Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 43, No. 11, 2004
ISSN 0735-1097/04/$30.00
doi:10.1016/j.jacc.2004.01.036

Hydroxyl Radical Generation, Levels of Tumor Necrosis Factor-Alpha, and Progression to Heart Failure After Acute Myocardial Infarction

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OBJECTIVES We used acetylsalicylic acid (ASA) as a probing agent to quantify hydroxyl radical (·OH) in Controls and patients with coronary artery disease and to prospectively investigate ·OH production in patients with myocardial infarction (MI) complicated by heart failure (HF).

BACKGROUND Oxidative stress status (OSS) is a mechanism for transition to HF in experimental heart injury models, but evidence for its causal role in humans is still limited.

METHODS Thirty healthy subjects (Controls), 12 patients with stable angina (Group 1), and 74 patients with ST-segment elevation MI (Group 2) were enrolled. A dose of 250 mg Flectadol was given intravenously before each blood collection to determine the 2,3-dihydroxybenzoic acid/salicylic acid (DHBA/SA) ratio. We also quantified vitamin E and coenzyme Q_{10} to monitor antioxidant reserve, as well as tumor necrosis factor (TNF)-alpha, TNF-soluble receptors, interleukin (IL)-6, and IL-1ra to assess inflammatory status. All measurements were repeated at month 6 in Group 2.

RESULTS There were no differences between Controls and Group 1. Group 2 showed increased ·OH production, peaking at 24 h, whereas vitamin E and coenzyme Q_{10} progressively declined. Group 2 patients developing HF during hospitalization (Group 2Bi) presented with an increase of both ·OH production at discharge and inflammatory status, as compared with patients without HF (Group 2Ai), persisting at month 6 in post-MI patients with HF (Group 2Bii).

CONCLUSIONS We found a distinct pattern of ·OH generation in post-MI patients who show progression to HF. The interplay between OSS and inflammatory status should be targeted as a possible mechanism of progression to post-MI left ventricular dysfunction. (J Am Coll Cardiol 2004;43:2000–8) © 2004 by the American College of Cardiology Foundation

An imbalance of oxidative stress status (OSS) has been reported to occur in acute coronary syndromes and heart failure (HF) (1). Elevation of reactive oxygen species (ROS) during myocardial ischemia occurs both at an early stage—during reperfusion (2), contributing to stunning (3,4) and reperfusion injury (5,6)—and at a late phase—days or weeks after myocardial infarction (MI) (7). Experimental studies have shown that ROS production at this later stage is convincingly associated with post-MI left ventricular (LV) remodeling and progression to HF (8–11).

An increase of lipid peroxidation markers and encroachment on antioxidant reserves in chronic HF have been widely reported in humans (12–14), but no prospective data are currently available on the association between (increased) OSS and progression to HF in post-MI patients (15).

A radical probing technique for in vivo OSS measurement, based on the ability of hydroxyl radical (·OH) to attack the benzene ring of aromatic molecules, such as acetylsalicylic acid (ASA), and to produce hydroxylated compounds, such as 2,3-dihydroxybenzoic acid (DHBA), has been previously employed in humans (Fig. 1) (16,17). This method seems particularly interesting in patients with coronary artery disease, in whom ASA is indicated for primary and secondary prevention of acute cardiovascular events (18).

We aimed to prospectively investigate the pattern of ·OH elevation in MI patients with and without transition to HF by using a recommended intravenous dose of ASA (19) as a probe for ·OH production in vivo. Healthy subjects and stable patients with coronary artery disease were similarly investigated.

Endogenous antioxidant molecules—vitamin E and coenzyme Q_{10}—and inflammatory status—tumor necrosis factor (TNF)-alpha, related soluble receptors of tumor necrosis factor-alpha (sTNFR), interleukin (IL)-6, and IL-1ra—were also monitored.

METHODS

Study population. Thirty healthy subjects and 86 patients were enrolled. Their clinical and biochemical profiles are presented in Table 1. The local Ethics Committee on Human Research approved the study protocol, and all participants gave their written, informed consent to participate in the study.

Three groups were considered: *Controls* comprised 30

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Manuscript received December 16, 2003; accepted January 7, 2004.

Abbreviations and Acronyms

ASA = acetylsalicylic acid
DHBA = dihydroxybenzoic acid
HF = heart failure
IL = interleukin
LV = left ventricular
MI = myocardial infarction
·OH = hydroxyl radical
OSS = oxidative stress status
ROS = reactive oxygen species
SA = salicylic acid
sTNFR = related soluble receptors of tumor necrosis factor-alpha
TNF = tumor necrosis factor

healthy subjects who were matched in terms of gender and age (19 men; mean age 65 ± 6 years) to the patients. None had clinical signs of acute or chronic illness or was receiving any treatment. *Group 1* comprised 12 patients (8 men; mean age 68 ± 11 years) with angiographically documented coronary atherosclerotic lesions and stable angina (Canadian Cardiovascular Society classes II and III). They were treated with beta-blockers (n = 11), statins (n = 12), angiotensin-converting enzyme (ACE) inhibitors (n = 9), and calcium channel blockers (n = 5). *Group 2* comprised 74 patients (49 men; mean age 67 ± 10 years) consecutively admitted for acute MI, defined as the occurrence of typical chest pain at rest lasting >20 min, accompanied by persistent ST-segment elevation of ≥1 mm in at least two standard electrocardiographic leads or ≥2 mm in at least two contiguous precordial leads. In all Group 2 patients, MI was confirmed by a rise in serum creatine kinase by twofold or more than the upper normal limit during hospitalization.

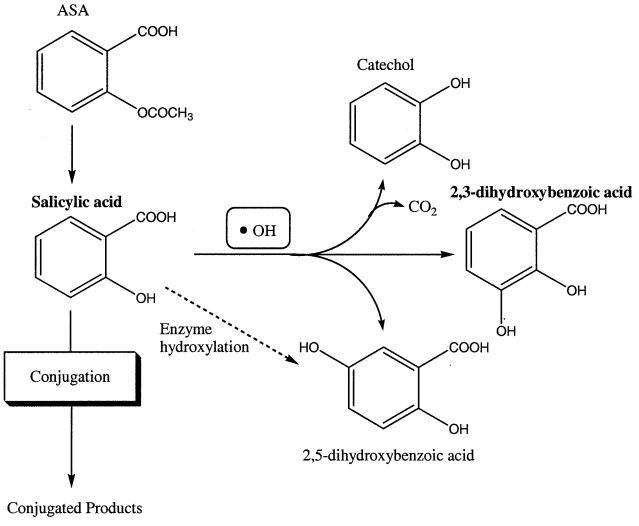

Figure 1. Oxidative metabolism of salicylic acid: acetylsalicylic acid (ASA) is rapidly hydrolyzed to SA by esterases and for 60% remains unmodified and can undergo hydroxyl radical (·OH) attack to produce two derivates—namely, 2,3-dihydroxybenzoic acid (DHBA) and 2,5-DHBA. Enzymatic pathways through the cytochrome P-450 system can also produce the latter acid, whereas the former acid is solely formed by direct ·OH attack. Therefore, measurement of 2,3-DHBA or the 2,3-DHBA/SA ratio, after administration of ASA, is recognized as a sensitive and specific method to determine ·OH production in vivo.

Exclusion criteria were symptoms lasting >12 h before hospitalization, history of HF before hospitalization, presence of any known neoplastic disease, diseases affecting the immune system, and ongoing infectious diseases. The infarct location was anterior in 36 patients. Fifty-five patients (74%) received accelerated tissue plasminogen activator. All Group 2 patients were receiving a standard regimen of beta-blocker, statin, and ACE inhibitor.

The occurrence of HF was defined as the presence of rest or effort dyspnea and at least one of the following: pulmonary rales at lung auscultation, evidence of pulmonary congestion on the chest X-ray, new appearance of peripheral edema, and use of diuretics.

Blood processing. PLASMA. Antecubital venous blood was collected in K^3 EDTA-containing tubes, immediately centrifuged at 1,700*g* at 4°C for 15 min, and subsequently stored at −80°C. Plasma vials that would be used to measure 2,3-DHBA/SA were also frozen in liquid nitrogen before storage.

SERUM. Antecubital venous blood was collected in empty tubes and, after 45 min, centrifuged at 1,700*g* at 4°C for 15 min. The serum obtained was stored at −80°C.

·OH probing in vivo. The principle behind ·OH quantification is schematically shown in Figure 1 (16,17). A dose of 250 mg Flectadol was given intravenously 5 min before each blood collection. The time lag between Flectadol administration and blood collection was chosen after pilot observations in patients (n = 13) or Controls (n = 10), showing no significant changes in the 2,3-DHBA/SA ratio in the range of 3 to 20 min.

The kinetics of ·OH production was established in five Controls, all Group 1 patients, and 10 Group 2 patients by sampling at 6, 12, 24, 36, 48, and 72 h after symptom onset and then after 5 and 7 days and at discharge (9 ± 3 days). Based on the results obtained, the remaining 25 Controls and 64 Group 2 patients were studied at entry and at 24, 48, and 72 h after symptom onset and at discharge (10 ± 4 days).

Extraction and quantification of 2,3-DHBA and 2,5-DHBA. Aliquots of standard solutions or plasma samples (500 μl) were mixed with 20 μl of 5 μmol/l 3,4-DHBA (internal standard) and acidified with 25 μl of concentrated HCl (37%) in glass tubes. The samples were vortexed for 1 min and centrifuged at 2,000 *g* for 15 min. Ether (%ml) was added to the supernatant. The samples were vortexed for 1 min and centrifuged at 2,000*g* for 15 min. The ether phase was extracted. The extraction was repeated (with 3 ml of ether), and the ether phases were recollected. The ether phase was then dried under nitrogen steam. The dry residue was reconstituted in 500 μl of mobile phase and filtered on 0.22-μm filters (Millipore USA), and 100 μl was injected into the column (XTerra RP_{18}—3.5 μm, 4.6 × 150-mm cartridge columns; Waters, Milford, Massachusetts). Chro-