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ARTICLE *in* JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY · SEPTEMBER 1998

Impact Factor: 16.5 · DOI: 10.1016/S0735-1097(98)00270-8 · Source: PubMed

JACC Vol. 32, No. 2
August 1998:373–9

Production of Oxidative Products of Nitric Oxide in Infarcted Human Heart

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Objectives. **We sought to assess whether oxidation products of nitric oxide (NO), nitrite (NO_2^-) and nitrate (NO_3^-), referred to as NO_x, are released by the heart of patients after acute myocardial infarction (AMI) and whether NO_x can be determined in peripheral blood of these patients.**

Background. **Previously we reported that in experimental myocardial infarction (rabbits) NO_x is released mainly by inflammatory cells (macrophages) in the myocardium 3 days after onset of ischemia. NO_x is formed in heart muscle from NO; NO originates through the activity of the inducible form of nitric oxide synthase (iNOS).**

Methods. **Eight patients with acute anterior MI and an equal number of controls were studied. Coronary venous blood was obtained by coronary sinus catheterization; NO_x concentrations in coronary sinus, in arterial and peripheral venous plasma were measured. Left ventricular end-diastolic pressure was determined. Measurements were carried out 24, 48 and 72 h after onset of symptoms. The type and location of coronary arterial lesions were determined by coronary angiography. Plasma NO_3^- was reduced to NO_2^- by nitrate reductase before determination of NO_2^- concentration by chemiluminescence.**

Results. **The results provided evidence that in patients with acute anterior MI, the myocardial production of nitrite and nitrate (NO_x) was increased, as well as the coronary arterial–venous difference. Increased NO_x production by the infarcted heart accounted for the increase of NO_x concentration in arterial and the peripheral venous plasma. The peak elevation of NO_x occurred on days 2 and 3 after onset of the symptoms, suggesting that NO_x production was at least in part the result of production of NO by inflammatory cells (macrophages) in the heart.**

Conclusions. **The appearance of oxidative products of NO (NO_2^- and NO_3^-) in peripheral blood of patients with acute MI is the result of their increased release from infarcted heart during the inflammatory phase of myocardial ischemia. Further studies are needed to define the clinical value of these observations.**

(J Am Coll Cardiol 1998;32:373–9

Nitric oxide (NO) is a free radical with an unpaired electron; it is an important physiologic messenger, produced by nitric oxide synthases, which catalyze the reaction L-arginine to citrulline and NO. There are two main categories of NO synthase, the constitutive and the inducible forms. The constitutive isoforms exists in neuronal and endothelial cells and is calcium dependent (1,2). Calcium binds to calmodulin and the calcium calmodulin complex activates the constitutive NO synthase that releases NO, relaxing smooth muscle cells through activation of guanylate cyclase and the production cGMP (3). Therefore, the NO produced has a negative inotropic effect on the heart and is instrumental in the autoregulation of the coronary circulation (4,5).

In contrast, the inducible form of NO synthase (iNOS) is mostly produced in macrophages activated by cytokines and endotoxin (6). It eliminates intracellular pathogens, damaging cells by inhibiting ATP production and oxidative phosphorylation and DNA synthesis (7). In infection, lipopolysaccharide released from bacterial walls, stimulates production of iNOS primarily in macrophages (8). The large amount of NO produced causes extensive vasodilation and hypotension. The iNOS is also activated in heart muscle under pathologic conditions, such as in heart failure, dilated cardiomyopathy and cardiac allograft rejection (9,10). It has been found that in infarcted rabbit heart muscle after ligation of a coronary artery, iNOS activity increases on the first postoperative day and persists for at least 14 days, declining to control levels 3 weeks after the onset of ischemia. iNOS was also localized in infiltrating macrophages of the human heart (11). Animals with myocardial ischemia showed consistent elevation of

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Manuscript received July 25, 1997; revised manuscript received April 2, 1998, accepted April 17, 1998.

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0735-1097/98/$19.00
PII S0735-1097(98)00270-8

Abbreviations and Acronyms

AMI = acute myocardial infarction
CPK = creatine kinase
CS = coronary sinus
iNOS = inducible form of nitric oxide synthase
LAD = left anterior descending coronary artery
NO = nitric oxide
NO_2^- = nitrite
NO_3^- = nitrate
NO_x = sum of nitrite and nitrate
RPP = rate pressure product

plasma nitrite (NO_2^-) and nitrate (NO_3^-) concentration 3 days after onset of myocardial infarction (12). Several findings suggest a causal relationship between NO production by the heart and elevated plasma levels of NO_x, such as a relationship in time between NO_x plasma concentration and NO_x production and between coronary arteriovenous difference of NO_x and myocardial iNOS activity.

It is the purpose of this study to demonstrate that after occlusion of the anterior descending coronary artery in humans, NO_x plasma levels are elevated and that this is caused by increased NO_x production in the infarcted human heart muscle.

Methods

Patients and methods. All participants in the study were informed of the procedure and gave written consent. The protocol was approved by the local ethics committees and is in line with the recommendations of the Declaration of Helsinki.

The study involved 16 patients, of these eight (6 men, 2 women), aged 48 to 80 years (mean 65.0 ± 3.3 years) with no evidence of acute myocardial infarction (AMI) served as control (Table 1). These patients were undergoing coronary arteriography using a Judkins catheter (6F) for evaluation of nonspecific chest pain and had no angiographic evidence of significant coronary artery stenosis (Table 1). All control patients were studied after an overnight fast and received 5,000 units of intravenous heparin at the initiation of the study. Antianginal and antihypertensive medications were discontinued at least 48 hours before this study. Routine cardiac catheterization was performed using a femoral approach and a biplane cineangio system (Toshiba). After a Cordis infinity micro pigtail catheter (6F) was inserted into the left ventricle, left ventricular end-diastolic pressure was recorded. Left ventriculography was performed and left ventricular ejection fraction was calculated (Table 1). A coronary sinus catheter (5F; Baxter) was placed in the coronary sinus (CS) through the left subclavian vein and the position was confirmed by injection of the contrast medium (iopamilone) and by typical position on fluoroscopy (13). Blood samples for analysis were drawn from the CS, femoral artery and femoral or brachial vein (henceforth referred to as the peripheral vein) before performing coronary angiography. A Cordis infinity Judkins type catheter (6F) was used for diagnostic coronary angiography.

Patients with acute myocardial infarction (AMI) (6 men, 2 women), aged 46 to 81 years (mean 66.0 = 4.5 years) (Table 1) displayed evidence of infarction as documented by the following criteria: 1) chest pain lasting ≥30 min; 2) electrocardiographic ST-segment elevation of ≥0.1 mV in at least two leads in either anterior or lateral distribution; and 3) elevated creatine kinase MB isoenzymes within 24 h after the onset of

Table 1. Patient Characteristics and Hemodynamic Variables (Control and AMI)

	Age (y)	Sex	Smoking	Medication	HR (beats/min)	Mean BP (mm Hg)	LVEDP (mm Hg)	LVEF (%)	Peak CPK (IU/liter)	CPK-MB (IU/liter)	Culprit Vessel† (Coronary Segment)	Angio‡
Control group												
1	59	M	+	None	67	100	7	74	—	—	None	0
2	71	M	+	None	70	102	8	63	—	—	None	0
3	65	M	−	None	59	112	12	68	—	—	None	0
4	80	M	−	None	79	105	10	53	—	—	None	0
5	66	F	−	None	90	110	13	72	—	—	None	0
6	69	F	−	None	74	92	6	60	—	—	None	0
7	48	M	−	None	70	125	8	68	—	—	None	0
8	62	M	+	None	75	109	11	71	—	—	None	0
AMI group*												
1	74	M	+	Ca	74	96	9	—	2,989	774	LAD (6)	2
2	48	M	+	ACE	65	110	19	36	391	43	LAD (6)	1
3	81	M	+	ACE, Diu	87	82	14	52	1,112	173	LAD (7)	1
4	65	M	+	ACE, Ca	64	103	14	37	3,514	476	LAD (6)	2
5	68	F	−	ACE, Ca, Diu	76	100	15	39	1,546	289	LAD (6)	1
6	75	M	−	ACE, Ca, Diu	82	90	5	43	2,543	263	LAD (8)	2
7	46	M	+	ACE, β	85	106	11	52	4,648	468	LAD (6)	1
8	71	F	−	ACE, Diu	78	97	19	38	3,219	317	LAD (7)	1

*All patients had anterior myocardial infarction. †Location of obstruction in left anterior descending artery according to American Heart Association classification. ‡Number of stenosed coronary arteries. CPK = creatine kinase; CPK-MB = MB isoenzyme of creatine kinase; Culprit vessel = left anterior descending coronary artery; Ca = calcium antagonist; ACE = angiotensin-converting enzyme inhibitor; Diu = diuretic (furosamide); β = beta-blocker; Angio = coronary angiography.

symptoms. Excluded were patients with electrocardiographic or historic evidence of previous myocardial infarction, coronary artery bypass surgery or percutaneous transluminal coronary angioplasty.

Tissue plasminogen activator or urokinase were not administered. Therapy included heparin and morphine when indicated. In most cases patients with AMI had sublingual nitrate once in the emergency room or catheterization laboratory before or after coronary angioplasty. Blood was drawn for determination of NO_x >12 h after coronary angioplasty and the administration of nitroglycerine. Patients with AMI were subjected to cardiac catheterization within 6 h after onset of symptoms. Coronary angiography and angioplasty were performed using a standard femoral approach. All patients underwent direct coronary angioplasty (mean time from onset of chest pain to first inflation was 3.9 ± 0.6 h). The culprit artery was identified by coronary angiogram (Table 1). A significant coronary artery stenosis was defined as >75% luminal narrowing by visual estimate after intracoronary nitroglycerin (100 to 200 μg). The culprit arteries were revascularized using Scimed Bandit coronary angioplasty dilation catheter (2.5 to 3.5 mm by 20 mm, Boston Scientific) to Thrombolysis in Myocardial Infaction flow grade 3 until residual stenosis was <50% (14). Electrocardiographic ST-segment elevation decreased to normal levels in one patient (No. 2) after coronary angioplasty (Table 1). The remaining patients displayed continuous ST-segment elevation after coronary angioplasty. Sustained ventricular tachycardia, ventricular fibrillation or atrial fibrillation/flutter were not observed.

During hospitalization no patient had cardiogenic shock, reinfarction or infection. At time of discharge coronary angiography revealed that all culprit arteries had remained patent.

Measurement of plasma NO_2^- and NO_3^-. Blood was drawn in heparinized tubes for NO_2^- and NO_3^- determination, and immediately centrifuged at 1,200 *g* for 10 min for removal of the formed elements by plasma separation. The plasma samples were ultrafiltered using a micropartitioning device at 4,000 *g* for 1 h (Centrifree Micropartition System) to remove residual proteins. The deproteinized plasma samples were frozen at −20°C until analysis by chemiluminescence (15).

Analysis of NO_2^- + NO_3^- required reduction of NO_3^- to NO_2^- with aspergillus nitrate reductase (Sigma Chemical). All samples were run in duplicate. Nitrate reductase 50 μl (0.2 U), FAD 5 μl (5 mmol/liter), NADPH 5 μl (6 mmol/liter) and phosphate buffer (1.2 mmol/liter) were added to 50 μl of deproteinized plasma to yield a final volume of 150 μl and subsequently incubated at 36°C for 1 h to allow for sufficient conversion of NO_3^- to NO_2^-. After incubation, 50 μl of the sample were injected into the reaction vessel. The NO_3^- content of the sample was calculated by subtracting the amount of NO_2^- in untreated samples from the amount of NO_2^- in the reduced samples (12). The sample containing nitrate reductase contains basal NO_2^- plus NO_2^- derived from reduction of NO_3^-.

Chemiluminescence was used for detection of NO_2^- after its reduction to NO. We used a reducing medium consisting of a ferrocene derivative dissolved in acetonitrile containing 1% perchloric acid (Sigma). In this solution, NO is generated from NO_2^- by a one-electron reduction pathway. In the reaction vessel, helium is used as the carrier gas of NO to the NO analyzer, at a rate of 35 ml/min. Reducing solutions (30 mg of 1,1-dimethylferrocene in 3 ml of acetonitrile, acidified with 50 μl of 70% perchloric acid) were freshly prepared and used within 2 h (15). Under these conditions, NO_2^- in the injected samples (50 μl) was reduced to NO and detected by chemiluminescence with a NO analyzer. Data were recorded automatically on a chart recorder (Soltec Corporation) as previously described (15).

NO_2^- and NO_3^- standard curves. Standard curves for NO_2^- (0 to 80 μmol/L) and NO_3^- (0 to 80 μmol/liter) were calculated by adding small aliquots (10 μl/50 μl sample) of sodium nitrite or sodium nitrate solution (in degassed ultrapure water) (12). A standard curve relating the luminescence produced by the added NO_2^- or NO_3^- was constructed, and the data were fitted to a straight line (linear regression) (12). All standard curves had r >0.96.

Calculation of NO_x production equivalent. To estimate the production of NO_x by the heart, the arterial–venous difference of NO_x and coronary flow had to be known. NO_x production by the heart was calculated as: Output of NO_x from the heart (CS_{NO_x} × coronary flow) − Input of NO_x into the heart ($Arterial_{NO_x}$ × coronary flow) (16). Because coronary flow was not measured directly in these patients, data were used from patients with AMI, in which coronary flow was calculated from positron emission tomography imaging, using [^{13}N]ammonia (17). Czernin et al. (17) established a regression correlation (r = 0.74) relating the rate pressure product (RPP) to myocardial blood flow as defined by the equation: $y = 0.00009x - 0.07$, where y = myocardial blood flow and x = RPP.

Using this equation we calculated mean coronary flow in our patients. The production equivalent of NO_x by the heart could then be calculated. The term production equivalent was used in preference to production because coronary flow was not determined directly (12).

Statistical analysis. Two analyses were performed to evaluate the change in mean NO_x levels over time. A two-way analysis of variance with repeated-measures was used to analyze the difference in mean NO_x levels over time in the venous, arterial and coronary sinus plasma in patients with AMI. The repeated measures model used a general covariance matrix, allowing for unequal correlations over time. The design of this study was an unbalanced two-way repeated measures analysis of variance. All parameters (differences, means, p values) were estimated via maximum likelihood. A second analysis was performed comparing the mean NO_x levels in the control patients to the patients with AMI at 24, 48 and 72 h based on between-group repeated measures variances for the peripheral venous, arterial and coronary sinus blood.

To compare the three sources of mean NO_x levels to each other within each time period (control 24, 48 and 72 h), post-hoc *t* tests (based on the covariance structure of the

analysis of variance model) were performed using the Tukey-Fisher least significant difference method. The within-subject variances were used to calculate the standard errors.

A similar method was used to compare the mean NO_x level of the control group to the mean NO_x levels at 24, 48 and 72 h. Post-hoc *t* tests (based on the covariance structure of the analysis of variance model) using the Tukey-Fisher least significant difference method were used to compare these means. The total variance estimated from the analysis of variance model using a general covariance matrix was used to calculate these standard errors. All variances used to calculate the standard errors for the within-subject and between-subject comparisons were estimated from the repeated measures of the analysis of variance model using a general covariance matrix, which allowed for unequal correlations over time. A p value of 0.05 or less was considered statistically significant. Data processing was performed using the SAS (version 6.11) software package.

Results

Hemodynamic data (heart rate, mean blood pressure, left ventricular end-diastolic pressure and left ventricular ejection fraction in patients with and without myocardial infarction are shown in Table 1. The infarction group had higher left ventricular end-diastolic pressure and lower left ventricular ejection fraction. The respective means for left ventricular end-diastolic pressure in the two groups were 9.38 ± 0.89 mm Hg in the control and 13.25 ± 1.70 mm Hg in patients with myocardial infarction ($p < 0.05$); left ventricular ejection fraction was 66.13 ± 2.49% in the control and 42.43 ± 2.61% in patients with infarction ($p < 0.05$). The possibility must be considered that some of the changes in patients with myocardial infarction were attributable to factors other than coronary occlusion such as cigarette smoking or medication. Three of the patients of the control group and five patients with myocardial infarction were smokers. Smoking as a factor, however, could be excluded. There were no statistical difference within each group for mean left ventricular end-diastolic pressure or left ventricular ejection fraction or for coronary sinus NO_x concentration. There was also no difference in heart rate or mean blood pressure. Diuretics and calcium antagonists also did not influence NO_x concentration in CS plasma. The effect of other factors such as angiotension-converting enzyme inhibitors or beta-adrenergic blocking agent could not be evaluated because all but one patient were given angiotension-converting enzyme inhibitors and only one patient was given beta-blockers.

Table 2 shows that after onset of symptoms the values for NO_x in CS plasma are close to significance at 48 h ($p < 0.07$) as compared to arterial and peripheral venous NO_x concentrations. At 24 h, CS NO_x concentrations still exceed those in arterial and peripheral venous plasma (Table 2). Compared to the control group, all patients with AMI showed statistically significant increase in NO_x concentrations in arterial, CS and peripheral venous plasma at 48 and 72 h after onset of symptoms (Fig. 1). These statistical differences were absent 24 h after onset of symptoms. In Figure 2 the NO_x levels in arterial blood are plotted in each individual patient as a function of time from the presentation through the remainder of the study. The values of the control series are also shown. A general increase in arterial NO_x concentrations in individual patients is noted. Changes in individual CS and peripheral venous plasma followed a similar trend (not shown).

The coronary arterial–venous differences of NO_x were significantly greater when compared to their control and the systemic arterial–venous NO_x differences (Table 2). Forty-eight hours after onset of symptoms the mean difference between the coronary and systemic arterial–venous NO_x difference was 6.0 μmol/L ($p < 0.05$). In control patients this difference was not noted (Table 2). This suggests a cardiac origin of NO_x.

The output of NO_x from the heart is represented as the product of coronary flow and the NO_x concentration in the CS plasma (12,16). Because coronary flow was not determined directly, it was calculated from the rate–pressure product as presented in Table 3, according to methods of Czernin et al. (17). From the coronary flow thus calculated the production equivalent of NO_x by the heart was obtained (12). The cardiac NO_x production equivalent is listed in Table 3. A multiple comparison test showed that at 48 h after onset of symptoms the NO_x production equivalent was significantly greater than at control, again pointing to the heart as the source of NO_x production (control 137 ± 37 nmol/100 g/min; AMI at 48 h = 472 ± 104 nmol/100 g/min; $p < 0.05$; Table 3).

Discussion

Patients. Patients in this study represent a clinically homogenous population; none of them had cardiogenic shock or congestive heart failure, and all had anterior myocardial infarction with the left anterior descending coronary artery as the culprit vessel. Furthermore all AMI patients had higher left ventricular end-diastolic pressure and lower left ventricular ejection fraction when compared to controls. Heart rate and blood pressure did not differ (Table 1). Other factors such as smoking, diuretics or calcium antagonists did not influence either hemodynamics or NO_x plasma concentration in patients with AMI (Tables 1 and 2). The effect of angiotension-converting enzyme inhibitors could not be evaluated, because all but one patient was given angiotension-converting enzyme inhibitors.

The angiotension-converting enzyme inhibitors may have influenced NO production as inhibition of angiotension-converting enzyme localized in the luminal site of the vascular endothelium results in increased synthesis of NO and prostaglandin I_2 by accumulation of endogenous bradykinin. This demonstrates the potential ability to increase NO production through the kinin pathway (18).

Formation of NO_x. It is well known that NO is produced in infarcted heart muscle primarily by the activity of iNOS (19). Activation of iNOS occurs in cardiomyocytes and during the

Table 2. Plasma NO_x in Control and AMI Patients

	Control			AMI Group									
						24 H*			48 H*			72 H*	
No.	Per. V. (μmol/liter)	Arterial (μmol/liter)	CS (μmol/liter)	No.	Per. V (μmol/liter)	Arterial (μmol/liter)	CS (μmol/liter)	Per. V. (μmol/liter)	Arterial (μmol/liter)	CS (μmol/liter)	Per. V. (μmol/liter)	Arterial (μmol/liter)	CS (μmol/liter)
1	59.2	60.2	60.9	1	50.7	50.1	54.2	53.8	53.0	58.3	52.4	53.8	53.8
2	31.6	29.2	30.3	2	33.0	33.6	35.6	84.4	86.4	93.0	76.1	75.8	78.4
3	32.7	32.3	33.7	3	53.3	50.6	57.8	60.4	61.3	70.6	—	—	—
4	41.8	41.8	42.9	4	29.8	26.9	34.4	62.9	61.2	64.5	—	—	—
5	52.6	52.7	56.5	5	38.4	35.7	36.3	68.0	63.2	69.0	84.0	89.8	93.5
6	38.4	38.1	38.8	6	38.1	39.7	42.2	—	—	—	57.8	55.2	61.5
7	41.8	39.1	41.8	7	49.4	48.1	51.3	62.7	60.2	72.2	56.4	56.8	59.3
8	42.2	42.5	44.9	8	41.0	41.6	41.7	42.2	47.2	49.0	52.7	55.3	56.0
Mean ± SE	42.5 ± 3.3	42.0 ± 3.6	43.7 ± 3.7		41.7 ± 3.0	40.8 ± 3.0	44.2 ± 3.2	62.1 ± 4.9‡	61.8 ± 4.6‡	68.1 ± 5.2‡	63.2 ± 5.5‡	64.5 ± 6.1‡	67.1 ± 6.4‡
ACSD			1.7 ± 0.4				3.4 ± 1.0			6.3 ± 1.3†			2.6 ± 0.9
APVD			0.6 ± 0.5				0.9 ± 0.6			0.3 ± 1.2			−1.2 ± 1.2

*After onset of symptoms. †$p < 0.05$ versus control group and APVD. ‡$p < 0.01$ versus control group. Arterial versus CS blood (control, NS; AMI, NS); peripheral venous versus CS blood (control, NS; AMI, NS); arterial versus peripheral venous blood (control, NS; AMI, NS). Per. V. = peripheral venous; Mean ± SEM = Mean ± standard error; ACSD = mean arterial–coronary sinus NO_x difference; APVD = mean arterial–peripheral venous NO_x difference; NS = not significant.

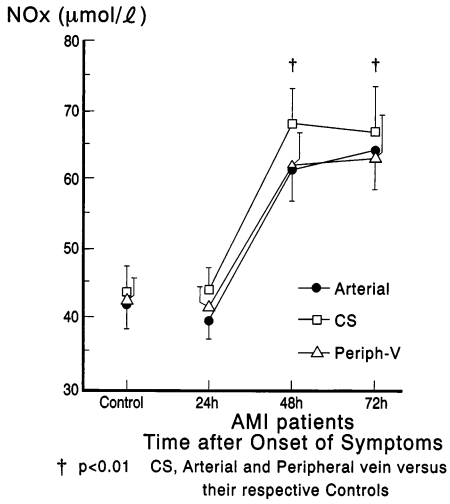

Figure 1. Mean plasma concentrations (μmol/L) of NO_x from arterial **(closed circles)**, peripheral venous **(open triangles)** and coronary sinus **(open squares)** plasma in control patients and in patients with AMI. Plasma NO_x concentrations are significantly elevated above control values, at 48 and 72 h after onset of symptoms ($p < 0.01$). Values for NO_x in coronary sinus plasma significantly exceed those in peripheral venous plasma from 24 to 72 h after onset of symptoms ($p < 0.05$). The NO_x values in coronary sinus plasma also significantly exceed those in arterial plasma from 24 to 72 h after onset of symptoms ($p < 0.05$).

inflammatory phase in activated macrophages (11). iNOS shows no requirement for Ca^{2+} and calmodulin, although calmodulin exists as a highly bound subunit (20). Activation of iNOS depends on the release of endogenous substances, including cytokines (tumor necrosis factor-alpha, interleukin-1 beta, interferon) (1). NO itself has an unpaired electron, and is physiologically and pharmacologically active (21). In contrast, as shown by Stuehr and Nathan (22), NO_2^- and NO_3^- are inactive, except at acidic pH when NO_2^- is converted into more reactive species. NO_2^- is formed by a reaction of NO

Figure 2. NO_x concentrations in arterial plasma of individual AMI patients increased from 24 to 48 h after onset of symptoms. The peak NO_x concentration usually occurred from 48 to 72 h after onset of symptoms.

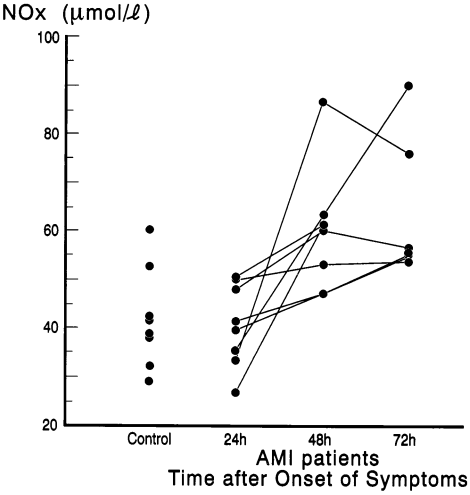

Table 3. Calculation of Cardiac NO_x Production Equivalent

Control Group		AMI Group			
No.		No	24 H	48 H	72 H
1					
HR	67	1	88	86	72
BP	130		112	115	120
RPP	8710		9856	9890	8640
CS flow	71.39		81.7	82.01	70.76
NO_x Eq	48.55		333.34	435.47	173.36
2					
HR	70	2	72	68	86
BP	120		128	119	120
RPP	8400		9216	8092	10320
CS flow	68.6		75.95	65.83	85.88
NO_x Eq	74.77		151.14	436.45	227.58
3					
HR	59	3	96	88	—
BP	140		101	110	—
RPP	8260		9696	9680	—
CS flow	67.34		80.26	80.12	—
NO_x Eq	91.58		574.66	741.11	—
4					
HR	79	4	68	74	—
BP	144		120	134	—
RPP	11376		8160	9916	—
CS flow	95.38		66.44	82.24	—
NO_x Eq	103.01		495.64	270.57	—
5					
HR	90	5	82	74	82
BP	124		105	103	100
RPP	11160		8610	7622	8200
CS flow	93.44		70.49	61.6	66.8
NO_x Eq	350.4		47.93	356.66	249.83
6					
HR	74	6	84	80	90
BP	100		110	116	108
RPP	7400		9240	9280	9720
CS flow	59.6		76.16	76.52	80.48
NO_x Eq	40.53		207.16	—	511.85
7					
HR	70	7	80	84	82
BP	150		118	112	116
RPP	10500		9440	9408	9512
CS flow	87.5		77.96	77.67	78.61
NO_x Eq	238		247.91	930.49	202.03
8					
HR	75	8	70	74	72
BP	130		134	121	122
RPP	9750		9380	8954	8784
CS flow	80.75		77.42	73.58	72.06
NO_x Eq	192.19		6.19	135.39	53.32
Mean HR	73.0 ± 3.2		80.0 ± 3.4	78.5 ± 2.5	80.7 ± 3.0
Mean BP	130 ± 6		116 ± 4	117 ± 3	114 ± 4
Mean RPP	9444 ± 519		9200 ± 198	9105 ± 298	9196 ± 322
Mean CS flow	77.9 ± 4.6		75.6 ± 1.9	74.9 ± 2.7	75.8 ± 2.9
Mean NO_x Eq	137 ± 37		240 ± 77	472 ± 104*	236 ± 62

*$p < 0.05$ versus control. HR = heart rate (beats/min); BP = blood pressure (mm Hg); RPP = rate pressure product; CS flow = coronary sinus flow (ml/100 g/min); NO_x Eq = cardiac NO_x production equivalent (nmol/100 g/min).

with oxygen. The primary metabolite of NO is NO_3^- with <10% of the total appearing as NO_2^- (23); NO_2^- concentrations remain constant, whereas those of NO_3^- vary widely (12,16). The sum of NO_2^- and NO_3^- has been designated as NO_x (12). The product of the reaction of NO with oxygen, superoxide and transition metals support additional nitrosative reactions (24). Hibbs et al. (8) showed that L-arginine is required for activation of macrophages to a bactericidal and tumoricidal state. Apparently NO is an intermediate in the oxidation of L-arginine to NO_2^- and NO_3^- and the citrulline pathway (25).

NO_x and myocardial infarction. The results of the present study provide evidence that in patients with anterior AMI at 48 h after onset of symptoms, NO_x production equivalent by the heart is increased together with its coronary arterial–venous difference (Tables 2 and 3). This accounts for the increase of NO_x in arterial and peripheral venous plasma (Table 2, Fig. 1). In experimental myocardial infarction activated macrophages are a major source of NO (26). An elevated NO_x production by the heart and increased concentrations of NO_x in peripheral venous blood have been noted previously in rabbits 3 days after occlusion of a coronary artery (16). This may have been the result of formation of NO by activated macrophages (19). It is possible that both cardiomyocytes and inflammatory cells, primarily activated macrophages, are involved (11).

Although it is likely from the evidence presented here that the elevated levels of NO_x in peripheral plasma observed after myocardial infarction are the result of increased production of NO_x by macrophages in infarcted heart muscle, the possibility must be considered that other factors are involved. Part of plasma NO_x originates as a consequence of the activity of the endothelial form of NO synthase. Evidence for this is the finding of Akiyama et al. (12) that *S*-methylisothiourea, which primarily inhibits iNOS, only partially lowers plasma levels of NO_x. It is unlikely that coronary angioplasty influences the results as collections of plasma for NO_x determination were carried out ≥18 h after angioplasty. Another possible reason for the increase of NO_x in plasma is accumulation of nitrate anion if the production of NO_x exceeds its elimination within the half-life of NO_x (3.8 h) (16). Similarly, diminished clearance of NO_x by the kidney can lead to increased NO_x plasma levels. This may be the case in severe congestive heart failure as reported by Winlaw et al. (27). However, none of the patients in this series had signs of congestive heart failure.

Another possible explanation for increased appearance of NO_x in CS plasma is enhanced NO production originating in coronary vascular endothelium beginning 24 h after reperfusion. Kim et al. (28) have shown that brief ischemic episodes are responsible for enhanced coronary artery endothelial function. This effect is delayed in onset and prolonged in duration. This may be one of the factors responsible for the increased concentration of NO_x in CS plasma of patients after onset of acute myocardial infarction. It also suggests that in certain instances involving multiple factors NO_x in plasma may not be the exclusive reflection of iNOS in heart muscle.

Our observations of increased arteriocoronary sinus difference and of NO_x myocardial production equivalent strongly point to the heart as a source of increased plasma levels of NO_x in myocardial infarction.

Nitric oxide and cardiac disorders. Cardiac diseases other than myocardial infarction are accompanied by changes in plasma concentration of NO_3^- (29,30). Winlaw et al. (27) reported that plasma NO_3^- increased significantly in patients with chronic congestive heart failure; however, because only peripheral plasma concentrations were measured, decrease in renal function may have been partially responsible. Haywood et al. (31) found increased expression of inducible form of nitric oxide synthase messenger RNA in the hearts of patients with chronic congestive heart failure. Langrehr et al. (29) demonstrated in allografted rats a significant increase in NO_2^-/NO_3^- levels in peripheral blood before critical signs of rejection were noted. Treatment with FK-506, an inhibitor of transplant rejection, abolished this increase. Winlaw et al. (32) found in rats an eightfold increased excretion of urinary NO_2^- in untreated allograft rejection. Akiyama et al. (12) also reported reduction of plasma levels of NO_x by *S*-methylisothiourea, a selective inhibitor of iNOS in experimental myocardial infarction in rabbits.

Conclusions. From data presented in this report, no definite conclusions on future applications of NO_x determinations in peripheral blood of patients with myocardial infarction can be drawn. More patients need to be studied and the various parameters responsible for increased NO_x production should be further defined. However, the evidence of a cardiac origin in the increase in plasma NO_x concentration in patients with myocardial infarction appears convincing.

We acknowledge the support of the Sidney Stern Memorial Trust, Pacific Palisades, California, and an unrestricted grant from Pfizer Inc., New York, New York. We also thank the U.C.L.A. Biomathematics Department for their help in carrying out the statistical analysis.

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