Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular and Cellular Cardiology 43 (2007) 726–732
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original article

Alterations in both death and survival signals for apoptosis in heart failure due to volume overload

Melissa R. Dent, Samarjit Das, Naranjan S. Dhalla *

Institute of Cardiovascular Sciences, St. Boniface Hospital Research Centre, Winnipeg, Canada
Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada

Received 13 July 2007; received in revised form 9 August 2007; accepted 4 September 2007
Available online 12 September 2007

Abstract

Heart failure is known to be associated with an increase in cardiomyocyte apoptosis; however, neither its occurrence nor the mechanisms involved in hearts failing due to volume overload are completely understood. This study examined some of the signal pathways, which are known to regulate pro- or anti-apoptotic proteins, in heart failure due to volume overload induced by arteriovenous (AV) shunt in male Sprague–Dawley rats. Animals were assessed for cardiac function at 16 weeks of the operation and the left ventricle was used for studying apoptosis and associated signal transduction mechanisms. Hemodynamic and echocardiographic data indicated the presence of severe heart failure in AV shunt rats. A marked elevation in the amount of tumor necrosis factor-α and increased occurrence of apoptosis were detected in the volume overloaded myocardium. Western blot analysis revealed a significant increase in BAX and caspases 3/9 proteins in the failing hearts whereas the levels of phosphorylated Akt and Bcl-2 proteins were decreased. These data suggest that there is a downregulation in the Akt-dependent survival signal involving anti-apoptotic protein, Bcl-2, whereas the signals for the pro-apoptotic protein, BAX, are upregulated and these alterations may play a role in cardiomyocyte apoptosis in heart failure due to volume overload.

Keywords: Volume overload; Cardiomyocyte apoptosis; Congestive heart failure; Tumor necrosis factor-α; Apoptotic proteins

1. Introduction

Several investigators have shown that cardiomyocyte apoptosis is associated with heart failure due to myocardial infarction [1–4], pressure overload [5,6], as well as in other non-ischemic models of heart failure [7]. It has been demonstrated that mitochondria generate the initial apoptotic cascade by releasing various proteins including cytochrome c [8–11], which activate caspase 3, 8 and 9 and result in cardiomyocyte apoptosis. This intrinsic pathway for apoptotic cell death [12] has been suggested as one of the major mechanisms for the cardiac cell death due to different pathophysiological stimuli [13–16]. It has also been shown that tumor necrosis factor (TNF)-α binds to its membrane receptors for activating downstream caspases and initiating the receptor-dependent cell death pathway [17]. Furthermore, TNF-α has been observed to provoke inhibition of the mitochondrial function [18] and induce apoptosis [19]. Recent studies have suggested that BAX, a member of the apoptotic signal protein family [8,20], enters the outer mitochondrial membrane and forms a large conductance channel, which allows the release of cytochrome c [10,20]. In addition, BAX has been shown to induce the transport of cytochrome c in liposomes [21]. On the other hand, Bcl-2 and other proteins of the anti-apoptotic family are required for the binding of glucokinase to the mitochondrial complex to protect the release of cytochrome c [11]. One of the important upstream targets of this Bcl-2 super family of proteins is protein kinase-B (Akt), a serine/threonine protein kinase, that is activated by a number of growth factors and cytokines as a consequence of phosphatidylinositol 3-kinase (PI3-kinase) dependent phosphorylation [22]. The role of Akt in cell survival has been suggested to be mediated through an anti-apoptotic pathway which directly antagonizes mitochondria-directed apoptosis [22,23].

Although heart failure has been reported to occur due to volume overload in a rat model of arterial venous (AV) shunt following 12–16 weeks of operation [24–26], very little information regarding the presence of apoptosis in this type of failing

* Corresponding author. Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, 351 Tache Avenue, Winnipeg, Manitoba, Canada R2H 2A6. Tel.: +1 204 235 3417; fax: +1 204 233 6723.
E-mail address: nsdhalla@sbrc.ca (N.S. Dhalla).

0022-2828/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.yjmcc.2007.09.001

hearts is available in the literature. It should be mentioned that cardiac hypertrophy induced by volume overload in pigs for 24 to 96 h was found to be associated with apoptosis due to oxidative stress; however, the status of apoptosis at the heart failure stage in this experimental model was not examined. Furthermore, volume overload produced in a mouse model for 8 weeks showed the occurrence of endothelial apoptosis due to the activation of matrix metalloproteinase 9. Thus, in view of the lack of information regarding the development of apoptosis as well as its mechanisms in the failing heart due to volume overload, the aim of the present study was to determine whether cardiomyocyte apoptosis occurs at the heart failure stage in the AV shunt model of volume overload. In addition, this study examined the mechanisms of cardiomyocyte apoptosis occurring in heart failure in 16 weeks post-AV shunt rats. This study shows that elevated levels of TNF-α in the failing heart are associated with the activation of BAX as well as the cell death pathway involving caspase 3 and 9 whereas the Akt-Bcl-2 survival signal is downregulated. Such alterations in both the death and survival signals are suggested to produce cardiomyocyte apoptosis in congestive heart failure due to volume overload.

2. Methods

2.1. Experimental animals

All experimental protocols for animal studies were approved by the Animal Care Committee of the University of Manitoba, following the guidelines established by the Canadian Council on Animal Care. An AV shunt was performed in male Sprague–Dawley rats (weighing 150–200 g) according to the procedure described earlier [24–26]. Briefly, the animals were anesthetized with 5% isoflurane at a flow rate of oxygen (2 l/min) and an abdominal laparotomy was performed. Following exposure of the abdominal aorta and inferior vena cava, the descending aorta was temporarily occluded proximal to the intended puncture site. An 18-gauge needle was inserted and withdrawn across the medial wall of the descending aorta three times to ensure the size and presence of the shunt. The puncture site was then immediately sealed with a drop of isocynate (Krazy glue). The creation of the shunt was visualized by the pulsatile flow of oxygenated blood into the vena cava from the abdominal aorta. Age-matched, sham-operated animals served as controls and were treated similarly, except that the puncture into the descending aorta was not performed. The circulation system was only occluded for 0.5–1 min and the entire procedure was finished within 10 min. It is pointed out that there was no mortality in the control group but the mortality rate of the AV shunt animals as the result of the surgical procedure was less than 4% during 6 h after the surgery. Both control and AV shunt animals were used at 16 weeks following the surgery.

2.2. Measurement of cardiac performance

Echocardiographic assessment [27] was performed using an ultrasound imaging system (SONOS 5500 ultrasonograph; Agilent Technologies, Mississauga, ON, Canada) for the measurement of cardiac output, heart rate, left ventricular (LV) wall size and internal diameters during systole and diastole as well as ejection fraction of the sham and shunt rat hearts. Echocardiographic measurements were conducted in rats anesthetized using 2.5% isoflurane in 2 l/min of oxygen. Briefly, the transthoracic short-axis measurements were performed using a 12-MHz annular array ultrasound transducer. The M-mode images of posterior wall of the LV at the level of the papillary muscle were obtained for wall thickness and chamber dimensions. Images were stored in digital format on a magnetic optical disk for analysis. The LV function of animals was assessed by anesthetizing the rats using an injection of ketamine–xylazine (100:10 mg/kg i.p.) [24–26]. The right carotid artery was exposed, and a micromanometer-tipped catheter (2-0; Millar SPR-249) was inserted, advanced into the LV and secured with a silk ligature around the artery. After a 15-min stabilization of the heart function, LV pressures and maximum rates of isovolumic pressure development ($+dP/dt_{max}$) and decay ($-dP/dt_{max}$) were recorded. Hemodynamic data were computed instantaneously and displayed on a computer data-acquisition workstation (Biopac, Harvard Apparatus, PQ, Canada). Following hemodynamic assessment the LV tissue will be frozen in liquid N_2 and stored at −80 °C.

2.3. Detection of TNF-α levels in the myocardium

Ventricular tissue from 16 weeks sham and AV shunt groups was homogenized in 10 volumes of phosphate-buffered saline that contained 1% w/v Triton X-100 and a protease inhibitor cocktail (Roche Applied Science, PQ, Canada). The homogenate was centrifuged at 2500×*g* for 20 min at 4 °C. TNF-α content in the myocardial tissue was measured using a sandwich ELISA kit for rat TNF-α (R&D Systems, MN, USA) [28].

2.4. Western blot analysis

LV tissue (50 mg) was homogenized in buffer containing 50 mmol/L Tris–HCl (pH 7.5), 0.25 mol/L sucrose, 10 mmol/L EGTA, 4 mmol/L EDTA and protease inhibitor cocktail and 1%

Table 1
General characteristics and cardiac function of sham controls and arteriovenous shunt groups at 16 weeks

Parameter	Sham	Shunt
Body wt, g	622±21	591±11
LV wt, mg	1170±13	1381±31*
LV/Body wt ratio, mg/g	1.9±0.2	2.3±0.2*
Lung wet wt, g	1.8±0.06	2.7±0.08*
Liver wet wt, g	21±2.2	28±1.3*
Heart rate, beats/min	322±14	327±13
LVEDP, mm Hg	5.9±1.4	12.6±1.3*
LVSP, mm Hg	124±11	106±8
$+dP/dt_{max}$, mm Hg/s	2850±88	2290±108*
$-dP/dt_{max}$, mm Hg/s	2681±55	2280±52*

Data are mean±SE of 7 different animals for each group. The left ventricular weight (LV wt) of the experimental animals refers to the weight of the LV plus septum. LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; $+dP/dt_{max}$, maximum rate of contraction; $-dP/dt_{max}$, maximum rate of relaxation; *$P<0.05$ vs. control value.