ORIGINAL ARTICLE

Effect of Cyclosporine on Reperfusion Injury in Acute Myocardial Infarction

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ABSTRACT

BACKGROUND

Experimental evidence suggests that cyclosporine, which inhibits the opening of mitochondrial permeability-transition pores, attenuates lethal myocardial injury that occurs at the time of reperfusion. In this pilot trial, we sought to determine whether the administration of cyclosporine at the time of percutaneous coronary intervention (PCI) would limit the size of the infarct during acute myocardial infarction.

METHODS

We randomly assigned 58 patients who presented with acute ST-elevation myocardial infarction to receive either an intravenous bolus of 2.5 mg of cyclosporine per kilogram of body weight (cyclosporine group) or normal saline (control group) immediately before undergoing PCI. Infarct size was assessed in all patients by measuring the release of creatine kinase and troponin I and in a subgroup of 27 patients by performing magnetic resonance imaging (MRI) on day 5 after infarction.

RESULTS

The cyclosporine and control groups were similar with respect to ischemia time, the size of the area at risk, and the ejection fraction before PCI. The release of creatine kinase was significantly reduced in the cyclosporine group as compared with the control group (P=0.04). The release of troponin I was not significantly reduced (P=0.15). On day 5, the absolute mass of the area of hyperenhancement (i.e., infarcted tissue) on MRI was significantly reduced in the cyclosporine group as compared with the control group, with a median of 37 g (interquartile range, 21 to 51) versus 46 g (interquartile range, 20 to 65; P=0.04). No adverse effects of cyclosporine administration were detected.

CONCLUSIONS

In our small, pilot trial, administration of cyclosporine at the time of reperfusion was associated with a smaller infarct by some measures than that seen with placebo. These data are preliminary and require confirmation in a larger clinical trial.

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Myocardial infarction is a disabling disease that is common in the United States, with more than 1.5 million new cases diagnosed each year.[1,2] Infarct size is a major determinant of mortality in myocardial infarction.[3,4] Limitation of infarct size has therefore been an important objective of strategies to improve outcomes. Currently, the most effective way to limit infarct size is to reperfuse the jeopardized myocardium as soon as possible with the use of coronary angioplasty or thrombolysis and to prevent reocclusion of the coronary artery with the use of antiplatelet therapy.

Although reperfusion is undoubtedly beneficial, it has detrimental effects, including myocardial stunning, ventricular arrhythmias, and microvascular dysfunction.[5-7] Accumulating evidence suggests that reperfusion may also cause irreversible myocardial injury, possibly through a form of mitochondrial dysfunction that has been designated permeability transition.[8-12] The opening of a nonspecific high-conductance channel (called the mitochondrial permeability-transition pore) in the inner mitochondrial membrane results in the collapse of the membrane potential, the uncoupling of the respiratory chain, the efflux of cytochrome *c* and other proapoptotic factors, and the hydrolysis rather than synthesis of ATP; these metabolic alterations may lead to cardiomyocyte death.[13,14] Calcium overload and excessive production of reactive oxygen species in the early minutes of reflow trigger the opening of the mitochondrial permeability-transition pore.[10,12,14] Griffiths and Halestrap found that in the isolated rat heart, the permeability-transition pore remains closed during ischemia but opens at the time of reperfusion.[15]

In addition to its well-known immunosuppressive properties, cyclosporine is a potent inhibitor of mitochondrial permeability transition, and several reports indicate that it can limit ischemia–reperfusion injury under experimental conditions.[8,15-21] The objective of the present study was to determine whether the administration of cyclosporine at the onset of reperfusion reduces the infarct size in patients with ongoing acute myocardial infarction.

Methods

Trial

This study was a prospective, multicenter, randomized, single-blind, controlled trial. The trial was designed, the data were collected and analyzed, and the manuscript was written solely by the authors. Cyclosporine for the trial was purchased with institutional grant support; the manufacturer had no role in the study. The trial was performed in accordance with the Declaration of Helsinki (revised version, 1996), the European Guidelines for Good Clinical Practice (version 11, July 1990), and French laws. In accordance with French law, the study protocol was approved by the ethics committee of the institution of the principal investigator (Dr. Ovize) acting on behalf of all the institutions involved in this trial. All subjects gave written informed consent before being included in the study.

Study Population

Men and women, 18 years of age or older, who presented within 12 hours after the onset of chest pain, who had ST-segment elevation of more than 0.1 mV in two contiguous leads, and for whom the clinical decision was made to treat with percutaneous coronary intervention (PCI) were eligible for enrollment. Patients were eligible for the study whether they were undergoing primary PCI or rescue PCI. Occlusion of the culprit coronary artery (Thrombolysis in Myocardial Infarction [TIMI] flow grade 0) at the time of admission was also a criterion for inclusion.[22]

Patients with cardiac arrest, ventricular fibrillation, cardiogenic shock, stent thrombosis, previous acute myocardial infarction, or angina within 48 hours before infarction were not included in the study. Patients with occlusion of the left main or left circumflex coronary artery or with evidence of coronary collaterals to the region at risk on initial coronary angiography (at the time of admission) were excluded.[23] Patients with known hypersensitivity to cyclosporine, known renal failure or a serum creatinine level of 120 μmol per liter (1.36 mg per deciliter) or more at admission, liver failure, or uncontrolled hypertension and women who were pregnant or who were of childbearing age and were not using contraception were not included. Finally, patients who had any disorder that is associated with immunologic dysfunction (e.g., cancer, lymphoma, a positive serologic test for the human immunodeficiency virus, or hepatitis) more recently than 6 months before presentation were excluded.

Angiography and PCI

Left ventricular and coronary angiography was performed with the use of standard techniques, just

before revascularization. The size of the area at risk, a major determinant of infarct size,[24] was estimated for each patient by measuring the circumferential extent of abnormally contracting segments, according to the method of Feild et al.[25] Revascularization was performed with the use of direct stenting.[26]

EXPERIMENTAL PROTOCOL

After coronary angiography was performed but before the stent was implanted, patients who met the enrollment criteria were randomly assigned to either the control group or the cyclosporine group. Randomization was performed with the use of a computer-generated randomization sequence. Numbered, sealed envelopes that contained the study group assignment were distributed to each catheterization laboratory and were opened after informed consent had been obtained.

Less than 10 minutes before direct stenting, the patients in the cyclosporine group received an intravenous bolus injection of 2.5 mg of cyclosporine (Sandimmune, Novartis) per kilogram of body weight. Cyclosporine was dissolved in normal saline (final concentration, 25 mg per milliliter) and was injected through a catheter that was positioned within an antecubital vein. The patients in the control group received an equivalent volume of normal saline. The dose of cyclosporine was chosen arbitrarily, based on experimental data of Argaud et al.,[17,27] as well as on our experience in the treatment of heart-transplant recipients, for whom this dose would be a typical loading dose.

INFARCT SIZE

The primary end point was the size of the infarct as assessed by measurements of cardiac biomarkers. Blood samples were obtained at admission and repeatedly over the next 3 days. The area under the curve (AUC) (expressed in arbitrary units) for creatine kinase and troponin I release (Beckman kit) was measured in each patient by computerized planimetry (Image J1.32j).[26,28,29]

The principal secondary end point was the size of the infarct as measured by the area of delayed hyperenhancement that was seen on cardiac magnetic resonance imaging (MRI), assessed on day 5 after infarction.[30-33] Because MRI facilities were available in only one of the three study centers, this estimation of infarct size could be performed in only a subgroup of patients. Imaging was performed on a 1.5-T whole-body MRI scanner (Magnetom Avanto, Siemens). For the late-enhancement analysis, 0.2 mmol of gadolinium–tetrazacyclododecanetetraacetic acid (DOTA) per kilogram was injected at a rate of 4 ml per second and was flushed with 15 ml of saline. Delayed hyperenhancement was evaluated 10 minutes after the injection of gadolinium–DOTA with the use of a three-dimensional inversion-recovery gradient–echo sequence. The images were analyzed in short-axis slices covering the entire left ventricle. Myocardial infarction was identified by delayed hyperenhancement within the myocardium, defined quantitatively by an intensity of the myocardial postcontrast signal that was more than 2 SD above that in a reference region of remote, noninfarcted myocardium within the same slice. For all slices, the absolute mass of the infarcted area was calculated according to the following formula: infarct mass (in grams of tissue) = $\sum$ (hyperenhanced area [in square centimeters]) × slice thickness (in centimeters) × myocardial specific density (1.05 g per cubic centimeter).

OTHER END POINTS

The whole-blood concentration of cyclosporine was measured at 1 and 20 minutes and at 3 and 12 hours after injection with the use of a radioimmunoassay kit (DiaSorin). Blood pressure and serum concentrations of creatinine and potassium were measured on admission and 24, 48, and 72 hours after PCI. Serum concentrations of bilirubin, γ-glutamyltransferase, and alkaline phosphatase, as well as white-cell counts, were measured on admission and 24 hours after PCI.

We recorded the cumulative incidence of major adverse events that occurred within the first 48 hours after reperfusion, including death, heart failure, acute myocardial infarction, stroke, recurrent ischemia, the need for repeat revascularization, renal or hepatic insufficiency, vascular complications, and bleeding. We also specifically assessed infarct-related adverse events, including heart failure and ventricular fibrillation. In addition, 3 months after acute myocardial infarction, cardiac events were recorded, and global left ventricular function was assessed by echocardiography (Vivid 7 systems; GE Vingmed).

STATISTICAL ANALYSIS

To calculate the target sample size for the present trial, we used the available database of the study of myocardial postconditioning by Staat et al.[26]

Table 1. Baseline Characteristics of the Study Population.*

Characteristic	Cyclosporine Group (N = 30)	Control Group (N = 28)	P Value
Age (yr)	58±2	57±2	0.54
Sex (M/F)	25/5	21/7	0.32
Body-mass index†	26±1	27±1	0.62
Hypertension (no.)	15	13	0.50
Smoking (no.)	17	16	0.59
Dyslipidemia (no.)	14	12	0.49
Diabetes (no.)	4	4	0.60
History of coronary artery disease (no.)	4	4	0.60
Angiographic findings			
Infarct-related artery (no.)			0.48
Left anterior descending coronary artery	13	11	
Right coronary artery	14	14	
Left circumflex coronary artery	3	3	
Left ventricular ejection fraction (%)	50±2	49±3	0.88
Abnormally contracting segments (%)	37±2	35±3	0.44
Ischemia time (min)	292±37	302±28	0.70
Percutaneous coronary intervention			
Stenting of culprit lesion (no.)	30	28	1.00
Postprocedure TIMI flow grade	2.7±0.2	2.7±0.1	0.87
TIMI flow grade <2 (no.)	3	1	0.61
Treatment before PCI (no.)			
Intravenous nitrates	18	17	0.58
Morphine	13	13	0.78
Thrombolytic agents (failed)	5	8	0.22
Treatment at time of PCI (no.)			
Heparin	30	28	0.78
Aspirin or clopidogrel	29	24	0.30
Glycoprotein IIb/IIIa inhibitor	11	10	0.99

* Plus–minus values are means ±SD. PCI denotes percutaneous coronary intervention, and TIMI Thrombolysis in Myocardial Infarction.
† The body-mass index is the weight in kilograms divided by the square of the height in meters.

We hypothesized that cyclosporine would reduce the AUC for creatine kinase release by 30%. For a statistical power of 80% and a probability of a type I error of 0.05 using a two-sided test, we calculated that the sample size should be 62 subjects (31 per group).

All analyses were performed by independent experts who were unaware of the treatment-group assignments. Between-group comparisons of AUCs for serum creatine kinase or troponin I release, the time of ischemia, the area at risk, and the infarct size as assessed by MRI were performed with the use of the Wilcoxon rank-sum test. We performed an analysis of covariance to test for equality of the slopes of the regression of infarct size on the area at risk in the cyclosporine and control groups. A comparison of the incidence of cumulative adverse clinical events between the groups was performed by means of Fisher's exact test. All values are expressed as medians and interquartile ranges. All reported P values are two-sided.

RESULTS

CHARACTERISTICS OF THE STUDY POPULATION

From July 2005 to October 2006, a total of 340 patients were hospitalized at the three study centers for management of acute myocardial infarction; 230 of these patients underwent PCI. Among these 230 patients, 24 were not evaluated for enrollment because study personnel were not available. Another 148 were evaluated and excluded for the following reasons: onset of chest pain more than 12 hours before presentation (20 patients), preadmission ventricular fibrillation (3), cardiac arrest before PCI (14), occlusion of the left main or circumflex coronary artery (27), stent thrombosis (16), previous myocardial infarction in the same territory (12), TIMI flow grade of more than 0 at admission (49), or evidence of coronary collaterals on initial angiography (7). Data are thus presented for 58 patients (28 in the control group and 30 in the cyclosporine group).

There was no significant difference between the two groups with respect to baseline characteristics (Table 1). The mean age of the trial participants was 58 years, and almost 80% were men. The two study groups were similar with respect to ischemia time, the size of the area at risk, and the ejection fraction before PCI. Thrombolytic therapy before PCI failed in 13 patients (8 in the control group and 5 in the cyclosporine group).

Stenting of the culprit lesion was performed in all patients (Table 1). No patient underwent PCI on arteries other than the infarct-related artery. In four patients, TIMI 2 flow was not achieved after PCI.

INFARCT SIZE

The AUC for serum creatine kinase release after reperfusion was significantly reduced in the cyclosporine group as compared with the control group, with a median of 138,053 arbitrary units (interquartile range, 114,008 to 283,461) in the cyclosporine group versus 247,930 (interquartile range, 145,639 to 404,349) in the control group ($P=0.04$ for the difference), which represents a reduction in infarct size of approximately 40% (Fig. 1A). The median AUC for troponin I release was 112,312 arbitrary units (interquartile range, 48,680 to 153,956) in the cyclosporine group and 129,320 arbitrary units (interquartile range, 65,019 to 224,116) in the control group. This difference was not significant ($P=0.15$) (Fig. 1B).

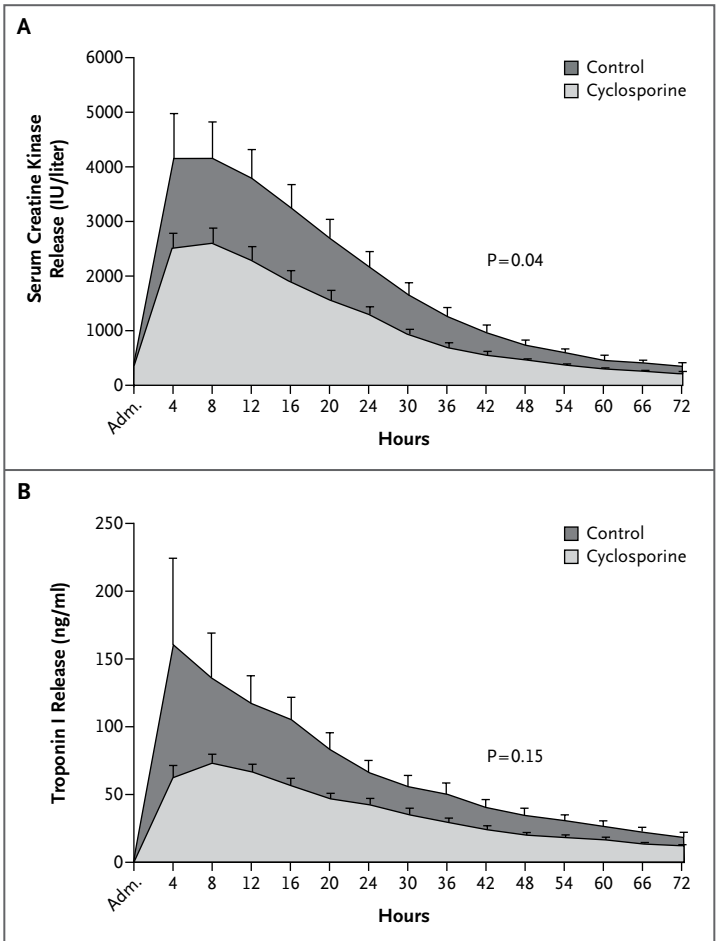

Figure 1. Assessment of Infarct Size by Biomarker Measurement.

Serum creatine kinase was measured every 4 hours on day 1 and every 6 hours on days 2 and 3 after coronary reperfusion. Curves for the control and cyclosporine groups are shown in Panel A. Cyclosporine administration (Adm.) resulted in a significant reduction in infarct size of approximately 40% as measured by creatine kinase release. Serum troponin I was measured every 4 hours on day 1 and every 6 hours on days 2 and 3 after coronary reperfusion. Curves for the control and cyclosporine groups are shown in Panel B. Cyclosporine administration did not result in a significant reduction in infarct size as measured by troponin I release. T bars denote standard errors

In the control group, there was a significant correlation between the AUC for serum creatine kinase release and the original area at risk (as defined by the circumferential extent of abnormally contracting segments on initial left ventricular angiography). As shown in Figure 2A, the regression line for the cyclosporine group had a smaller slope than the regression line for the control group, indicating that for any given size of area at risk, smaller infarcts developed in the cyclosporine-treated patients. This difference in the

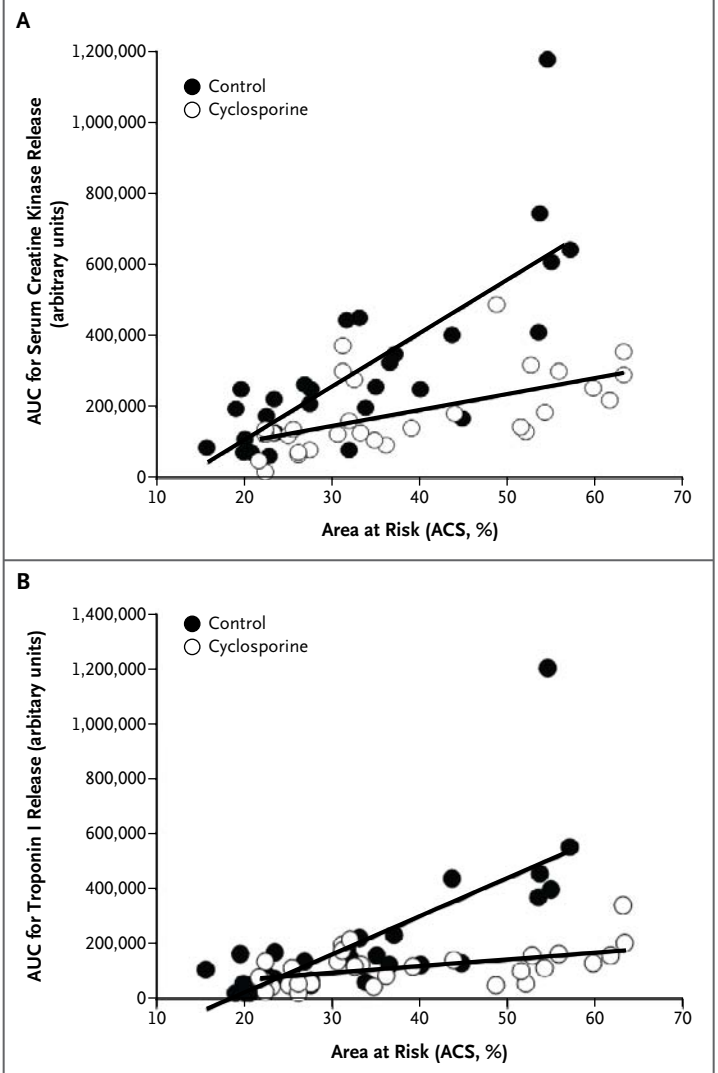

Figure 2. Infarct Size as a Function of the Area at Risk.

The area under the curve (AUC) for serum creatine kinase release was expressed as a function of the circumferential extent of abnormally contracting segments (ACS), an estimate of the area at risk, as shown in Panel A. There was a significant correlation between the two variables in the control group ($r^2=0.60$). Data points for the cyclosporine group ($r^2=0.34$) lie below the regression line for the control group. These data indicate that, for any given area at risk, cyclosporine administration was associated with a reduction in the resulting infarct size as measured by creatine kinase release. This difference was significant by analysis of covariance (P=0.006). There was also a significant correlation between the AUC for troponin I release and the area at risk in the control group ($r^2=0.54$), as shown in Panel B. Data points for the cyclosporine group ($r^2=0.26$) lie below the regression line for the control group. These data indicate that, for any given area at risk, cyclosporine administration was associated with a reduction in the resulting infarct size as measured by troponin I release. This difference was confirmed to be significant by analysis of covariance (P=0.002).

slope was significant by analysis of covariance (P=0.006). An analysis of the data for troponin I provided similar results, including a significant correlation between the AUC for troponin I release and the area at risk, with a smaller slope of the regression line for the cyclosporine group than for the control group (P=0.002 by analysis of covariance) (Fig. 2B).

In a subgroup of 27 patients, the absolute mass of the area of hyperenhancement on MRI was significantly reduced in the cyclosporine group as compared with the control group, with a median of 37 g (interquartile range, 21 to 51) versus 46 g (interquartile range, 20 to 65; P=0.04) (Fig. 3). This 20% reduction in the area of hyperenhancement on MRI corresponded to the 26% and 36% reductions in AUCs for creatine kinase and troponin I release, respectively, that were observed in this subgroup of patients (baseline characteristics of the subgroup can be found in the Supplementary Appendix, available with the full text of this article at www.nejm.org).

OTHER END POINTS

The whole-blood concentration of cyclosporine reached a peak level (mean ±SE, 6272±714 ng per milliliter) 1 minute after injection (Fig. 4). None of the treated patients had any clinical symptoms after the administration of cyclosporine. There were no significant changes in blood pressure; in serum concentrations of creatinine, potassium, bilirubin, γ-glutamyl-transpeptidase, or alkaline phosphatase; or in the white-cell count.

During the first 48 hours after reperfusion, seven adverse clinical events were recorded in the control group: one episode of ventricular fibrillation and six episodes of heart failure. There were three adverse clinical events in the cyclosporine group: one episode of ventricular fibrillation, one episode of heart failure, and one episode of recurrent ischemia (P=0.11). When only infarct-related events were considered (i.e., ventricular fibrillation and heart failure), seven events were observed in the control group versus two in the cyclosporine group (P=0.05).

Three months after infarction, three patients in the control group and one in the cyclosporine group required rehospitalization for heart failure (P=0.28). These four patients were among those who had had heart failure within the first 2 days

after acute myocardial infarction. There were no other adverse events during the interval from 48 hours to 3 months. At 3 months, the mean left ventricular ejection fraction as measured by echocardiography was 47±3% in the control group and 50±2% in the cyclosporine group (P=0.32).

DISCUSSION

In our small, proof-of-concept trial, the administration of cyclosporine in patients with acute myocardial infarction at the time of reperfusion was associated with a smaller infarct size, as assessed by some measures, than that seen with placebo. Infarct size was assessed both by measuring the release of the cardiac biomarkers creatine kinase and troponin I and by measuring the area of late hyperenhancement of the reperfused myocardium on MRI on day 5. The AUC for the creatine kinase concentration suggests that the administration of cyclosporine was associated with a reduction in infarct size of approximately 40%. This finding was confirmed by a significant reduction in the area of late hyperenhancement on MRI in the cyclosporine-treated patients. However, the AUC for the troponin I concentration did not differ significantly between the two groups.

We investigated these observations further by comparing the infarct size, as measured by the release of cardiac biomarkers, with the size of the area at risk, as determined by left ventricular angiography. The slope of this relationship was not as steep in the cyclosporine group as in the control group, regardless of whether measurements of creatine kinase or of troponin I were used to assess infarct size.

The fact that cyclosporine reduced the infarct size, as estimated by the release of creatine kinase, when administered at the time of reperfusion suggests that lethal reperfusion injury occurs in humans.[23,34,35] This observation supports the argument that reperfusion necrosis is a major component of infarct size after prolonged ischemia and reperfusion and raises the possibility that lethal reperfusion injury may be an important new pharmacologic target for the treatment of patients with ongoing acute myocardial infarction.[26,34]

The rationale for evaluating the ability of cyclosporine to reduce infarct size in patients with ongoing acute myocardial infarction was based on experimental evidence that indicated a crucial role of the opening of the mitochondrial permeability-transition pore in lethal reperfusion injury.[9,10,14,17-20] Under physiologic conditions, the inner mitochondrial membrane is impermeable to almost all metabolites and ions, and the permeability-transition pore is in a closed conformation.[9,10,12,36,37] At the time of reperfusion after prolonged ischemia, abrupt matrix accumulation of calcium and overproduction of reactive oxygen species trigger the opening of the pore.[14,15,38] The resulting collapse of the membrane potential, uncoupling of the respiratory chain, efflux of proapoptotic factors, and hydrolysis of ATP may ultimately cause irreversible damage.

Cyclosporine probably inhibits the mitochondrial permeability transition by preventing the calcium-induced interaction of cyclophilin D with a pore component.[14,39] Whether the reduction in infarct size that we observed by some measures in

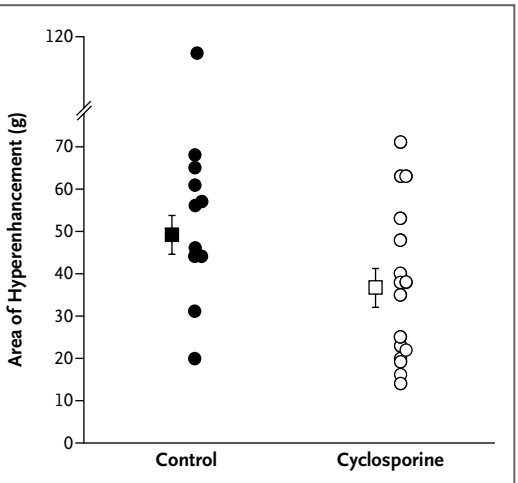

Figure 3. Assessment of Infarct Size by Magnetic Resonance Imaging (MRI).

The size of the area of late hyperenhancement on MRI is presented for 11 patients in the control group (black circles) and 16 patients in the cyclosporine group (white circles). The mean infarct size as assessed by MRI was significantly greater in the control group (black square) than in the cyclosporine group (white square). The size of the area of late hyperenhancement was calculated with the use of the following formula: infarct mass (in grams of tissue) = Σ (hyperenhanced area [in square centimeters]) × slice thickness (in centimeters) × myocardial specific density (1.05 g per cubic centimeter). P=0.04 for the comparison with the control group.

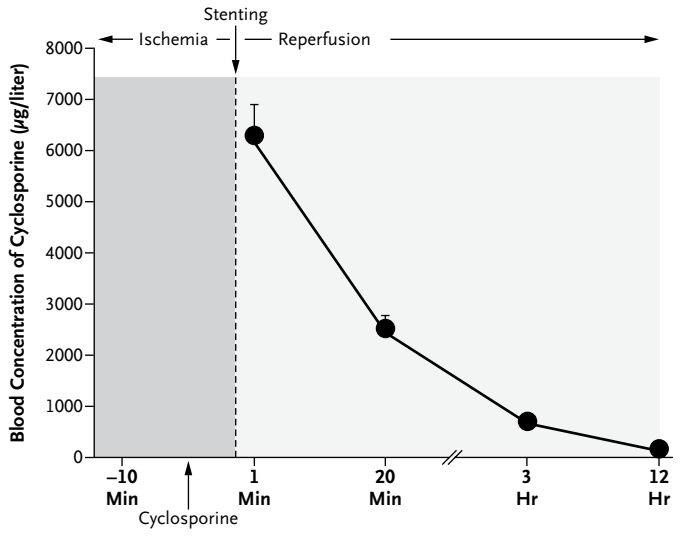

Figure 4. Blood Concentration of Cyclosporine during Reperfusion.
The whole-blood concentration of cyclosporine is shown at various times during early reperfusion. T bars denote standard errors.

the present study is related to this mechanism is uncertain, since cyclosporine is not specific for mitochondrial cyclophilin D but has other intracellular effects as well.[40-42] However, data from in vivo studies further support the hypothesis that specific inhibition of the opening of the permeability-transition pore may reduce infarct size. NIM811, a nonimmunosuppressive derivative of cyclosporine that also binds to the matrix cyclophilin D, significantly reduced infarct size when administered at the time of reperfusion in a rabbit model.[43] Moreover, mice that lack cyclophilin D have an enhanced capacity to retain mitochondrial calcium and a delayed opening of the transition pore when calcium overload is present, and they have smaller infarcts after prolonged ischemia and reperfusion.[44,45] The reduction in infarct size that was observed in the present study was similar to that seen with the use of ischemic postconditioning by means of angioplasty in patients with ongoing acute myocardial infarction, as described by Staat et al.[26] Postconditioning, in which an angioplasty balloon is inflated repeatedly in the infarct-related artery after reperfusion has been achieved, is also believed to reduce the extent of reperfusion injury by inhibiting the opening of the permeability-transition pore.[16,17]

Cyclosporine is widely used as an immunosuppressive agent for the prevention of acute allograft rejection. Long-term use of cyclosporine has several potentially detrimental effects, including renal and hepatic toxicity and increased susceptibility to infections and cancers. In the present study, cyclosporine was administered as a single intravenous bolus. Although we cannot exclude the possibility of delayed toxicity, there was no evidence of acute renal or hepatic injury, hypertension, or other short-term adverse effects.

In summary, we evaluated the effect of cyclosporine in a small pilot study of patients with acute myocardial infarction who were undergoing PCI. The administration of cyclosporine at the time of reperfusion was associated with a reduction in infarct size as measured by the release of creatine kinase and delayed hyperenhancement on MRI. Release of troponin I, however, was not significantly reduced by the administration of cyclosporine. These data are preliminary and require confirmation in a larger clinical trial.

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Dr. Ovize reports serving as a consultant to Novartis. No other potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Antman EM, Anbe DT, Armstrong PW, et al. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1999 Guidelines for the management of patients with acute myocardial infarction). J Am Coll Cardiol 2004;44(3):e1-e211.
2. Ergin A, Muntner P, Sherwin R, He J. Secular trends in cardiovascular disease mortality, incidence, and case fatality rates in adults in the United States. Am J Med 2004;117:219-27.
3. Gibbons RJ, Valeti US, Araoz PA, Jaffe AS. The quantification of infarct size. J Am Coll Cardiol 2004;44:1533-42.
4. Burns RJ, Gibbons RJ, Yi Q, et al. The relationships of left ventricular ejection fraction, end-systolic volume index and infarct size to six-month mortality after hospital discharge following acute myocardial infarction treated by thrombolysis. J Am Coll Cardiol 2002;39:30-6.
5. Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? J Clin Invest 1985;76:1713-9.
6. Kloner RA, Ganote CE, Jennings RB. The "no-reflow" phenomenon following temporary coronary occlusion in the dog. J Clin Invest 1974;54:1496-508.
7. Moens AL, Claeys MJ, Timmermans JP, Vrints CJ. Myocardial ischemia/reperfusion injury: a clinical view on a complex pathophysiological process. Int J Cardiol 2005;100:179-90.
8. Nazareth W, Yafei N, Crompton M. Inhibition of anoxia-induced injury in heart

myocytes by cyclosporin A. J Mol Cell Cardiol 1991;23:1351-4.
9. Duchen MR, McGuinness O, Brown LA, Crompton M. On the involvement of a cyclosporin A sensitive mitochondrial pore in myocardial reperfusion injury. Cardiovasc Res 1993;27:1790-4.
10. Crompton M, Costi A. A heart mitochondrial Ca2(+)-dependent pore of possible relevance to reperfusion-induced injury: evidence that ADP facilitates pore interconversion between the closed and open states. Biochem J 1990;266:33-9.
11. Di Lisa F, Canton M, Menabò R, Dodoni G, Bernardi P. Mitochondria and reperfusion injury: the role of permeability transition. Basic Res Cardiol 2003;98:235-41.
12. Hunter DR, Haworth RA. The Ca-induced membrane transition in mitochondria. Arch Biochem Biophys 1979;195:468-77.
13. Bernardi P, Petronilli V. The permeability transition pore as a mitochondrial calcium release channel: a critical appraisal. J Bioenerg Biomembr 1996;28:131-8.
14. Javadov S, Karmazyn M. Mitochondrial permeability transition pore opening as an endpoint to initiate cell death and as a putative target for cardioprotection. Cell Physiol Biochem 2007;20:1-22.
15. Griffiths EJ, Halestrap AP. Mitochondrial non-specific pores remain closed during cardiac ischaemia but open upon reperfusion. Biochem J 1995;307:93-8.
16. Hausenloy DJ, Maddock HL, Baxter GF, Yellon DM. Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? Cardiovasc Res 2002;55:534-43.
17. Argaud L, Gateau-Roesch O, Raisky O, Loufouat J, Robert D, Ovize M. Postconditioning inhibits mitochondrial permeability transition. Circulation 2005; 111:194-7.
18. Di Lisa F, Menabò R, Canton M, Barile M, Bernardi P. Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic NAD(+) and is a causative event in the death of myocytes in postischemic reperfusion of the heart. J Biol Chem 2001; 276:2571-5.
19. Qian T, Nieminen AL, Herman B, Lemasters JJ. Mitochondrial permeability transition in pH-dependent reperfusion injury to rat hepatocytes. Am J Physiol 1997;273:C1783-C1792.
20. Kim JS, Jin Y, Lemasters JJ. Reactive oxygen species, but not Ca2+ overloading, trigger pH- and mitochondrial permeability transition-dependent death of adult rat myocytes after ischemia-reperfusion. Am J Physiol Heart Circ Physiol 2006;290: H2024-H2034.
21. Oka N, Wang L, Mi W, Zhu W, Honjo O, Caldarone CA. Cyclosporine A prevents apoptosis-related mitochondrial dysfunction after neonatal cardiac arrest. J Thorac Cardiovasc Surg 2008;135:123-30.
22. Chesebro JH, Knatterud G, Roberts R, et al. Thrombolysis in Myocardial Infarction (TIMI) trial, phase 1: a comparison between intravenous tissue plasminogen activator and intravenous streptokinase. Circulation 1987;76:142-54.
23. Rentrop KP, Cohen M, Blanke H, Phillips RA. Changes in collateral channel filling immediately after controlled coronary artery occlusion by an angioplasty balloon on human subjects. J Am Coll Cardiol 1985;5:587-92.
24. Reimer KA, Jennings RB. The "wavefront phenomenon" of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. Lab Invest 1979; 40633-44.
25. Feild BJ, Russell RO Jr, Dowling JT, Rackley CE. Regional left ventricular performance in the year following myocardial infarction. Circulation 1972;46:679-89.
26. Staat P, Rioufol G, Piot C, et al. Postconditioning the human heart. Circulation 2005;112:2143-8.
27. Argaud L, Gateau-Roesch O, Chalabreysse L, et al. Preconditioning delays Ca^{2+}-induced mitochondrial permeability transition. Cardiovasc Res 2004;61:115-22.
28. Roberts R, Henry PD, Sobel BE. An improved basis for enzymatic estimation of infarct size. Circulation 1975;52:743-54.
29. Hackel DB, Reimer KA, Ideker RE, et al. Comparison of enzymatic and anatomic estimates of myocardial infarct size in man. Circulation 1984;70:824-35.
30. Mahrholdt H, Wagner A, Holly TA, et al. Reproducibility of chronic infarct size measurement by contrast-enhanced magnetic resonance imaging. Circulation 2002; 106:2322-7.
31. Kim RJ, Fieno DS, Parrish TB, et al. Relationship of MRI delayed contrast enhancement to irreversible injury, infarct age, and contractile function. Circulation 1999;100:1992-2002.
32. Rehwald WG, Fieno DS, Chen EL, Kim RJ, Judd RM. Myocardial magnetic resonance imaging contrast agent concentrations after reversible and irreversible ischemic injury. Circulation 2002;105:224-9.
33. Shan K, Constantine G, Sivananthan M, Flamm SD. Role of cardiac magnetic resonance imaging in the assessment of myocardial viability. Circulation 2004;109: 1328-34.
34. Zhao ZQ, Corvera JS, Halkos ME, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol 2003; 285:H579-H588. [Erratum, Am J Physiol Heart Circ Physiol 2004;286:H477.]
35. Kloner RA. Does reperfusion injury exist in humans? J Am Coll Cardiol 1993; 21:537-45.
36. Crompton M. The mitochondrial permeability transition pore and its role in cell death. Biochem J 1999;341:233-49.
37. Zoratti M, Szabò I. The mitochondrial permeability transition. Biochim Biophys Acta 1995;1241:139-76.
38. Piper HM, Garcia-Dorado D, Ovize M. A fresh look at reperfusion injury. Cardiovasc Res 1998;38:291-300.
39. Woodfield K, Rück A, Brdiczka D, Halestrap AP. Direct demonstration of a specific interaction between cyclophilin-D and the adenine nucleotide translocase confirms their role in the mitochondrial permeability transition. Biochem J 1998; 336:287-90.
40. Obasanjo-Blackshire K, Mesquita R, Jabr RI, et al. Calcineurin regulates NFAT-dependant iNOS expression and protection of cardiomyocytes: co-operation with Src tyrosine kinase. Cardiovasc Res 2006; 71:672-83.
41. De Windt LJ, Lim HW, Taigen T, et al. Calcineurin-mediated hypertrophy protects cardiomyocytes from apoptosis in vitro and in vivo: an apoptosis-independent model of dilated heart failure. Circ Res 2000;86:255-63.
42. Bueno OF, Lips DJ, Kaiser RA, et al. Calcineurin Aβ gene targeting predisposes the myocardium to acute ischemia-induced apoptosis and dysfunction. Circ Res 2004; 94:91-9.
43. Argaud L, Gateau-Roesch O, Muntean D, et al. Specific inhibition of the mitochondrial permeability transition prevents lethal reperfusion injury. J Mol Cell Cardiol 2005;38:367-74.
44. Nakagawa T, Shimizu S, Watanabe T, et al. Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. Nature 2005;434:652-7.
45. Baines CP, Kaiser RA, Purcell NH, et al. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. Nature 2005;434:658-62.