Journal of Clinical and Basic Cardiology

An Independent International Scientific Journal

Journal of Clinical and Basic Cardiology 2000; 3 (3), 201-203

Evidence for myocyte apoptosis in the heart

Olivetti G, Bertani N, Cigola E, Graiani G

Indexed in
Chemical Abstracts
EMBASE/Excerpta Medica

Krause & Pachernegg GmbH · VERLAG für MEDIZIN und WIRTSCHAFT · A-3003 Gablitz/Austria

Evidence for Myocyte Apoptosis in the Heart

G. Olivetti, E. Cigola, N. Bertani, G. Graiani

In the heart myocyte loss is an important factor in the genesis, development and progression of end-stage failure. Myocyte apoptosis has been seen in a variety of experimental and human conditions and it seems to play an important and unrecognized role in the loss of contractile material explaining, at least in part, functional deterioration. Since apoptosis is an active process regulated by several genes efforts have to be made to control the phenomenon with appropriate interventions. *J Clin Basic Cardiol 2000; 3: 201–3.*

Key words: apoptosis, necrosis, myocyte, heart failure

Apoptosis or programmed cell death is a form of cell death that was described several years ago and has been found relatively recently in different cardiac disorders and has been considered of primary importance in the onset and progression of cardiac dysfunction and failure in animals and men. This is because the loss of contractile cells in the heart poses an additional workload on the remaining viable myocytes that may be unbearable, resulting in pathologic stimuli and death signals. The evidence that supports this view is derived from several data, mostly quantitative, collected under different conditions that will be reviewed here.

Since 1994, the only form of myocyte death in the myocardium has been attributed to the well known process described as necrosis [1]. However, several studies have demonstrated that myocyte apoptosis may also be present in the heart. Apoptotic myocyte cell death has been found in embryogenesis [2], during postnatal growth [3], hypoxia *in vitro* [4], after ischaemic and reperfusion injury [5, 6], stretching of the papillary muscle *in vitro* [7], myocardial infarction in animals [8] and humans [9–12], in congenital heart defects [13], normal aging [14], rapid ventricular pacing [15], heart failure after coronary embolization [16], and more recently, in hearts in end-stage failure [17, 18], arrhytmogenic right ventricular dysplasia [19, 20], cardiac allograft rejection [21] pressure overload cardiac hypertrophy [22] and acromegaly [23].

Myocyte Apoptosis

Apoptosis is activated by an endogenous endonuclease able to cleave DNA in the linker region resulting in single or multiple DNA fragments of 200 bp [24, 25]. At this relatively early stage of the process there are minor morphological changes in the nuclei described as chromatin margination [24], not always detectable in the heart. In order to visualize in the tissue nuclear DNA strand breaks specific methods are needed [3, 7, 8, 10, 11, 14, 17]. In later stages, when DNA is fully compacted, the morphologic recognition of the residual apoptotic bodies is much easier [15]. Such a pattern of DNA cleavage can also be seen by agarose gel electrophoresis after extraction of DNA from the tissue which results in a typical nucleosomal laddering [7, 8, 14, 17]. Although both techniques have inherent limitations they have been used in combination to demonstrate the occurrence of the apoptotic process in the heart.

In contrast to apoptosis, the morphological criteria indicative of necrotic myocyte cell death have been extensively described [1]. Initially, the damage is limited to the mitochondria, then to the cytoplasmic components and finally to the sarcolemma compartment. In this scheme, the appearance of sarcolemma discontinuities is the landmark of an irreversible damage [1]. Unfortunately these structural changes are difficult to be seen by standard morphologic techniques and are apparent ultrastructurally only at the completion of the biochemical events, limiting their use in the detection of the process.

Myocyte Necrosis

A more direct approach to recognize necrotic myocytes has been developed by injecting monoclonal antibody specific to cardiac myosin *in vivo* and detecting its localization in the tissue with fluorescein labeled secondary antibody [26, 27]. It has been shown that the anti-myosin IgG binds to myofibrillar myosin only in myocytes with ruptured plasma membranes, whereas intact myocytes remained unlabeled. With this technique the presence of irreversible damaged myocytes after the infusion of isoproterenol [27] or angiotensin II [28] has been established and finally, the relative contribution of myocytes necrosis and apoptosis during the evolution of myocardial infarction has been quantified [8]. Indium-111-labeled antimyosin monoclonal antibody has also been applied in the nonivasive detection of myocardial damage in different pathologic conditions in humans [28–31].

Mechanisms of Myocyte Cell Death

The possibility that myocyte cell death may be elicited either by necrosis or apoptosis raises some considerations on the mechanisms responsible for these processes. The appearance of apoptosis before necrosis in a carefully planned experiment [8] supports the notion that a decrease in oxygen tension may activate the suicide program of myocytes. This possibility has been described in neonatal myocytes in culture [4], after ischaemic-reperfusion injury [5, 6] and after myocardial infarction in rats [8] and humans [11, 12]. In the infarcted myocardium, however, the affected and surviving muscles are both subjected to a significant elevation in diastolic overload [32, 33] and a direct relationship between mechanical forces and apoptotic myocyte cell death has been clearly demonstrated *in vitro* [7]. A diastolic overloading was also seen with aging [34], during rapid ventricular pacing [15], and in end-stage cardiac failure [17], all conditions in which apoptotic myocyte cell death has been documented.

From the Department of Pathology and Laboratory Medicine, University of Parma, Italy
Correspondence to: Giorgio Olivetti, MD, Department of Pathology and Laboratory Medicine, Pathology Section, University of Parma, Via Gramsci 14, I-43100 Parma, Italy; E-mail: olivetti@ipruniv.cce.unipr.it

In addition, abnormal resting tension levels imposed on papillary muscles result in an increased oxygen consumption, leading to the generation of superoxide anion which may activate the suicide program of myocytes [7]. Similarly, the formation of reactive oxygen species has been claimed to be the initial event of apoptotic myocyte cell death in the ischemia-reperfusion injury model [5]. Although a cause and effect relationships between apoptosis and Fas molecule cannot be completely established, Fas overexpression has been found in conditions associated with myocyte programmed cell death [4, 5]. The Fas gene belongs to the tumor necrosis factor and nerve growth factor receptor family, and ligand activation of Fas receptor can trigger apoptosis [7]. It is of interest to remember that several molecules that are accumulating in the circulation in patients with heart failure are able to induce myocyte cell death by apoptosis. Atrial neatriuretic peptide [35], angiotensin II [36, 37], catecholamines [38, 39] have been found to increase the number of myocytes dying by DNA fragmentation *in vitro* and *in vivo*. Finally, the role played by calcium accumulation in myocytes, assumed to be mediated by alterations in the sarcolemmal transport of this cation following ischaemia [40], may be an additional trigger for apoptosis. In fact, it has been demonstrated that manipulations leading to free calcium accumulation in the cytoplasm are able to initiate apoptosis in several cell systems [24, 41]. In summary, the mechanism by which the apoptotic cell death in myocytes is activated is still obscure and the available evidences of its occurrence in many different pathologic conditions cannot allow a definite answer to this question.

Bcl-2 and Apoptosis

Apart from the described morphologic characteristics, apoptosis differs from necrosis because several genes are activated during apoptosis. This is important since apoptosis is an active process and could be prevented or modified by appropriate intervention. The expression of some members of the Bcl-2 family has been studied recently in the myocardium. During postnatal maturation of the heart, the expression of Bcl-2, which prevents apoptosis [42], is up-regulated in myocytes when apoptotic cell death is decreased and *vice versa* [3]. Acutely, after experimental myocardial infarction, myocyte expression of Bcl-2 is enhanced in correspondence of the onset and peak of apoptotic myocyte cell death. At the same intervals, Bax, which promotes apoptosis [43], is unchanged [8]. Furthermore, Bcl-2 expression is moderately increased in human hearts with end-stage failure, where apoptosis is present in a large number of myocytes [17]. In infarcted ventricles in men [9, 44] apoptosis is accompanied by myocyte expression of Bcl-2 with overexepression of Bax. These contrasting findings can depend upon the interaction of different genes with Bcl-2.

Defects in Bcl-2 production in Bcl-2 deficient transgenic mice are coupled with cell death in different organs [45]. However, the interaction of Bcl-2 with other members of the same family may originate different results. For example, Bcl-2 forms heterodimers with Bax [46]. If Bax homodimers predominate cell death will occur, but when Bcl-2 and Bax heterodimerization prevails cells can survive [47]. Bcl-2 production may also interfere with the apoptotic process induced by Fas antigen [8]. In fact, it has been demonstrated that Fas protein is upregulated in more than 50 % of myocytes present in the area at risk after the occlusion of the left coronary artery despite the overexpression of Bcl-2 [8].

Conclusion

In summary, there are convincing evidences that myocyte cell loss may be induced by apoptosis and necrosis. Ischaemia and overload are essential in the generation of the cell death signal although changes in the induction of genes promoting or opposing apoptosis may modulate the total amount of myocyte damage. However, there is still a need to clarify the role played by different genetic and environmental factors implicated in cell death or survival.

References

1. Reimer KA, Jennings RB. Myocardial ischemia, hypoxia and infarction. In: Fozzard HA, Haber E, Jennings RB, Katz AM, Morgan HE (eds). The Heart and Cardiovascular System. Raven Press, New York, 1986; 2: 1133–201.
2. Takeda K, Yu Z, Nishikawa T, Tanaka M, Hosoda S, Ferrans VJ, Kasajima T. Apoptosis and DNA fragmentation in the bulbus cordis of the developing heart. J Mol Cell Cardiol 1996; 28: 208–13.
3. Kajstura J, Mansukhani M, Cheng W, Reiss K, Krajewski S, Reed JC, Quaini F, Sonnenblick EH, Anversa P. Programmed cell death and the expression of the protooncogene Bcl-2 in myocyte during postnatal maturation of the heart. Exp Cell Res 1995; 219: 110–21.
4. Tanaka M, Ito H, Adachi S, Akimoto H, Nishikawa T, Kasajima T, Marumo F, Hiroe M. Hypoxia induces apoptosis with enhanced expression of fas antigen messenger RNA in cultured neonatal rat cardiomyocytes. Circ Res 1994; 75: 426–33.
5. Gottlieb RA, Burleson KO, Kloner RA, Bablor BM, Engler RL. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. J Clin Invest 1994; 94: 1621–8.
6. Buerke M, Murohara T, Skurk S, Nuss C, Tomaselli K, Lefer AM. Cardioprotective effect of insulin-like growth factor I in myocardial ischemia followed by reperfusion. Proc Natl Acad Sci 1995; 92: 8031–5.
7. Cheng W, Li B, Kajstura J, Li P, Wolin MS, Sonnenblick EH, Hintze TH, Olivetti G, Anversa P. Stretch-induced programmed myocyte cell death. J Clin Invest 1995; 96: 2247–59.
8. Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa P. Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. Lab Invest 1996; 74: 86–107.
9. Itoh G, Tamura J, Suzuki M, Suzuki Y, Ikeda H, Koike M, Nomura M, Jie T, Ito K. DNA fragmentation of human infarcted myocardial cells demonstrated by the nick end labelling method and DNA agarose gel electrophoresis. Am J Pathol 1995; 146: 1325–31.
10. Quaini F, Cigola E, Sala R, Andreoli AM, Giordano G, Lagrasta C, Maestri R, Olivetti G. Apoptosis in the infarcted human heart. BAM 1996; 6: 240–50.
11. Olivetti G, Quaini F, Sala R, Lagrasta C, Corradi D, Bonacina E, Gambert S, Cigola E, Anversa P. Acute myocardial infarction in humans is associated with activation of programmed myocyte cell death in the surviving portion of the heart. J Mol Cell Cardiol 1996; 28: 2005–16.
12. Bardales RH, Shea Hailey L, Xie SS, Schaefer RF, Hsu S. In situ apoptosis assay for the detection of early acute myocardial infarction. Am J Pathol 1996; 149: 821–9.
13. James TN, Martin ES, Willis PW, Lohr TO. Apoptosis as a possible cause of gradual development of complete heart block and fatal arrhythmias associated with absence of the AV node, sinus node and internodal pathways. Circulation 1996; 93: 1424–38.
14. Kajstura J, Cheng W, Sarangarajan R, Li P, Li B, Nitahara JA, Chapnick S, Reiss K, Olivetti G, Anversa P. Necrotic and apoptotic myocyte cell death in the aging heart of Fischer 344 rats. Am J Physiol 1996; 271: H1215–H1228.
15. Liu Y, Cigola E, Cheng W, Kajstura J, Olivetti G, Hintze TH, Anversa P. Myocyte nuclear mitotic division and programmed myocyte cell death characterize the cardiac myopathy induced by rapid ventricular pacing in dogs. Lab Invest 1995; 73: 771–87.
16. Sharov VG, Sabbah HN, Shimoyama H, Goussev AV, Lesch M, Goldstein S. Evidence of cardiocyte apoptosis in myocardium of dogs with chronic heart failure. Am J Pathol 1996; 148: 141–9.
17. Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, Quaini E, Di Loreto C, Beltrami CA, Krajewsky S, Reed JC, Anversa P. Programmed myocyte cell death affects the failing human heart. Circulation 1996; 94 (Suppl I): I-157.
18. Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, Schmidt U, Semigran MJ, Dec GW, Khaw B. Apoptosis in myocytes in end-stage heart failure. N Engl J Med 1996; 335: 1182–9.
19. Mallat Z, Tedgui A, Fontaliran F, Frank R, Durigon M, Fontaine G. Evidence of apoptosis in arrhythmogenic right ventricular dysplasia. N Engl J Med 1996; 335: 1190–6.
20. Valente M, Calabrese F, Thiene G, Angelini A, Basso C, Nava A, Rossi L. In vivo evidence of apoptosis in arrhythmogenic right ventricular cardiomyopathy. Am J Pathol 1998; 152: 479–84.
21. Szabolcs M, Michler RE, Yang XC, Aji XC, Roy D, Athan E, Sciacca RR, Minanov OP, Cannon PJ. Apoptosis of cardiac myocytes during cardiac allograft rejection. Relation to the induction of nitric oxide synthase. Circulation 1996; 4: 1665–73.
22. Teiger E, Dam TV, Richard L, Wisnewsky C, Tea BS, Gabouri L, Tremblay J, Schwartz K, Hamet P. Apoptosis in pressure overload induced heart hypertrophy in the rat. J Clin Invest 1996; 97: 2891–7.

23. Frustaci A, Chimenti C, Setoguchi M, Guerra S, Corsello S, Crea F, Leri A, Kajstura J, Anversa P, Maseri A. Cell death in acromegalic cardiomyopathy. Circulation 1999; 99: 1426–34.
24. Wyllie AH, Morris RG, Smith AL, Dunlop D. Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. J Pathol 1984; 142: 67–77.
25. Arends MJ, Morris RG, Wyllie AH. Apoptosis: The role of endonuclease. Am J Pathol 1990; 136: 593–60.
26. Nolan A, Clark W, Karwoski T, Zak R. Patterns of cellular injury in myocardial ischemia determined by monoclonal antimyosin. Proc Natl Acad Sci USA 1983; 80: 6046–50.
27. Benjamin IJ, Jalil JE, Tan LB, Cho K, Weber KT, Clark WA. Isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis. Circ Res 1989; 67: 657–70.
28. Tan LB, Jalil JE, Pick R, Janicki JS, Weber KT. Cardiac myocyte necrosis induced by angiotensin-II. Circ Res 1991; 69: 1185–95.
29. Khaw BA, Strauss KW, Moore R, Fallon JT, Yasuda T, Gold HK, Haber E. Myocardial damage delineated by indium-111 antimyosin Fab and technetium-99m pyrophosphate. J Nucl Med 1987; 28: 76–82.
30. Obrador D, Ballester M, Carrio I, Berna L, Pons G. High prevalence of myocardial monoclonal antimyosin antibody uptake in patients with chronic idiopathic dilated cardiomyopathy. J Am Coll Cardiol 1989; 13: 1289–93.
31. Tamaki N, Yamada T, Matsumori A, Yoshida A, Fujita T, Obtani H, Watanabe Y, Yonekura Y, Endo K, Konishi J, Kawai C. Indium-111-antimyosin antibody imaging for detecting different stage of myocardial infarction: comparison with technetium-99m pyrophosphate. J Nucl Med 1990; 31: 136–42.
32. Olivetti G, Capasso JM, Sonnenblick EH, Anversa P. Side-to-side slippage of myocytes partecipates in ventricular wall remodeling acutely after myocardial infarction in rats. Circ Res 1990; 67: 23–34.
33. Olivetti G, Capasso JM, Meggs LG, Sonnenblick EH, Anversa P. Cellular basis of chronic ventricular remodeling after myocardial infarction in the rat. Circ Res 1991; 68: 856–69.
34. Capasso JM, Palackal T, Olivetti G, Anversa P. Severe myocardial dysfunction induced by ventricular remodeling in aging rat hearts. Am J Physiol 1990; 259: H1086–H1096.
35. Wu CF, Bishopric NH, Pratt RE. Atrial natriuretic peptide induces apoptosis in neonatal rat cardiac myocytes. J Biol Chem 1997; 272: 14860–6.
36. Cigola E, Kajstura J, Li B, Meggs LG, Anversa P. Angiotensin II activates programmed myocyte cell death in vitro. Exp Cell Res 1997; 231: 363–71
37. Kajstura J, Cigola E, Malhotra A, Li P, Cheng W, Meggs LG, Anversa P. Angiotensin II induces apoptosis in adult ventricular myocytes in vitro. J Mol Cell Cardiol 1997; 29: 859–70.
38. Communal C, Singh K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the beta-adrenergic pathway. Circulation 1998; 98: 1329–34.
39. Shizukuda Y, Buttrick PM, Geenen DL, Borczuk AC, Kitsis RN, Sonnenblick EH. Beta-adrenergic stimulation causes cardiocyte apoptosis: influence of tachycardia and hypertrophy. Am J Physiol 1998; 275: H961–H968.
40. Jennings RB, Reimer KA. Factors involved in salvaging ischemic myocardium. Effects of reperfusion of arterial blood. Circulation 1983; 68: 125–36.
41. Albritton NL, Verret CR, Wolley RC, Eisen HN. Calcium ion concentration and DNA fragmentation in target cell destruction by murne cloned cytotoxic T lymphocytes. J Exp Med 1988; 167: 514–27.
42. Reed JC. Bcl-2 and the regulation of programmed cell death. J Cell Biol 1994; 124: 1–6.
43. Krajewsky S, Krajewska M, Shabaik A, Miyashita T, Wang HG, Reed JC. Immunohistochemical determination of in vivo distribution of Bax, a dominant inhibitor of Bcl-2. Am J Pathol 1994; 145: 1323–36.
44. Misao J, Hayakawa Y, Ohno M, Kato S, Fujiwara T, Fujiwara H. Expresssion of bcl-2 protein, an inhibitor of apoptosis, and Bax, an accelerator of apoptosis, in ventricular myocytes of human hearts with myocardial infarction. Circulation 1996; 94: 1506–12.
45. Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ. Bcl-2 deficient mice demonstrate fulminant lymphoid apoptosis, polycistic kidneys and hypopigmented hair. Cell 1993; 75: 229–40.
46. Oltvai ZN, Millirman CL, Korsmeyer SJ. Bcl-2 heterodimerized in vivo with a conserved homolog bax, that accelerates programmed cell death. Cell 1993; 73: 609–19.
47. Oltvai ZN, Korsmeyer SJ. Checkpoints of dueling dimers foil death wishes. Cell 1994; 79: 189–92.

J Clin Basic Cardiol 2000; 3: 204

Mitteilungen aus der Redaktion

Besuchen Sie unsere

zeitschriftenübergreifende Datenbank

☑ **Bilddatenbank** ☑ **Artikeldatenbank** ☑ **Fallberichte**

e-Journal-Abo

Beziehen Sie die elektronischen Ausgaben dieser Zeitschrift hier.

Die Lieferung umfasst 4–5 Ausgaben pro Jahr zzgl. allfälliger Sonderhefte.

Unsere e-Journale stehen als PDF-Datei zur Verfügung und sind auf den meisten der marktüblichen e-Book-Readern, Tablets sowie auf iPad funktionsfähig.

☑ **Bestellung e-Journal-Abo**