Primary research

Design of a trial evaluating myocardial cell protection with cariporide, an inhibitor of the transmembrane sodium–hydrogen exchanger: the Guard During Ischemia Against Necrosis (GUARDIAN) trial

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Received: 31 May 2000
Accepted: 19 July 2000
Published: 8 August 2000

Curr Control Trials Cardiovasc Med 2000, **1**:59–67

Current Controlled Trials Ltd
(Print ISSN 1468-6708; Online 1468-6694)

Abstract

Inhibition of the sodium–hydrogen exchanger (NHE) is a powerful experimental tool to inhibit sodium and calcium accumulation within the ischemic myocyte and halt progression of cell ischemia to cell necrosis. This paper describes the protocol and rationale of a first large-scale clinical trial designed to evaluate the safety and efficacy of cariporide, a novel specific and potent inhibitor of the exchanger.

Keywords: cariporide, coronary angioplasty, coronary artery bypass graft surgery, myocardial cell protection, sodium/hydrogen exchanger

Synopsis

Background: Direct myocardial cell protection in patients with unstable angina or evolving myocardial infarction (MI) could prevent cell necrosis or reduce its extent, and minimize the risk of MI and death associated with percutaneous coronary interventions (PCIs) and coronary artery bypass surgery. The myocardial NHE plays a critical role in mediating the progression of ischemia to necrosis by promoting intracellular accumulation of sodium and calcium in exchange for hydrogen. Blockage of the system in various experimental models of ischemia and reperfusion had a strong antinecrotic effect. The present paper describes a trial that was intended to investigate the potential clinical benefit of cariporide, a potent and selective inhibitor of the NHE, in a large spectrum of at-risk patients.

Trial design: The GUARDIAN trial was a multicenter, double-blind, randomized, four-arm trial that compared three

CABG = coronary artery bypass grafting; CK = creatine kinase; DSMB = Data and Safety Monitoring Board; ECL = Electrocardiography Core Laboratory; EVC = End-point Validation Committee; IC_{50} = 50% inhibitory concentration; MI = myocardial infarction; NHE = sodium–hydrogen exchanger; PCI = percutaneous coronary intervention; UA/NSTEMI = unstable angina and non-ST-segment elevation myocardial infarction.

cariporide dosages with placebo in patients with unstable angina and non-ST-segment elevation myocardial infarction (UA/NSTEMI) and in patients undergoing a high-risk PCI or coronary artery bypass surgery. A total of 11 590 patients with one of the three possible entry diagnoses were enroled in 23 countries. The trial was designed as a combined phase 2/phase 3 study. The primary objective was to evaluate the efficacy of cariporide in reducing all-cause mortality and/or MI across the various entry populations 36 days after randomization. Three different doses of cariporide were compared with placebo. Secondary end-points were death or non-fatal MI at 10 days and 6 months, and cardiac events related to left ventricular dysfunction. The extent of MI was also assessed by peak elevation in creatinine kinase (CK)-MB and a ratio of peak elevation to normal values. The sample size was driven by a total event rate of 1200 patients experiencing a primary end-point, powered to detect a 25% risk reduction in any of the three treatment groups compared with placebo at a significance level of 0.02, accounting for the three pairwise comparisons.

Full article

Introduction

The acute coronary syndromes involve a cascade of events that can culminate in MI and death [1]. Current therapy targets control of thrombus formation on an active culprit coronary plaque. Antithrombotic therapy with or without a reperfusion procedure significantly reduces the ischemic event rate. Treatment, however, often fails to prevent irreversible cell damage, and procedures can lead to cell necrosis. The left ventricular damage results in a variable amount of left ventricular dysfunction, which is the major determinant of subsequent prognosis and cardiac disability.

Although direct cell protection has been a long-term concern and the focus of numerous investigations, no intervention has so far gained widespread clinical application [2]. Meanwhile, the cellular mechanisms involved in cell ischemia and cell necrosis have become acknowledged, leading to the development of new protective agents and opening new therapeutic perspectives.

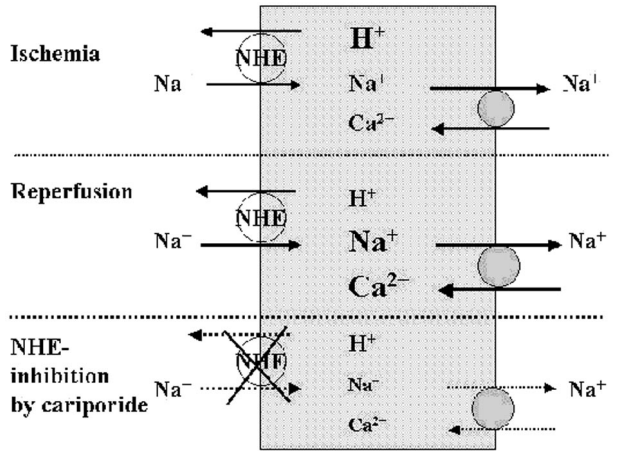
In this line, the role of the NHE in ischemia and reperfusion injury has recently been characterized [3]. The NHE exchangers comprise a family of membrane proteins that are involved in the transport of hydrogen in exchange for sodium, utilizing the transmembrane sodium gradient as the driving force [4]. They are regulated by the intracellular pH through interaction of hydrogen with a sensor site of the exchanger protein and by many other driving forces that are generally receptor mediated.

Six NHE isoforms have been recognized [5]. NHE-1 is ubiquitously distributed in tissue, and is the predominant NHE isoform in the myocardial cell. It is rapidly activated during ischemia by intracellular hydrogen accumulation that is exchanged for sodium. The intracellular accumulation of sodium in turn leads to an increase in intracellular calcium concentrations via sodium–calcium exchange as the ATP-dependent sodium–potassium exchanger becomes inoperative (Fig. 1) [6]. Elevated intracellular calcium concentration directly mediates cell death by activation of various proteases and by causing cell contracture and membrane rupture. The activity of the NHE system is self-limited during prolonged ischemia once an ionic equilibrium is reached between the extracellular and intracellular spaces, but is intensified upon reperfusion by washout of the acidic extracellular fluid [7]. The massive entry of calcium into the cell during reperfusion precipitates cell contracture and contraction band necrosis [3].

Cariporide (HOE642A) was developed recently as a selective inhibitor of the NHE exchanger [8]. The drug is a benzoylguanidine with a molecular weight of 379.46 Da. Inhibition of the activity of the system has been documented experimentally in endothelial cells, myocytes, erythrocytes and platelets [8]. Internal data has shown a dose-dependent inhibition of the NHE system in platelets, with 100% inhibition reached within 15 min after the injection of a bolus dose of 60 mg, declining to approximately 50% after 4 h. The elimination half-life is about 3.5 h.

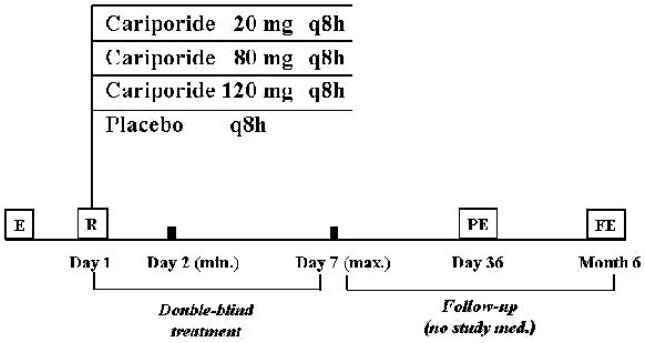
Cardioprotective effects of cariporide have consistently been documented in various experimental models of ischemia/reperfusion, with marked reduction in infarct size when NHE inhibitors were administered before or early during ischemia [9]. Higher doses of the inhibitors administered before reperfusion are still effective [10,11]. Cariporide was administered safely in 20 patients undergoing aortic valve surgery and bypass surgery. In another pilot study [12], in patients with an evolving anterior MI undergoing direct revascularization, administration of this drug has shown promising results.

Figure 1



Ions exchange during ischemia and reperfusion and consequence of inhibition of the NHE. Ischemia results in rapid intracellular acidosis as the metabolism becomes anaerobic, activating the pH-regulating ion transporters and the NHE. This results in a large influx of sodium (Na^+) into the cell. As the ATP supply for the Na^+/potassium (K^+) pump falls, Na^+ accumulates within the cell, reducing calcium (Ca^{2+}) efflux and/or promoting reverse mode Ca^{2+} entry ($3Na^+/Ca^{2+}$). The exchange is self-limited as extracellular protons accumulate, but is reactivated upon reperfusion by the washout of the acidotic extracellular fluid, reactivating the NHE system for an accumulation of ions within the cell. NHE inhibition markedly reduces this Na^+ and Ca^{2+} overload. H^+, hydrogen.

Figure 2



Study protocol. E, enrolment; FE, follow-up evaluation; PE, primary evaluation; R, randomization.

The GUARDIAN trial was the first large-scale trial designed to assess the potential protective effect of NHE inhibition in humans. The four-arm trial was double-blind, randomized, and placebo-controlled. It was designed as a combined phase 2/phase 3 study to evaluate the potential of cariporide to prevent necrosis across various situations that involve myocardial ischemia, such as UA/NSTEMI, PCI, and coronary artery bypass grafting (CABG).

Trial organization

The study was sponsored by Aventis Incorporated (New Jersey, USA). The Steering Committee supervised the organization and scientific conduct of the GUARDIAN study. An independent Data and Safety Monitoring Board (DSMB), composed of four physicians and one statistician, was responsible for safety, side effects, critical event monitoring and ethical aspects. The Chairman of the DSMB received and reviewed online individual reports of all unexpected associated serious adverse events. Quarterly overall safety reports were generated by the DSMB and submitted to the Steering Committee and the sponsor. An End-point Validation Committee (EVC) and an Electrocardiography Core Laboratory (ECL) located at St Louis University, St Louis, Missouri, USA, shared the responsibility of adjudicating all end-points of the trial, including secondary end-points. A total of 382 clinical centers from 23 countries actively participated in the study. Worldwide data collection, study monitoring, and data management were performed by Quintiles Incorporated (Research Triangle Park, NC, USA).

Study design

A general overview of the trial design is provided in Fig. 2.

Patient selection

Patients of either sex and of any race, aged 18 years or more, presenting with UA/NSTEMI or undergoing a PCI or CABG could be enroled, as long as some high-risk features were present (as described in detail under Inclusion criteria, below). Approval by regulatory agencies of the various countries, as well as from all Institutional Review Boards and Ethical Committees concerned, was sought before the start of the study, and signed informed consent was obtained from all patients before enrolment. It was expected that patients with UA/NSTEMI would represent 40–50% of the trial population, patients undergoing a high-risk PCI 30–40%, and patients undergoing high-risk CABG 10–20%.

Inclusion criteria

The inclusion criteria for the UA/NSTEMI entry category were repetitive (≥ two episodes of ≥5 min duration) or prolonged (≥ one episode of ≥20 min duration) angina chest pain at rest or on minimal exercise within 12 h before randomization, with one of the following electrocardiographic or laboratory findings: ST-segment depression >1 mm in at least two contiguous leads; T-wave inversion >1.5 mm in at least three contiguous leads; transient ST-segment elevation >1 mm with duration of <30 min in at least two contiguous leads; total CK and/or CK-MB >1.5 times upper limit of the normal range; and troponin I >2.0 μg/l or troponin T >0.15 μg/l. Patients presenting with angina at rest or on minimal exercise 12 h to 4 weeks after an acute MI could also be included in the study, regardless of whether they had received immediate PCI or thrombolysis or met any of the above criteria for acute ischemia.

The criteria for inclusion in the PCI entry category were history of angina at rest or on minimal exercise within the past 4 weeks, with any approved PCIs planned in the following hours for a lesion with at least two type B or one type C characteristic [13].

For CABG, the criteria were an urgently required intervention (eg following failed PCI), repeat CABG, and candidates with a history of angina at rest or minimal exercise within the past 4 weeks and with the presence of two or more of the following features: age >65 years; female sex; diabetes mellitus; left ventricular ejection fraction <35%; and left main or three-vessel disease.

Exclusion criteria

The protocol criteria for exclusion were persistent (≥30 min) ST-segment elevation (>1 mm), or new ST-segment elevation MI, a secondary cause of unstable angina (eg anemia, hyperthyroidism, arrhythmias), and cardiogenic shock or pulmonary edema refractory to medical treatment. Other exclusion criteria were as follows: previous exposure to the drug; history of hypersensitivity to amiloride (a structurally related compound); noncardiac progressive fatal disease; known ALAT and ASAT levels exceeding three times the upper limit of normal or known bilirubin >1.75 mg/dl (>30 μmol/l); known serum creatinine levels >2.0 mg/dl (>177 μmol/]); or clinical evidence of severe hepatic or renal impairment. Pregnancy or potential for pregnancy in the absence of reliable contraception, breast-feeding, alcohol abuse, potential for noncompliance, and mental inaptitude were also exclusion criteria.

Randomization and study drug administration

The study ran four parallel groups: one placebo group and three treatment arms with doses of cariporide of 20, 80, or 120 mg, respectively, administered in a volume of 50 ml in a 60 min infusion three times daily at intervals of 8 h. The randomization schedule linked sequential numbers to treatment codes allocated at random on a 1:1:1:1 basis. The subjects were numbered at each center, in the order in which they were enroled. The study drug was initiated as soon as possible after hospital admission in patients randomized for an UA/NSTEMI, and between 15 min and 2 h before the intervention in patients randomized for planned PCI or CABG.

The treatment duration could vary from 2 days (six doses) to 7 days (21 doses) to cover the period of risk of MI, as evaluated by the treating physician and the local investigators. The recommendation was made to discontinue the drug when the patient was clinically stable with a symptom-free period of 12–24 h, with no further intervention planned. The drug could also be discontinued before 2 days in patients discharged earlier after an uneventful percutaneous procedure.

In patients with impaired renal function, the dosage of the drug was to be adjusted downward. Patients with serum creatinine level between 1.5 and 2.0 mg/dl (133–177 μmol/l) before randomization received a full dose at the first injection and half the doses subsequently. The dose of the drug was halved in patients who developed this increase in creatinine during the course of the study. Patients with a baseline creatinine level >2.0 mg/dl (>177 μmol/l) were excluded, whereas the study drug was discontinued during the study when the creatinine levels increased to >3.0 mg/dl (>265 μmol/l).

Study end-points

The primary end-point of the trial was the combined incidence of all-cause mortality or MI measured 36 days after randomization. The secondary end-points were as follows: the rate of the primary composite end-point at 10 days; events related to left ventricular dysfunction (defined as cardiac-related mortality, cardiogenic shock, overt congestive heart failure, and life-threatening arrhythmia) evaluated 36 days and 6 months after randomization; the extent of infarction as assessed by peak CK-MB elevation and a ratio of elevation; and occurrence of refractory ischemia within 36 days. End-points were both analyzed for the overall population and for the three entry categories (UA/NSTEMI, PCI and CABG).

Serial electrocardiographs and CK-MB determinations were obtained as part of the protocol. A 12-lead electrocardiograph was obtained before randomization and after 24 h, 10 days (or at discharge if earlier), and 36 days. All centers had to determine the CK-MB values, at least when the total CK values were elevated. Routine determinations were performed before randomization and at 4, 8, 12, and 24 h after randomization in patients with UA/NSTEMI and in those undergoing PCI, and at 8, 12, 16, and 24 h after surgery in patients undergoing CABG. The sequence of blood sampling was repeated whenever the patient experienced chest pain with duration of 20 min or longer during the hospital stay. The 12-lead electrocardiograph was also repeated during chest pain and 24 h later. When patients had suspect MI events after hospital discharge, the investigator was requested to supply the more diagnostic electrocardiograph(s) and results of serum markers associated with the event.

Cardiac-related mortality was defined as sudden cardiac death, death due to circulatory failure, or death due to another cardiovascular disease, such as perioperative death, or aortic, cerebral, mesenteric or peripheral vascular disease. Noncardiac mortality included all other causes of death that were not directly the consequence of the underlying cardiac disease or of an intervention procedure, such as infection, respiratory failure, trauma, and suicide.

The diagnosis of MI was based on electrocardiograph or CK-MB criteria, with or without chest pain. Infarcts were classified as Q-wave or non-Q-wave MI. Q-wave MI was defined by appearance of new Q-waves that occurred after randomization (between 24 h and 36 days). Non-Q-wave MI was defined as the appearance of abnormal CK/CK-MB in the absence of Q-wave criteria. Criteria for abnormal CK/CK-MB elevation were more than two times the upper limit of normal in patients with UA/NSTEMI, and, if within 24 h of a revascularization procedure, more than three times the upper limit of normal after PCI and more than 100 units/l within 24 h after CABG. For enzyme elevation that occurred more than 24 h after a PCI or CABG, the criteria for UA/NSTEMI were used.

All electrocardiograph tracings were analyzed at the ECL using the Minnesota code [14]. Serial comparisons of consecutive electrocardiographs were performed using an adaptation of the Novacode for Q-wave items [15,16]. The St. Louis University ECL uses supplemental Q-wave codes to detect the lesser degrees of Q-wave abnormality, as well as criteria for true posterior infarction (new appearance of a 40-ms R wave and R/S >1 in lead V2). Grade 1 or grade 2 ST- or T-wave worsening were classified according to the Minnesota code and assigned to the anterior (leads V1–V5), inferior (leads 2, 3, aVF), lateral (leads 1, aVL, V6), and posterior (leads V1, V2).

In order to differentiate an entry from outcome MI in patients with UA/NSTEMI, patients with normal CK/CK-MB measurements before randomization and 8 h after randomization were specifically examined. When the measurements were normal before randomization and up to 8 h after randomization, an outcome MI was diagnosed if CK/CK-MB values exceeded two times the upper limit of normal after 8 h or more in the UA/NSTEMI group, or if they exceeded two times the upper limit of normal in the 24 h after a PCI. When CK-MB values were abnormal at baseline or up to 8 h, the patient was diagnosed as having an entry MI. Special criteria were used to define a reinfarction in this setting. For those with elevated CK-MB, more than two times the upper limit of normal within 8 h after randomization, and with a decrease in CK-MB of more than 50% from the previous peak value, reinfarction was defined as an elevation of the value of CK-MB at or after 8 h to more than two times the upper limit of normal (three times in the case of PTCA) and 75% more than the preceding trough CK-MB values. When baseline CK-MB was more than two times the upper limit of normal, a new episode of chest pain and new ST-segment shifts were required in addition to the 50% elevation over the preceding trough and an increase to more than two times the upper limit of normal (three times after PCI). In subjects in whom CK-MB was elevated at baseline or during the first 8 h after randomization, and the CK-MB value decreased by more than 50% from the peak value, chest pain and new ST-segment changes were not required. The diagnostic criterion for MI related to CABG was an elevation of the CK-MB values to more than 100 units/l within the 24 h that followed surgery. Other information, such as autopsy reports, could also be used for the diagnosis of MI.

Estimates of infarct size were obtained by using the peak CK-MB elevation values observed during the first 24 h after randomization and the distribution of a ratio formed by the peak value to the baseline values. For the purpose of comparing infarct sizes between the various entry diagnoses, an equivalence table was constructed using the twofold increase criterion for the diagnosis of MI in patients with UA/NSTEMI, and the threefold increase with PCI-related MI; for a CABG-related MI, the peak values were used (Table 1). To account for the wide variation observed in the upper limit of normal between the different laboratories, further *post hoc* analyses were defined also to compare peak CK-MB values in CABG patients as ratios to the upper limit of normal.

End-point validation

All suspected end-point events occurring during the study were documented on an end-point form and validated by the EVC and/or by the ECL. In order to facilitate procedures, the EVC was divided into American and European subcommittees. The homogeneity of the validation process by the two subcommittees was regularly checked in open meetings between the two co-Chairmen and also by blind cross-adjudication of randomly selected case report forms. The ECL screened all case report forms of randomized patients for presence of myocardial ischemia and of MI at entry and during the 36-day follow up in order to validate reported events and to detect the unreported ones, helped by the source documents. Two members of the EVC independently reviewed all other clinical events, as well as unreported MIs identified by the ECL. Any discrepancy in adjudication was referred to one of the two Chairmen of the EVC for final adjudication. The Chairmen also reviewed all investigator-reported MIs validated or not by the ECL. The Chairmen of the ECL and of the EVC met regularly to reconcile, by consensus decision, any remaining queries in the adjudication process. The sponsors, members of the Steering Committee, of the EVC and of the ECL, were blinded to the treatment assignment until the database was cleaned after completion of the study.

Study performance

After discharge from hospital, the subjects entered a 6-month follow-up phase, during which they kept a diary documenting further clinical events. The patients were requested to return to the hospital 36–46 days after randomization for a follow-up visit and recording of a 12-lead electrocardiograph. They were contacted 6 months after randomization to establish their life status and whether they had suffered a MI or been rehospitalized. The discharge form and a summary of rehospitalization were obtained.

Table 1

Scores used to compare the extent of infarction between the various entry diagnoses

Score	UA/NSTEMI	High-risk PCI	High-risk CABG*
0	≤ULN	≤ULN	≤60 U/l
1	>ULN, ≤2×ULN	>ULN, ≤3×ULN	>60 U/l to ≤100 U/l
2	>2*ULN, ≤5×ULN	>3×ULN, ≤5×ULN	>100 U/l to ≤200 U/l
3	>5*ULN, ≤10×ULN	>5×ULN, ≤10×ULN	>200 U/l to ≤300 U/l
4	>10×ULN	>10×ULN	>300 U/l

Patients who died from an acute MI were given a score of 4. *For CABG-related MIs, the peak value during the first 24 h after surgery was also used, as was the mean of a ratio between the peak values during the first 24 h to the laboratory upper limit of normal. ULN, upper limit of normal of local laboratories for the CK-MB values.

Statistics

Sample size

The sample size of 2250 patients per group in the GUARDIAN trial was calculated on the basis of an estimated 15% primary end-point event rate for the placebo group (10–15% in UA/NSTEMI and PCI, and 20–25% in CABG patients), an expected 25% relative risk reduction in the active treatment group, and a target significance level of 0.017 (0.05/3, accounting for the three pairwise comparisons) detected with 90% power. Assuming that the higher two doses of cariporide would be effective and that the lowest dose would be similar to placebo, approximately 1200 patients with primary end-point events were to be expected with a total sample size of 9000 patients. The total information on 1200 patients with a primary outcome event was required to determine the actual number of patients recruited. Upon a planned blinded event rate re-estimate, the overall sample size was adjusted to 11 500 patients because of a lower than expected event rate.

Statistical analyses

For the primary composite end-point of all-cause mortality and MI at day 36 after randomization, a nonparametric covariance adjustment analysis method [17] was used to calculate the *P* value and the 98% confidence limits. This analysis requires only the randomization assumption, maintains the same interpretation for the estimators of the event rate differences across different specifications of covariates, and reduces variance of these estimators compared with an unadjusted analysis. The variables predefined before unblinding as covariates for this analysis were as follows: age, sex, MI at entry, ST-segment depression, congestive heart failure, diabetes, previous MI, and cerebrovascular or peripheral vascular disease. The entry diagnosis groups were treated in the analysis as strata. In support of this primary efficacy analysis, the square root of the unadjusted χ^2 statistic was calculated and the times to the first primary end-point event data were displayed using Kaplan–Meier survival curves.

The secondary incidence data were analyzed by the same covariance adjustment method as for the primary end-point, and the extent of infarction, as assessed using peak CK-MB scores, was evaluated by the extended Mantel–Haenszel statistic with standardization within strata ranks. The test statistic applied the proportional odds model, with diagnosis group and treatment as independent variables.

In addition to the predefined analyses on the primary and secondary efficacy end-points, it was planned to perform a number of exploratory analyses in accordance with the phase 2 design of the trial, in order to understand better the modalities for potential benefit of the drug and of the new pharmacologic approach. Such analyses considered were an efficacy analysis, including only treated patients, all patients who underwent PCI or CABG while on treatment, patients with an MI or not at the time of randomization, and patients with some degree of renal failure.

Two interim analyses by the DSMB were planned after 400 and 800 subjects with primary end-point events had been identified. If the DSMB contemplated giving a recommendation concerning the future conduct of the trial, these results were also to be reviewed blindly by the Chairman of the Steering Committee and representatives of the sponsor. The statistical decision guidelines to be used for early stopping of the trial were based on the proposals of Hwang *et al* [18] and Chang *et al* [19]. Early stopping for a specific dose group could occur due to overwhelming efficacy or as a result of the lack of efficacy.

Adverse events

All adverse events were coded according to the Hoechst Adverse Reactions Terminology System, and were classified by the main body system involved. Fisher's exact tests were used for comparison of active treatment and placebo groups. Predefined events of special interest were studied separately. These were arrhythmia, congestive heart failure and shock, myocardial ischemia, altered mental status, acute renal failure, allergic reactions, liver toxicity, hypotension, stroke, and transient ischemic attack. Because of a concern raised in phase 1 studies of a possible effect of cariporide on blood pressure, the first 400 patients underwent serial blood pressure measurements during the treatment phase.

Discussion

Most agents that protect myocardial cells against ischemic injury act indirectly by favorably influencing the balance between oxygen demand and supply, the latter being particularly effective in modifying the prognosis of the disease [2]. Agents that could directly protect the ischemic cell against necrosis would have an important role in treatment, and could magnify the benefit of various other treatment

forms. A number of interventions that target different pathophysiologic mechanisms involved in cell necrosis are currently under evaluation for this purpose. The GUARDIAN trial was designed to investigate one of these promising interventions, the inhibition of the NHE by cariporide.

The NHE is believed to play an important role in contributing to cell necrosis by promoting sodium and calcium entry within the cell in exchange for the accumulating protons [3–5,9]. Giving the novelty of the approach and of the pharmacologic agent evaluated, the trial design required many assumptions. This was primarily reflected in its phase 2/phase 3 design.

Study design and end-points

A major challenge of the GUARDIAN trial was its phase 2/phase 3 design. The phase 2 components included selection of various doses in an attempt to define a dose–response relationship for safety and efficacy, and of patients with heterogeneous entry diagnoses, assuming an important role of the NHE across various clinical situations involving ischemic tissue that is at risk of necrosis. Overall, the study had four treatment arms and three different entry diagnoses for a total of 12 groups.

The phase 3 component included the clinical end-points of death or MI in order to document drug efficacy. Selection of these convincing end-points was motivated by the absence of a direct marker of myocardial effects of the drug and of surrogate markers of efficacy, such as hemodynamic or antithrombotic parameters. Although the experimental data have measured efficacy in terms of infarct size reduction rather than infarct prevention, the latter was selected as the primary end-point considering the wide variability in infarct size in humans in the absence of reliable markers of area at risk and area of necrosis. Other valuable clinical markers of efficacy could be improved global and regional left ventricular function in states of ischemia and reperfusion and of reversible left ventricular dysfunction, such as stunning and hibernation that can be favorably influenced by treatment [9]. No effects on recurrent ischemia were expected. The relatively infrequent occurrence of MI in unstable angina and the wide spectrum in severity when it occurs mandates a large sample size to detect drug effect. The trial was powered to detect a 25% reduction in risk, and to permit a reliable evaluation of drug safety and of the modalities for a potential benefit in a large spectrum of patients with coronary disease and an immediate risk of MI and death. Infarct size, which was assessed as a secondary end-point, was measured by the distribution of outcome MIs as non-Q-wave versus Q-wave MI and by the peak elevation in CK-MB values.

Entry diagnoses

A strong incentive to the choice of the primary end-point of prevention of death or MI was the experimental findings that NHE inhibition yielded maximal benefit when applied before coronary occlusion or early during ischemia rather than before reperfusion [8,10,11]. Accordingly, target populations of patients who were at risk for myocardial cell necrosis either as a result of the disease process or of an intervention procedure were selected. Unstable angina and non-ST-segment MI are situations of repeated periods of ischemia. These alternate with periods of reperfusion related to distal microembolization and progression of the occlusion at the plaque level, yielding microinfarcts and larger infarcts respectively, and infarct extension. In PCI and CABG, the drug was administered immediately before the period of risk. An algorithm was developed to differentiate an outcome MI occurring after randomization from evolving MIs at the time of randomization. Although the various categories of patients had a common background of unstable symptoms (within 12 h for unstable angina and non-ST-elevation MI, and within 30 days for patients undergoing an intervention), they were nevertheless quite heterogeneous. The assumption was made that once ischemia is present, the mechanisms that lead to cell death are similar independently of the immediate trigger to ischemia, with major participation of the NHE.

Risk features were selected for each of the entry categories in order to ensure an event rate greater than 10% in each. In UA/NSTEMI, the severity of the presenting chest pain, ischemic ST–T changes, post-MI angina, and elevated serum levels of CK-MB and of troponin T or I are markers of risk [1]. The criteria defined by the American College of Cardiologists/American Heart Association [13] were used to define high-risk lesions for PCI; the criteria used for high-risk CABG are generally well accepted. Troponin T and I were not used as markers of an end-point MI, because very few hospitals were using the test at the time of the study.

As a consequence of patient selection and study design, the results of the GUARDIAN trial may not apply to many other clinical situations for which cell protection is warranted. Such situations are evolving ischemia and MI, with or without reperfusion, reversible ischemic left ventricular dysfunction, ischemia and reperfusion arrhythmias, and left ventricular remodeling. Trials with different study designs will be required to answer these very important clinical issues.

Dose selection

In the absence of adequate surrogate end-points of efficacy in the indications studied in the present trial, the dose selection was based on preclinical data and phase 1 studies in humans. Thus, the low and high ranges of the presumably effective doses were extrapolated from *in vivo* and *in vitro* experimental data that had shown dose-related inhibition of the NHE system and a correlation between doses, blood concentration, and infarct size reduction [8]. In the rabbit model of 30-min ischemia, infarct size was reduced by 50% at IC_{50} (57 ng/ml) and by

Table 2

Trial performance

	Total	UA/NSTEMI	PCI	CABG
Enrolment				
Projected	9 000	40–50%	20–35%	10–20%
Actual*	11 590	45%	30%	25%
Event rate				
Projected	13.12%	10–15%	10–15%	20–25%
Actual	12.95%	12.72%	10.91%	15.78%

*The total number of patients recruited was readjusted followed a blinded interim analysis that showed an event rate slightly lower than expected.

80% at three to four times the IC_{50} concentration (200 ng/ml) [10]. Similar IC_{50} values were found in *ex vivo* human platelet studies (45–72 ng/ml), with concentrations of 200 ng/ml producing 80% inhibition (internal data; Aventis); the 80 and 120 mg doses in these studies inhibited NHE exchange by 90–100% shortly after administration. With an 8-h (three times a day) dosing interval, trough concentrations of 129 ng/ml were achieved with the 80-mg dose, and of 278 ng/ml with the 120-mg dose. Accordingly, trough NHE inhibition levels of 74 and 85%, respectively, were expected with the two high doses. The 20-mg dosage arm was introduced to cover the full range of the expected dose/response, because clinical effects of lower dosages could not be excluded. It was expected that this low-dose arm would be dropped during the course of the trial at one interim analysis. Such an action was not undertaken because it was not deemed appropriate by the DSMB.

Doses higher than 120 mg were not studied. This is because phase 1 studies had suggested that the maximally tolerated dose in humans was 140 mg, higher doses administered within 30 min being associated with frequent side effects, such as nausea, flushing, taste disturbance, paresthesia, vertigo, and diaphoresis, occurring shortly after drug administration (internal data, Aventis), and an increase in blood pressure. In order to avoid high peak drug concentrations and optimize tolerability, the drug was administered over a period of 1 h in the GUARDIAN trial. The drug regimens used resulted in no excess significant side effects compared with placebo and no changes in blood pressure [20]. A report on the preliminary results of the trial showed no effects of the two lower doses, suggesting undertitration of the drug was used and, also, a need for a near complete inhibition of the exchanger for clinical benefit [20].

Duration of treatment

The intent of the protocol was to administer the study drug for the duration of the period of high risk without unduly prolonging the administration of an experimental drug and the duration of hospitalization stay. In UA/NSTEMI, the period of higher risk extends only for a couple of days in the absence of recurrent ischemia, and the risk decreases after a 24-h pain-free period. A new risk is introduced when an

Table 3

Concomitant cardiovascular illnesses and risk factors in the overall population

			Cariporide		
	All (*n*=11 587)	Placebo (*n*=2909)	20 mg (*n*=2908)	80 mg (*n*=2887)	120 mg (*n*=2883)
Median age (years)	66.0	65	66	66	66
Sex (% male)	69.9	70.0	70.2	69.3	69.9
Smoking status (%)					
Current	22.8	23.7	22.1	23.0	22.5
Ex smoker	44.7	44.6	45.1	44.4	44.6
MI at study entry (%)	10.4	10.0	11.1	10.4	10.1
History of (%)					
Stable angina	24.1	23.8	23.9	23.6	25.0
Unstable angina	58.8	58.0	59.9	59.4	58.0
MI	44.5	44.0	45.4	44.6	43.9
Congestive heart failure	7.7	7.1	8.3	7.4	7.9
Hypertension	53.1	51.8	53.7	53.3	53.5
Cerebrovascular disease	6.1	5.4	6.0	6.2	6.7
Peripheral vascular disease	13.5	12.9	13.9	13.9	13.1
Cardiac arrhythmia	12.2	11.7	13.0	11.5	12.4
Hypercholesterolemia	55.0	54.3	55.1	55.9	54.8
NIDDM	19.7	19.2	20.2	20.5	18.9
IDDM	7.1	6.1	7.2	7.1	8.0

IDDM, insulin-dependent diabetes mellitus; NIDDM, noninsulin-dependent diabetes mellitus.

intervention is performed. With PCI and CABG the high-risk period extends for 12–24 h after the procedure. In order to accommodate these different situations, the duration of treatment was left open, relying on the medical judgment of the investigator and the treating physician to define the at-risk period for individual patients. It was recommended to administer the drug for the duration of the stay in the intensive or coronary care unit and for at least 2 days (six doses). The drug could be discontinued earlier after an uneventful percutaneous intervention.

Status of the trial

A total of 11 950 patients have been recruited into the trial from May 15 1997 to August 15 1998. This rapid pace in recruitment was not matched by the performance in study monitoring and end-point validation. Table 2 summarizes the final versus expected performance with regard to recruitment, entry diagnosis, and event rates. Table 3 describes the baseline characteristics of the populations enroled in the four study groups.

Conclusion

The GUARDIAN trial had many challenging features. It originally tested a novel pharmacologic agent (cariporide) and the hypothesis that inhibition of the NHE that is believed to play a fundamental in producing cell necrosis during ischemia (NHE) would prevent MI. The trial was designed as a phase 2/phase 3 trial, evaluating the dose/response relationship for safety and efficacy. It was also designed to identify evidence of benefit across a wide spectrum of at-risk patients, presumably because of an important and common role of the NHE. To achieve these aims, the study employed convincing end-points and prevention of MI rather than reduction in infarct size, a sample size driven by the event rate, a tight validation process for these end-points, and a nonparametric covariance adjustment analysis method.

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