TRIAL DESIGN

PERindopril—Function of the Endothelium in Coronary Artery Disease Trial: The PERFECT Study—Sub Study of EUROPA: Rationale and Design

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Summary. Background. ACE inhibition reduces morbidity and mortality among a variety of patients. Among mechanisms explaining these beneficial effects are the effects on the sympathetic system and on local vasodilating substances such as nitric oxide and bradykinins at the level of the endothelium. The PERFECT study was designed to verify the above mentioned pathophysiological concepts.

Methods. The PERFECT study is a study nested within the EUROPA trial, a three year double-blind, multi-centre, placebo-controlled randomised study that aims at studying the effect of the ACE-inhibitor Perindopril on morbidity and mortality in over 12,000 patients with stable coronary artery disease without clinical heart failure. The PERFECT study is designed as a parallel group randomised placebo controlled trial to determine the effect of Perindopril (8 mg) on brachial artery endothelial function in patients with stable coronary artery disease without clinical heart failure. In the PERFECT study, B-mode ultrasonography of the brachial artery is used as a model for changes in the coronary arteries. Endothelial function in response to ischaemia (reactive hyperaemia) and to vasoconstriction (cold pressor test) is assessed. The ischemia test is used a model to assess the effects of ACE inhibition on nitric oxide/bradykinine mediated vasodilatory response to ischaemia, whereas the cold pressor test is applied to assess the effect of ACE inhibition on the neurohormonal response. The recruitment for the PERFECT study started in May 1998 en was completed in June 1999. 345 patients were recruited in 20 European centers. The Vascular Imaging Center Utrecht, an ultrasound core laboratory, is performing the endothelial function measurements. The primary study outcomes are (1) percentage change in flow-mediated vasodilatation of the brachial artery between the 36 month measurement and the baseline measurement and (2) percentage change in neurohormonal mediated vasoconstriction of the brachial artery between the 36 month measurement and the baseline measurement. The size of the study allows detection of an absolute difference in FMD of 2.0% with a 90% power and a two-sided alpha of 5%.

Conclusion. The findings of the PERFECT study may help to understand and explain the effects of ACE inhibition, in particular Perindopril, on cardiovascular morbidity and mortality.

Key Words. ACE inhibition, brachial reactivity, endothelial function, cardiovascular disease prevention, atherosclerosis, coronary heart disease

Introduction

ACE inhibition has been shown to reduce morbidity and mortality among a variety of patients, i.e. patients with congestive heart failure [1–3], survivors of a myocardial infarction [4,5], asymptomatic patients with left ventricular dysfunction [6,7], patients with acute myocardial infarction [8–11], post-myocardial infarction patients [6,7,12–14] and older high risk patients with documented coronary heart disease without heart failure and, in some, no left ventricular dysfunction [15]. In addition, the EUROPA trial addresses the issue of prevention of cardiovascular morbidity and mortality by ACE inhibition with Perindopril in a broader, low-risk population with documented coronary heart disease without heart failure and irrespective of left ventricular dysfunction [16,17]. Several mechanisms may explain the beneficial effects of ACE inhibition on morbidity and mortality [18].

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The EUROPA-PERFECT investigators are listed in the Appendix.

227
ACE inhibitors antagonise the effects of angiotensin II (Fig. 1). Angiotensin II has direct effects on cytokines: higher angiotensin levels resulting in higher cytokine production. In addition, elevated angiotensin II increases free radical production through an effect on induced nitric oxide synthase (iNOS). It has been shown that increased in iNOS leads to an increase in free radicals, a process that can be attenuated by angiotensin II blocking agents, ACE inhibitors or AT1 antagonists, indicating that angiotensin II is indeed responsible for elevated levels of free radicals [19,20]. Therefore, it may be assumed that ACE inhibition, leading to lower levels of angiotensin II, results in reduction of oxidative stress on the endothelium. Indeed, angiotensin-converting enzyme inhibitors have been shown to counteract the vasoconstriction of the atherosclerotic coronary arteries and therapy with ACE inhibitors has been demonstrated to prevent endothelial dysfunction in animal [21–25] as well as human experiments [26–30].

ACE inhibitors increase local vasodilating substances such as nitric oxide and bradykinin on the level of the endothelium (Fig. 1) [18]. Increasing bradykinin levels results in improved vasodilatory capacity. Through an increase in bradykinin, the ecNOS production of nitric oxide is stimulated. When the bradykinin receptor is blocked, NO production is reduced. Bradykinin induces the production of nitric oxide and prostaglandins, which are powerful vasodilators. In addition, bradykinin/nitric oxide has growth inhibitory properties and modifies platelet aggregation.

One important pathway is through affecting the sympathetic system activation [31]. The sympathetic nervous system may be involved in the process of atherosclerosis through platelet activation and subsequent platelet-derived growth factor formation and by inducing mechanical injury to the vascular wall as a result of increased blood pressure and increased flow velocity. In addition, sympathetic control of coronary vasomotor tone, which under normal conditions is not important, becomes functionally significant once coronary artery disease and endothelial dysfunction have occurred. Under these circumstances, increased sympathetic adrenergic tone may lead to coronary vasoconstriction and, as myocardial oxygen demand increases concomitantly, myocardial ischaemia may ensue. Furthermore, myocardial ischaemia activates several neurohormonal systems, such as the sympathetic and the circulating renin-angiotensin system [32]. This leads to systemic and, possibly, coronary vasoconstriction and thus to further myocardial ischaemia. ACE inhibitors affect myocardial ischaemia by reducing neurohormonal activation and related systemic and coronary vasoconstriction [33,34]. These acute effects may become more important over time, as coronary endothelial function improves following long-term ACE inhibition.

The PERFECT study, as a substudy to the EUROPA trial was designed to verify the above mentioned pathophysiological concepts [17]. In the PERFECT study, B-mode ultrasonography of the brachial artery will be used as a model for changes in the coronary arteries, and to assess endothelial function in response to ischaemia (reactive hyperaemia) and to vasoconstriction (cold pressor test). The first test is used as a model to assess the effects of ACE inhibition on nitric oxide/bradykinine mediated vasodilatation in response to ischaemia, whereas the cold pressor test is applied to assess the effect of ACE inhibition on the neurohormonal response, i.e. sympathetic activity.

The present paper describes the rationale and design of the PERFECT study.

**Methods**

**General**

The PERFECT study is a study nested within the EUROPA trial, a three year double-blind, multicentre, placebo-controlled randomised study that aims at studying the effect of the ACE-inhibitor Perindopril on morbidity and mortality in over 12,000 patients with stable coronary artery disease without clinical heart failure [17]. The PERFECT study is designed as a parallel group randomised placebo controlled trial to determine the effect of Perindopril (8 mg/day) on brachial artery endothelial function in patients with stable coronary artery disease without clinical heart failure. After a run-in period of 4 weeks with 4 mg/day Perindopril, participants will be randomised to Perindopril or placebo. The recruitment for the PERFECT study started in May 1998. Both the EUROPA trial and the PERFECT study finished recruitment in June 1999. In the PERFECT study 345 patients were recruited in 20 European centers (8 in Czech Republic, 1 in Germany, 2 in Greece, 4 in Netherlands, 4 in Poland and 1 in Sweden). Endothelial function, measured as flow-mediated vasodilatation and as cold pressor induced vasoconstriction was assessed at visit M-1, just before the run-in period. At visits M-1 and M36, an endothelial independent vasodilatation test using nitroglycerine sublingually, was performed. Follow-up B mode ultrasound
assessments were performed at 6, 12, 24 and 36 months after randomisation.

Approval for the conduct of the PERFECT study was obtained from the Institutional Review Boards of the participating clinics and written informed consent was obtained from all participants. Both studies are carried out according the International Conference for Harmonisation—Guidelines of Good Clinical Practice (ICH-GCP).

**Study population**

The population enrolled in the PERFECT study is similar to that enrolled in the EUROPA trial [17]. In short, the main inclusion criteria are 18 years of age or above; documented coronary artery disease, not scheduled for re-vascularisation and informed consent obtained. Documented coronary heart disease includes a history of previous myocardial infarction (confirmed by ECG demonstrating Q waves in 2 continuous leads and/or changes in cardiac enzymes more than or equal to twice the normal values) of at least three months prior to the selection visit, or, a history of PTCA or CABG of at least six months prior to the selection visit, or, history of stroke or cerebral transient ischaemic attacks within the preceding 3 months; child-bearing potential without contraception; previously not treated hypertension; systolic blood pressure ≥ 100 mmHg; use of ACE inhibitors or angiotensin II receptor inhibitors within one month prior to the selection visit; renal failure with serum creatinin > 3 times upper normal values; history of stroke or cerebral transient ischaemic attacks within the preceding 3 months; child-bearing potential without contraception; previously not tolerated ACE-inhibitor.

**Objectives of the PERFECT Study**

The primary objective of the PERFECT study is to assess the effects of Perindopril on endothelial function of the brachial artery, measured by B-mode ultrasound in patients with stable coronary artery disease without clinical heart failure in a double blind randomised placebo-controlled trial with a duration of three years.

The primary study outcomes are (1) percentage change in flow-mediated vasodilatation of the brachial artery between the 6 month measurement and the baseline measurement and (2) percentage change in neurohormonal mediated vasoconstriction of the brachial artery between the 36 month measurement and the baseline measurement.

Secondary study outcomes are (1) Percentage change in flow-mediated vasodilatation of the brachial artery between the 6 month measurement and the baseline measurement, (2) percentage change in neurohormonal mediated vasoconstriction of the brachial artery between the 6 month measurement and the baseline measurement, (3) Rate of change in flow-mediated vasodilatation in 36 months and (4) Rate of change in neurohormonal vasoconstriction in 36 months.

**Ultrasound Examinations**

**General**

Participating centres needed to have access to a Duplex scanner with a > 7 MHz or 7–10 MHz linear array transducer, on line ECG recording and a (S-)VHS video recorder and preferably experience with non-invasive measurement of flow-mediated vasodilatation of the brachial artery. Preceding the PERFECT study, a detailed training programme was implemented to ensure standardisation of the brachial reactivity measurements. This took place at the local centres and was done by the staff of the central core laboratory, i.e., the Vascular Imaging Center, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands. During this course, sonographers learned the theory, and, in particular, the practicalities involved in these brachial reactivity measurements. Based on the performance during and after the training programme all sonographers entered the certification procedure. This meant that after the training, sonographers needed to practice at their institution and sent the Vascular Imaging Center in Utrecht the examinations for review. Between 10–20 ultrasound studies were needed that show sufficient proficiency to be certified depending on the prior skills of the sonographer and the time allocated for training. The certification examinations were reviewed Vascular Imaging Center staff.

During the PERFECT Study, ultrasound examinations were evaluated by the Vascular Imaging staff in the same way as during the certification period in order to assess whether the performance of the sonographer was kept at the highest level possible. Around 10% of ultrasound examinations were evaluated in this QC/QA programme. Emphasis was on protocol execution, focus, segment positioning, quality of the boundaries, gain and technique. Scans were rated as excellent (A), good/moderate (B) and insufficient (C). When a scan was rated as C, the following scan of the same sonographer was additionally evaluated. When that scan was again rated as C, a third scan was evaluated and a contact was made with the sonographer to discuss reasons of insufficient scans and ways of improvement. When a sonographer made C scans repeatedly, the sonographer was decertified and removed from the study.
**B-mode acquisition**

The ultrasound examination included an ‘ischemia’ test, a ‘cold pressor’ test and a ‘nitroglycerine’ test (the latter only at visits m-1 and m36). Patients were studied in supine position. Three ECG leads were attached. The arm was placed in a specifically developed splint to reduce arm movements during the procedure as much as possible and to allow for fixation of the ultrasound transducer (Fig. 2). The blood pressure cuff was placed just below the elbow. After a 10 minute rest, the brachial artery at the elbow was visualised using a ultrasound machine with a > 7 MHz or 5–10 MHz linear array transducer. When a satisfactory longitudinal optimal image of the brachial artery was obtained, the position of the transducer was fixed in the holder. Three B-mode images showing the lumen diameter were frozen on the R-wave of the ECG to provide information for off-line measurement of the ‘baseline’ lumen diameter for the ischemia test. Then the blood pressure cuff was inflated to suprasystolic levels (50 mmHg above) for a four-minute period. During that time the sonographer checked whether the optimal image was steady. After deflation of the blood pressure cuff, ultrasound examination continued for 5 minutes. Every 15 seconds a B-mode image was frozen on the R-wave of the ECG (end-diastole) for off-line lumen diameter measurements. The ultrasound examination is recorded on (S-)VHS videotape for off line analysis [35,36].

After 15 to 60 minutes rest, the patient was re-examined in supine position to assess the effect of the ‘cold pressor test’ in a similar procedure as described above. When a satisfactory longitudinal optimal image of the brachial artery was obtained, three B-mode images showing the lumen diameter were frozen on the R-wave of the ECG to provide information for off-line measurement of the ‘baseline’ lumen diameter for the cold-pressor test. Then the other arm was put in an ice cold water bath for 4–5 minutes, depending on the tolerability of the patient. The ultrasound examination continued during and after the cold pressor test for 10 minutes. Every 15 seconds a B-mode image was frozen on the R-wave of the ECG (end-diastole) for off-line lumen diameter measurements. The ultrasound examination was recorded on videotape for off line analysis. After 15 to 60 minutes rest, the patient was re-examined in supine position to assess the effect of the ‘nitroglycerine test’ in a similar procedure as described above. When a satisfactory longitudinal optimal image of the brachial artery was obtained, three B-mode images showing the lumen diameter were frozen on the R-wave of the ECG to provide information for off-line measurement of the ‘baseline’ lumen diameter for the nitroglycerine test. Then 400 micrograms of nitroglycerine were sublingually administered. The ultrasound examination continued for another 5 minutes. Every 15 seconds a B-mode image was frozen on the R-wave of the ECG (end-diastole) for off-line lumen diameter measurements. The ultrasound examination was recorded on videotape for off line analysis [36,37]. The videotapes containing up to studies of 4 participants were then sent to the Vascular Imaging Center in Utrecht, The Netherlands by express mail.
Quantification of Lumen Diameter (Off-Line Reading)

Off-line analysis is performed at the Vascular Imaging Center in Utrecht, The Netherlands. Preceding the reading process, a detailed training programme was implemented to ensure standardisation of the brachial reactivity measurements. The training programme included, apart from instructions, having the readers read through a set of images twice. This provided for readers information on between reader variability and within reader variability in the measurement. When a reader showed to be reading consistently, the reading of the main study started. To assess whether reading behaviour changed over time, the readers completed a library image set at regular intervals.

At present, between reader readings of the same B-mode images (n = 36 patients) show Intraclass correlation coefficients for the ischemia test of around 0.65, for the cold pressor test of 0.91 and for the nitroglycerine test of 0.92. Within reader readings (repeat) reading of the same B-mode images (n = 36 patients) showed Intraclass correlation coefficients for the ischemia test of 0.70, for the cold pressor test of 0.94 and for the nitroglycerine test of 0.93.

The reading of the PERFECT Study ultrasound examinations is being performed using Brachial ToolsR, version 3.2.6 (Medical Imaging Applications, Iowa, USA). In short, first the frozen images on videotape are digitised and put into a time sequence. Then the reader manually identifies the part of the brachial artery in which the boundaries are good and stable visualised in all these images. Then the Brachial ToolsR software detects the boundaries through all these images and provides numerical data on lumen diameters of all the images and provides a graphical plot. Ultrasound interfaces from which the lumen diameter is measured is are the trailing edge of the adventitia-media interface on the near wall and the leading edge of the media-adventitia interface at the far wall. An example of the lumen diameter measurements from a cold pressor test is presented in Figure 3, and from a nitroglycerine test in Figure 4. The raw data are being processed using SAS statistical software. The diameter at baseline is based on the average of the three frozen images. The following calculations are then being performed: Flow mediated vasodilatation as marker for endothelial function, is estimated as \[ \frac{\text{maximal lumen diameter after ischemia} - \text{diameter at baseline}}{\text{diameter at baseline}} \]. The neurohormonal vasoconstriction induced by cold pressor test is estimated as \[ \frac{\text{minimal lumen diameter after cold exposure} - \text{diameter at baseline}}{\text{diameter at baseline}} \]. The nitroglycerine response (flow independent vasodilatation) is estimated as \[ \frac{\text{maximal lumen diameter after nitroglycerine} - \text{diameter at baseline}}{\text{diameter at baseline}} \].

Sample Size Considerations

At the start of the preparations of the PERFECT study in 1998, an estimate of what was considered a
clinically relevant difference in flow mediated vasodilatation from baseline varied between 1.8% and 6.7% in the published literature. In a study among subjects who underwent coronary angiography, the positive predictive value of FMD less than 3% in predicting coronary endothelial dysfunction was 95% [38]. It had been argued that in clinical trials the number of subjects needed should be such that at least a 2% difference in FMD is detectable [39]. In an earlier study performed by our group, among 32 healthy volunteers the mean FMD was 7.7% (SD 5.0) [37]. Since the present study is multi-centre which generally increases the variability in the FMD measurements slightly compared to single-centre studies, the sample size calculation is to be considered conservative.

With a 90% power, a two-sided alpha of 5%, an absolute difference in FMD of 2.0% can be assessed with a sample size of 131 subjects in each arm of the trial. With an anticipated 10% drop-out rate in the EUROPA trial, a total of 288 subjects needed to be randomised.

**Data Analysis**

Patient demography, risk factors and relevant clinical variables (e.g. cardiovascular history and medication) will be summarised by the appropriate descriptive statistics to characterise the study population and to judge baseline comparability of the treatment groups. Because this is a randomised study, baseline characteristics will not be compared among the treatment groups using a statistical test. All patients will be included in the primary analysis as randomised, regardless of the actual treatment received: i.e., intention-to-treat analysis. The principal analysis of efficacy data will employ analysis of variance (ANOVA). A model including treatment group as the single independent variable will test the null hypothesis of no differences between treatment group means. This strategy will apply to both 36 months (primary endpoint) and 6 months endpoints (secondary endpoint). Because some patients may not complete the study as planned, numerical values for missing data will be imputed by the last observation carried forward (LOCF). This strategy will be employed for all efficacy endpoints expressed as change from baseline.

To evaluate differences in rate of change of flow-mediated vasodilatation for the treatment group the following analysis will be done. For each individual patient the rate of change in flow mediated vasodilatation will be estimated by fitting the measurement data points from baseline, 6 months, 12 months, 24 months and 36 months into a regression model weighted for time and number of measurements and possibly other covariates. The individual rates (slopes) are then used to estimate a mean rate of change for each treatment group. The mean rates of change will be compared using an ANOVA analysis. A similar approach is used for neurohormonal vasoconstriction.

The size of the study group is relatively small for subgroup analyses. Explanatory analyses will be performed in strata of diabetes mellitus (yes/no); of previous myocardial infarction (yes/no); of hypertension...
(yes/no); of statins (yes/no). Consistency of the treatment effects on the efficacy across these subgroups will be investigated primarily by using a multiplicative interaction term for treatment by subgroup in the statistical model.

**Discussion**

The PERFECT study, as a substudy to the EUROPA trial was designed to verify the pathophysiological concepts regarding the mechanism through which ACE inhibition with Perindopril prevents cardiovascular morbidity and mortality. We selected the flow-mediated vasodilatation of the brachial artery after ischemia as a reflection of the nitric oxide pathway. In addition, we use the cold pressor test as a reflection of neurohormonal status of the patient.

There is abundant evidence showing that nitric oxide is the main mechanism through which arterial dilation occurs after ischemia in the brachial artery [40,41]. Indirect measurement of bioavailable nitric oxide, through its vasodilating properties, is an extensively investigated surrogate of endothelial (vasomotor) function in clinical and experimental studies. A large number of studies has shown that non-invasively assessed endothelial function can be measured accurately and reliably in populations at large by measurement of flow mediated vasodilatation of the brachial artery (FMD) [38,39]. Furthermore, the forearm circulation has been shown to exhibit functional changes very similar to those in coronary circulation in patients at risk of coronary heart disease [39,42]. An impaired flow mediated vasodilatory response to ischaemia has been related to unfavourable levels of cardiovascular risk factors [38,43–45]. Recently, the predictive value of coronary endothelial dysfunction was demonstrated [46–48]. Measurement of endothelial function in accessible peripheral vessels, such as the brachial artery, may therefore be a useful surrogate for coronary endothelial vasomotor function and may be measured by flow mediated dilation using high resolution ultrasound [49].

Information on the vasoreactivity of the brachial artery induced by a cold pressor test is much more limited [50–52]. Normal subjects have shown an increase in lumen diameter whereas coronary heart patients appear to react with vasoconstriction. A clear relationship with coronary reactivity has been shown [52]. These limited data point towards a useful surrogate for coronary endothelial function.

In conclusion, the findings of the PERFECT study may help to understand and explain the effects of ACE inhibition, in particular Perindopril, on cardiovascular morbidity and mortality.

**Appendix**

**Participating PERFECT centers**

**Czech Republic:** CESKY KRUMLOV: J. Florian, MD (principal investigator), V. Kuchar, MD; BRNO: B. Semrad, MD (principal investigator), J. Ziembova, MD, J. Schildberger, MD; PILSEN, H. Rosolova, MD (principal investigator), V. Jankovych, MD; PRAQUE: R. Spacek, MD (principal investigator), P. Stanka; PRAQUE: J. Hradec, MD (principal investigator), J. Malik, MD; TÁBOR: J. Charouzek, MD (principal investigator) V. Jirka, MD; PRAQUE: P. Jansky (principal investigator), Koelbel, MD; SLANY: G. Marcinek, MD (principal investigator), M. Votyeka-Pecha; BRNO: L. Groh (principal investigator), I. Hofrek, L. Nechvatel. **Germany:** MUNCHEN: C. von Schacky, MD, PhD (principal investigator), S. Stoerk, MD, P. Markov. **Greece:** ATHENS: Gialavos, MD (principal investigator), A. Androulakis, MD; ATHENS: J. Lekakis, MD, C. Papamichael, MD. **Netherlands:** HARDERWIJK: R. Dijkgraaf, MD (principal investigator), Y. Jansen-Timmer, H. Bralts; HENGELO: J.J.J. Bucx, MD (principal investigator until 01/01/2000), H. Droste, MD (principal investigator from 01/01/2000), G. Assink; EMEN: L.F.M. van de Markhof, MD (principal investigator), E. Vinke; ROTTERDAM: van Nieuwenhoven, MD (principal investigator), IM. Toonder, E. Korten. **Poland:** KRAKOW: A. Gackowski, MD (principal investigator); KRAKOW: T. Zielinsky, MD (principal investigator), T. Rywik, MD; KATOWICE: A.M. Wnuk-Wonjar, MD (principal investigator), C. Vitas; KATOWICE: M. Tendera, MD (principal investigator), M. Kazmierski. **Sweden:** MALMO: J. Persson, MD (principal investigator), G. Ousting.

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References


