From endothelial dysfunction to clinical events

Concept and update on the ENCORE trials

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In Western Countries morbidity and mortality are still mainly related to coronary artery disease and its complications, such as angina pectoris and myocardial infarction. An early event in atherosclerosis is endothelial dysfunction. For this reason, therapeutic interventions aiming to restore coronary endothelial dysfunction may be clinically relevant.

A number of studies have been performed in surrogate circulations, such as the human forearm, although not much is known about the effects of intervention in the coronary circulation. For the ENCORE I trial 343 patients with coronary artery disease undergoing percutaneous transluminal angioplasty, with or without stenting, have been randomized. After the coronary interventions, endothelial function was assessed by intracoronary (i.c.) infusion of increasing dosages of acetylcholine in a coronary segment without stenotic lesions. Quantitative coronary angiography (QCA) and Doppler flow velocity measurements were used to measure coronary responses to acetylcholine. Endothelium-independent responses are tested by i.c. adenosine and nitroglycerine. Patients were randomly assigned in a double-blind fashion to four treatment groups: placebo, nifedipine at 30–60 mg . day$^{-1}$, cerivastatin at 400 mg . day$^{-1}$ or their combination. Studies have been repeated in 247 patients after an interval of 6 months and the trial was completed in August 2000. This trial will determine whether or not endothelial function in patients with coronary artery disease is improved within 6 months by calcium antagonists and/or a statin alone or in combination.

The ENCORE II trial is scheduled to run for 2 years. It examines the correlation between endothelial function and structural atherosclerosis (as assessed by QCA and intravascular ultrasound [IVUS]) in 200 patients each treated with cerivastatin at a dose of 200 or 800 mg . day$^{-1}$ compared with 200 patients treated with a combination of cerivastatin at a dose of 800 mg . day$^{-1}$ and nifedipine at a dose of 30–60 mg . day$^{-1}$. Endothelium-dependent responses of epicardial coronary arteries to acetylcholine at baseline as well as structural vascular changes, as assessed by IVUS, will be correlated and followed over 2 years. After 2 years the acetylcholine test, QCA and IVUS are to be repeated. Over 150 patients have so far been enrolled in the trial.

The ENCORE trials will show at the clinical level whether or not calcium antagonists and statins, alone or in combination, reverse early coronary endothelial dysfunction. In addition, these trials will address the question whether endothelial dysfunction and its pharmacological improvement are associated with progression or regression of atherosclerotic coronary artery disease. Finally, it may provide evidence whether this is reflected in fewer clinical events as suggested by several small observational studies.

Key Words: Coronary artery disease, atherosclerosis, endothelial dysfunction, nifedipine, cerivastatin.

Introduction

The results of atherosclerosis are functional and structural vascular changes and, in later stages, obstruction of large conduit arteries in the heart, brain, kidney and legs$^{[1,2,3]}$ (Fig. 1). These alterations are clinically associated with angina pectoris, myocardial infarction, transient ischaemic cerebrovascular attacks and stroke, in addition to ischaemic events in the peripheral circulation. Functional changes occur both in epicardial coronary arteries and in the microcirculation. These precede atherosclerotic lesion formation, but become more pronounced as the disease progresses$^{[2,3]}$. The atherosclerosis process implies a complex interaction of...
vascular cells, such as the endothelium and smooth muscle, and blood components, such as monocytes, lymphocytes, platelets and coagulation products. Because of the important anatomical position of endothelial cells between the circulating blood (i.e. platelets, monocytes) and vascular smooth muscle, dysfunction of the endothelium mediates abnormal coronary vasomotion, adhesion of monocytes and platelets as well as migration and proliferation of vascular smooth muscle cells\(^2,3\) (Fig. 2).

A major part of research into vascular disease concentrates on abnormalities in the production, release or breakdown of endothelial factors (i.e. nitric oxide [NO] and endothelin [ET]). It is hypothesized that endothelial dysfunction initializes abnormal coronary vasomotion, adhesion of monocytes and platelets and eventually of smooth muscle cell migration and proliferation\(^2,3\). Endothelial dysfunction reflects a ‘response to injury’ to oxidized low-density lipoproteins (LDL)\(^3,4\), hypertension\(^5\–\(^7\), high glucose\(^8\) and oxygen-derived free radicals\(^9\).
Lipids have been known to promote atherosclerosis since Ajetmanov fed rabbits with high cholesterol at the beginning of the last century. The 'lipid theory' regards atherosclerosis and, later on, thrombosis as separate, although related, processes\(^1-3\). The accumulation of LDL in the vessel wall is thought to be related to increased endothelial permeability and elevated levels of LDL. In particular, oxidized LDL reduces the endothelial release of NO\(^4\) and stimulates the expression of adhesion molecules. Monocytes then adhere to the endothelium, get into the subintima, absorb oxidized LDL via the scavenger receptor and transform into foam cells, the major cell type in fatty streak lesions\(^2-3\) (Fig. 1). With the lesion growing over time, plaque fissure can lead to local haemorrhage and exposure of subendothelial structures, which in turn provoke rapid aggregation of platelets, thrombosis and finally infarction of cardiac muscle cells. Smooth muscle cells are also responsible for the formation of the lesion by proliferation and migration of the cells as well as connective tissue\(^1-3\). Denudation or dysfunction of the endothelium depletes the vessel wall from the release of NO which, in turn, inhibits smooth muscle proliferation and migration. Additionally, platelets release growth factors, i.e. platelet-derived growth factor (PDGF) and transforming growth factor beta-1 (TGF\(\beta\)). There is also a release of other growth factors by the endothelial cell itself, activated macrophages and vascular smooth muscle\(^6\).

There is close interaction between nervous, endocrine and paracrine regulatory systems in coronary vasomotion. The effects of the sympathetic nervous system activating alpha- and beta-receptors are generally set off by endothelium-derived NO. An impaired release of NO in atherosclerosis explains, in large part, the paradoxical vasoconstriction to sympathetic activation (such as the cold pressor test) in patients with coronary artery disease\(^10,11\). Furthermore, abnormal vasoconstrictor responses to various receptor-operated endothelium-dependent agonists such as acetylcholine (Fig. 3), serotonin and histamine occur in the coronary circulation of patients with atherosclerosis. This paradoxical vasoconstriction can also be observed during physical exercise\(^12\). Flow-dependent vasodilation is lost in patients with coronary artery disease. This may imply ischaemic events in these patients and occasionally lead to the rupture of plaques. Intensive physical activation, which causes paradoxical coronary vasoconstriction, can lead to acute coronary syndromes\(^13\). The clinical importance of endothelial dysfunction as a predictor of coronary events, particularly of those in the microcirculation, as measured by the acetylcholine test, has recently been shown in patients with coronary artery disease\(^14\) (Fig. 4). In this trial, the majority of the patients with clinical events at follow-up showed an impaired increase in coronary blood flow velocity after acetylcholine infusion.

It has yet to be determined whether or not early functional changes of the endothelium in the coronary circulation initiate later atherosclerotic vascular lesions\(^31\). Moreover, the implications of cardiovascular drugs have been studied in trials with only small numbers of patients and with an emphasis either on coronary vasomotion\(^15-18\) or on angiographic changes only\(^19-21\), but not both. Studies on the effects of pharmacological interventions on the responsiveness to acetylcholine in the coronary circulation have been mainly with statins\(^17,18\) or with an ACE inhibitor\(^19\). Trials with statins so far have studied only a small patient sample and have shown contradictory results\(^22\) (Ganz P., personal communication). The CARATS study\(^22\) showed no significant effect on coronary endothelial vasomotor function in a study population of 60 patients with coronary disease and mildly elevated cholesterol levels after 6 months of cholesterol-lowering therapy (Fig. 5). On the assumptions of the authors, the statistical power of the study is less than 70%. The number of patients was only 60 implying a 67% statistical power; however, if they had chosen 80 patients a statistical power of 80% would have been reached.

Statins, ACE inhibitors and dihydropyridine calcium antagonists have been investigated in the forearm circulation with patients with hypercholesterolaemia\(^23-25\). All studies showed endothelium-dependent vasodilatation to acetylcholine. Angiographic trials, such as the INTACT trial\(^29\) (Fig. 6) and the Montreal Heart study\(^19\), initially focused on dihydropyridine calcium antagonists while more recent trials evaluated statins\(^22,26\). An improved prognosis in patients without left ventricular dysfunction, but with high risk for coronary artery disease, was found for ACE inhibitors in the HOPE trial\(^27\). A relative risk reduction was achieved for cardiovascular death, myocardial infarction and stroke (Fig. 7). This effect followed without reduction of blood pressure, which may give insights into a direct mechanism of ACE inhibitors on endothelial dysfunction.

It thus appeared appropriate to initiate a major clinical trial that investigated endothelial function and atherosclerotic structural changes with modern quantitative techniques with assessment of vascular structure by both quantitative coronary angiography (QCA) and intravascular ultrasound (IVUS) and of vascular function by an acetylcholine test (QCA and flow measurements). The ACTION trial, a double-blind, placebo-controlled study of the effects of nifedipine on morbidity and mortality in 6000 patients with coronary artery disease\(^28\), investigated the beneficial action of nifedipine on the ‘early’ stages of coronary artery disease. However, evidence is still lacking that dihydropyridines can improve endothelium function and, in turn, the atherosclerotic process in the human coronary circulation. A possible interaction of dihydropyridines with statins, which are now increasingly used in patients with coronary artery disease, is of major clinical importance. Such a possibility has been raised by the REGRESS trial\(^29\). Hence, statins and calcium antagonists may have supplementary beneficial effects on vascular function and structure, which may prove clinically
important to avoid the progression of atherosclerosis and subsequent ischaemic events such as unstable angina and myocardial infarction.

A trial needed to be designed in which the long-acting formulation of nifedipine GITS (=gastro-intestinal therapeutic system) and a statin, as well as their combination, were compared with placebo. Change in endothelial function as assessed by intracoronary infusion of acetylcholine was selected as the major end-point of this trial. Atherosclerotic vascular changes and their relationship to endothelial dysfunction were also seen as important. Such a trial, as a consequence, will (1) determine the effects of nifedipine on endothelium-dependent coronary vasomotion, alone and in combination with a statin, (2) give important clues to a possible interaction between calcium antagonists and statins on atherosclerotic vascular changes, and (3) for the first time, relate changes in endothelial function with

Figure 3 Angiographic response of a human circumflex coronary artery to acetylcholine intracoronary infusion: (a) markedly constricted coronary segment after intracoronary infusion of acetylcholine, (b) the same vessel after intraglycerine intracoronary infusion.
those seen in the atherosclerotic process as assessed by IVUS and QCA.

**The ENCORE I trial**

Recruitment for the ENCORE I trial began in 1998 and was completed by 15 February 2000. Three hundred and forty-three patients were randomized who were scheduled to undergo percutaneous transluminal coronary angioplasty (PTCA) with or without stent implantation\(^{29}\). Patients with one left coronary artery branch with minimal or no lesions (<40%) were followed. After successful coronary angioplasty of one branch with a significant stenosis, patients could enter into the ENCORE I trial. Coronary vasomotion was assessed by QCA and measurement of flow velocities with a FloWire\(^{30}\) in a left coronary branch with no or minimal coronary lesions. In all patients, acetylcholine was selectively infused at increasing dosages (10\(^{-7}\), 10\(^{-6}\), 10\(^{-5}\) mol . 1\(^{-1}\) in the target artery). After acetylcholine infusion adenosine was infused (2·4 mg.m in) and changes in coronary flow monitored. Patients who did not show an increase in coronary diameter of the target segment in response to acetylcholine were randomized to placebo, nifedipine at 30–60 mg . day\(^{-1}\), cerivastatin at 400 \( \mu \)g. day\(^{-1}\) or a combination of both (Fig. 8). Patients were in consequence clinically examined after 14 days, 1 and 3 months. After 6 months repeat coronary angiography was performed in most patients, including quantitative coronary angiography and

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**Figure 4** Kaplan–Meier analyses demonstrating proportion of patients without cardiovascular events during long-term follow-up. Acetylcholine-induced vasodilation is divided into vasodilator and vasoconstrictor responses\(^{14}\).

**Figure 5** Effect of acetylcholine on epicardial coronary diameter before and after treatment in the CARATS study. Diameter changes during intracoronary acetylcholine infusion were assessed at baseline and after 6 months of treatment with simvastatin at 40 mg . day\(^{-1}\). Acetylcholine produced dose-dependent coronary vasoconstriction at baseline in both groups. After 6 months of treatment, the constrictor response improved to a similar extent in both groups\(^{28}\).

**Figure 6** Effects of nifedipine on the development of new coronary artery lesions in patients with coronary artery disease over a period of up to 6 years (INTACT trial). (Modified with permission\(^{20}\).)

**Figure 7** Kaplan–Meier estimates of the composite outcome of myocardial infarction, stroke, or death from cardiovascular causes in the ramipril group and the placebo group. The relative risk of the composite outcome in the ramipril group compared with the placebo group was 0·78 (95% confidence interval, 0·70–0·86)\(^{29}\).
therapy. The database closed in the autumn of 2000. The baseline results clearly show that the acetylcholine test in quantitative angiography (Fig. 9) and flow velocities measurement using FloWire® (Fig. 10) is a suitable tool for the assessment of coronary vasomotion and flow in patients with coronary artery disease.

The ENCORE II trial

The ENCORE II trial will adopt similar entry criteria to those used in the ENCORE I trial[29]. Patients scheduled to undergo PTCA will be selected and currently 135 patients have been recruited (Fig. 11). They are considered for inclusion if they have only one or two vessel coronary disease and one coronary artery has minimal (<40% stenosis) or no disease (target artery). Patients then undergo PTCA with or without stenting and subsequently an acetylcholine test and IVUS will be performed, using an automatic pull-back device, in the artery with no or minimal disease. Patients who did not show an increase in coronary artery diameter in response to acetylcholine will be randomized to either 200 µg . day⁻¹ of cerivastatin, 800 µg . day⁻¹ of cerivastatin alone or in combination with 30–60 mg . day⁻¹ of nifedipine GITS[29] (Fig. 12). Patients will be followed up at 14 days, 1, 3, 6, 12 and 18 months. At 24 months, control angiography and an acetylcholine test will be performed as well as an IVUS study of the target segment.

The primary end-point of this study will be the assessment of plaque volume (measured in the IVUS pull-back tracing) between the baseline (pre-randomization) investigation and that at 24 months in parallel with changes of endothelial function as assessed by QCA. Comparisons of the net changes in plaque volume after 24 months of treatment between the group of patients receiving cerivastatin at 200 µg . day⁻¹, cerivastatin at 800 µg . day⁻¹ or the combination of cerivastatin at 800 µg . day⁻¹ with nifedipine at GITS 30–60 mg . day⁻¹ will also be made.

Clinical importance of the ENCORE trials

The ENCORE trials will show at the clinical level whether calcium antagonists and statins, alone or in combination, ameliorate or normalize endothelial dysfunction in coronary arteries of patients at an early stage of the disease (ENCORE I). In addition, the ENCORE trials address the clinically important issue of whether endothelial dysfunction and its improvement during treatment with either a statin alone, or a statin in combination with a calcium antagonist, is associated with progression or regression of the disease process respectively. As endothelial dysfunction and structural atherosclerosis have not yet been linked, this aspect
will be of crucial importance in understanding the interrelationship between functional and structural alterations in atherosclerosis.

References


Appendix

ENCORE Steering Committee

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