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(71) Applicant and
(72) Inventor: **OVIZE, Michel** [FR/FR]; 68 Chemin des Fonts, F-69110 Sainte-Foy-Les-Lyon (FR).

(74) Agents: **LITTAYE, THIBON, Annick** et al.; BP 19, F-78164 Marly-le-Roi Cedex (FR).

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(54) **Title:** USE OF CYCLOSPORIN A OR MELLE4-CYCLOSPORIN FOR THE TREATMENT OF ACUTE MYOCARDIAL INFARCTION

(57) **Abstract:** The invention relates to a composition for the treatment of acute myocardial infarction, comprising Cyclosporin A or Melle^{4}- cyclosporin as an active component, in a pharmaceutically suitable vehicle. A method for the treatment of acute myocardial infarction comprises administering to a patient such a composition of Cyclosporin A or Melle^{4}-cyclosporin, at least starting said administration before proceeding to a reperfusion process after a myocardial ischemia. A preferred composition contains from 5 mg to 6 g of active component in a vehicle suitable to be administered intra-venously, at a dose of from 0.1 to 50 mg per kg of body weight of a human patient.

USE OF CYCLOSPORIN A OR MELLE4-CYCLOSPORIN FOR THE TREATMENT OF ACUTE MYOCARDIAL INFARCTION

The invention relates to a composition and a method for the treatment of acute myocardial infarction, especially in humans.

BACKGROUND OF THE INVENTION

Acute myocardial infarction (AMI) is frequent (about 1,000,000 cases per year in the United States of America) and remains a leading cause of cardiac death in Western countries. Heart failure is an increasingly common outcome of myocardial infarction, and a frequent cause of cardiovascular morbidity and mortality, with approximately 400,000 new cases reported annually in the United States of America. Survival 5 years after the diagnosis of heart failure is poor, ranging as low as 25-35%.

Infarct size is a major determinant of post-infarction mortality: the largest the infarct, the worse the prognosis. Besides treatment of heart failure per se, limitation of infarct size appears the best-suited strategy to improve survival.

Acute myocardial infarctions are consecutive to ischemia, which are generally caused by insufficient blood circulation toward the cardiac tissues, leading to reduced oxygen supply to the myocardial tissue.

The treatment of acute myocardial infarction is primarily based on reperfusion of jeopardized myocardium, i.e. re-opening of the occluded coronary artery, either by thrombolysis or by percutaneous coronary intervention (PCI), in order to restore blood circulation in the myocardial tissue suffering from ischemia. Although this re-opening of the culprit coronary artery does salvage part of the jeopardized myocardium, recent evidence indicates that reperfusion per se also kills a significant amount of cardiac tissue. Thus, the final damage of cardiac tissue represents the addition of an irreversible damage occuring before the re-opening of the

occluded coronary artery (ischemic damage), to an irreversible damage occuring just at the time of reflow (reperfusion damage).

Despite extensive research, none of the currently available pharmacological agents used to treat AMI patients (e.g. aspirin, β-blockers, angiotensin converting enzyme inhibitors, statins...) can limit infarct size. The demonstrated beneficial effects of these treatments in AMI patients are related to other mechanisms.

Recent experimental studies indicate that brief episodes of ischemia and reperfusion, performed within the early minutes of reflow, i.e. just after the period of ischemia, can drastically reduce infarct size (by approximately 50%). This powerful protection has been termed " ischemic postconditioning " (Zhao and al., Am. J. Physiol. Heart Circ. Physiol., 2003; 285(2): H579-88 ; Kin and al., J. Cardiovasc. Res., 2004; 62(1): 74-85). A recent proof of concept study by the present inventor indicates that postconditioning protects the human heart in patients with ongoing AMI (Staat and al., Circulation, 2005; 112: 2143-2148). This was the first demonstration of the existence of reperfusion infarction in humans. It also demonstrated that a given intervention at the time of reflow can reduce infarct size in man. Unfortunately, ischemic postconditioning can only be performed in a limited number of AMI patients.

Therefore, there remains a need for a pharmacological treatment that would replace ischemic postconditioning and that would be available to all patients with AMI, as an adjunct treatment to reperfusion therapy by either thrombolysis or PCI.

It is an object of the invention to provide a pharmaceutical composition and a method for the treatment of acute myocardial infarction, which limit the infarct size when used as an adjunct therapy to a reperfusion process after a prolonged myocardial infarction.

The invention relates to a composition for the treatment of acute myocardial infarction, comprising Cyclosporin A or Melle^4–cyclosporin as an active principle, in a pharmaceutically suitable vehicle.

The composition of the invention is more particularly intended to be used in humans. The vehicle is then chosen so as to be suitable for such an application.

The invention also relates to the use of Cyclosporin A or $Melle^4$–cyclosporin for the preparation of a composition for the treatment of acute myocardial infarction, and to a method for such a treatment with such a composition, said method comprising at least starting said administration before proceeding to a reperfusion process after a prolonged myocardial ischemia.

Cyclosporin A (CsA) is a cyclic undecapeptide poly-N-methylated, of the structure (in its usual nomenclature) :

-MeBmt- αAbu-Sar-MeLeu-Val-MeLeu-Ala-(D)Ala-MeLeu-MeLeu-MeVal-
1 2 3 4 5 6 7 8 9 10 11

where :

Abu is L-α-aminobutyric acid; Ala is L-alanin; MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonin; MeLeu is N-methyl-L-leucin; MeVal is N-methyl-L-valin; Leu is L-leucin; Sar is sarcosin; Val is L-Valin.

CsA may be prepared by known methods, such as described for example by Kobel and al., European Journal of applied microbiology and biotechnology, 1982; 14: 237-240, or in US 4,108,985.

It is an immunosuppressive agent, that has been widely used in humans for many years to prevent acute graft rejection following transplantation.

In cardiology, Cyclosporin A is used on a daily basis to prevent acute cardiac graft rejection in patients that experienced heart transplantation: this currently represents its only therapeutic use. Its immunosuppressive action is due to the binding of CsA to the cytosolic cyclophilin A, that inhibits an enzyme named

calcineurin, which results in blocking the transcription of early activation genes (O'Keefe and al., Nature, 1992; 357: 692-694).

Melle^4-cyclosporin is a CsA derivative, of the formula:

-MeBmt- αAbu-Sar-Melle-Val-MeLeu-Ala-(D)Ala-MeLeu-MeLeu-MeVal-

1 2 3 4 5 6 7 8 9 10 11

where :

Abu is L-α-aminobutyric acid; Ala is L-alanin; MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonin; MeLeu is N-methyl-L-leucin; Melle is N-methyl-L-isoleucin; MeVal is N-methyl-L-valin; Leu is L-leucin; Sar is sarcosin; Val is L-Valin.

In Melle^4–cyclosporin, the molecule is modified, as compared to CsA, at the fourth amino acid (from N-methyl-leucin to N-methyl-isoleucin: Melle).

Melle^4–cyclosporin may be obtained by known methods, such as fermentation of the fungus *Tolypocladium niveum* (also designated *Beauveria nivea*), followed by extraction and purification. The compound obtained is > 98% pure as determined by analytical high-pressure liquid chromatography (Rosenwirth B and al., Antimicrobal agents and chemotherapy, 1994;38 (8):1763-1772).

The present inventor has discovered that, surprisingly, CsA and Melle^4–cyclosporin show potent anti-infarct properties, when administered at the time of reperfusion.

In particular, Cyclosporin A or Melle^4–cyclosporin, administered a few minutes before reperfusion, advantageously efficiently reduce infarct size of patients with ongoing myocardial infarction. They also show a beneficial effect on arrhythmias induced by ischemic insults.

Melle^4–cyclosporin is particularly prefered according to the invention, because it is devoid of the immunosuppressive effect, as

well as of the potential nephrotoxicity (deleterious for the kidney function) of CsA.

Indeed, in the Melle^4–cyclosporin molecule, the fourth residue, which is responsible for the interaction with calcineurin, is modified (from N-methyl-leucine to Melle), whereas the binding sites for the cyclophilins, covering residues 1-3, 10 and 11, are unchanged, resulting in prevention of calcineurin binding and retention of the ability to interact with cyclophilins (Hansson and al., Curr. Med. Chem., 2003;10(16):1485-506).

In consequence, Melle^4–cyclosporin does not show the immunosuppressive effects of CsA.

The composition of the invention may be presented in the form of a solution, a dispersion, or any other suitable pharmaceutical form.

A composition according to the invention contains from about 5 mg to about 6 g of Cyclosporin A, in a vehicle suitable to be administered intravenously at a dose of from 0.1 to 50 mg of Cyclosporin A per kg of body weight of a patient.

Another composition according to the invention contains from about 5 mg to about 6 g of Melle^4–cyclosporin in a vehicle suitable to be administered intravenously at a dose of from 0.1 to 50 mg of Melle^4–cyclosporin per kg of body weight of a patient.

In a use and a method according to the invention, the administration is carried out at a concentration of 0.1 to 50 mg of CsA or Melle^4–cyclosporin, per kg of body weight.

The administration may be carried out intravenously, orally or by intra-coronary injection.

In the case of intra-coronary injection, the administration is carried out at a concentration of 1 mg to 50 mg of CsA or Melle^4–cyclosporin per kg of body weight.

According to the invention, the administration starts at least at the time of reperfusion, after a prolonged ischemia.

The choice of this time-window, which is similar to that used in ischemic postconditioning, together with the potent anti-infarct properties of CsA and Melle^4–cyclosporin, allow for a very efficient therapeutic effect, which results in a significant limitation of the infarct size.

The significant infarct size reduction observed with CsA and Melle^4–cyclosporin may be explained by their ability to inhibit mitochondrial permeability transition during reperfusion. CsA and Melle^4–cyclosporin, by binding to the mitochondrial cyclophylin D, inhibit the opening of a mega-channel (called the " mitochondrial permeability transition pore ", or mPTP), located within the inner membrane of mitochondria. This pore, which is in a closed state in normal conditions, opens in conditions of severe stress, e.g. after prolonged ischemia and reperfusion. Evidence indicates that the transition pore opens at the time of reperfusion after a prolonged myocardial ischemia. Opening of the transition pore triggers various mechanisms that may kill the cell. It represents a " point of no return " to cell death. CsA or Melle^4–cyclosporin, administered at the concentration range of the invention, i. e. 0.1 to 50 mg per kg of body weight, by blocking the opening of the transition pore at the time of reflow, limit lethal myocardial reperfusion injury, and thus the infarct size.

According to the invention, administration of CsA or Melle^4–cyclosporin is carried out a few minutes before reperfusion. It may preferably be administered within the thirty minutes that precede reperfusion. Yet, according to conditions of first medical care, administration of CsA or Melle^4–cyclosporin may be performed earlier, i.e. within six hours prior to reperfusion.

The object of the invention is also achieved when administration is carried out continuously, from thirty minutes before reperfusion until 72 hours after reperfusion.

A composition of the invention may be administered together with at least one other active principle, such as anti-ischemic agents, anti-aggregants (e.g. aspirin), anti-thrombotic agents (e.g. heparin), angiotensin converting enzyme inhibitors, or statins.

The method according to the invention is especially suitable to be carried out in humans. Administration of CsA or Melle^4–cyclosporin is carried out before re-opening of the culprit occluded coronary artery responsible for the ischemia, either by thrombolysis or by percutaneous coronary intervention, according to standard methods.

The object of the invention is achieved particularly efficiently when the method for the treatment of acute myocardial infarction in humans comprises administering intravenously to a patient a composition of Melle^4–cyclosporin as an active principle in a suitable vehicle, at a concentration of 0.1 to 50 mg of Melle^4–cyclosporin per kg of body weight, starting the administration before proceeding to re-opening of the culprit occluded coronary artery after a prolonged myocardial ischemia.

The invention may be applied to all cases of acute myocardial infarction, in particular to AMI related to a thrombotic occlusion of a coronary artery that occurs upon a ruptured atherosclerotic plaque. The invention may also be applied to AMI related to any other cause of coronary occlusion, whatever the associated diseases and cardiovascular consequences.

The method of the invention may also be applied for the treatment of other diseases, in particular for the treatment of ischemic stroke, which shows an analogy to AMI both in terms of the disease and of the pathophysiology.

The invention will now be further specified in terms of its preferred features and its advantageous results, through the detailed description of specific embodiments which are the subject of the examples hereafter. Unless otherwise stipulated, all indications

will be expressed, firstly, in accordance with international normalization and, secondly, as amounts by mass.

Example I

Melle[4]–cyclosporin (50mg) was dissolved by stirring in a mixture of Cremophor EL (polyethoxylated castor oil) (available from SIGMA, catalog n° C5135) (0.65g) made to a volume of 1.0 ml in ethanol-94%. Melle[4]–cyclosporin may take several hours to dissolve.

Before infusion, it is diluted with 10 volumes of 0.9% saline.

Example II

CsA (50mg) was dissolved by stirring in a mixture of Cremophor EL (polyethoxylated castor oil) (available from SIGMA, catalog n° C5135) (0.65g) made to a volume of 1.0 ml in ethanol-94%.

Before infusion, it is diluted with 10 volumes of 0.9% saline.

Example III

Use of CsA and of Melle[4]–cyclosporin in animal models of acute myocardial infarction.

The general objective of this study was to determine whether CsA and Melle[4]–cyclosporin may reduce infarct size when administered at the time of reperfusion following a prolonged ischemia.

Surgical Preparation

Anesthetized New Zealand White rabbits, weighing 2.2 to 2.5 kg, underwent a tracheotomy and were ventilated with room air. A marginal branch of the left circumflex coronary artery was occluded for 30 minutes and reperfused for 4 hours.

Experimental design

Rabbits were randomly assigned to one of the following three groups. One minute before reperfusion, the rabbits received an intravenous bolus of either: vehicle (i.e. Cremophor EL) (control), CsA (10 mg/kg) as prepared in Example II, Melle^4–cyclosporin (10 mg/kg) as prepared in Exemple I. At the end of the 4 hour reperfusion period, the animals were euthanasized and hearts were excised for further assessment of area at risk and infarct size, using the triphenyltetrazolium chloride technique.

Results

Area at risk was comparable in all three groups, thus allowing comparison of infarct size.

In the control group, infarct size averaged 60 ± 6 % of the area at risk. Both CsA and Melle^4–cyclosporin were able to reduce infarct size that averaged 24 ± 4 % and 25 ± 3 % of the area at risk ($p<0.05$ versus control for both groups).

Example IV

Clinical trial - Use of CsA in patients with ongoing acute myocardial infarction.

The study was performed according to the declaration of Helsinki (revised version of Somerset West, Republic of South Africa, 1996) and according to the European Guidelines of Good Clinical practice (version 11 July 1990) and French Laws.

General objective

The aim of the study was to determine whether CsA, administered immediately before reperfusion, could decrease infarct size in patients with ongoing acute myocardial infarction that undergo with percutaneous transluminal coronary angioplasty (PTCA).

Study population

Male and female patients, aged more than 18 years, presenting within 6 hours of the onset of chest pain (consistent with ischemia lasting more than 30 minutes), who had ST segment elevation > 0.1 mV in two contiguous leads of the ECG, in whom the clinical decision was made to treat with percutaneous transluminal coronary angioplasty (PTCA), were eligible for enrollment.

Experimental design

Eligible patients had coronary angiography at hospital admission. Patients were randomly allocated to either a control group (placebo) or cyclosporin A (CsA) group. CsA (Sandimmun®, Novartis) (2.5 mg/kg) or placebo (saline) were administered intravenously, before the opening of the occluded coronary artery by the angioplasty balloon. In both groups, coronary angioplasty was performed according to the direct stenting technique, in order to reperfuse in one time the coronary artery.

Analysis

Patients with the following characteristics were excluded from the study: (1) evidence of coronary collaterals (Rentrop grade ≥ 1) to the risk region as assessed by coronary angiography, (2) pre-infarction angina within 48 hours, (3) failure to obtain a reperfusion 2-3 TIMI flow grade.

LV angiography

LV angiography (30° RAO) was performed just before coronary angioplasty. It was used to evaluate the size of the risk region, a major determinant of infarct size, according to validated techniques.

Study endpoint: serum Creatine Kinase release during the first 72 hours after PTCA

Blood samples were taken at admission, every 4 hours following opening of the coronary artery during day 1, and every 6

hours on days 2 and 3. Area under the curve (arbitrary units) of serum CK release (Beckman® Kit, expressed in IU/l) was measured in each patient by computerized planimetry (Image J® 1.29x) and used as a surrogate marker of infarct size.

Results

With 25 patients included, mean area at risk averages 32.1 ± 1.8 % and 29.3 ± 1.7 % in control and CsA groups, respectively (p = ns). This absence of difference for areas at risk allows a comparison for infarct size between the two groups. Mean area under the curve (i.e. infarct size) averages 278,422 ± 24,008 in controls. CsA-treated patients display a significantly reduced infarct size that averages 178,837 ± 27,151 (arbitrary units) ($p<0.05$ versus control group).

These results demonstrate that CsA, administered intravenously before the opening of the occluded coronary artery, efficiently reduces infarct size of patients with ongoing myocardial infarction, and improves patient's outcome.

Example V

Clinical trial - Use of cyclosporin A (CsA) in patients with ongoing acute myocardial infarction treated by coronary angioplasty.

The study was performed according to the declaration of Helsinki (revised version of Somerset West, Republic of South Africa, 1996) and according to the European Guidelines of Good Clinical practice (version 11 July 1990) and French Laws.

General objective

The aim of the study was to determine whether CsA, administered immediately before reperfusion, could decrease infarct size in patients with ongoing acute myocardial infarction that undergo with percutaneous transluminal coronary angioplasty.

Study population

Male and female patients, aged more than 18 years, presenting within 12 hours of the onset of chest pain, who had ST segment elevation > 0.1 mV in two contiguous leads, in whom the clinical decision was made to treat with coronary angioplasty, were eligible for enrollment. Patients with cardiac arrest, cardiogenic shock, previous AMI or pre-infarction angina within 48 hours were not included. The culprit coronary artery had to be occluded at the time of admission (TIMI 0 flow grade), and adequately reperfused (TIMI 2-3 flow grade) following angioplasty. Patients with evidence of coronary collaterals to the risk region or recurrent ischemia within 72 hours of reflow were excluded. Patients with failed pre-hospital thrombolysis were eligible for the study. Patients with known hypersensitivity to Cyclosporin A, immunosuppressive disease < 6 months (cancers, lymphomas, positive serology for HIV, hepatitis,...), known renal failure or serum creatinine > 120 µmole/l at admission, liver failure, uncontrolled hypertension, current pregnancy or woman without contraception, were not included.

LV angiography and coronary angioplasty

LV and coronary angiography were performed using a standard Seldinger technique. Estimation of the size of the area at risk, a major determinant of infarct size, was performed by LV angiography. Coronary angioplasty was performed according to the direct stenting technique.

Experimental protocol

This was a prospective, multi-center, randomized, single-blinded, controlled study. Patients were randomly allocated to either the control or the cyclosporin A group. In the cyclosporin A group, within 10 minutes before direct stenting (i.e. reperfusion), patients received an intravenous bolus injection of 2.5 mg/kg of cyclosporin A (Sandimmun®, Novartis). Sandimmun® was dissolved in saline (volume proportion: ½) and injected via a catheter positioned within an antecubital vein. In the control group, patients received an equivalent volume of saline.

Tolerance of cyclosporin A

Blood concentration of cyclosporin A was measured at 1 and 20 minutes, 3 and 12 hours after injection (RIA kit; Diasorin®). Blood pressure, serum concentrations of creatinine, potassium, bilirubine, γ-glutamyl-transpeptidase (γGT) and alcaline phosphatases were measured repeatedly after cyclosporin A administration.

Study endpoints

1. Primary endpoint: infarct size

a. Serum creatine kinase and troponin I release

Blood samples were taken at admission, every 4 hours following opening of the culprit coronary artery during day 1, and every 6 hours on days 2 and 3. Area under the curve (arbitrary units) of the release of creatine kinase release and troponin I (Beckman® Kit, expressed in IU/L) were measured in each patient by computerized planimetry (Image J® 1.29x).

b. Magnetic Resonance Imaging

At day 5 after infarction, MRI was performed to estimate infarct size. Myocardial infarction was identified by late hyper-enhancement within the myocardium. Infarct size was quantified by planimetry of the hyper-enhanced myocardium with the post-processing software Argus (Siemens, Erlangen, Germany). For all slices infarct absolute mass in grams was measured according to the following formula:

Infarct mass (g) = $\sum$ (hyperenhanced area (cm^2)) × slice thickness (cm) × myocardial specific density (1,05 g/cm^3).

2. Secondary endpoint: Adverse clinical events

The cumulative incidence of major adverse events that occurred within the first 48 hours of reperfusion, including: death, heart failure, acute myocardial infarction and stroke, was recorded.

Data analysis and statistics

Analysis of cardiac enzyme release curves, LV and coronary angiograms, and MRI data were performed by independent experts unaware of the treatment groups. Comparison between the area under the curve of serum creatine kinase or troponin I release, time of ischemia, area at risk, and MRI infarct size was performed using a student t test. For analysis of the difference between groups in the relationship between creatine kinase release and area at risk, an analysis of covariance, with a post-hoc Tukey's test (Statistica® software) was used. Comparison of the incidence of cumulative adverse clinical events between groups was performed by Chi-square test. All values are expressed as mean ± standard error (SEM).

Results

Study population

Fifty-three patients were included into this trial (27 control; 26 cyclosporin A). There was no significant difference between the two groups with respect to baseline characteristics, including age, sex distribution, body mass index (BMI), or incidence of hypertension (HBP), smokers, dyslipidemia or diabetes mellitus. Before re-opening of the culprit coronary artery by angioplasty, comparable medical treatment had been given to both groups of patients including heparin, aspirin and clopidogrel, anti-GPIIb/IIIa, thrombolytics. Distribution of the culprit coronary artery, LV ejection fraction (LVEF), estimate of the area at risk (ACS) and ischemia time were comparable between the two groups, as shown in table 1.

Table 1: Baseline characteristics

	Control (n=27)	**Cyclosporin A (n=26)**
Age (y)	57±2	59±2
Sex (M/F)	20/7	22/4
BMI (kg/m^2)	27±1	26±1
HBP (%)	12/27	14/26

Smokers (%)	16/27	15/26
Dyslipidemia (%)	12/27	14/26
Diabetes (%)	4/27	4/26
LV and coronary angiography		
Culprit artery (LAD,RCA/CX)	10/14/3	10/13/3
LVEF (%)	50 ± 3	50 ± 2
ACS (%)	35 ± 3	38 ± 2
Ischemia time (min)	286 ± 26	272 ± 20
Treatment at time of angioplasty (%)		
heparin	100	100
aspirin/clopidogrel	84	96
Anti-GPIIb/IIIa	36	36
morphine	48	44
thrombolytics (failed)	28	12

Endpoints

Cardiac enzyme release

The area under the curve of serum creatine kinase release during the first 72 hours of reperfusion was significantly reduced in the cyclosporin A group when compared to the control group, averaging 180936 ± 16134 (arbitrary units) in cyclosporin A, versus 325548 ± 48136 in control, which represents a 44 % reduction in infarct size ($p<0.01$).

Area under the curve of troponin I release averaged 126199 ± 11857 in cyclosporin A, versus 238580 ± 47932 IU/L in control, representing a 47% reduction in troponin I time-curve area ($p<0.05$).

MRI infarct size

In a subset of 27 patients, MRI area of hyper-enhancement was significantly reduced in the cyclosporin A versus control group, averaging 39 ± 5 and 53 ± 5 g, respectively ($p<0.05$). This 29% reduction in MRI area of hyper-enhancement corresponded to the

26% and 36% reduction in time-curve areas of creatine kinase and troponin I release observed in that unselected subset of patients.

Tolerance

There was a major peak of blood concentration of cyclosporin A one minute after the intravenous bolus injection (mean: 6272 ± 714 ng/ml), with a subsequent decline to a mean value of 165 ± 23 ng/ml at 12 hours post-injection. None of the treated patients developed any clinical or biological symptoms after administration of cyclosporin A. Blood pressure (SBP; DBP) was not modified by cyclosporin A and remained comparable between the two groups throughout the first three days of reperfusion. Serum creatinine, potassium (K^+), leukocytes, bilirubine, alkaline phosphatases (Alc-Ph) and γGT were not changed either (Table 2).

Table 2: Tolerance to Cyclosporin A

	Control		Cyclo A	
	before treatment	24 hrs after treatment	before treatment	24 hrs after treatment
SBP (mmHg)	123±4	110±3	121±7	116±4
DBP (mmHg)	73±2	68±3	71±4	72±3
creatinine (µM/l)	82±5	80±5	77±6	80±6
K+ (mM/l)	3.6±0.2	3.8±0.2	3.4±0.2	3.6±0.2
Leukocytes (Giga/l)	11.9±0.8	11.9±0.8	10.9±0.9	10.6±1.0
bilirubine (µM/l)	9.7±1.0	11.7±1.0	9.1±1.1	11.2±1.0
Alc. Ph (IU/l)	60.4±4.0	59.9±4.2	59.2±4.9	59.9±4.9
γGT (IU/l)	67.0±10.5	65.7±10.9	66.4±11.0	65.7±11.4

Adverse clinical events

During the first 48 hours after reperfusion, seven adverse clinical events, including one ventricular fibrillation and six episodes of heart failure were recorded in the control group versus two, including one ventricular fibrillation and one episode of heart failure, in the cyclosporin A group ($p<0.05$ for cumulative endpoint).

Conclusion

These results demonstrate that cyclosporin A, given as an intravenous bolus immediately before reperfusion, to patients with ongoing myocardial infarction, can dramatically reduce infarct size as measured by cardiac enzymes release and magnetic resonance imaging.

These first data in humans indicate that CsA, an inhibitor of mPTP, can be of major help for the treatment of patients with ongoing acute myocardial infarction, as described above.

Similar results may be obtained with Melle^4–cyclosporin, given before reperfusion to patients with ongoing AMI.

Melle^4–cyclosporin, a CsA derivative, retains, via its interaction with the mitochondrial cyclophilin D, the ability to inhibit mPTP opening. It is also as powerful as CsA to reduce infarct size in experimental models.

Furthermore, Melle^4–cyclosporin has the advantage of lacking the immunosuppressive activity and nephrotoxicity of CsA.

Example VI

Effect of CsA on the incidence of reperfusion-induced ventricular fibrillation in the pig model

The effect of CsA on arrhythmias following an ischemic insult was studied using the pig model of ischemia-reperfusion-induced ventricular fibrillation.

Methods

59 male farm pigs, male (20-35 kg) were anesthetized and ventilated. A thoracotomy was performed and the left anterior descending (LAD) coronary artery was dissected for further ischemia and reperfusion.

Protocol I: Infarct size determination

Twelve pigs underwent 40 min of LAD occlusion followed by 120 min of reperfusion. They were randomly allocated into two groups: Control (n=6) or CsA: intravenous bolus of 5 mg/kg, one minute before reperfusion (n=6). Extent of the area at risk was similar between groups. Infarct size was significantly reduced in the CsA group (17.5 ± 6.1 % of the area at risk) versus the control group (39.9 ± 9.7 %; $p<0.05$).

Protocol II: incidence of ventricular fibrillation

Twenty-nine pigs underwent 15 min of LAD occlusion followed by 60 min of reperfusion. They were randomly allocated into a control (no additional intervention; n = 15), or a CsA group (intravenous bolus injection of 5 mg/kg of CsA; n=14). During the reperfusion period, the incidence of ventricular fibrillation was assessed. The incidence of ventricular fibrillation at reperfusion averaged 71% in the control group versus 38% in the CsA group ($p<0.05$).

Conclusion

This in vivo study in the pig model demonstrates that cyclosporin A, administered immediately before reperfusion, can significantly reduce infarct size and attenuate reperfusion-induced ventricular fibrillation. This is a demonstration of the anti-arrhythmic property of cyclosporin A.

Similar results may be obtained with Melle[4]-cyclosporin, given immediately before reperfusion.

CLAIMS

1. A composition for the treatment of acute myocardial infarction, comprising Cyclosporin A or Melle^4–cyclosporin as an active component, in a pharmaceutically suitable vehicle.

2. A composition as claimed in Claim 1, containing from about 5 mg to about 6 g of Cyclosporin A or Melle^4–cyclosporin in a vehicle suitable to be administered intravenously at a dose of from 0.1 to 50 mg of Cyclosporin A or Melle^4–cyclosporin per kg of body weight of a patient.

3. The use of Cyclosporin A or Melle^4–cyclosporin in a pharmaceutically suitable vehicle for the preparation of a composition for the treatment of acute myocardial infarction.

4. The use as claimed in claim 3, wherein the composition is administered at least before proceeding to a reperfusion process after a myocardial ischemia, preferably within the six hours that precede reperfusion, more preferably within the thirty minutes that precede reperfusion, and more preferably a few minutes before reperfusion.

5. The use as claimed in claim 3 or 4, wherein the composition is administered continuously, from the thirty minutes that precede reperfusion until 72 hours after reperfusion.

6. The use as claimed in any of claims 3 to 5, wherein the concentration of Cyclosporin A or Melle^4–cyclosporin in the composition is 0.1 to 50 mg per kg of body weight of a patient.

7. The use as claimed in any of claims 3 to 6, wherein the composition is administered intravenously, intra-coronarily or orally.

8. The use as claimed in any of claims 3 to 7, wherein the composition is administered together with at least one other active component.

9. The use as claimed in any of claims 3 to 8, wherein the composition is administered to a human.

10. The use as claimed in any of claims 3 to 9, wherein the composition is administered before re-opening the occluded coronary artery responsible for said ischemia.

11. A method for the treatment of acute myocardial infarction, comprising administering to a patient a composition comprising Cyclosporin A or Melle^4–cyclosporin, as an active component, and a pharmaceutically suitable vehicle, wherein the composition is administered at least before proceeding to a reperfusion process after a myocardial ischemia.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2007/000402

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/13 A61P9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2006/072639 A (DEBIOPHARM SA [CH]; SCALFARO PIETRO [CH]; DUMONT JEAN-MAURICE [CH]; VU) 13 July 2006 (2006-07-13) claims 1-10 page 7, line 17 - line 21 page 9, line 10 - line 12 examples 1,2 the whole document	1-11
P,X	THIBAULT HÉLÈNE ET AL: "Acute myocardial infarction in mice: assessment of transmurality by strain rate imaging." AMERICAN JOURNAL OF PHYSIOLOGY. HEART AND CIRCULATORY PHYSIOLOGY JUL 2007, vol. 293, no. 1, July 2007 (2007-07), pages H496-H502, XP002444339 ISSN: 0363-6135 the whole document	1-11

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 August 2007	21/09/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fayos, Cécile

INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	DE 197 11 795 A1 (APOGEPHA ARZNEIMITTEL GMBH [DE]) 24 September 1998 (1998-09-24) claims 1,2 column 1, line 3 - line 8 column 2, line 58 - line 62	1-11
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2007/000402

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2007/000402

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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