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Endothelin-1 and acute myocardial infarction: a no-reflow mediator after successful percutaneous myocardial revascularization

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Aims No-reflow after a primary percutaneous coronary intervention (PCI) is associated with a high incidence of left ventricular (LV) failure and a poor prognosis. Endothelin-1 (ET-1) is a potent endothelium-derived vasoconstrictor peptide and an important modulator of neutrophil function. Elevated systemic ET-1 levels have recently been reported to predict a poor prognosis in patients with acute myocardial infarction (AMI) treated by primary PCI. We aimed to investigate the relationship between systemic ET-1 plasma levels and no-reflow in a group of AMI patients treated by primary PCI.
Methods and results A group of 51 patients (age 59 $\pm$ 9.9 years, 44 males) with a first AMI, undergoing successful primary or rescue PCI, were included in the study. Angiographic no-reflow was defined as coronary TIMI flow grade $\leq$2 or TIMI flow 3 with a final myocardial blush grade $\leq$2. Blood samples were obtained from all patients on admission for ET-1 levels measurement. No reflow was observed in 31 patients (61%). Variables associated with no-reflow at univariate analysis included culprit lesion of the left anterior coronary descending artery (LAD) (67 vs. 29%, $P = 0.006$) and ET-1 plasma levels (3.95 $\pm$ 0.7 vs. 3.3 $\pm$ 0.8 pg/mL, $P = 0.004$). At multivariable logistic regression analysis, ET-1 was the only significant predictor of no-reflow ($P = 0.03$) together with LAD as the culprit vessel ($P = 0.04$).
Conclusion ET-1 plasma levels predict angiographic no-reflow after successful primary or rescue PCI. These findings suggest that ET-1 antagonists might be beneficial in the management of no-reflow.

Introduction

Reperfusion therapy for acute myocardial infarction (AMI) by a primary percutaneous intervention has been shown to improve the clinical outcome compared with thrombolisys.[1] Yet, in as many as 40% of patients, myocardial perfusion in the infarct-related artery territory is insufficient, thus negating the potential advantages of a successful restoration of epicardial coronary artery patency.[2,3] This phenomenon, known as no-reflow,[4] is associated with a high incidence of left ventricular (LV) failure and a poor prognosis.[3,5] Several mechanisms responsible for no-reflow have been identified in experimental models, including extravascular compression, microvascular vasoconstriction, and platelet–leukocyte capillary plugging.[4,6]

Endothelin-1 (ET-1) is a potent endothelium-derived vasoconstrictor peptide,[7] an important modulator of neutrophil, leucocyte function,[8–10] and a stimulator of surface expression of adhesion molecules.[11] Enhanced ET-1 release in AMI, therefore, might aggravate reperfusion injury by increasing microvascular vasoconstriction and leukocytes adhesiveness to microvessel. Of note, ET-1 plasma levels have been found to be elevated in the first hours after AMI[12] and unstable plaques display a high content of ET-1.[13] Furthermore, elevated systemic ET-1 levels have recently been reported to predict a poor prognosis in patients admitted for AMI treated by primary percutaneous coronary intervention (PCI),[14] and selective ET-1 receptor antagonists have been found to reduce mortality and improve coronary microvascular function and LV remodelling in experimental models of AMI.[15–17] Finally, an up-regulation of ET-1 receptor has been demonstrated in the heart of AMI patients and transcardiac extraction of ET-1 seems to play a significant role in modulating post-infarct LV remodelling.[18,19]

However, whether ET-1 may exert its detrimental effects in AMI patients by favouring the no-reflow phenomenon is still unknown. Thus, in this study, we investigated the relationship between systemic ET-1 plasma levels and

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occurrence of no-reflow in a group of AMI patients treated by successful primary or rescue PCI.

Methods

Patient selection

Consecutive patients admitted to our hospital from January 2005 to June 2005 because of a first AMI were considered for the study. Inclusion criteria were prolonged chest pain (>30 min), ST-segment elevation >0.2 mV in two or more adjacent leads on standard electrocardiogram, and successful primary or rescue (after failed thrombolysis) PCI (residual coronary stenosis <20%) performed within 12 h of the onset of chest pain.

During the study period, 70 patients were admitted to our coronary care unit with a first AMI. Overall, 19 patients were excluded from the study, due to time from symptom onset >12 h ($n = 15$), refusal to undergo PCI ($n = 2$), residual stenosis after PCI >20% ($n = 1$), or missing of blood sample for ET-1 measurement ($n = 1$). Thus, 51 patients (age 59 ± 9.9 years, 44 males) were eventually included in the study.

The study was approved by the Ethics Committee of the Catholic University and all patients gave their consent to use part of their blood for scientific purposes.

PCI procedure

All PCI procedures were performed through a femoral approach with a 6 french guiding catheter. A bolus of 5000 IU of heparin was administered. After conventional wire crossing, direct stenting implantation was performed whenever possible, preceded by balloon predilatation if necessary. Use of either intracoronary or systemic bolus of Reopro, followed by a 12-h continuous infusion, and of a device for thrombus aspiration (Diver CE, Invatec) were left at the operator's discretion. Intracoronary nitrates were always administered after vessel recanalization.

Angiographic analysis

Angiographic analyses included coronary TIMI flow grading, corrected TIMI frame count, final myocardial blush grade (MBG), collateral grading, and thrombus scoring. Angiographic assessment was always performed by two independent angiographers (G.N. and E.R.) who were unaware of ET-1 results, and final agreement was 90%, with discordances being resolved by consensus. All angiographic endpoints were evaluated before and after PCI.

TIMI flow was assessed according to previous studies.[20] MBG was assessed according to van 't Hof *et al.* criteria.[21] We defined angiographic no-reflow as a coronary TIMI flow grade ≤2 after vessel reopening or TIMI flow 3 with a final MBG ≤2 (modified from Gibson *et al.*).[22] Collateral grading was done according to the Rentrop grading system that ranges from 0 (no collateral filling) to 3 (complete vessel opacification by retrograde flow).[23] Thrombus score was modified from Gibson *et al.*[24]

Laboratory assays

Blood samples were drawn from a brachial vein in all patients on admission. Blood was collected in EDTA tubes or tubes without any anticoagulant, and centrifuged. Plasma and serum aliquots were stored at −80°C in appropriate cuvettes until assayed.

C-reactive protein serum levels were measured using an immunonephelometric high-sensitivity method (DADE Behring, Milan, Italy), the lower detection limit of which is 0.175 mg/L.

Serum ET-1 levels were measured by radio-immunoassay using a synthetic human/porcine ET-1 (Sigma) and a highly specific rabbit antibody against synthetic ET-1 (Peninsula Laboratories), and ^{125}I-ET-1 (Amersham), according to a previously described method.[25,26] All measures were performed in duplicate and averaged. Radioactivity of the pellets was determined by a gamma counter (Canberra Packard, Zurich, Switzerland) and the RIA data processed using machine software. The lower limit of ET-1 detection with this method is 0.16 pg/tube.

Serum cardiac enzymes (creatine kinase [CK] and CK-MB fraction) and troponin T-levels were measured every 4 h during the first day and every 24 h in the following 3 days using standardized methods.

In all patients, LV ejection fraction was measured on admission in coronary care unit after the PCI procedure by 2D-echocardiography (Simpson method).

Statistical analysis

Comparisons between groups were done by *t*-test or Mann–Whitney *U* test (as indicated) for continuous variables and by Fisher's exact test for discrete variables. Correlation analyses were done by Pearson test or Spearman test, as indicated.

Table 1 General characteristics and laboratory data

Characteristics	Data
Age (years)	59 ± 9.9
Male gender, *n* (%)	44 (86)
Hypertension, *n* (%)	23 (46)
Smoking, *n* (%)	42 (82)
Hypercholeterolemia, *n* (%)	25 (50)
Family History, *n* (%)	17 (34)
Diabetes, *n* (%)	9 (18)
Previous ischaemic heart disease, *n* (%)	3 (6)
Primary angioplasty, *n* (%)	42 (82)
Time to PCI (min)	205 (120–360)
Heart rate (bpm)	74.8 ± 12
Systolic blood pressure (mmHg)	135 ± 30
Diastolic blood pressure (mmHg)	81 ± 17
LV ejection fraction (%)	50.6 ± 10
CK-MB peak (ng/mL)	227 ± 155
C-reactive protein (mg/L)	3.5 (2.2–6.4)
ET-1 (pg/mL)	3.7 ± 0.8

Table 2 Angiographic and procedural data

Number of diseased vessels, *n* (%)	
3	7 (14)
2	13 (25)
1	31 (61)
Culprit vessel, *n* (%)	
LAD	26 (51)
RCA	19 (37)
LCX	6 (12)
Reopro usage, *n* (%)	40 (78)
Diver usage, *n* (%)	38 (74)
Direct stenting, *n* (%)	26 (51)
Initial TIMI flow 0, *n* (%)	33 (65)
Initial thrombus score 4, *n* (%)	32 (63)
Rentrop collaterals grading, *n* (%)	
0	33 (65)
1	14 (27)
2	3 (6)
3	1 (2)
Final TIMI 3, *n* (%)	40 (78)
Final MBG 3, *n* (%)	23 (45)
No-reflow, *n* (%)	31 (61)

RCA, right coronary artery; LCX, left circumflex.

Multivariable logistic regression analysis was applied to identify whether ET-1 was independently associated with coronary no-reflow. At this scope, in the model we included variables showing a significant or borderline association with no-reflow at univariate analysis (P values ≤ 0.1); furthermore, also non-significant variables that were suggested to have an association with coronary no-reflow in some previous studies (Diver use, Reopro use, thrombus score) were also forced in the multivariable model. The assumption of linearity for continuous variables included in the model was confirmed by logit step test.

Data are reported as mean $\pm$ standard deviation, unless otherwise indicated. A $P < 0.05$ was always required for statistical significance. The software SPSS 12.01 (SPSS Italia, Florence, Italy) was used for statistical analyses.

Results

General characteristics of patient population are listed in *Table 1*. PCI was a primary intervention in 42 patients (82%), whereas PCI was performed after failed thrombolysis (rescue PCI) in nine (18%). Pre-PCI time was 335 $\pm$ 446 min (median 205 min). Angiographic and procedural data are listed in *Table 2*. A final TIMI flow 3 was achieved in 39 patients (76%), whereas a final MBG 3 was observed in 23 patients (45%). No-reflow was observed in 31 patients (61%), whereas reflow was observed in 20 patients (39%).

Table 3 summarizes the results observed in patients with or without no-reflow. Variables associated with no-reflow at univariate analysis included culprit lesion involving the left anterior descending coronary artery (LAD) (67 vs. 29%, $P = 0.006$) and ET-1 plasma levels (3.95 $\pm$ 0.7 vs. 3.3 $\pm$ 0.8 pg/mL, $P = 0.004$) (*Figure 1*).

At multivariable logistic regression analysis, ET-1 was a significant predictor of angiographic no-reflow ($P = 0.03$) together with LAD as the culprit vessel ($P = 0.04$) (*Table 4*). Furthermore, ET-1 plasma levels were significantly increased in patients with final TIMI flow ≤ 2 compared with those with final TIMI flow 3 (4.1 $\pm$ 0.8 vs. 3.5 $\pm$ 0.7 pg/mL, $P = 0.03$, respectively) (*Figure 2*), and in patients with MBG ≤ 2 compared with those with MBG 3 (3.95 $\pm$ 0.7 vs. 3.4 $\pm$ 0.8 pg/mL, $P = 0.02$, respectively) (*Figure 3*).

No clinical or laboratory variable showed a significant association with ET-1 plasma levels, including the PCI type

Table 3 Univariate predictors of angiographic no-reflow (TIMI flow ≤ 2 and/or TIMI 3 with MBG ≤ 2)

	No-reflow ($n = 31$)	Reflow ($n = 20$)	P-value
Age (years)	59 $\pm$ 10	56 $\pm$ 8	0.26
Male, *n* (%)	25 (83)	18 (90)	0.46
Hypertension, *n* (%)	12 (38)	10 (50)	0.56
Smokers, *n* (%)	24 (79)	17 (86)	0.72
Hypercholesterolmia, *n* (%)	17 (55)	9 (43)	0.57
Family history, *n* (%)	9 (31)	8 (38)	0.54
Diabetes, *n* (%)	6 (21)	3 (14)	1.0
Type of PCI			
Primary, *n* (%)	23 (76)	18 (91)	0.28
Rescue, *n* (%)	8 (24)	2 (9)	
Time to PCI (min)	300 (175–465)	180 (120–220)	0.07
Heart rate (bpm)	74 $\pm$ 13	75 $\pm$ 11	0.78
Systolic blood pressure (mmHg)	129 $\pm$ 24	141 $\pm$ 36	0.16
Diastolic blood pressure (mmHg)	79 $\pm$ 15	83 $\pm$ 18	0.20
LV ejection fraction (%)	49 $\pm$ 6	51 $\pm$ 11	0.40
Number of diseased vessel, *n* (%)			
$=3$	4 (13)	3 (14)	1.0
<3	27 (87)	17 (86)	
Culprit vessel, *n* (%)			
LAD	21 (67)	6 (29)	
RCA	7 (23)	11 (57)	0.006
LCX	3 (10)	3 (14)	
Reopro use, *n* (%)	24 (79)	15 (76)	1.0
Diver use, *n* (%)	10 (31)	8 (38)	0.76
Direct stenting, *n* (%)	15 (50)	10 (52)	1.0
Initial TIMI flow, *n* (%)			
$=0$	19 (63)	13 (66)	1.0
$=1$	12 (37)	7 (34)	
Thormbus score, *n* (%):			
$=4$	19 (60)	13 (66)	1.0
<4	12 (40)	7 (34)	
Rentrop collaterals grading, *n* (%):			
$=0$	20 (64)	13 (65)	1.0
>0	11 (36)	7 (35)	
CK-MB peak (ng/mL)	235 $\pm$ 139	215 $\pm$ 179	0.67
C-reactive protein (mg/L)	3.5 (1.9–6.4)	2.8 (2.2–8.7)	0.86
ET-1 (pg/mL)	3.95 $\pm$ 0.7	3.30 $\pm$ 0.8	0.004

RCA, right coronary artery; LCX, left circumflex.

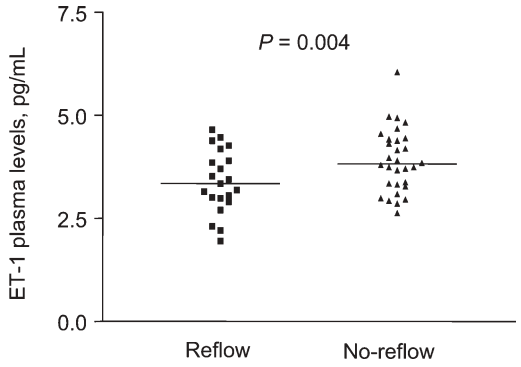

Figure 1 Individual values of ET-1 plasma levels according to no-reflow occurrence. No-reflow was defined as a final TIMI flow ≤ 2 and/or a final TIMI flow $= 3$ with an MBG ≤ 2. Data are expressed as individual point value and median in pg/mL. Horizontal line across individual values represents the median.

Table 4 Multivariable predictors of angiographic no-reflow

	OR	95% confidence interval	*P*-value
ET-1	2.76	1.1–7.1	0.03
Reopro use	1.61	0.3–7.6	0.54
Diver use	1.45	0.3–5.9	0.60
Thrombus score	1.25	0.6–2.2	0.45
Culprit LAD	0.36	0.1–0.9	0.04
Time to PCI	1.0	0.9–1.0	0.46

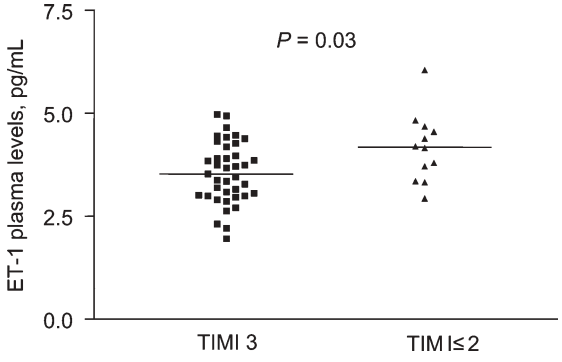

Figure 2 Individual values of ET-1 plasma levels according to final TIMI flow. Data are expressed as individual point value and median in pg/mL. Horizontal line across individual values represents the median.

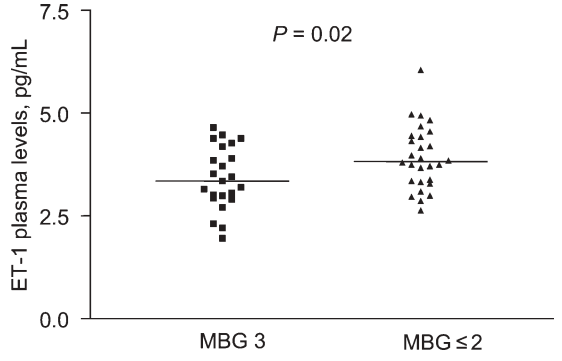

Figure 3 Individual values of ET-1 plasma levels according to final MBG. Data are expressed as individual point value and median in pg/mL. Horizontal line across individual values represents the median.

(primary vs. rescue), the culprit coronary vessel (LAD vs. other vessels), and LV function (*Tables 5 and 6*).

Discussion

In this study, we show that in patients admitted with ST-elevation AMI, ET-1 plasma levels predict angiographic no-reflow after successful primary or rescue PCI, independent of the most important clinical, haemodynamic, and laboratory variables. Our population was too small for clinical outcome assessment, but the demonstration of an association between ET-1 levels and no-reflow suggests that the negative impact of ET-1 on outcome reported in previous studies may, at least partially, be mediated by no-reflow.

The difference in ET-1 levels between patients with or without no-reflow might seem small (*Table 3*), but it should be considered that it is very likely underestimated for at least two reasons: first, ET-1 released in the coronary circulation is diluted in the blood of the systemic

Table 5 Plasma ET-1 levels according to the most important clinical/haemodynamic variables

Dichotomous variables	ET-1 levels	*P*-value
Gender		
Male	3.6 ± 0.8	0.2
Female	3.3 ± 0.8	
Hypertension		
Yes	3.4 ± 0.7	0.17
No	3.8 ± 0.8	
Smokers		
Yes	3.6 ± 0.8	0.3
No	3.4 ± 0.6	
Hypercholesterolmia		
Yes	3.5 ± 0.9	0.5
No	3.7 ± 0.6	
Diabetes		
Yes	3.7 ± 0.7	0.8
No	3.6 ± 0.8	
Type of PCI		
Primary	3.6 ± 0.7	0.6
Rescue	3.7 ± 1.1	
LAD involvement		
Yes	3.8 ± 0.8	0.18
No	3.5 ± 0.7	

Table 6 Plasma ET-1 levels according to the most important clinical/haemodynamic variables

Continuous variables	*R*-value	*P*-value
Age	−0.008	0.9
Heart rate	−0.19	0.17
Systolic blood pressure	−0.25	0.08
Diastolic blood pressure	−0.16	0.26
Time to PCI	0.01	0.9
LV ejection fraction	−0.22	0.12
CK-MB peak	0.22	0.13
C-reactive protein	−0.02	0.8

circulation; second, only a small part of ET-1 produced by the endothelium is released in the blood, whereas most is released in the non-luminal side of the endothelial cells.[27,28]

There are several mechanisms by which ET-1 can favour no-reflow. ET-1 is the most potent vasoconstrictor produced by the human body and is mainly synthesized by vascular endothelium, in particular, following endothelial activation.[7] The vasoconstrictor effects of ET-1 are mainly exerted on small-resistance coronary arteries;[29] hence, enhanced release of ET-1 from ischaemia-reperfusion injured endothelium may result in intense and sustained microvascular constriction.[30] Furthermore, ET-1 has also relevant effects on polymorphonuclear (PMN) leukocytes which could account for its association with no-reflow. Indeed, ET-1 stimulates adhesion of PMN leukocytes to the endothelium, thus favouring PMN plugging by increasing the expression of the integrin CD11b/18 on PMN surface,[8] by inducing elastase release,[9] which may also mediate tissue injury and oedema, and by increasing endothelium expression of ICAM-1.[10] Furthermore, ET-1 enhances microvascular permeability with consequent oedema resulting in microvascular compression.[31]

In our study, an anterior location of AMI also was an independent predictor of no-reflow, in keeping with previous studies.[32] This might be due to a greater area at risk and in turn to more extensive microvascular damage. In line with this interpretation, peak CM-MB tended to be higher in patients compared with those without no-reflow (although this difference did not achieve statistic significance).

We did not find a significant association between inflammation and no-reflow, although previous studies showed a detrimental effect of systemic inflammation on epicardial and microvascular vasoreactivity,[33–35] as well as on neutrophil function.[36] Indeed, in our study C-reactive protein serum levels were similar in patients with or without no-reflow. However, a potential confounder in our study was the inclusion of patients admitted up to 12 h after pain onset. Indeed, C-reactive protein serum levels obtained more than 6 h after pain onset mainly reflect the inflammatory response to myocardial necrosis. Thus, further studies are needed to better define the possible role of inflammation in the pathogenesis of no-reflow.

Study limitation

This study has some limitations. First, the sample size is small and, therefore, our data need confirmation in future studies. Yet, statistical significance in our study was achieved despite the limited sample and independent of several clinical and laboratory variables, thus suggesting that ET-1 can actually have a role in no-reflow occurrence. A second limitation is the inclusion of rescue PCI patients, who had previously received fibrinolytic therapy, which might have possibly influenced ET-1 levels. However, the type of PCI, i.e. primary or rescue, was not a predictor of no-reflow and ET-1 plasma levels were similar in these two groups of patients.

Furthermore, ongoing necrosis might influence ET-1 levels, but we did not find any correlation between ET-1 levels and CK-MB levels or time to angiography. Finally, in our study, the prevalence of no-reflow seems higher than previously reported,[21] however, comparable data have been shown in studies that utilized a definition of no-reflow similar to ours.[22]

Conclusion

In conclusion, this study demonstrates that in patients admitted with ST-elevation AMI, ET-1 plasma levels predict angiographic no-reflow after successful primary or rescue PCI. Our data may have relevant clinical implications. Indeed, they suggest that a trial of the effects of an ET-1 antagonist on no-reflow and outcome is warranted.

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Conflict of interest: none declared.

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