated with the use of these pharmacological agents in CABG is the prevention of atherosclerosis and ischemia-reperfusion injury.58-61

In isolated arteries and veins CA are potent vasodilators.30,56,62,63 Calcium antagonists are less effective in inhibiting receptor-mediated, compared to depolarisation-induced contraction, also in SV and IMA (this thesis).9,30,64,65 In addition, CA are more effective vasodilators in arteries when compared to veins, which also holds true for SV and IMA (Table 4) (this thesis).42 The potency of the three classes of CA in SV and IMA varies; 1,4-dihydropyridines CA are the most potent vasodilators followed by the phenylalkylamines (verapamil) and the benzothiazepines (diltiazem) CA, respectively.31,66 In SV the new compound, mibefradil is a less effective inhibitor of depolarisation-induced contractions when compared to verapamil, and its effect at SMC of SV demonstrated to be potassium channel-independent (this thesis).
**Table 5: Comparative vasoreactivity of native and grafted saphenous vein preparations.**

<table>
<thead>
<tr>
<th></th>
<th>Native (N) versus grafted (G) saphenous vein reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenergic responses</td>
<td>N &gt; G</td>
</tr>
<tr>
<td>Angiotensin II-mediated responses</td>
<td>N = G</td>
</tr>
<tr>
<td>Other vasoconstrictor responses</td>
<td></td>
</tr>
<tr>
<td>- Serotonin</td>
<td>N &gt; G</td>
</tr>
<tr>
<td>- Histamin</td>
<td>N &gt; G</td>
</tr>
<tr>
<td>- Endothelin-1</td>
<td>N = G</td>
</tr>
<tr>
<td>Nitrates</td>
<td>N = G</td>
</tr>
<tr>
<td>Endothelium-dependent relaxation</td>
<td>N &gt;&gt; G</td>
</tr>
</tbody>
</table>

**III. Reactivity of venous grafts**

Functional studies of venous grafts demonstrate the time-dependent changes in reactivity compared to native veins (Table 5). Nine years’ old venous grafts demonstrated reduced maximal contractile responses to potassium, noradrenaline, serotonin and histamin. The sensitivity of the response to these compounds was decreased for noradrenaline, however increased for histamine and unchanged for serotonin. The endothelium-dependent relaxation in response to acetylcholine was decreased. In addition, responses to angiotensin II, endothelin-1, and organic nitrates remained intact in venous grafts. The sensitivity of the graft to endothelin-1 increased with the age of the graft.
Coronary artery bypass graft failure

Epidemiology

Saphenous vein grafting can be considered as a palliative intervention for a progressive disease due to the accelerated atherosclerosis developing within grafted saphenous vein conduits.\(^{21,69,70}\) One year after CABG 15% of the venous grafts appears to be occluded. Until 6 years postoperatively the graft attrition rate is 1-2% per year, and from 6 to 10 years it is 4% annually. Consequently, 60% of saphenous vein grafts is patent at 10 years of follow up. Another 50% of the open grafts display a significant degree of stenosis. Besides this accelerated atherosclerosis in implanted saphenous vein, the native coronary artery disease progresses during this period in ±5% of the patients per year.\(^{21,71}\) The recurrence of clinical symptoms after CABG shows a similar pattern. Angina reoccurs in up to 20% of patients during the first year and in the following years this number increases by approximately 4% annually.\(^{21}\) Approximately 33% of the patients need a repeat revascularisation procedure (PTCA and/or CABG) by 20 years after the initial operation, with an increasing incidence after 7 years.\(^{70}\) A higher morbidity and mortality rate and a diminished clinical benefit accompany the ‘redo’ interventions.\(^{21,70}\) Mortality increases from 3-7% after 1 year, to 23-34% after 10 years to 59-71% 20 years after CABG.\(^{70,72}\) After 5 years the mortality rises and it is higher than in a matched Dutch population not subjected to CABG, indicating that CABG with venous grafts does not provide a normal life expectancy. Only patients with single vessel disease and normal left ventricular function are excepted from this general tendency.

Compared to 60% patent saphenous vein grafts at 10 years of angiographic follow up, IMA grafts display a patency rate of 90% at 10 years and even so at 15-21 years.\(^{73,74}\) Also the use of other arterial grafts (gastroepiploic-, inferior epigastric-, and radial artery) is accompanied by better patency rates, although follow-up studies for periods longer than 5 years are not yet available.\(^1\) At 18 years 61% of the patients with IMA grafts were alive and had not suffered cardiac events.\(^{75}\)

Pathogenesis

Graft spasm is an early occurring phenomenon leading to reduced flow and ischemic complications, in particular in arterial grafts. Spasm of venous grafts during their removal, needs high pressure distension to be neutralised. Subsequent damage to the integrity of the vessel wall might lead to graft failure. The pathogenesis of graft spasm remains to be elucidated. However, the interaction between different constrictor stimuli such as surgical trauma, locally released vasoconstrictors, neural factors, and circulating hormones and passive distension from arterial pressure, are likely to cause abnormal
Graft failure is especially relevant in SV grafts, since the IMA displays a special feature compared to venous grafts, that is the relative immunity against atherosclerosis. Therefore, the recurrence of symptoms in patients treated with IMA conduits is mainly based on the progression of atherosclerosis in the native coronary arteries. In contrast to the IMA grafts, the venous grafts are subject to an accelerated form of atherosclerosis. Three pathophysiologically linked processes form the basis of this SV graft disease: thrombosis, intimal hyperplasia and atherosclerosis. The three processes are involved in graft failure at distinct time periods of the postoperative phase. Thrombosis determines graft failure during the first months after CABG by acute occlusion, and serves as a stimulus for intimal hyperplasia. Intimal hyperplasia developed from 1 month after arterialisation only modestly compromises the lumen of graft, but renders the graft susceptible for atherosclerosis. After more than one year venous graft disease is characterised by atherosclerosis.

Thrombosis is caused by alterations in the vessel wall, blood rheology and flow dynamics. The main alteration of the vessel wall brought about by CABG is the injury of the endothelial monolayer (Figure 2). For SV the decrease in endothelial function results in enhanced coagulation. Fibrin is accumulated on the luminal surface, platelets and neutrophils are adhered, the extrinsic coagulation cascade becomes activated by the exposition of tissue factor, and the activity of thrombomodulin (an anti-thrombotic regulatory protein) is attenuated. Furthermore, denuded SV is highly responsive to circulating vasoconstrictor stimuli. Blood rheology changes during CABG by an increased level of factors influencing hemostasis, especially plasma fibrinogen. The altered flow dynamics to which the SV is subjected will further increase the risk of thrombosis. The introduction of the SV into the arterial circulation and the subsequent pressure-induced venodilation provoke a graft flow reduction. Low fluid shear stress reduces the shear stress-dependent release of anticoagulant factors (tissue plasminogen activator, NO, and prostacyclin).

Intimal hyperplasia involves the accumulation of SMC and extracellular matrix in the intimal compartment. The native vein exhibits mild intimal or medial fibrosis, whereas the grafted SV develops intimal thickening within 5 weeks after grafting. Intimal hyperplasia follows a pathogenic sequence. At first SMC from the media will proliferate, followed by their migration into the intima. Growth factors and cytokines released from endothelial cells, platelets, and macrophages induce these processes. Secondly, the SMC in the intima produce an extracellular matrix which is deposited in the intima, thereby
Figure 2: Pathogenesis of graft occlusion (adapted from Cox 1991)
enhancing intima fibrosis, and the reduction of the number of cells. The loss of endothelial function does impair growth-modulation and it is one of the underlying factors inducing intimal hyperplasia. However, after the formation of a new endothelial layer, the intimal hyperplasia will progress, which indicates that other factors play a role in promoting intimal hyperplasia. SMC proliferation caused by ischemia-reperfusion, the formation of superoxide radicals, and the translocation of perivascular fibroblasts into the neointima that differentiate into myofibroblasts are examples of the factors involved. The altered flow dynamics caused by the implantation of the SV into the arterial circulation further stimulate intimal hyperplasia. A reduction in shear stress preceeds the enhanced production of mitogens (platelet-derived growth factor, bFGF, endothelin-1) and diminishes the production of growth inhibitors (transforming growth factor-β, NO).

Atherosclerosis as reflected by atheromatous plaques in the venous grafts can be found one year after CABG. The fundamental processes of plaque formation are equal in comparison to atherosclerosis found in native coronary arteries. However, differences exist with respect to certain temporal, histological and topographic characteristics. The most striking finding is the accelerated form of atherosclerosis found in venous grafts, that is mainly caused by endothelial dysfunction (see above). Histologically, the atheroma contains numerous foam and inflammatory cells indicating an immune-mediated form of atherosclerosis. Furthermore, morphologically the atherosclerosis in vein grafts appears to be diffuse, concentric and friable compared to the focal eccentric, and non-friable lesions demonstrated in native coronary arteries.

The process of atherosclerosis may lead to lesions with clinical significant obstruction, whereas thrombosis in degenerated grafts (so called “late thrombosis”) with advanced atherosclerosis may occur as well.

Factors influencing coronary artery bypass graft failure
Patient-related factors. Several clinical trials and basic scientific publications have demonstrated cigarette smoking, hyperlipidaemia, hypertension, diabetes mellitus, female sex, and increased plasma levels of lipoprotein (a), homocysteine and fibrinogen to be factors influencing the function and morphology of the vessels used as grafts and predispose to the angiographic demonstrated development of (venous) graft stenosis, and/or the recurrence of clinical symptoms (Table 6).
Table 6: Patient-related factors for reduced graft patency and adverse clinical outcome after CABG

<table>
<thead>
<tr>
<th>Factor</th>
<th>Functional changes</th>
<th>Pathological process</th>
<th>↑ rate angina/MI</th>
<th>↑ late (&gt;3y) cardiac mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR ER &lt;1y &gt;3y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking&lt;sup&gt;87&lt;/sup&gt;</td>
<td>↓ Yes Yes</td>
<td>ET/ATH/LT</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hypertension&lt;sup&gt;94-96&lt;/sup&gt;</td>
<td>↑ ↓ No No</td>
<td>NIH</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>↑ Lipid&lt;sup&gt;91,93,103&lt;/sup&gt;</td>
<td>↑ ↓ No Yes</td>
<td>ATH/LT</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Diabetes&lt;sup&gt;98&lt;/sup&gt;</td>
<td>= ↓ No Yes/No</td>
<td>ATH</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Female sex&lt;sup&gt;101&lt;/sup&gt;</td>
<td>&lt; Yes</td>
<td>ET</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>↑ Lipoprotein (a)</td>
<td>No Yes</td>
<td>ATH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ Fibrinogen</td>
<td>Yes ET/ATH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑ Homocystein</td>
<td>No ?ATH</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CR = contractile responsiveness, ER = endothelium-dependent relaxation, ET = early thrombosis, NIH = neointimal hyperplasia, ATH = atherosclerosis, LT = late thrombosis. (adapted from Motwani 1998)

Heart failure. The number of patients with congestive heart failure (CHF) subjected to myocardial revascularisation is increasing<sup>6,104</sup>. The activation of compensatory mechanisms such as the sympathetic nervous system and the renin-angiotensin-aldosterone system and the subsequent changed levels of neurohumoral factors<sup>105</sup> induce alterations in vascular responsiveness in patients with CHF. As a consequence the graft patency, partially dependent on this responsiveness, might be influenced by these changes. In the current thesis it is for instance demonstrated in a rabbit model of CHF that in SV contractile responses are increased, whereas dilatory responses were decreased in the condition of CHF.

Vessel-related factors. Certain intrinsic properties of saphenous vein make the blood vessel more sensitive to graft failure. Besides the aforementioned factors (“Characteristics of saphenous vein and internal mammary artery”) ischemia due to disturbance of the vasa vasorum<sup>106</sup>, incomplete recovery of the endothelial function<sup>80,107</sup> and enhanced SMC proliferation in SV compared with IMA<sup>108</sup> are additive to the susceptibility to failure of venous grafts.
Surgical procedural factors. The exposure to pharmacological agents such as anaesthetics and cardiac stimulants applied perioperatively will influence vasoactive responses of the grafts (see Pharmacological properties).\textsuperscript{109,110} Cardiopulmonary bypass during aortocoronary bypass surgery is accompanied by a change in vessel wall reactivity as a result of the different circulating vasoactive substances, the elicited inflammatory response, and the nonpulsatile flow.\textsuperscript{111} Elevated levels of angiotensin II\textsuperscript{112}, catecholamines\textsuperscript{113}, endothelin\textsuperscript{114}, thromboxane A\textsubscript{2}\textsuperscript{12} during and after cardiopulmonary bypass have been described. Circulating angiotensin II may unfavourably influence the patency of bypass grafts, due to the stimulation of SMC proliferation.\textsuperscript{80,115} Furthermore, heparin used during cardiopulmonary bypass, may displace active bFGF, a potent mitogen, which subsequently may be preferentially deposited on injured vessel wall, thus contributing to the pathogenesis of restenosis.\textsuperscript{116} As mentioned previously the surgical manipulation of the vessel influences the functional properties of the SV and IMA. Rough dissection techniques, excessive distension, low storage temperatures and the composition of solutions used for irrigation and storage have all been implicated in damaging the endothelium of SV grafts (this thesis).\textsuperscript{39,78} In addition, vascular SMC vasoactive responses might be influenced by the aforementioned factors. Responses to depolarisation and \(\alpha\)-adrenoceptor stimulation were reduced in harvested, distended vein segments.\textsuperscript{117,118} The type of manipulation was not decisive for the demonstrated injury of SV (this thesis) and IMA\textsuperscript{119}. The exposure to cold preservation and/or cardioplegic solutions of SV and to a lesser extent IMA, during the harvest and prior to implantation might alter vascular SMC responsiveness as well (this thesis).\textsuperscript{37,78} Other surgery-related predisposing factors for graft failure, although controversial, are the diameter of the grafted coronary artery, the type of grafted coronary artery\textsuperscript{120}, and the severity of the proximal stenosis bypassed\textsuperscript{121}. Furthermore, the manifestation of vasospasm might depend on the segments of the vessel used, since segmental differences in contractility of SV and IMA exists.\textsuperscript{12,122} As described previously, the placement of the SV into the arterial circulation will disturb the balance of factors influencing hemostasis and SMC proliferation.\textsuperscript{21,84} In addition the responsiveness of the SV might alter under arterial pressure.\textsuperscript{123}

Harvesting techniques

Saphenous vein harvesting is usually performed through a longitudinal lower extremity incision. This open technique can result in wound complications that may occur in 1%
to 44% of the patients. Commonly reported leg wound complications include dermatitis, cellulitis, greater saphenous vein neuropathy, chronic non-healing wounds and lymphocele. Major wound complications requiring surgical treatment are rare. The occurrence of wound complications is correlated with risk factors such as female gender, diabetes, peripheral vascular disease and the need for an intraaortic balloon pump (IABP).

Newer techniques utilising minimally invasive harvesting have been developed to reduce the postoperative wound complications. The skin-bridging techniques described include the venectomy by using a standard bridge technique, mediastinoscope, retractor coupled to a light source, Mayo stripper, or endoscope-assisted techniques. The initial reports demonstrate clinical benefit due to a decrease in wound complications, early mobilisation, and reduced hospital stay. In addition to the clinical benefit, it has been shown that the vein quality is not impaired. Histological studies have shown that the injury of the endothelium is comparable to that of SV segments dissected by the conventional method. Functional studies provide evidence for an abnormal, however comparable endothelial function, and a normal vascular SMC responsiveness (this thesis).

Strategies to improve long-term outcome of bypass grafts

General
Arterial bypass conduits display a much better long-term outcome than venous grafts. Theoretical reasons for the better performance of arterial grafts are their structure and endothelial function that make them more suitable coronary bypass grafts, conferring favourable flow characteristics, the ability to adapt to changes in myocardial oxygen demand and resistance to atherosclerosis. To improve clinical outcome after CABG the use of arterial grafts should be stimulated. However, the arterial supply is not always sufficient for complete myocardial revascularisation and revascularisation by the use of arteries cannot be performed in 30% of the patients. Therefore, saphenous vein graft performances should be enhanced by strategies that will be discussed below.

Risk factors
Only 15-25% of patients with CABG or PTCA undergo risk factor management. In a recent survey in Europe in patients 6 months after an intervention or myocardial infarction, it was shown that 19% continued to smoke, 53% had an elevated blood pressure, 44% had high levels of cholesterol and 25% were obese.
A marked reduction in risk for mortality, myocardial infarction and/or reintervention can be realised by the cessation of smoking. Pharmacological intervention can reduce the long-term incidence of fatal and nonfatal cardiac events, as shown for aggressive lipid-lowering, and antiplatelet aggregation therapy in several trials. Antiplatelet aggregation agents, such as aspirine, indomethacin and ticlopidine, exhibit beneficial effects on graft patency and clinical outcome during the first postoperative year, when commenced no later than one day after surgery. Oral anticoagulants administered postoperatively were demonstrated to cause some reduction of long-term mortality. Since the explanation for this observation remains to be elucidated, the use of oral anticoagulants is controversial.

**Operation technique**

To minimise the injury to the grafts' vessel wall during aortocoronary bypass surgery, certain measures can be taken. ”No touch” dissection together with the prevention of vein spasm, that allows gentle distension of the SV, is the first step. The prevention of vein spasm can be achieved by pharmacological relaxation during its dissection. The topical and intraluminal application during the harvest, and addition to the bathing solution are all routes for introducing the vasodilator substance. Prevention of spasm and morphological preservation of the wall integrity can be achieved by using papaverine. Early postoperative graft patency was improved by this strategy. A glyceryl trinitrate-verapamil containing solution proved superior to papaverine in preserving the endothelium. In addition, a solution with the lipophilic calcium antagonist lacidipine was able to prevent spasm for more than 24 hours (this thesis). Besides the prevention of spasm, CA (and ACE-inhibitors) have proven to exert a beneficial influence on the endothelium and to possess anti-atherogenic properties as well.

When vein integrity is evaluated morphologically, the solutions used to distend and store the vein during the operation, should preferably be kept at room temperature. The experiments testing the functional properties of SV in the present investigation, demonstrate no alteration in functional properties after storage at 4°C in the solutions used at present during CABG. The composition of the solutions used to irrigate and store the veins is another point of attention. Blood may be an adequate preservation medium, although the platelets and leukocytes possibly interact with the endothelium. Balanced electrolyte solution or even culture medium, or organ preservation solutions might be an alternative. Saline, however, causes serious morphological and functional damage to the SV. Also the composition of the cardioplegic solution for reinfusion after anastomosis, must be considered in this respect (this thesis).
Potential strategies

The understanding of vein graft failure has stimulated the development of approaches to counteract specific pathogenic mechanisms. Gene therapy by gene transfer of replication-defective adenoviral vectors or plasmid liposome vectors, encoding a soluble inhibitory form of vascular cell adhesion molecule-1 (VCAM-1), endothelial nitric oxide synthase, antisense oligonucleotides, or tissue inhibitor of metalloproteinase-3 has proven successful in animal models or cultured human veins. Synthetic grafts, that have thus far demonstrated low patency rates because of early thrombotic occlusion, are a target for genetic modulation as well. Other fields of intervention preventing venous graft failure currently under investigation are for instance: platelet-selective NO-donors (S-nitrosogluthation), modulation of growth factors (bFGF, VEGF), tissue factor antagonism, and external stenting.


122. He G-W, Acuff TE, Yang C-Q, Ruan WH, Mack MJ. Middle and proximal sections of the human internal mammary artery are not "passive conduits". *J Thorac Cardiovasc Surg* 1993;106:406-11.


Aim of the present thesis

Long-term follow-up studies concerning the patency of aortocoronary bypass grafts have yielded disappointing results for venous bypass grafts. Venous grafts deteriorate with time, thus resulting in decreased survival and the necessity of revascularisation procedures for patients provided with venous grafts. By contrast, arterial bypass conduits display a much better long-term outcome. Therefore, to improve clinical outcome after CABG the use of arterial grafts should be stimulated and saphenous vein graft performances should be enhanced.

In the present study several factors influencing the functional properties of saphenous vein were investigated, and a first step towards an improvement of the currently applied harvesting method by prevention of spasm by pharmacological measures was made. Accordingly, pharmacological, functional methods were used to evaluate the quality of saphenous vein, and on a smaller scale that of internal mammary artery preparations. The functional, pharmacological methods applied to estimate the quality of the blood vessels may offer important additional information beyond morphological investigations, which can only demonstrate the characteristics and presence of structures, without giving a clue to their functional relevance in vivo.

The presence of pre-existent diseases in the patient might alter the responsiveness of saphenous vein. Accordingly, the changes of saphenous vein functional responses provoked by the condition of congestive heart failure were determined in two distinct rabbit models. With respect to the peri-operative factors, the influence of exposure to manipulation and preservation/cardioplegic solutions were studied. The effect of surgical manipulation was investigated by comparison of human saphenous vein preparations obtained after three distinct harvesting techniques. Accordingly, in rabbit saphenous vein and rat aorta preparations stored in several preservation solutions the time-dependent and compound-dependent alterations of contractile responses were analysed.

The prevention of spasm of saphenous vein and internal mammary artery by pharmacological agents was investigated with an emphasis on calcium antagonists. Verapamil and mibefradil were compared to establish the mechanism of action of calcium antagonists in human saphenous vein. A new approach to prevent graft spasm was introduced by means of a very long acting calcium antagonist, lacidipine. In a superfusion model, this procedure was investigated for the long-term prevention of graft spasm in human saphenous vein and internal mammary artery preparations.