Time Course of Free Radical Production After Primary Coronary Angioplasty for Acute Myocardial Infarction and the Effect of Vitamin C

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Reactive oxygen species are thought to mediate reperfusion injury after rapid revascularization for acute myocardial infarction (AMI) and 8-epi prostaglandin (PG) F₂, a free-radical catalyzed product of arachidonic acid, has been proposed as an indicator of oxidative stress in vivo during myocardial reperfusion. The time course of urinary 8-epi PGF₂ excretion after primary coronary angioplasty (PTCA) for AMI was investigated, as well as the effect of prior administration of vitamin C. Urine samples, 1 before and 5 after primary PTCA (0–30, 30–60, 60–90, 90–120 and 120–150 min), were collected in 11 patients with AMI undergoing primary PTCA (Group 1), 10 patients with AMI treated with water-soluble vitamin C at an initial dose of 2.0 g followed by a constant infusion at 20 mg/min prior to primary PTCA (Group 2), and 6 patients with stable effort angina undergoing elective PTCA (Group 3). 8-epi PGF₂ was measured by enzyme immunoassay. There were no significant differences in urinary 8-epi PGF₂ excretion at baseline among the 3 groups. In Group 1, urinary 8-epi PGF₂ excretion (ng/mmol creatinine) significantly increased from 60±8 at baseline to 122±16 at 60–90 min ($p<0.001$), and declined to the baseline level at 120–150 min after primary PTCA. In Group 2, it also increased from 72±12 to 123±15 at 60–90 min ($p<0.01$), and the percent increase did not differ from that in Group 1. In Group 3, it remained unchanged during the study period. The free radical production is rapidly and transiently enhanced after primary PTCA for AMI, and vitamin C fails to suppress it. (*Jpn Circ J* 1999; 63: 924–928)

Key Words: Free radical; Myocardial infarction; Prostaglandin; Reperfusion injury

Early reperfusion is the major management strategy for acute myocardial infarction (AMI). However, the process of reperfusion always encounters an adverse process known as reperfusion injury: reperfusion-induced death of cells due to calcium overload, an expanding area of no reflow, stunned myocardium, and reperfusion arrhythmia.¹ Oxygen-derived free radicals, which are produced at least in part by stimulated leukocytes, are thought to mediate the reperfusion injury.

Arachidonic acid is subjected to oxidative modification, and converted to isomeric species such as isoprostane.² Recently, 8-epi prostaglandin (PG) F₂ (class IV of isoprostane) was shown to be potentially useful as a specific, chemically stable, noninvasive index of free radical generation in vivo.^{3–5} Reilly et al demonstrated that urinary 8-epi PGF₂ excretion was increased in the first 6 h after thrombolysis with or without subsequent coronary angioplasty (PTCA).⁵ However, the time course of the urinary 8-epi PGF₂ excretion during the first 6 h still remains unclear. Furthermore, the effect of primary PTCA on the urinary 8-epi PGF₂ excretion was not necessarily investigated.

On the other hand, the efficacy of antioxidant treatment in reperfusion injury is a matter of controversy.^{6,7} It is unclear whether the discrepancy is related to a lack of ther-

apeutic effect of the agent, the dose selected, the nonspecific index of oxidant stress, or the relative unimportance of oxidant stress in its clinical setting. Remarkably, the most appropriate antioxidant dosing regimen for vitamin C remains to be determined. Thus, we investigated the time course of urinary 8-epi PGF₂ excretion after primary PTCA for AMI and evaluated the effect of an antioxidant regimen of vitamin C in this setting.

Methods

Study Patients

The study protocol was approved by the Ethics Committee of the University Hospital, and informed consent was obtained from all patients. Patients with AMI and stable effort angina were included in this study (Table 1). The patients with AMI were randomly divided into 2 groups. All patients had typical chest pain with ST-segment elevation on their initial electrocardiogram. In Group 1 (11 patients), urinary excretion of 8-epi PGF₂ after primary PTCA was characterized. In Group 2 (10 patients), after treatment with water-soluble antioxidant vitamin C, which was intravenously infused at an initial dose of 2000 mg followed by a constant infusion at 20 mg/min, primary PTCA was performed and excretion of 8-epi PGF₂ was characterized. The constant infusion of vitamin C was continued throughout the study period. The duration from the onset of AMI to the admission to hospital was 166±18 min in Group 1 and 192±26 min in Group 2. In 6 patients with stable effort angina (Group 3), excretion of 8-epi PGF₂ after elective PTCA was characterized. The PTCA

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Table 1 Clinical and Angiographic Characteristics of the Study Groups

Characteristics	AMI undergoing direct PTCA		Angina pectoris
	Group 1 (vitamin C (-)) (n=11)	Group 2 (vitamin C (+)) (n=10)	Group 3 (elective PTCA) (n=6)
Age (years)	68±4	63±3	63±4
Sex (M/F)	8/3	6/4	5/1
Risk factors			
Hypertension (n)	4/11	4/10	3/6
Diabetes (n)	3/11	2/10	3/6
Cigarette smoking (n)	4/11	4/10	3/6
Previous MI (n)	4/11	3/10	2/6
Time to PTCA (min)*	85±19	91±28	—
Culprit vessel (n)			
LAD	3/11	4/10	2/6
LCx	3/11	2/10	1/6
RCA	5/11	4/10	3/6
Peak creatine kinase (U/L)	4193±839	3622±724	—
Stent implantation (n)	6/11	3/10	4/6
Killip class at admission ≥II (n)	2/11	1/10	—
Forrester subset at admission ≥II (n)	7/11	4/10	—

LAD, left anterior descending coronary artery; LCx, left circumflex artery; RCA, right coronary artery; MI, myocardial infarction; AMI, acute MI.

*Time from arrival in emergency department.

Numerical variables are mean ± 1 standard error.

procedure was successful in all patients and TIMI (Thrombolysis in Myocardial Infarction) grade 3 flow was obtained by the procedure. There was no acute vessel closure in any of the study patients.

PTCA and Urine Sampling

All patients with AMI were administered oral aspirin (162 mg) at the time of admission. The patients with stable effort angina were administered oral aspirin (81 mg) at least 1 week before the study. PTCA was performed with the use of standard over-the-wire balloons and floppy guidewires after bolus injection of heparin sulfate (5000 units). When the optimal angiographic result, including residual stenosis <25% and absence of major dissection of the intima, was not obtained, intracoronary stent implantation was performed. Neither cardiogenic shock nor ventricular fibrillation developed during the procedure in any of the patients.

For serial urine sampling, a balloon catheter was inserted into the bladder in all patients. Urine was collected for every 30-min period at baseline and after the first balloon inflation for 150 min.

8-Epi PGF₂ Analysis

The urinary 8-epi PGF₂ level was determined with enzyme immunoassay after extraction by C-18 solid column and separation by thin-layer chromatography.^{3,8} Urine samples were collected in polyethylene tubes containing 0.1% of the antioxidant butylated hydroxyanisole, kept refrigerated during the collection period, and immediately aliquoted and stored at -80°C until the assay. Urine samples were acidified to pH below 3.0 with HCl, and centrifuged to discard the pellets. The supernatant was applied to the C-18 cartridge column, and the 8-epi PGF₂ fraction was eluted with 5 ml ethyl acetate containing 1% methanol. After being evaporated in vacuo to dryness, the sample and standard were applied to a silica gel thin-layer chromatography plate (Whatman International Ltd, USA) and developed using a mobile phase of chloroform, methanol, acetic acid, and water (80:18:1:0.8 v/v). After the authentic standard was colored with 3.5% phosphomolybdic acid, the band corresponding to 8-epi PGF₂ was

scraped and eluted with ethanol. After the supernatant was evaporated in vacuo to dryness, urinary 8-epi PGF₂ level was quantified by an enzyme immunoassay kit (Cayman Chemical Company, USA) and corrected with the recovery rate, which was determined by the addition of internal standard 8-epi PGF₂ to each sample before the extraction procedure. Urinary creatinine was measured in each sample by spectrophotometry and urinary 8-epi PGF₂ excretion was expressed as nanograms per millimole of creatinine.

Statistical Analysis

All data are expressed as mean ± 1 standard error. The change in urinary 8-epi PGF₂ excretion after PTCA was analyzed by one-way ANOVA for repeated measures in each group. Comparison of the percent changes in urinary 8-epi PGF₂ excretion after PTCA among the 3 groups was carried out by one-way ANOVA. A p value <0.05 was considered to be statistically significant.

Results

Comparison of Clinical Profiles Among the 3 Groups

There were no significant differences in age, sex and risk factors, such as hypertension, diabetes, smoking, and previous history of myocardial infarction, among the 3 groups (Table 1). Neither distribution of the culprit vessel nor the frequency of stent implantation differed among the 3 groups. The peak values of creatine kinase, Killip classes, and Forrester subsets were similar between groups 1 and 2. The left ventricular ejection fraction (LVEF) measured by left ventriculography at admission was similar between group 1 (49±4%) and group 2 (51±4%) (p=NS). The LVEF at 3–4 weeks after the onset was also similar between group 1 (51±4%) and group 2 (55±6%) (p=NS). The cardiac index (L min⁻¹ m⁻²), measured by thermodilution procedure at admission, was similar between group 1 (2.4±0.2) and group 2 (2.3±0.1) (p=NS), and that at 3–4 weeks after the onset was also similar between group 1 (2.8±0.2) and group 2 (2.9±0.2) (p=NS). Neither the incidence of ST elevation >0.1 mV or development of cardiac arrhythmia immediately after primary PTCA differed between groups 1 and 2.

Fig 1. The percent change in urinary 8-epi prostaglandin (PG) F₂ excretion from the baseline value in the 3 groups.

Urinary 8-epi PGF₂ Excretion

There was no significant difference in urinary 8-epi PGF₂ excretion at baseline among the 3 groups. In group 1, urinary 8-epi PGF₂ excretion was 60 ± 8 ng/mmol creatinine at baseline, 81 ± 15 at 0–30 min, 93 ± 13 at 30–60 min, and 122 ± 16 at 60–90 min ($p < 0.001$ vs the baseline value) after direct PTCA. Then the levels declined to the preprocedural values at 120–150 min after direct PTCA (82 ± 8). The percent changes in urinary 8-epi PGF₂ at each sampling period from the baseline value are shown in Fig 1. There was a heterogeneity in the increment in urinary 8-epi PGF₂ excretion among individual patients, and the maximal fold-increases over the respective baseline values ranged from 1.1 to 3.2. The fold-increase did not correlate with any of the peak values of creatine kinase, the site of culprit vessel, or the duration from the onset of AMI to the successful reperfusion.

In group 2, the time course of urinary 8-epi PGF₂ excretion was almost similar to that in group 1. The level of urinary 8-epi PGF₂ excretion was 72 ± 12 ng/mmol creatinine at baseline, 98 ± 26 at 0–30 min, 104 ± 13 at 30–60 min and 123 ± 15 at 60–90 min ($p = 0.0014$ vs the baseline value) after direct PTCA. The levels promptly decreased to the baseline values at 120–150 min after the procedure. The maximal fold-increases over the respective baseline value ranged from 1.1 to 3.9.

In group 3, urinary 8-epi PGF₂ excretion unchanged during the study period. Urinary 8-epi PGF₂ level was 67 ± 10 at baseline, 79 ± 14 at 0–30 min, 82 ± 14 at 30–60 min, 72 ± 9 at 60–90 min, 69 ± 9 at 90–120 min, and 79 ± 15 at 120–150 min after the elective PTCA.

The percent changes in urinary 8-epi PGF₂ excretion after PTCA from the baseline value were compared among the 3 groups (Fig 1). The percent increases in urinary 8-epi PGF₂ excretion at 60–90 min after PTCA in groups 1 and 2 were both significantly greater than that in group 3. However, there was no statistical difference between groups 1 and 2.

Discussion

The present study showed that urinary 8-epi PGF₂

excretion was increased in patients with AMI after direct PTCA and peaked at 60–90 min after the procedure and that pretreatment with vitamin C had no influence on the time course of urinary 8-epi PGF₂ excretion after primary PTCA. Because elective PTCA in patients with stable effort angina did not affect urinary 8-epi PGF₂, the increase in urinary 8-epi PGF₂ excretion was not induced by the PTCA procedure but by acute reperfusion of the occluded coronary artery.

Although 8-epi PGF₂ could be generated in a free radical-dependent manner, it was also reported to be a minor product of cyclooxygenase-1 in activated human platelets^{3,4} and of cyclooxygenase-2 in human monocytes *in vitro*.⁹ Inhibition of platelet COX-1 was shown to have no effect on urinary 8-epi PGF₂, despite a reduction in serum 8-epi PGF₂ and serum thromboxane B₂.¹⁰ Furthermore, there was a positive correlation between 8-epi PGF₂ (class IV) and isoprostane F₂ (class I) which is not formed by cyclooxygenase *in vitro* and *in vivo*.⁵ These data suggest that although the capacity for cyclooxygenase-dependent formation of 8-epi PGF₂ may be expressed *ex vivo*, this pathway contributes in an undetectable manner to the urinary level of the compound. The increased excretion of urinary 8-epi PGF₂ has been reported *in vivo* in an animal model of free radical injury,¹¹ in humans after poisoning with acetaminophen and paraquat,¹² in patients with alcoholic liver disease,¹³ and in chronic cigarette smokers.¹⁴ In addition, 8-epi PGF₂ has been detected in human atherosclerotic plaque and in macrophages and smooth muscle cells within the atherosclerotic lesion.¹⁵

Recently, the urinary 8-epi PGF₂ excretion during the first 6 h after thrombolysis with or without subsequent PTCA was reported to be increased.⁵ In the present study, we further characterized the time course of 8-epi PGF₂ excretion after primary PTCA for AMI using an enzyme immunoassay method. The results demonstrated that the urinary excretion of 8-epi PGF₂ peaked at 60–90 min and returned toward the baseline at 120–150 min after primary PTCA in patients with AMI. This indicates that the generation of free radicals after PTCA is prompt, but transient, as reported in the animal model of coronary occlusion and reperfusion. No increase in urinary 8-epi PGF₂ excretion

was observed in patients undergoing elective PTCA, suggesting that the procedure of catheterization per se is not associated with the isoprostane generation. The present results failed to find a relationship between the increase in its compound and the peak value of creatine kinase, as consistent with the previous report.⁵ A possible explanation is that regardless of the size of the AMI, the free-radical production after reperfusion therapy may be stimulated maximally.

Vitamin C could directly scavenge superoxide anion, and thereby decrease nitric oxide inactivation and suppress peroxynitrite-mediated inactivation of target enzymes.¹⁶⁻¹⁹ Thus, we assessed the efficacy of water-soluble antioxidant vitamin C by measuring urinary 8-epi PGF₂ excretion of serially collected samples. However, the effect of water-soluble antioxidant vitamin C was not found in patients undergoing PTCA for AMI. The reaction rate constant between vitamin C and superoxide anion is estimated to be $3 \times 10^5 \text{ mol L}^{-1} \text{ s}^{-1}$,²⁰⁻²² whereas that between superoxide dismutase and superoxide anion is $2 \times 10^9 \text{ mol L}^{-1} \text{ s}^{-1}$.²²⁻²⁴ Despite a 10^4 times slower reaction rate observed with vitamin C, tissue vitamin C concentration is 10^4 times higher than that of superoxide dismutase,^{20,23,25} enabling vitamin C to competitively scavenge superoxide anion. In the present study, vitamin C was infused at a constant dose of 20 mg/min after the initial bolus injection of 2000 mg to achieve a local concentration of 1–10 mmol/L in the coronary artery. This concentration of vitamin C has been shown to completely protect human plasma from free-radical mediated lipid peroxidation in vitro²⁶ and to improve endothelium-dependent vasodilation in noninsulin-dependent diabetes,¹⁸ smokers,²⁷ coronary artery disease,²⁸ hypertension,²⁹ and hypercholesterolemia³⁰⁻³² in vivo. Thus, the lack of efficacy of vitamin C seems to be due not to the dose selected, but to the therapeutic ability of vitamin C. One possible explanation for the lack of the efficacy is that the acute, abundant production of free radicals induced by the reperfusion therapy is too potent to be scavenged by vitamin C.^{4,8,33} Further studies are required to elucidate this issue.

The candidates for the increase in free-radical production include nicotinamide adenine dinucleotide phosphate oxidases;³⁴ xanthine oxidase;³⁵ cytosolic forms of phospholipase A₂^{36,37} and mitochondrial oxidative phosphorylation defects.³⁸ Because vitamin C could directly affect the superoxide production by nicotinamide adenine dinucleotide phosphate oxidase,^{17,19} mechanisms other than nicotinamide adenine dinucleotide phosphate oxidase may be important in the production of free radicals during reperfusion therapy. The major source of superoxide in postischemic tissues has been shown to be xanthine oxidase.³⁹ Although the enzyme is initially synthesized as xanthine dehydrogenase, its conversion to xanthine oxidase occurs in vivo in ischemic tissues. Concomitantly, the depletion of intracellular adenosine triphosphate results in an elevated concentration of adenosine monophosphate, and it is catabolized to hypoxanthine, a substrate for xanthine oxidase. Further studies on the effect of inhibiting the superoxide-producing enzymes, especially xanthine oxidase, are required.

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