8-Oxo-dGTPase, Which Prevents Oxidative Stress-Induced DNA Damage, Increases in the Mitochondria From Failing Hearts

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Background—Reactive oxygen species (ROS) can cause an oxidative modification of nucleotides, such as 8-oxo-7,8-dihydrodeoxyguanosine triphosphate (8-oxo-dGTP), which can lead to defects in DNA replication. The misincorporation of 8-oxo-dGTP into DNA is prevented by 8-oxo-dGTPase, which hydrolyzes 8-oxo-dGTP into 8-oxo-dGMP. The changes in this defensive system have not yet been examined in failing hearts, in which the generation of ROS increases.

Methods and Results—Myocardial infarction (MI) was created in mice by ligating the left coronary artery. Four weeks later, the left ventricle was dilated and contractility was diminished on echocardiography. The generation of ROS, as measured by electron spin resonance spectroscopy with 4-hydroxy-2,2,6,6-tetramethyl-piperidine-N-oxyl, increased in the noninfarcted left ventricle from MI mice. The formation of thiobarbituric acid–reactive substances also increased in the mitochondria from MI mice. 8-Oxo-dGTPase was detected in the mitochondrial fractions isolated from MI mice using a Western blot analysis with an antibody to its human homologue (hMTH1). Immunohistochemistry showed positive staining for hMTH1 was localized in the cardiac myocytes.

Conclusions—The level of 8-oxo-dGTPase increased in the mitochondria isolated from post-MI hearts as oxidative stress increased, thus suggesting that a preventive mechanism is activated against ROS-induced DNA damage. As a result, 8-oxo-dGTPase is considered a useful marker of mitochondrial oxidative stress in heart failure. (Circulation. 2001;104:2883-2885.)

Key Words: free radicals ■ heart failure ■ DNA ■ mitochondria

R

Reactive oxygen species (ROS) can damage various cellular components, such as proteins, lipids, and DNAs. Specifically, ROS produce 8-oxo-7,8-dihydrodeoxyguanosine triphosphate (8-oxo-dGTP), which modifies the bases in DNA and generates 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG).1 As a result, 8-oxo-dGTP is considered a potent mutagenic substrate for DNA replication.2 Oxidative DNA damage has been detected in such clinical settings as cancer and neurodegenerative diseases.3 However, limited data are available regarding hearts, except for an increased level of 8-oxo-dG formation in aged hearts3 and in superoxide dismutase 2 mutant mice.4

Escherichia coli MutT protein (8-oxo-dGTPase), which hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP, prevents the misincorporation of 8-oxo-dGTP into DNA.2,5 MutM and mutY correct the mis-pairs caused by the incorporation of 8-oxo-dG into DNA.5 Recently, a human functional homologue of the MutT protein (hMTH1 for mutT homologue 1) was shown to increase in the mitochondria of substantia nigral neurons from patients with Parkinson’s disease in association with oxidative stress.6 We recently showed that the increased generation of ROS was associated with mitochondrial DNA damage and dysfunction in failing hearts after myocardial infarction (MI).7 However, the expression of 8-oxo-dGTPase, which can prevent oxidative DNA damage, has not yet been examined in failing hearts. In view of the increasing evidence of oxidative stress in myocardial remodeling and failure,8,9 it is of considerable interest to examine whether changes in 8-oxo-dGTPase may exist in this setting. Therefore, the objective of the present study was to examine the changes in 8-oxo-dGTPase in a murine model of heart failure due to MI.7,9

Methods

Animal Model

This study was approved by our Institutional Animal Research Committee and conformed to the animal care guidelines of the American Physiological Society. MI was created in male CD-1 mice (5 to 8 weeks old; 25 to 35 g of body weight) by ligating the left coronary artery.

Received August 29, 2001; revision received October 23, 2001; accepted October 23, 2001.

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Circulation is available at http://www.circulationaha.org

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Left Ventricular Function
On the day of the terminal study, 4 weeks after surgery, in vivo left ventricular (LV) function studies, including echocardiography and pressure measurements, were performed.

Myocardial Tissue Preparation
The myocardial tissue specimens with MI were carefully dissected into 2 parts: one consisting of the infarcted LV with the peri-infarct rim (a 0.5- to 1-mm rim of normal-appearing tissue) and the remaining, noninfarcted LV. In all subsequent assays, comparisons were made between noninfarcted LV from MI animals and control LV from sham-operated animals.

To isolate the mitochondrial fraction, myocardial tissue specimens (70 to 80 mg) were minced and homogenized at 4°C for 30 s in 12 volumes of HES buffer (HEPES, EDTA, and sucrose), which consisted of 10 mmol/L HEPES-NaOH (pH 7.4), 1 mmol/L EDTA, and 250 mmol/L sucrose, with a Teflon pestle in a glass homogenizer. The homogenate was centrifuged at 4°C and 700 000 g for 10 minutes to remove any nuclear and myofibrillar debris, and the resultant supernatant was then subsequently centrifuged at 7000 g for 10 minutes. The resultant pellet was then resuspended and washed 3 times with HES buffer (∼70 μg of mitochondrial protein).

ROS and Thiobarbituric Acid–Reactive Substances
ROS were quantified using electron spin resonance (ESR) spectroscopy with 4-hydroxy-2,2,6,6-tetramethyl-piperidine-N-oxyl (hydroxy-TEMPO) as described previously. Thiobarbituric acid–reactive substances (TBARS) were measured in the mitochondrial fractions.

Western Blot Analysis and Immunohistochemistry of hMTH1
For a Western blot analysis of hMTH1, the mitochondrial proteins were separated by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After blocking, the blots were incubated with a polyclonal antibody (dilution 1:3000) produced by immunizing rabbits with synthetic peptides corresponding to the amino acid sequences of Gln 140 to Val 156 of hMTH1 at 4°C overnight. This polyclonal antibody (dilution 1:200) and peroxidase-labeled streptavidin 0.05% Tween-20 and a reaction with biotin-labeled goat anti-rabbit IgG antiserum were separated by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Echocardiographic Data, Hemodynamic Data, Organ Weights, and Oxidative Stress

<table>
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<th>MI (n=9)</th>
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<tbody>
<tr>
<td>Echocardiographic data</td>
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<tr>
<td>LV end-diastolic diameter, mm</td>
<td>3.4±0.1</td>
<td>6.1±0.3†</td>
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<tr>
<td>LV end-systolic diameter, mm</td>
<td>1.9±0.1</td>
<td>5.1±0.3†</td>
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<td>Fractional shortening, %</td>
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<td>Hemodynamic data</td>
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<td>LV end-diastolic pressure, mm Hg</td>
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<td>LV dP/dt max, mm Hg</td>
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<td>9620±362*</td>
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<td>Pleural effusion, %</td>
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<td>44*</td>
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<td>Oxidative stress</td>
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<tr>
<td>Rate of ESR signal decay, min⁻¹</td>
<td>0.061±0.003</td>
<td>0.091±0.005†</td>
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<td>Mitochondrial TBARS, nmol/g wet weight</td>
<td>3.3±1.1</td>
<td>12.0±2.3*</td>
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</tbody>
</table>

Data are expressed as means±SEM. *P<0.05 and †P<0.01 vs sham.

All MI mice showed moderate to severe immunostaining for hMTH1 in the cardiac myocytes, but no other cells (such as vascular smooth muscle cells and endothelial cells) were stained (data not shown).

Discussion
8-Oxo-dGTPase was highly expressed in cardiac myocytes from post-MI failing hearts, thus suggesting that this enzymatic system preventing oxidative DNA damage may be activated in response to increased oxidative stress.

We recently demonstrated that increased ROS were associated with mitochondrial DNA (mtDNA) damage and dysfunction in post-MI failing hearts. DNA is one of the most important targets of ROS-mediated damage, and 8-oxo-dG, which is produced by the ROS-mediated 8-hydroxylation of the guanine base, is a well-established marker of oxidative DNA damage. 8-oxo-dGTPase acts as the first line of defense against ROS-mediated DNA damage and also plays a major role in the prevention of oxidative DNA modifications. As a result, the increase of 8-oxo-dGTPase in post-MI failing hearts may be a reactive response to enhanced oxidative stress. This increase was not observed in MI mice when treated with antioxidant, dimethyliourea (data not shown), suggesting an important role of ROS in the induction of this enzyme. In...
In this regard, it was recently shown that 8-oxo-dG and 8-oxo-dGTPase are increased in the mitochondria of substantia nigral neurons from patients with Parkinson’s disease.6

8-Oxo-dGTPase was increased in the mitochondrial fractions from MI mice. Further, 8-oxo-dGTPase seemed to be localized within the cytoplasm of cardiac myocytes, even though we could not exclude the possible contribution of myocyte apoptosis. These results may be important in light of evidence that the mitochondria are a potential source of ROS in failing hearts.7 The increase of 8-oxo-dGTPase might play an important role in protecting mtDNA from an ROS attack, because the integrity of mtDNA is essential for maintaining the normal function of the mitochondria and their dysfunction may lead to a decline in the respiratory chain function.10 However, further studies are needed to clarify the functional significance of 8-oxo-dGTPase expression by using mice with targeting or overexpression of this gene. Recently, Ballinger et al11 demonstrated that mitochondrial DNA damage quantitated by polymerase chain reaction is a sensitive and specific marker of increased ROS. 8-Oxo-dGTPase could provide similar information regarding increased ROS, with a sensitivity and specificity comparable to that of mtDNA damage, because the decrease in the mtDNA copy number1 and the increase in the 8-oxo-dGTPase were concurrently demonstrated in the same animal model. Similar to 8-oxo-dG, which can be quantified as a measure of DNA oxidation, 8-oxo-dGTPase could also serve as an indicator for oxidative damage in situ.12 Although 8-oxo-dGTP could be a direct marker for mitochondrial oxidative stress, it is impossible to measure it in biological tissue due to its small amount. Further, DNA repair enzymes, such as DNA polymerase β, might also be altered in failing hearts.

In summary, 8-oxo-dGTPase may be induced as adaptive cardioprotective response to increased oxidative stress in post-MI failing hearts.

Acknowledgments

This study was supported in part by grants from the Ministry of Education, Science and Culture of Japan (No. 09670724 and 12670676). We also thank Brian Quinn for critically reading the manuscript.

References

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_Circulation._ 2001;104:2883-2885
doi: 10.1161/hc4901.101347

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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http://circ.ahajournals.org/content/104/24/2883

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