Oxidative Damage Precedes Nitrative Damage in Adriamycin-Induced Cardiac Mitochondrial Injury

Chaiswing L,1,2 Cole MP.,3 ST. CLAIR DK,3 Ittarat W,2 Szweda LI,4 and Oberley TD.1

1 Department of Pathology and Laboratory Medicine, William S. Middleton Memorial Veterans Administration Hospital and University of Wisconsin Medical School, Madison WI 53705, USA
2 Faculty of Medical Technology, Mahidol University, Bangkok, 10700, Thailand
3 Department of Toxicology, University of Kentucky, Lexington, KY 40506-0305, USA
4 Department of Physiology and Biophysics, School of Medicine, Case Western Reserve University Cleveland, OH 44106-4970, USA

Abstract

The purpose of the present study was to determine if elevated reactive oxygen (ROS)/nitrogen species (RNS) reported to be present in adriamycin (ADR)-induced cardiotoxicity actually resulted in cardiomyocyte oxidative/nitrative damage, and to quantitatively determine the time course and subcellular localization of these postulated damage products using an in vivo approach. B6C3 mice were treated with a single dose of 20 mg/kg ADR. Ultrastructural damage and levels of 4-hydroxy-2-nonenal (4HNE)-protein adducts and 3-nitrotyrosine (3NT) were analyzed. Quantitative ultrastructural damage using computerized image techniques showed cardiomyocyte injury as early as 3 hours, with mitochondria being the most extensively and progressively injured subcellular organelle. Analysis of 4HNE protein adducts by immunogold electron microscopy showed appearance of 4HNE protein adducts in mitochondria as early as 3 hours, with a peak at 6 hours and subsequent decline at 24 hours. 3NT levels were significantly increased in all subcellular compartments at 6 hours and subsequently declined at 24 hours. Our data showed ADR induced 4HNE-protein adducts in mitochondria at the same time point as when mitochondrial injury initially appeared. These results document for the first time in vivo that mitochondrial oxidative damage precedes nitrative damage. The progressive nature of mitochondrial injury suggests that mitochondria, not other subcellular organelles, are the major site of intracellular injury.

Keywords. Adriamycin; 4-hydroxy-2-nonenal protein adducts; nitrotyrosine; reactive oxygen species; reactive nitrogen species; cardiac injury.

Toxicologic Pathology. 2004; 32:536–547