Effect of Dexrazoxane on Homocysteine-Induced Endothelial Dysfunction in Normal Subjects

Haoyi Zheng, Clarito Dimayuga, Alhakam Hudaihed, Stuart D. Katz

Objective—Dexrazoxane is an antioxidant prodrug that on hydrolysis is converted into an intracellular iron chelator. We hypothesized that the antioxidant effects of dexrazoxane would prevent homocysteine-induced endothelial dysfunction in the brachial artery of normal human subjects.

Methods and Results—Ten healthy volunteers completed a randomized, double-blind, crossover study. Plasma homocysteine levels and brachial artery endothelium-dependent (flow-mediated dilation [FMD]) and endothelium-independent (sublingual nitroglycerin) responses were measured before and 4 hours after ingestion of L-methionine (100 mg/kg), preceded by intravenous administration of dexrazoxane (500 mg/m²) or placebo over 30 minutes. After placebo, oral methionine increased plasma homocysteine (from 5.1±0.4 μmol/L at baseline to 14.2±1.3 μmol/L at 4 hours, P<0.001) and decreased FMD (from 3.8±0.7% at baseline to 1.2±0.5% at 4 hours, P=0.02). Dexrazoxane did not change homocysteine concentrations after methionine administration (14.9±1.1 μmol/L at 4 hours, P=0.29 versus placebo) but did completely abrogate the homocysteine-induced reduction in FMD (from 3.5±0.5% at baseline to 5.9±1.1% at 4 hours, P<0.01 versus placebo). Endothelium-independent responses to sublingual nitroglycerin did not differ after the administration of placebo and dexrazoxane.


Key Words: methionine ■ vascular endothelium ■ oxidative stress ■ chelation ■ iron

Low molecular weight ferrous iron (LMW-Fe²⁺) catalyzes the production of hydroxyl radicals from hydrogen peroxide in the Fenton reaction.¹ The hydroxyl radical is a highly reactive species that leads to lipid peroxidation, oxidative damage of DNA, cell dysfunction, and death.² Dexrazoxane (ICFR-187), a cyclic derivative of the chelating agent EDTA, is approved by the Federal Drug Administration for the prevention of anthracycline cardiotoxicity in humans. In contrast to EDTA and deferoxamine, dexrazoxane is membrane permeable and chelates LMW-Fe²⁺ in the intracellular space, where hydrolysis of 2 imide bonds activates its binding sites.³ The cardioprotective action of dexrazoxane is due to its high-affinity binding of intracellular LMW-Fe²⁺, which reduces the formation of anthracycline-iron complexes and the consequent production of hydroxyl radical and other toxic reactive oxygen species.⁴,⁵

Hyperhomocysteinemia induced by oral methionine ingestion in normal subjects is associated with increased oxidative stress and transient vascular endothelial dysfunction.⁶–⁸ The present study was undertaken to test the hypothesis that the antioxidant effects of dexrazoxane would prevent vascular endothelial dysfunction induced by homocysteine in the brachial artery of normal human subjects in a double-blind, placebo-controlled, crossover study.

Methods

Study Population

Eight men and 2 women were studied. All subjects were normotensive nonsmokers with no history of chronic illness or chronic medication use. Criteria for exclusion were LDL >160 mg/dL, fasting blood sugar >110 mg/dL, plasma homocysteine >10 μmol/L, and pregnancy. Clinical characteristics of the study population are listed in Table 1. The study protocol was approved by the Institutional Ethical Review Committee. All subjects provided informed written consent before participation.

Brachial Artery Studies

Flow-mediated endothelium-dependent vasodilation in the brachial artery was determined with an ATL Apogee 800 duplex ultrasound imaging system connected to an 11-MHz high-resolution transducer by a single investigator (C.D.) who was blinded to the study conditions; published guidelines were adapted as previously described.⁹,¹⁰ The brachial artery was imaged longitudinally ~5 cm above the antecubital fossa. Arterial diameter (in millimeters) was

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derived from the average of 5 on-screen electronic caliper measurements. External landmarks and internal landmarks (veins, arterial branches, or distinctive soft tissue markings) were used to ensure that all measurements were derived from the same arterial segment. Brachial artery diameter and Doppler blood flow velocity were measured at rest and after transient arterial occlusion induced by 5-minute inflation of a forearm pneumatic cuff to 200 mm Hg. Flow-mediated dilatation was calculated as the percent increase in brachial artery diameter 60 seconds after release of the occluding cuff. Endothelium-independent vasodilation was determined as the percentage increase in brachial diameter 5 minutes after the administration of 0.4 mg sublingual nitroglycerin.

Biochemical Measurements
Plasma was separated from venous blood by cold centrifugation and stored at −80°C. Total plasma homocysteine was determined (in micromoles per liter) by high-pressure liquid chromatography.11

Study Protocol
The study followed a randomized, double-blind, placebo-controlled, crossover design. Each subject was studied in the fasting state with identical procedures at 2 study visits, separated by 7 to 10 days. Measurements of brachial artery diameter at rest and in response to increased flow and nitroglycerin and of venous blood for homocysteine levels were obtained before and 4 hours after oral methionine loading (100 mg/kg L-methionine in 300 mL fruit juice), immediately preceded by double-blind intravenous administration of 500 mg/m2 dexrazoxane (Zinecard, Pharmacia Upjohn) or matching placebo in 250 mL sterile saline over 30 minutes.

Statistical Analysis
Continuous variables are expressed as mean±SEM. Repeated-measures ANOVA was used to compare the effects of dexrazoxane versus placebo on flow-mediated dilatation, nitroglycerine-mediated dilatation, brachial artery diameter, brachial artery blood flow velocity, and plasma homocysteine. A value of P≤0.05 (2-tailed) was used to infer statistical significance.

Results
After placebo infusion, plasma homocysteine concentrations had increased 4 hours after oral methionine administration compared with baseline concentrations (from 5.1±0.4 to 14.2±1.3 μmol/L, P<0.001 versus baseline), and brachial artery flow-mediated dilatation decreased significantly at 4 hours after oral methionine (from 3.8±0.7% to 1.2±0.5%, P=0.02 versus baseline; Figure). The administration of dexrazoxane did not alter the elevation in homocysteine concentrations 4 hours after methionine (14.9±1.1 versus 14.2±1.3 μmol/L, P=0.29 versus placebo) but did completely abrogate the methionine-induced reduction in flow-mediated dilatation at 4 hours (from 3.5±0.5% at baseline to 5.9±1.1% at 4 hours; P=0.07 versus baseline, P<0.01 versus placebo). Brachial artery diameter at rest, brachial artery flow velocity at rest and during reactive hyperemia, nitroglycerin-mediated vasodilation, heart rate, and mean arterial pressure did not differ after the administration of placebo and dexrazoxane (Table 2). The study drug was well tolerated in all subjects, generating no adverse events or changes in safety laboratory parameters.

Discussion
The present data demonstrate that an acute elevation in plasma homocysteine concentration induced by oral methio-

### TABLE 1. Clinical Characteristics of Study Population

<table>
<thead>
<tr>
<th>Description</th>
<th>Mean±SEM</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>Age, y</td>
<td>34±2</td>
<td>21–44</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24±1</td>
<td>20–28</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>91±12</td>
<td>40–136</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>50±4</td>
<td>32–68</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>84±10</td>
<td>47–148</td>
</tr>
<tr>
<td>Blood glucose, mg/dL</td>
<td>92±4</td>
<td>79–110</td>
</tr>
</tbody>
</table>

Flow-mediated vasodilation (FMD, %) before (open bars) and 4 hours after (solid bars) 100 mg/kg oral methionine load with blinded coadministration of intravenous placebo and dexrazoxane. *P<0.02 vs placebo.
nine is associated with a rapid onset of endothelial dysfunction, which can be prevented by intravenous pretreatment with a single dose of dexrazoxane. Compared with placebo, dexrazoxane did not alter plasma homocysteine levels or the vasodilation response to nitroglycerin.

**Homocysteine and Oxidative Stress**

The effects of dexrazoxane on vascular endothelial function were determined in a model of endothelial dysfunction in normal subjects induced by transient hyperhomocysteinemia after oral methionine load. Our finding of decreased flow-mediated vasodilation 4 hours after methionine ingestion and placebo administration is in accord with previous reports in normal subjects and in subjects with chronic hyperhomocysteinemia. Increased generation of reactive oxygen species appears to be an important mechanism of homocysteine-induced endothelial cell injury. Homocysteine in plasma undergoes auto-oxidation to generate superoxide anion radical and hydrogen peroxide. In cultured human umbilical vein endothelial cells and isolated segments of rabbit aorta, homocysteine induces dose-dependent cellular injury, which can be prevented by catalase and superoxide dismutase. The role of oxidative stress in homocysteine-induced endothelial dysfunction is further supported by clinical studies demonstrating that ascorbic acid attenuates methionine-induced endothelial dysfunction in normal subjects.

**Iron-Based Oxidative Stress**

Homocysteine-mediated cellular toxicity may be dependent in part on interactions with intracellular LMW-Fe²⁺. Nearly all iron in the body is tightly bound to specific carrier proteins (transferrin), storage proteins (ferritin), or other heme iron–containing and nonheme iron–containing proteins. In nonerythrocyte tissues, a small intracellular labile pool of nonheme- and nonferritin-bound LMW-Fe²⁺ is maintained for incorporation into iron-containing proteins by a poorly characterized regulatory process that is sensitive to intracellular redox state and cellular iron stores. This pool of intracellular LMW-Fe²⁺ is exchangeable with ferritin-bound iron, is readily chelatable, and is accessible as a catalyst for the production of hydroxyl radical in the Fenton reaction. The hydroxyl radical is a highly reactive species that leads to lipid peroxidation, oxidative damage of DNA, cell dysfunction, and death. In cultured human dopaminergic neural cells, homocysteine-induced generation of reactive oxygen species is exacerbated by coincubation with ferrous iron. Inhibition of glutathione peroxidase by increased circulating homocysteine may promote hydroxyl radical production by maintaining metal ions in their reduced state.

**Antioxidant Effects of Dexrazoxane**

Dexrazoxane, a membrane-permeable derivative of EDTA, is the only approved cardioprotective agent against anthracycline-induced cardiotoxicity. The cardioprotective action of dexrazoxane is attributable to its high-affinity binding of intracellular LMW-Fe²⁺, which reduces the formation of anthracycline-iron complexes and the consequent generation of hydroxyl radicals, which are toxic to cardiomyocytes. Cytoprotective effects of dexrazoxane have also been reported in experimental models of oxidative injury in the lung, pancreas, and hepatic tissues. Our findings are consistent with its previously described antioxidant effects in other tissues and demonstrate that dexrazoxane administration is associated with increased bioavailability of endothelium-derived NO in response to increased flow.

**Comparison With Other Iron Chelators**

Deferoxamine, another iron chelation agent, has been reported to improve endothelium-dependent vasodilation in response to regional acetylcholine infusion in patients with coronary artery disease and to enhance coronary vasodilation in response to the cold pressor test in patients with type 2 diabetes mellitus. The mechanism of action of dexrazoxane is distinct from that of deferoxamine and other chelation agents. Compared with deferoxamine, dexrazoxane is a smaller and more lipid-soluble molecule, is more widely distributed within intracellular compartments, and is more effective in protection against anthracycline cardiotoxicity. Dexrazoxane is rapidly taken up by mammalian cells and hydrolyzed intracellularly to the open-ringed ADR-925 metabolite that strongly binds iron. Accordingly, dexrazoxane chelates iron primarily in the intracellular compartment, whereas deferoxamine chelates iron primarily in the extracellular compartment. The finding that dexrazoxane and deferoxamine are each associated with improved endothelium-dependent vasodilation suggests that intracellular and extracellular iron-dependent redox reactions may contribute to the decreased bioavailability of NO.

**Study Limitations**

The present findings were determined in an experimental model of transient endothelial dysfunction in normal subjects...
and may not be predictive of effects in patients with chronic vascular disease. The effects of dexrazoxane on iron metabolism were not characterized in the present study. In a previous study of oncology patients, dexrazoxane administration was associated with increased serum iron levels and increased urinary excretion of iron.31,32 Changes in serum iron provide limited insight into the proposed cellular mechanism of action of dexrazoxane, inasmuch as chelation of the intracellular LMW-Fe2+ iron pool may not be closely related to serum markers of iron stores. A meta-analysis of population-based studies failed to detect an association between serum markers of iron stores and coronary heart disease risk.33

Conclusions

The present study demonstrates that acute administration of dexrazoxane prevents endothelial dysfunction induced by transient hyperhomocysteinemia in normal subjects. Because iron-based production of hydroxyl radicals may contribute to endothelial dysfunction in response to diverse pro-oxidant stimuli, chelation of intracellular LMW-Fe2+ with dexrazoxane may have therapeutic potential in the amelioration of oxidative stress–induced endothelial dysfunction in cardiovascular diseases. Additional studies are warranted to determine whether the antioxidant action of dexrazoxane or related iron chelators may enhance endothelial function in populations with cardiovascular disease.

Acknowledgments

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References


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