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A model for cardiomyocyte cell death: Insights into mechanisms of oncosis

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ABSTRACT

It is now known that there are at least two basic patterns of cell injury progressing to cell death: cell injury with swelling, known as oncosis, and cell injury with shrinkage, known as apoptosis. Both types of cell death are "programmed" in the sense that the genetic information and many of the enzymes and other factors pre-exist in the cell. Previous investigation has pointed to cardiomyocyte ischemic injury evolving as the oncotic pattern of injury, although apoptosis has also been implicated. This study was designed, using a unique cell model system, to gain insight into the molecular events of anticancer agent-induced cardiomyocyte injury. Cardiomyocytes exposed for 2 h to 1.5 μg/ml sanguinarine consistently displayed the morphology of apoptosis in over 80% of cells, whereas a higher dose of 25 μg/ml at 2 h yielded the pattern of oncosis in over 90% of cells. Microarray analysis revealed altered expression of 2514 probes in sanguinarine-induced oncosis and 1643 probes in apoptosis at a level of significance of $p<0.001$. Some of the inductions such as perforin were found to be higher than 11-fold in oncosis. When perforin was blocked by perforin-specific siRNA we found a reduction in oncotic cell death. These results strengthen the notion that oncosis is not representative of nonspecific necrosis, but constitutes a genetically controlled form of "programmed cell death"; and also that oncosis might represent a pathogenetic mechanism of cardiomyocyte injury. This is also the first demonstration of the involvement of perforin in cardiomyocyte oncosis.

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Introduction

Oncosis, autophagy and apoptosis represent major modes of cell injury and cell death in a variety of pathological processes (Buja et al., 1993; Majno and Joris 1995). The intent of the present study was to use an *in vitro* cell culture system to establish proof of principle and to gain insights into molecular mechanisms involved in the response of cardiomyocytes to injury.

Cardiotoxicity and ischemia result in a characteristic pattern of metabolic and ultrastructural changes that can lead to irreversible injury in the heart and other organs. Recent studies have explored the relationship of myocardial ischemic injury to the major modes of cell death, and it appears that myocardial ischemic and reperfusion injury of cardiomyocytes involve the pathways of oncosis and apoptosis (Buja, 2005; Buja and Weerasinghe, 2008). Apoptosis is also prominent in other pathological processes, including viral myocarditis and heart failure (Abbate et al., 2009; Frustaci et al., 2006; Kawai, 1999; Saraste et al., 2003). The mode(s) of cell injury in chemotherapy-induced cardiotoxicity is under active investigation (Yeh and Bickford, 2009). Because we have shown that sanguinarine, a known anticancer agent, can induce oncosis and apoptosis in a variety of malignant cell lines (Ding et al., 2002; Hallock et al., 2007; Weerasinghe et al., 2001a, 2001b) and because certain pathobiochemical, ultrastructural and molecular effects of oncosis and apoptosis are known, we designed this study to determine if sanguinarine could induce similar changes in cardiac myocytes, and also gain insight into the molecular events of oncotic cell death.

Sanguinarine, a benzophenanthridine alkaloid biosynthesized from phenylalanine in plants of the Papaveraceae family, has been well studied in the laboratory and has been reported to display a wide spectrum of biological effects, such as antifungal, antimicrobial, analgesic and moreover, anti-cancer properties *via* the induction of oncosis and apoptosis (Ding et al., 2002; Hallock et al., 2007; Weerasinghe et al., 2001a, 2001b). Apoptosis in cardiomyocytes was initially characterized by cell morphology and further studies revealed the other characteristics and molecular markers (Buja et al., 1993; Majno and Joris 1995). In contrast, oncosis is a cell death process related to energy depletion leading to impairment of ionic pumps of the cell membrane, cell swelling and formation of cell surface blebs or blisters (Buja, 2005; Buja and Weerasinghe, 2008; Buja et al., 1993; Majno and Joris 1995). Molecular markers for oncosis are yet to be identified.

The ultimate objective of this study was to provide further insight into the pathogenetic mechanisms of oncosis, in comparison to apoptosis, in cardiomyocytes so that insights from the *in vitro* model could subsequently be directed toward design of appropriate interventions to minimize injury and preserve myocardial function in pathological processes including chemotherapy-induced cardiac injury, ischemic/reperfusion injury, myocarditis, and heart failure. Using

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the oncosis model we have previously characterized several morphological and biochemical parameters of these death modalities in several malignant cell lines (Ding et al., 2002; Hallock et al., 2007; Weerasinghe et al., 2001a, 2001b). In this report we have endeavored to gain insight into the molecular events of sanguinarine-treatment in cardiomyocytes. Thus far, oncosis/necrosis has been thought of as uncontrolled and accidental, and lacking the features of apoptosis and autophagy. However, our findings and that of others reported in the literature indicate that this occurrence is not accidental but a tightly regulated physiological process. To our knowledge, this is the first report to present a model for the comparative study of apoptosis *vs.* oncosis in primary cardiomyocytes.

Materials and methods

Primary mouse embryonic stem cell (ES)-derived cardiomyocytes were purchased from Reach Bio LLC (Seattle, WA) and neonatal rat ventricular cardiomyocytes from Lonza Walkersville Inc. (Walkersville, MD). Antibodies against β actin, perforin, caspase 3 breakdown products and precipitation assay (RIPA) buffer were obtained from Cell Signaling (Danvers, MA) and Santa Cruz Biotechnology (Santa Cruz, CA).

The drug sanguinarine-HCl, was purchased from Sigma Chemicals Co. (St. Louis, MO). Sanguinarine-HCl was maintained as a dry powder at room temperature. It was dissolved in deionized water at 1 mg/ml, and used as a stock solution. Sanguinarine was subsequently diluted with cardiomyocyte growth media (Reach Bio LLC, Seattle, WA). A total of 5 independent lots of sanguinarine-HCl were tested in these studies.

Cell culture

Mice embryonic stem cell (ES) derived cardiomyocytes and neonatal ventricular rat cardiomyocytes were prepared and grown in media using standard methods and procedures found in the supplier protocols. Preliminary dose–response studies utilizing these two cell types showed no differences in terms of induction of apoptosis and oncosis with sanguinarine exposure. Primary mouse embryonic stem cell (ES)-derived cardiomyocytes were used for the subsequent array experiments and transfection experiments, as these cells are

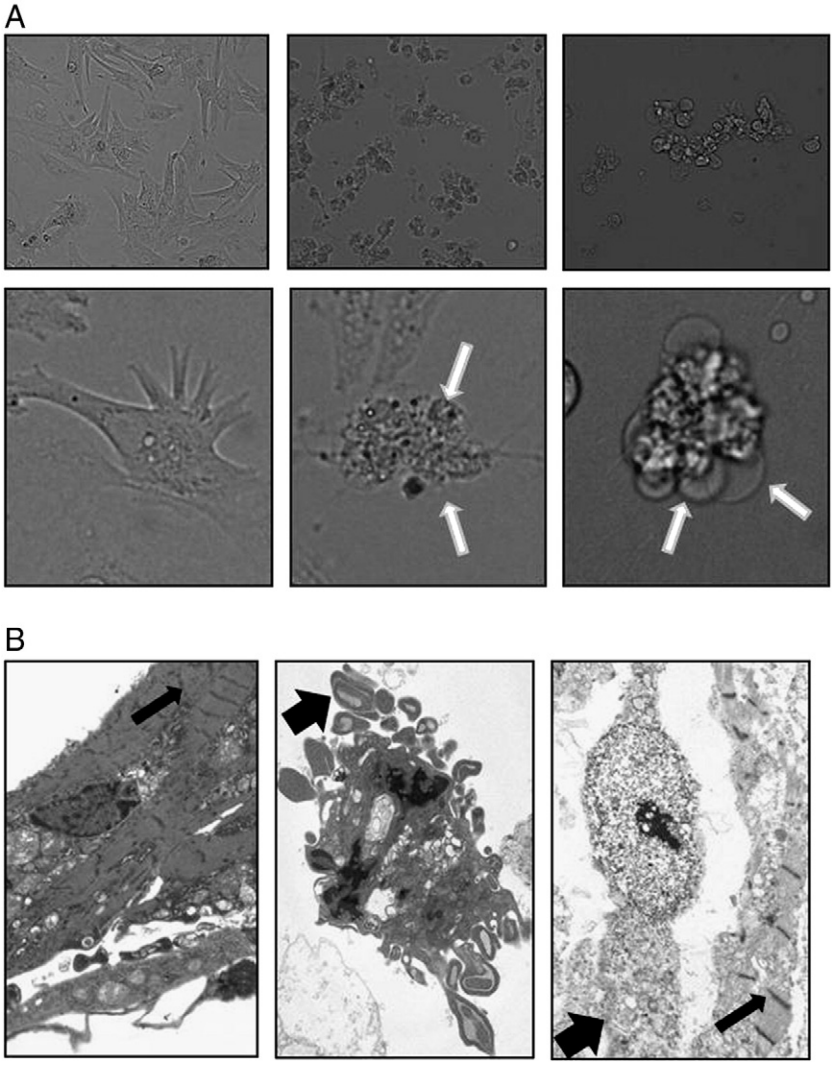

Fig. 1. Light and electron micrographs. A. Light micrographs (original magnification— ×60) of primary mouse cardiomyocytes that were untreated (left panel) or treated with concentrations of sanguinarine that induces apoptosis (1.5 μg/ml, middle panel) and oncosis (25 μg/ml, right panel). As seen, exposure to 1.5 μg/ml sanguinarine for 2 h resulted in the formation of apoptotic bodies (see arrows) and exposure to 25 μg/ml sanguinarine for 2 h resulted in single blister formation (see arrows). B. Electron micrographs (original magnification— ×10,000) of primary mouse cardiomyocytes, untreated (left panel) and treated with concentrations of sanguinarine that induces apoptosis (middle panel) and oncosis (right panel). When exposed to concentrations of sanguinarine that induces apoptosis (1.5 μg/ml), cells showed the classic morphological changes consisting of formation of apoptotic bodies containing organelles, chromatin condensation and nuclear fragmentation. When exposed to sanguinarine concentrations that induce oncosis (25 μg/ml), cells displayed single blister formation devoid of organelles, patchy chromatin condensation and an increase in cytoplasmic vacuolization.