BRIEF REPORTS

Myocardial Malondialdehyde and Uric Acid Release After Short-Lasting Coronary Occlusions During Coronary Angioplasty: Potential Mechanisms for Free Radical Generation

I. K. De Scheerder, MD, PhD, A. M. M. van de Kraay, MD, J. M. J. Lamers, PhD, J. F. Koster, PhD, J. W. de Jong, PhD, and P. W. Serruys, MD, PhD

The stunned myocardium has recently become the focus of considerable interest because of its potential role in negating the benefits of reperfusion. A critical but still unresolved issue relates to the mechanism responsible for this contractile abnormality. In recent years an increasing number of studies have provided indirect evidence that postischemic myocardial dysfunction may be mediated in part by the generation of reactive oxygen species, such as superoxide radical, hydrogen peroxide and hydroxyl radical. These oxygen-free radicals could arise from various sources, such as hypoxanthine conversion by xanthine oxidase, catecholamine degradation and mitochondrial electron transport. Direct evidence of injury by free radicals has yet to be shown in the human heart, but many studies of other mammals have linked reactive oxygen metabolites with myocardial injury.[1–5] During myocardial ischemia, xanthine dehydrogenase (which appears to be located in the endothelial cells)[6] is converted to xanthine oxidase, an enzyme that produces superoxide radical, hydrogen peroxide and uric acid from hypoxanthine or xanthine and molecular oxygen.[7] At the same time, ischemia is associated with rapid catabolism of adenosine triphosphate.[7] This degradation of adenosine triphosphate causes an efflux of breakdown products that are able to pass through the cell membrane, resulting in an accumulation of hypoxanthine, 1 of 2 substrates for xanthine oxidase. The other substrate (molecular oxygen) is provided by reperfusion, which results in a burst of free-radical generation.[8] These free radicals initiate chain reactions causing peroxidative breakdown of polyunsaturated fatty acids in the membrane bilayer.[9–12] The interaction among oxygen-free radicals with polyunsaturated fatty acids has been described as lipid peroxidation and can be measured by formation of malondialdehyde. Until recently, the assessment of alterations in myocardial metabolism in humans early after short and repetitive occlusions of a major coronary artery has not been feasible. However, percutaneous transluminal coronary angioplasty provides a unique opportunity to study the time course of these metabolic changes during transient interruption of coronary flow by the balloon-occlusion sequence in patients with 1-vessel disease and without angi-

ographically demonstrable collateral circulation.[13–16] In this report we studied the production of hypoxanthine, urate and malondialdehyde during percutaneous transluminal coronary angioplasty.

All patients met the following criteria: a brief history of angina pectoris (<1 year), an isolated obstructive lesion in the left anterior descending coronary artery, and an accessible stenosis of <1 cm in length. All patients were candidates for coronary artery bypass graft surgery because of disabling angina, but were selected for angioplasty rather than surgery because of their anatomy. Ten patients (8 men and 2 women, aged 37 to 72 years) were studied. Of these, 4 were in New York Heart Association class II, 5 in class III and 1 in class IV. In all, the ejection fraction was >50% and none of them had wall motion abnormalities on their left ventriculograms at rest. Four consecutive transluminal dilatations of 90 seconds each with deflation intervals of 3 minutes were performed. All patients gave informed consent and there were no complications directly related to the research procedure.

A Pepine catheter was inserted in the coronary sinus with the end-hole positioned at the origin of the great cardiac vein for blood sampling. Arteriovenous differences of blood hypoxanthine, urate and malondialdehyde were determined at baseline immediately after each balloon deflation and, finally, 5 and 15 minutes after the percutaneous transluminal coronary angioplasty procedure.

HYPOXANTHINE AND URATE: *Great cardiac vein and femoral arterial blood samples were collected in heparinized tubes with equal volumes of cold 154 mM sodium chloride, containing 20 μM dipyridamole (Boehringer, Ingelheim, Federal Republic of Germany), and 10 μM erythro-9-(2-hydroxy-3-nonyl) adenine hydrochloride (Wellcome, London, Great Britain). These drugs were used to inhibit adenosine uptake and breakdown. Mixtures were centrifuged in the cold. The supernatant fluids were stored at −80°C. On the day of analysis, these were thawed and mixed with cold 8% perchloric acid. Hypoxanthine and urate were assayed by high-performance liquid chromatography using the method of Huizer et al.[17]*

MALONDIALDEHYDE: *After separation of blood cells by centrifugation at 0 to 4°C, the plasma was frozen in liquid nitrogen and stored at −80°C. The malon-*

TABLE I Vein-Arterial Hypoxanthine Difference in the 10 Patients						
	1	2	3	4	5	6
BL	-0.224	0.473	-0.267	-0.446	-0.160	-0.320
Post 1	0.169	0.648	0.805	6.392	0.649	1.990
Post 2	2.606	0.522	2.367	28.410	0.747	1.117
Post 3	1.941	0.891	2.429	23.934	3.039	1.385
Post 4	0.815	3.070	4.163	2.080	0.467	1.510
R5	0.542	-0.069	5.940	0.938	0.562	-0.042
R15	0.336	0.205	6.114	0.415	-0.521	-0.364

	7	8	9	10	Mean ± SEM
BL	0.410	0.447	0.847	0.587	0.14 ± 0.15
Post 1	3.250	0.467	5.968	0.273	2.06 ± 0.75
Post 2	3.160	1.740	2.939	30.910	7.45 ± 3.72
Post 3	1.500	4.010	2.160	27.060	6.83 ± 3.13
Post 4	-0.978	0.840	1.160	7.441	2.06 ± 0.75
R5	-0.010	0.800	-0.660	5.900	1.39 ± 0.76
R15	-0.460	-0.300	-0.440	-0.460	0.45 ± 0.63

Individual hypoxanthine vein-arterial difference in the 10 patients during baseline (BL), immediately after the 4 percutaneous transluminal coronary angioplasty inflations and, finally, 5 and 15 minutes after the percutaneous transluminal coronary angioplasty procedure (R5, R15).
Post = after; SEM = standard error at the mean.

TABLE II Vein-Arterial Urate Difference in the 10 Patients						
	1	2	3	4	5	6
BL	10	7	6	21	-7	4
Postinfl 1	-15	2	6	5	11	5
Postinfl 2	-1	4	-2	2	5	-2
Postinfl 3	-5	6	2	6	19	10
Postinfl 4	5	11	29	6	-28	6
R5	-2	40	43	17	-7	2
R15	-4	5	17	4	-14	-3

	7	8	9	10	Mean ± SEM
BL	-6	-9	6.4	-9	2.34 ± 3.12
Postinfl 1	-2	-3	2	3.7	1.47 ± 2.22
Postinfl 2	3	3.6	5	-2	1.56 ± 0.95
Postinfl 3	8	9	-11	13.8	5.78 ± 2.76
Postinfl 4	18	19.8	-17	30	7.98 ± 5.87
R5	15	29	-14	52	17.50 ± 7.23
R15	3	10	-13	8.2	1.32 ± 3.12

See legend in Table I.
Postinfl = after inflation; other abbreviations as in Table I.

TABLE III Vein-Arterial Malondialdehyde Difference in the 10 Patients						
	1	2	3	4	5	6
BL	-0.32	-0.39	0.96	1.88	-1.67	1.30
Post 1	0.30	1.07	0.83	-0.55	-1.04	1.52
Post 2	1.27	-0.64	-0.1	0.79	1.07	-0.43
Post 3	-1.29	-0.65	3.47	2.73	-0.92	1.06
Post 4	-1.40	-1.00	2.67	2.38	1.5	-1.42
R5	-1.73	1.35	3.5	3.22	-1.48	-2.36
R15	-1.07	0.23	-0.96	-0.23	-0.37	1.39

	7	8	9	10	Mean ± SEM
BL	0.10	-1.88	-0.47	-1.71	-0.22 ± 0.41
Post 1	0.29	1.19	-1.94	1.73	0.34 ± 0.37
Post 2	0.66	1.37	0.31	0.23	0.45 ± 0.22
Post 3	0.93	3.60	2.44	-1.44	0.99 ± 0.63
Post 4	1.01	1.68	2.22	3.85	1.15 ± 0.58
R5	0.59	3.73	5.30	5.28	1.74 ± 0.91
R15	-0.63	1.06	-0.96	0.86	-0.07 ± 0.29

See legend in Table I.

dialdehyde content of the plasma samples was measured in the presence of 100 μM ethylene diaminetetraacetic acid as described by Jackson et al.[18] The extinction coefficient of the thiobarbituric acid product was taken as 1.56×10^5 M-1 cm-1 for all calculations of the malondialdehyde content.

Values are reported as mean ± standard error of the mean. Comparison between results before and after percutaneous transluminal coronary angioplasty and occlusion conditions were evaluated using analysis of variance for repeated measurements. When overall significance was found, multiple comparisons were considered significantly different at $p < 0.05$. The relation between uric acid and malondialdehyde arteriovenous difference was evaluated by linear regression analysis.

HYPOXANTHINE PRODUCTION: We studied hypoxanthine production by measuring vein arterial hypoxanthine concentration. Baseline value was +0.14 ± 0.15 μM. After the first dilatation, vein-arterial hypoxanthine concentration significantly increased to +2.06 ± 0.75 μM ($p < 0.01$ vs baseline), after the second to +7.45 ± 3.7, after the third to +6.83 ± 3.13, and after the fourth to +2.06 ± 0.75 μM. Five minutes after the fourth occlusion the vein-arterial difference remained significantly increased (+1.39 ± 0.76; $p < 0.05$ vs baseline), whereas after 15 minutes recovery vein-arterial difference returned to baseline values (+0.45 ± 0.63 μM; p = not significant [NS] vs baseline). Detailed hypoxanthine production figures of the 10 patients are listed in Table I.

URATE PRODUCTION: We observed a baseline urate vein-arterial difference of +2.34 ± 3.12 μM. After the first dilatation, urate vein-arterial difference

was +1.47 ± 2.22 μM (NS vs baseline), after the second +1.56 ± 0.95 μM (NS vs baseline), after the third +5.78 ± 2.76 μM ($p < 0.01$ vs baseline), and after the fourth +7.98 ± 5.87 ($p < 0.01$ vs baseline). After 5-minute reoxygenation, vein-arterial urate difference further increased to +17.50 ± 7.23 μM ($p < 0.001$), whereas 15 minutes after the last inflation, vein-arterial urate difference normalized (+1.32 ± 3.12 μM, NS). Detailed urate production figures of the 10 patients are listed in Table II.

MALONDIALDEHYDE PRODUCTION: Before percutaneous transluminal coronary angioplasty, vein-arterial malondialdehyde was -0.22 ± 0.41 μM, after the first dilatation it increased to +0.34 ± 0.37 μM (NS vs baseline), after the second occlusion to +0.45 ± 0.22 μM ($p < 0.05$ vs baseline), after the third to +0.99 ± 0.63 μM ($p < 0.01$ vs baseline), and after the fourth to +1.15 ± 0.58 μM ($p < 0.01$ vs base-

*line). After 5-minute reperfusion, vein-arterial ma-
londialdehyde further increased to 1.74 ± 0.91 µM
(p <0.01 vs baseline). Finally, after 15 minutes it re-
turned to baseline levels (−0.07 ± 0.29 µM, NS). De-
tailed malondialdehyde production figures of the 10
patients are shown in Table III.*

The present data show that short repetitive coronary
total occlusions of the left anterior descending artery
during percutaneous transluminal coronary angioplasty
procedure lead to a significant increase of great cardiac
vein-arterial concentrations of hypoxanthine, uric acid
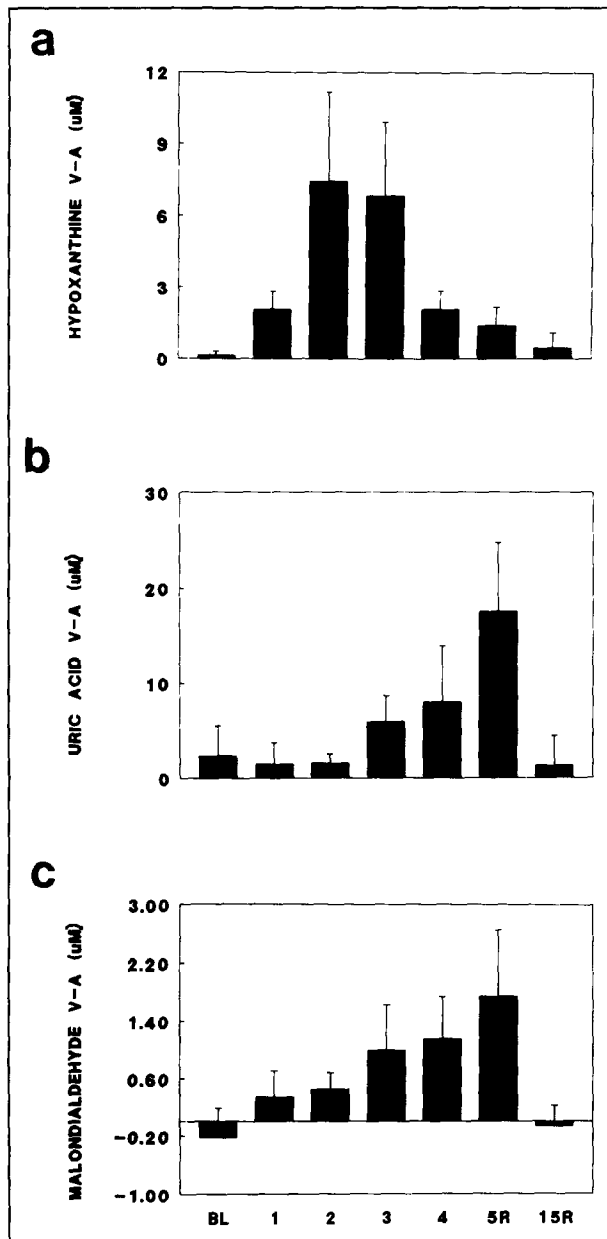and malondialdehyde (Figure 1). For malondialdehyde,

FIGURE 1. Hypoxanthine *(a)*, uric acid *(b)* and malondialde-
hyde *(c)* vein-arterial (V-A) differences during baseline (BL),
immediately after the 4 percutaneous transluminal coronary
angioplasty inflations and, finally, 5 and 15 minutes after the
percutaneous transluminal coronary angioplasty procedure
(5R, 15R).

vein-arterial difference showed a progressive increase
that peaked 5 minutes after the last inflation. The regres-
sion analysis between uric acid and malondialdehyde
vein-arterial difference was not significant (r = 0.34,
NS), which also suggests that other oxygen-free radical
sources may play a role. On average, uric acid vein-
arterial difference parallels that of malondialdehyde, in
contrast to hypoxanthine vein-arterial difference, which
peaked around the second and third inflations. Pro-
duction of malondialdehyde reflects lipid peroxidation,
which suggests production of oxygen-free radicals, so the
production of malondialdehyde in this model likely re-
sults from the generation of oxygen-free radicals.[9-12]
However, malondialdehyde is only one of many products
of lipid peroxidation. The reaction between thiobarbi-
turic acid and malondialdehyde produces a pink color
that can be measured by spectrophotometry. This assay
has been used to study circulating lipoperoxides in many
diseases.[19-21] The assay used in this study usually gives
higher malondialdehyde concentrations than are actually
present because not only is malondialdehyde measured
but also other aldehydes. Furthermore, it measures both
preexisting malondialdehyde and all other substances
that give rise to malondialdehyde during the assay (in
vitro and in vivo). In addition, several chemical species
other than malondialdehyde can react with thiobarbi-
turic acid to produce a pink to red color.[22] This relative
lack of specificity of the thiobarbituric assay has been
criticized, but malondialdhyde appears to be produced in
a relatively constant proportion to the rate of lipid peroxi-
dation[23]; thus, when all samples from 1 patient are as-
sayed simultaneously, the results should reflect the de-
gree of peroxidation. There are several potential sources
of oxygen-free radicals during human myocardial ische-
mia/reperfusion.[1,24] First, ischemia itself may produce
cytotoxic free radicals as a result of reactions involving
mitochondrial electron transfer[25] after catecholamine
degradation by monoamine oxidases, autooxidation or,
what is more likely at physiological pH, metal-catalyzed
oxidation.[1,26] Second, the univalent reduction of oxygen
by activated neutrophils,[1,27] though an important source
of radicals, is probably not relevant in transient ischemia
because it is likely that the chemotactic factors required
to promote neutrophil migration into ischemic tissue re-
quire several hours for full expression.[28] Third, the action
of endothelial xanthine oxidase on purine metabolites
accumulated during ischemia is another possible system
for generating oxygen-free radicals.[19,29,30] Published
data on xanthine oxidase in human myocardium are
controversial,[30] perhaps owing to the fact that the en-
zyme can inactivate itself.[31] We found urate production
after angioplasty,[15] but not by isolated perfused human
heart.[30] The parallel production of urate and malondial-
dehyde after repetitive coronary occlusions in the angio-
plastic procedure suggests the possible importance of this

pathway in the production of oxygen-free radicals in the human ischemic heart. These radicals may play a role in the persistence of regional left ventricle dysfunction after reperfusion.

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Waking Up Exhausted as Risk Indicator of Myocardial Infarction

Ad Appels, PhD, and Erik Schouten

During the last decade several studies have indicated that sleep problems might belong to the risk indicators for coronary artery disease (CAD). For example, a 6-year follow-up study of >10,000 subjects revealed a risk of 2.04 for CAD in "poor" versus "good" sleepers.[1] Little is known about the types of sleep complaints that are associated with future CAD. One may speculate that trouble falling asleep is

associated with CAD, because this is indicative of prolonged tension. Trouble staying asleep may be predictive for the same reason, or because it is indicative of a heart failure or nocturnal angina. Waking up tired may reflect an impaired sleep or an adverse effect of medication, angina pectoris or aging, and lose its predictive power when adjusted for these factors.

Waking up tired may also indicate depression. Recent meta-analyses of the vast literature of personality factors and CAD have shown that, of all personality attributes, depression is the one most strongly associated with disease outcome.[2] Because early-morning tiredness is a major characteristic of depression it is predicted that prob-