Apoptosis in the Infarcted Human Heart

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Abstract

Increased pressure loads and ischemia may trigger apoptosis in the heart. On this basis, the possibility was advanced that apoptotic myocyte cell death may be present in the infarcted area and in the surviving myocardium acutely after infarction. Ten myocardial samples were obtained from the necrotic tissue and from the region adjacent to and remote from infarction in 9 adults patients and one neonate who died shortly after the disease onset. Apoptosis was measured quantitatively on histologic sections stained by the terminal deoxynucleotidyl transferase assay and confirmed by DNA extraction and agarose gel electrophoresis. DNA strand breaks in myocyte nuclei were observed in all infarcted hearts involving most of myocyte nuclei in the necrotic tissue. In addition, the number of apoptotic myocytes was greater in the peri-infarcted region than that found far from the infarction. In these two regions of the heart evidences of apoptosis were confirmed by DNA laddering, whereas an aspecific DNA migration was present in the necrotic myocardium. Thus, apoptosis appears to be a significant complicating factor of acute myocardial infarction in humans increasing the magnitude of myocyte cell death in the non necrotic myocardium remote from the infarction.

Key words: apoptosis, myocardial infarction, human heart; DNA strand break.

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In recent years it has become apparent that the amount of viable functioning myocardium after a pathologic event is an important determinant in the development of cardiac dysfunction and intractable heart failure. Thus, the knowledge of the ongoing phenomena resulting in cell death is essential to develop strategies that can decrease myocyte cell loss. In the myocardium, in addition to the well known process of myocyte necrosis, the occurrence of myocyte cell death by apoptosis has been recently observed.

Apoptosis has been described in the heart during embryological [31, 32] and postnatal development [16]. This mechanism of death seems to play a role in the origin of myocardial injury following ischemia and reperfusion [4, 11], oxygen deprivation [29], overstretching papillary muscles [5], heart failure [19, 26] and myocardial infarction [13, 15]. In the infarcted heart myocyte apoptosis has been detected in area showing morphologic evidence of necrosis suggesting that both myocyte necrosis and apoptosis can affect the same cell [13, 15].

Acutely after ligation of the left coronary artery, most of the ischemic ventricle is functionally lost and destined to become necrotic whereas the remaining viable tissue is exposed to an abnormal diastolic load [21]. Thus, the infarcted heart represents a convenient model to determine whether ischemia and/or physical forces may elicit apoptotic cell death. In the necrotic area, ischemia is the prevailing factor whereas in the remote myocardium, in which blood supply is maintained, the passive stress predominates. The border zone located at the interface between the damaged and healthy myocardium represents an area where ischemia and stress are equally important [22]. In view of these observations, myocardial samples were obtained from adult patients who died shortly after acute infarction and the amount of apoptosis determined in the necrotic area, in the region bordering the infarction and in the tissue remote from the injured myocardium. A similar sampling was performed in a neonate with an extensive infarct of the right ventricle to determine whether age may have an influence on the apoptotic phenomenon. The results demonstrated that there is a gradient in the amount of apoptotic myocyte cell death, from the necrotic area to the remote myocardium, suggesting that apoptosis can be triggered by ischemia and mechanical forces alone or by a combination of both.

Materials and Methods

Patient population

Ten hearts, nine from adults and one from a neonate, with acute myocardial infarction were collected at autopsy from patients who died within 3 days from the onset of clinical symptoms. In each case a complete autopsy was performed within 13 hours after death. Specimens from the necrotic tissue and from the regions adjacent to and remote from the necrotic myocardium were fixed in 10% buffered formalin or frozen in liquid nitrogen and stored at -80°C. Seven hearts obtained from patients who died from causes other than cardiovascular diseases were used as controls. In these hearts, myocardial sampling was limited to the left ventricle. Since the infarction in the neonatal heart involved the right ventricle, the same ventricle from a control case was sampled.

Detection of DNA strand breaks

Myocardial samples were embedded in paraffin and sections, approximately 4 µm in thickness, were processed for routine staining and immunoenzymatic reactions. After dehydration in xylene, sections were exposed for 5 minutes to 0.3% H202 in methanol to block endogenous peroxidase activity. They were then incubated for 30 minutes at room temperature (RT) in PBS containing 0.1% saponin and 1 mM EGTA. Subsequently, sections were incubated for 30 minutes at 37°C in a solution containing 10 units per 100 µl of terminal-deoxynucleotidyl-transferase (TdT), 2.5 mM $CoCl_2$, 0.2 M potassium cacodylate, 25 mM Tris-HCl, 0.25% BSA and 0.5 nM biotinylated 2'-deoxyuridine-5'-triphosphate (biotin-16-dUTP). After rinsing in PBS, sections were immersed for 30 minutes at RT in a solution containing 4 X concentrated saline-sodium citrate buffer and 5% non-fat dry milk. Finally, the staining solution which contained 12.5% peroxidase conjugated Streptavidine and 10% Triton X-100 was applied for 10 minutes. Following incubation at 37°C with 3-amino-9-ethyl-carbazole the sections were counterstained with Mayer's hematoxylin. The counterstaining was omitted in some cases to obtain black and withe microphotograph. As a positive control, myocardial sections were exposed to DNase I before the staining reaction. Since this enzyme results in the formation of DNA strand breaks, all nuclei present in the tissue were positively labeled. Negative controls consisted of sections pretreated with DNase I in which the staining reaction was performed in the absence of biotin-16-dUTP or TdT.

Quantitative analysis of DNA strand breaks in myocytes

The number of myocyte nuclei labeled by biotinylated dUTP per mm^2 of tissue was measured by counting the number of positive nuclei per unit area of tissue in the necrotic myocardium, in the border zone and in the remote myocardium. Sections were examined at a magnification of X630 with an ocular reticle delineating a square area equal to 12,168 μm^2. An average of 200-220 fields in each sample were examined. In order to obtain the percentage of myocyte nuclei labeled by dUTP, the number of myocyte nuclei per unit area of tissue was also determined by counting, with the same grid, 50 fields at the same magnification in each area of the sampled myocardium stained by Hematoxylin & Eosin.

DNA extraction and gel electrophoresis

Frozen tissues were pulverized with a tissue dismembrator for 20 sec. The powder obtained from each sample was suspended in 500 µl of lysis buffer (10 mM Tris-HCl, pH 8.0, 10 mM NaCl, 10 mM EDTA, 100 µg/ml Proteinase K, 1% SDS) and incubated at 50°C for 1 hour. The extraction of DNA was achieved by the phenol/chloroform method and then precipitated overnight with sodium acetate 0.3 M and absolute ethanol at -20°C. The nucleic acids from each pellet were resuspended in a solution containing 10 mM Tris-HCl and 1 mM EDTA. After treatment with 20 µg/ml Rnase for 1 hour at 37°C, whole DNA was processed for electrophoresis. DNA concentration was determined by UV absorbency at 260 nm. Equal amounts of DNA (15 µg) from each the unseparated DNA and the supernatant fractions were mixed with loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol) and heated at 68°C for 5 minutes. Finally, ladder DNA markers and myocardial DNA were electrophoresed on agarose gel containing ethidium bromide. DNA was visualized under a UV transilluminator and the gels were photographed. Only sizes of DNA equivalent to 200 bp or multiples were considered to be representative of DNA laddering.

Data analysis

Data were collected blindly, and the code was broken at the end of the study. Results are presented as mean ± SD. Statistical significance for comparison between two measurements was determined by the unpaired two-tailed Student's t test. Values of $p < 0.05$ were considered to be significant.

Results

Tables 1 and 2 illustrates the characteristics of the patient population examined. Age, sex, body weight, heart weight and the intervals between initial symptoms and death and from death to autopsy are indicated. Death occurred within 2 ± 1 days from the initial clinical symptoms of myocardial infarction. The macroscopic examination of the heart showed that all patients had an acute myocardial infarction with tissue necrosis diffuse to the endomyocardium and midmyocardium of the anterior or posterior aspects of the left ventricular free wall, also extending to the interventricular septum. Three of these 10 infarcts were associated with rupture of the papillary muscles and sudden death. As illustrated in Table 2, the coronary vasculature was altered because of a severe and diffuse coronary atherosclerosis and in 4 instances was occluded by a recent thrombus. In adult patients heart weight varied from a minimum of 360 g to a maximum of 750 g. Total heart weight was increased above normal in 5 cases. In the neonatal heart a large acute

Table 1. Characteristics of the Patient Population

Patient Number	Age years	Sex	Body Weight Kg	Heart Weight g	Time Between Symptoms and Death, days	Time Between Death and Autopsy, h
1	2 days	M	1,92	15,3	2	7
2	58	F	75	470	3	10
3	57	M	76	650	1	12
4	51	M	77	560	1	11
5	54	M	72	570	1	16
6	48	M	57	750	3	14
7	39	M	66	390	3	17
8	42	M	65	360	3	12
9	55	M	72	420	0,5	13
10	60	M	67	360	1	9
	52 ± 7		70 ± 6	503 ± 130	2 ± 1	13 ± 2

Results are presentee as mean ± SD; M: Male; F: Female.

Table 2. Characteristics of the Myocardial Infarction

Patient Number	Location of Infarct	Site of Rupture	Atherosclerotic Involvement	Site of Coronary Occlusion
1	RV			
2	LV Posterior	PPM	One Vessel	LCA
3	LV Lateral		Several Vessels	
4	LV Anterior and Lateral		Several Vessels	
5	LV Posterior and Septum	PPM	Several Vessels	LCA
6	LV Anterior	APM	Several Vessels	LAD
7	Septum		Several Vessels	
8	LV Anterior and Lateral		Several Vessels	
9	LV Anterior and Septum		Several Vessels	LAD
10	LV Anterior		Several Vessels	

RV: Right Ventricle; LV: Left Ventricle; PPM: Posterior Papillary Muscle; APM: Anterior Papillary Muscle; LCA: Left Circumflex Coronary Artery; LAD: Left Anterior Descending Coronary Artery.

infarct of the right ventricle was present whereas the left ventricle and the interventricular septum appeared normal at gross examination. The right and left coronary arteries did not show acute thrombus formation or atherosclerosis. The clinical symptoms started few hours after delivery and ST depression was seen by ECG at one day after birth. Since cerebral hemorrhage was present at autopsy, a coagulation disorder in this premature sibling may have affected cerebral and coronary vessels. However, we cannot exclude a prolonged ischemia during the labour. Heart weight was within normal limits for this age.

Table 3 lists the characteristics of the patients used as controls and their cause of death. Age and body weight were not statistically different from that of infarcted patients. In addition, the interval between death and autopsy was similar in all groups of patients. Control hearts showed minimal coronary atherosclerosis of the three major epicardial vessels and the heart weight was not different from ischemic heart with no hypertrophy.

Light microscopic examination of tissue sections showed that myocardial necrosis, characterized by disappearance of the typical cytoplasmic structures, was present in all pathologic hearts. The definition of the border zone and

Table 3. Characteristics of Control Patients.

Patient Number	Age years	Sex	Body Weight Kg	Heart Weight g	Time Between Death and Autopsy, h	Causes of Death
1	2 days	M	1,8	18	7	Cerebral Hemorrhage
2	56	F	58	340	14	Cirrhosis
3	47	M	83	310	17	Cerebral Aneurysm
4	40	M	75	432	4	Trauma
5	51	M	68	380	5	Cerebral Aneurysm
6	57	F	77	380	12	Trauma
7	58	M	61	390	13	Cerebral Aneurysm
	52 ± 6		70 ± 9	372 ± 39	11 ± 5	

Results are presented as mean ± SD; M: Male; F: Female.

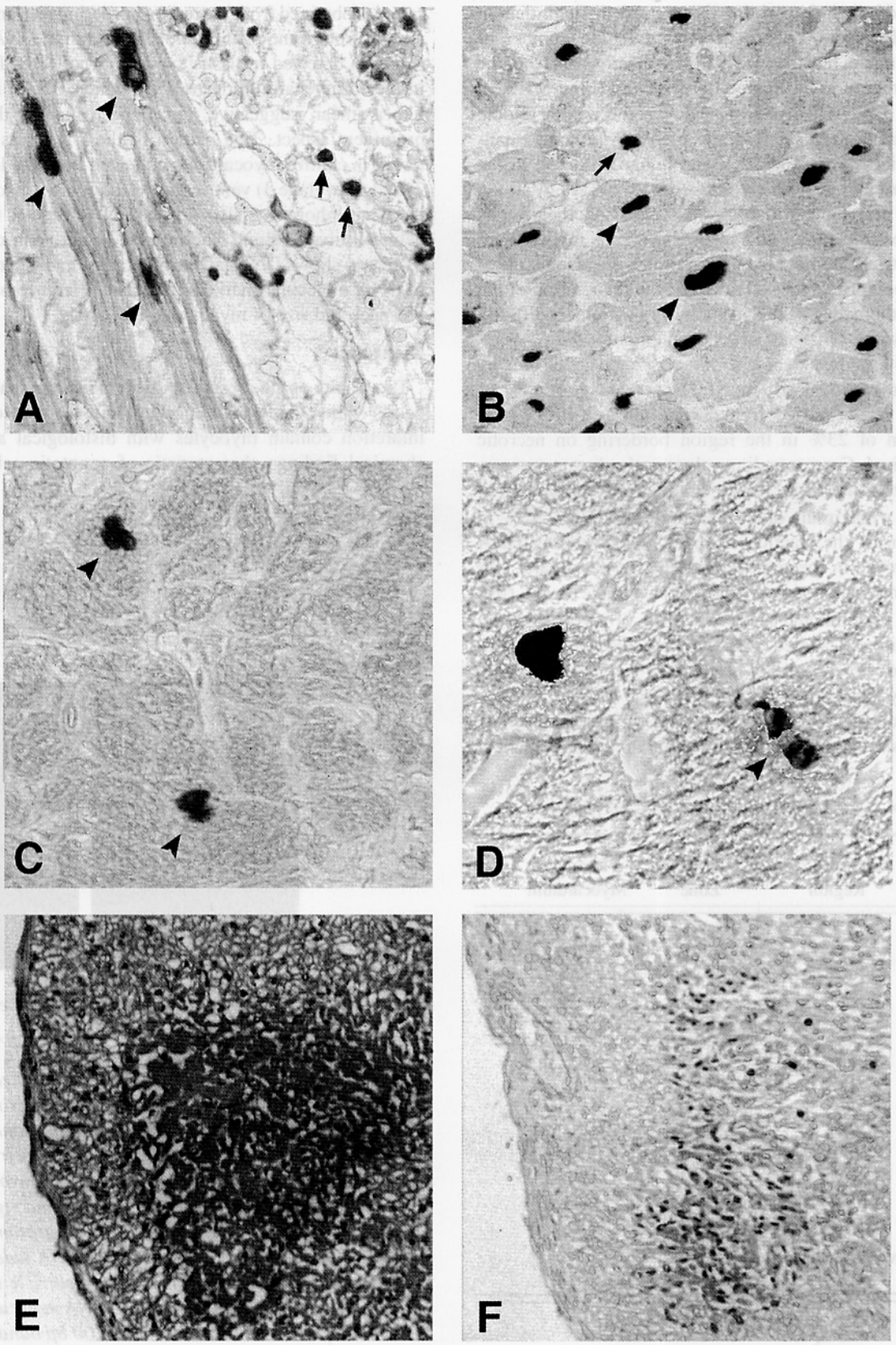
remote myocardium in the presence of an acute, necrotic infarct is difficult. Following the approach previously employed in our laboratory [22], the area of myocardium comprising 10% of the spared tissue adjacent to the necrotic portion of the wall was considered representative of the region bordering the infarct. An additional 10% of the left ventricular wall, between the edge of the border zone and the remote healthy tissue, was excluded from the analysis and was assumed to constitute a region of overlapping between these two portions of the surviving myocardium. The remaining left ventricular myocardium was sampled as tissue distant from the infarction. A similar procedure was used for the infarcted right ventricle of the neonate. In the infarcted adult hearts small foci of replacement fibrosis, less than 1 cm in diameter were observed across the left ventricular wall. Similar lesions characterized by reparative and interstitial fibrosis were also noted in myocardial samples of control hearts.

Sections of myocardium from control hearts did not show DNA strand breaks in myocyte nuclei and interstitial cells. The specificity of this technique to stain DNA strand breaks was documented by the ability to label nuclei following exposure of the normal tissue to DNase I (data not shown). In contrast, DNA strand breaks were not detected when biotin-16-dUTP or TdT was not included in the enzymatic reaction.

Figure 1A shows dUTP labelling in the necrotic region in which all myocyte nuclei are positive following the dUTP reaction whereas alterations in sarcomere striations and cytoplasmic membranes are apparent. Interstitial cell infiltrating the necrotic tissue also exhibit dUTP labeled nuclei. Figure 1B shows numerous myocytes with positively labeled nuclei surrounded by negative cells in the region bordering on an infarct of the left ventricle in a patient who died 2 days after an acute precordial pain indicative of myocardial ischemia. It should be emphasized that myocytes stained by dUTP in the border zone did not show alterations of cytoplasmic components, nor disruption of the plasma membrane, nor contraction bands in myofibrils. A few myocytes with DNA strand breaks were also present in the tissue remote from the infarction (Figure 1C) and again in all cases cellular morphology was preserved.

The progression of the apoptotic process leads to nuclear fragmentation. This phenomenon was observed in the necrotic myocardium as well as in the spared tissue and nuclear fragments of myocytes were found to be stained by

Figure 1. Myocardial sections of paraffin embedded ventricular tissue from infarcted hearts. A: In the necrotic region most myocytes with structural alterations show dUTP labeled nuclei (arrowheads). Interstitial cell nuclei (arrows) are also stained by dUTP. (Original Magnification [OM] : X 1000; dUTP reaction - not counterstained); B: Several myocytes with labeled nuclei (arrowheads) and negative cells both with normal morphology found in the region bordering on the necrotic area. dUTP labelling is seen in few interstitial cells (arrow). (OM: X 400; dUTP reaction - not counterstained); C: Scattered myocytes with labeled nuclei in the remote myocardium. The normal structural components of the cytoplasm are clearly identified. (OM: X 400; dUTP reaction - not counterstained); D: dUTP labeled nuclear fragmentation (arrowhead) is shown in an otherwise morphologically intact myocyte adjacent to a dUTP positive myocyte. (OM: X 1000, contrast phase; dUTP reaction - not counterstained); E: Myocardial section of the right ventricle from the neonatal heart showing an area of necrotic myocardium characterized by hyperchromatic staining. (OM: X 200; Hematoxylin & Eosin); F: Numerous myocyte nuclei in the same region depicted in E are clearly labeled by dUTP. (OM: X 100; dUTP reaction- not counterstained).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dUTP (Figure 1D). In the infarcted right ventricle of the neonate the myocardium demonstrated the typical findings associated with coagulative necrosis (Figure 1E). The border zone was of smaller size making the distinction between necrotic and surviving tissue more difficult than in adults. However, a sharp decrement in the number of myocyte nuclei labeled by dUTP was clearly seen from the necrotic region to the spared myocardium (Figure 1F). In addition, scattered myocyte nuclei in the region remote from infarction and small groups of myocytes corresponding to microscopic foci of tissue injury observed in the interventricular septum were labeled by dUTP. In all the infarcted hearts interstitial fibroblasts and endothelial cells in the necrotic tissue and in the surviving myocardium adjacent to and remote from infarction were also involved by the apoptotic process. However, the recognition of the origin of these dUTP labeled nuclei was extremely difficult in the absence of tissue perfusion - fixation.

Table 4 lists the percentage of myocyte nuclei labeled by dUTP in the 10 cases examined. The percentage of positive myocyte nuclei varied from a minimum of 1.85% to a maximum of 23% in the region bordering on necrotic myocardium. Corresponding values in the distant myocardium were consistently lower, varying from a minimum of 0.01% to a maximum of 0.9%, averaging 0.37 ± 0.32%. In all instances, the fraction of myocytes exhibiting DNA strand breaks in the region bordering on the infarct was significantly greater than that in the remote tissue ($p < 0.01$). In the infarcted region 94 ± 7% of myocyte nuclei were positively labeled. It should be noted that, occasionally, myocyte nuclei not stained by hematoxylin were positively labeled by the dUTP reaction.

The occurrence of DNA fragments on agarose gel electrophoresis of size equivalent to mono or oligonucleosomes confirmed the detection on histologic sections of apoptotic DNA strand breaks in myocyte nuclei. As illustrated in Figure 2, stretches of DNA of size equivalent to or multiples of 200 bp were identified in myocardial samples obtained from the region bordering on (lane 5) and remote from (lane 6) infarction. In addition, DNA laddering was detected in tissue specimens collected from the infarcted myocardium (lane 4) in which DNA diffusion, like a smear, suggestive of cell necrosis was the more prominent aspect. This pattern of the DNA was not detected in control myocardium collected from the left (lane 2) and right (lane 3) ventricular wall. Figure 2 also depicts the electrophoretic analyses of the DNA extracted from the neonatal myocardium. The infarcted right ventricle (lane 8) clearly shows DNA laddering. Lane 1 and 7 correspond to DNA molecular markers used as a reference bands for presence and size of myocardial laddering.

Table 4. Percentage of dUTP Labeled Myocyte Nuclei.

Patient Number	Necrotic Region	Border Zone	Remote Myocardium
1	98	3,7	0,010
2	79	23,00	0,430
3	82	1,85	0,022
4	99	10,94	0,340
5	97	13,19	0,880
6	100	5,17	0,830
7	95	10,00	0,074
8	98	10,66	0,010
9	92	10,30	0,600
10	97	9,00	0,500
	94 ± 7	9.78 ± 5.57	0.370 ± 0.318

Results are presented as mean ± SD.

Discussion

The results of the present study demonstrate that human hearts with clinical and morphologic features of acute infarction contain myocytes with histological and biochemical findings characteristic of apoptotic cell death.

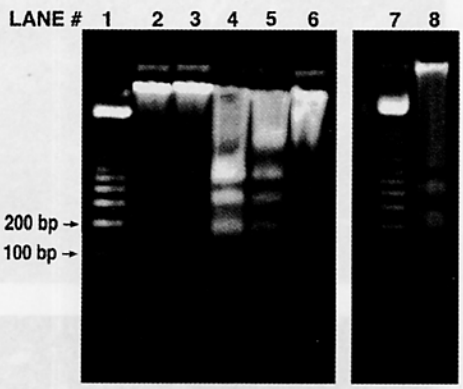

Figure 2. Electrophoretic pattern of the DNA extracted from the infarcted necrotic tissue (lane 4), from the border zone (lane 5) and from the remote myocardium (lane 6) of an adult patient who died 2 days after the disease onset. Note DNA laddering in the border zone and in the myocardium remote from infarction. In the necrotic region, DNA fragments assume the aspect of a smear. The myocardium obtained from control left (lane 2) and right (lane 3) ventricles do not show DNA migration. In the neonatal ischemic myocardium, DNA laddering is apparent in the infarcted right ventricle (lane 8). Lanes 1 and 7 corresponds to DNA molecular marker: arrows indicate 100 and 200 bp bands.

Apoptotic myocytes were detectable in the necrotic area, in the border zone and in the viable myocardium remote from these two regions. Similar magnitude of apoptosis was found in neonatal and adult ischemic heart, whereas this phenomenon was not detected in samples from patients who died without clinical or morphologic evidences of myocardial ischemia. No correlation was observed between the percentage of apoptotic myocytes and sex, body weight, heart weight and location and size of infarction. Thus, apoptotic cell death represent a significant component of acute myocardial infarction in humans.

The presence of DNA strand breaks in myocytes located in the region supplied by an occluded coronary artery is difficult to explain because necrosis and apoptosis are considered two distinct mechanisms of cell death. The term coagulative necrosis according to the textbooks is used to define a characteristic morphologic aspect of the myocardium several hours after a permanent occlusion of a large coronary vessel [24]. The initial response to myocardial ischemia includes inhibition of oxidative metabolism and reduction in contractile function. Cell death, however, develops when ATP levels are drastically reduced and anaerobic glycolysis stopped, probably when breaks in the plasmalemmal membrane develop [14]. This alteration in myocyte membrane is considered irreversible and an initial, although definite, marker of necrotic cell death.

The consequence of ischemic myocyte cell death is a reparative process in which necrotic myocytes are eliminated and replaced by fibroblasts and new vessels. Following deposition of collagen and ground substance, with time, scar formation takes place. The typical histologic aspect of coagulation necrosis consists of increased cytoplasmic eosinophilia and nuclear pyknosis, karyolysis and karyorrhexis that may be apparent within the first 2-4 hours or as late as 8-12 hours [24] after ischemia. Necrotic cell death is associated with the release of lysosomal proteases that lead to the degradation of histones in the nucleosomes so that the unprotected DNA can be cleaved by endonuclease [30]. This process results in the generation of DNA fragments with large variation in length and a diffuse DNA pattern, like a smear, on agarose gel electrophoresis. In summary, necrosis can be considered the final, irreversible morphologic outcome of multiple biochemical alterations induced by permanent ischemia.

Internucleosomal DNA fragmentation is considered a hallmark of apoptosis. In this process, activation of a Ca^{++}/Mg^{++} dependent endonuclease leads to DNA degradation in single or multiple 200-300 bp fragments. DNA strand breaks can be detected when tissue sections are exposed to TdT that catalyses the binding of biotinylated-dUTP to the 3' hydroxyl terminal of the opened DNA molecule [10]. This methodology can be complemented by the extraction of nucleic acids from the tissues and the demonstration of regular DNA laddering after agarose gel electrophoresis [13, 15, 19]. In addition, characteristic nuclear morphologic changes have been described in apoptotic cells. The nuclear chromatin collapses against the nuclear membrane and eventually the entire nucleus condenses in a single dense ball [8]. At the same time extensive cell blebbing can occur resulting in fragmentation of the entire cell in membrane enclosed vesicles, the so-called apoptotic bodies [17]. These structures are rapidly phagocytosed and destroyed by macrophages with no or minor signs of inflammation or immunological reaction in the surrounding tissue. Finally, there are many genes that can modulate apoptosis either favouring i.e. Bax, Bcl-x (short form), Bad, P53 and Ice, or preventing, i.e. Bcl-2, Bcl-x (long form), and Bag-1 [3, 12, 18, 20, 23, 27, 31, 32]. These gene products may act in concert with a variety of extracellular growth factors [4, 25] which in turn are opposed by other substances that predominantly induce cell death [7]. In summary, apoptosis is a very complicated event, that requires energy, is tightly regulated and under genetic control.

Although the above evidences seem to indicate that apoptosis and necrosis have to be considered two different types of cell death, as found here, a combination of necrosis and apoptosis has been described during the acute stages of myocardial infarction in human [13]. Results in the current study extend these previous observations in the infarcted region and document quantitatively that programmed myocyte cell death is also present independently from necrotic cell death, at least in the region remote from the infarcted myocardium. Experimental studies in rats have demonstrated that apoptosis precedes necrosis, and both phenomena may be present in the ischemic area at different time intervals [15]. Patients of the present study died within 2 ± 1 days from the initial symptoms of acute myocardial infarction and, in 3 out of 9, the death was sudden since occurred 60 minutes following symptoms. Although in humans is very difficult to establish with certainty the time of coronary artery occlusion, the present findings are in agreement with the notion that apoptosis is an early event in the infarcted myocardium. However, the presence of a large number of apoptotic myocytes in the necrotic area several days after the initial symptoms [13] may imply that this phenomenon persists for a long period of time. In cell cultures the length of the apoptotic process may be as short as 15-30 minutes [8], but this information is lacking in pathologic tissue, like the infarcted myocardium, in which no circulating blood is available immediately after the coronary occlusion. Furthermore, it should be noted that cell death by apoptosis or necrosis can share common pathways [2, 9]. In addition, the detection of these phenomena may be affected by the sensitivity and specificity of the method used. DNA strand breaks are present in both conditions, and only the regular DNA laddering composed of 200-300 bp fragments seem to be specific for the apoptotic process. Thus, further studies need to be done to clarify the significance of the association of necrosis and apoptosis in the infarcted myocardium.

The presence of apoptotic myocyte cell death found here in the border zone which is considered a portion of the myocardium at risk containing a mixture of dying and alive

myocytes may increase infarct size contributing to the development of intractable congestive heart failure and death. This conviction is supported by the absence of necrotic changes of myocytes in the presence of dUTP labeled nuclei, suggesting that the activation of apoptosis in the border as well as in the remote zone differs from the myocyte death signaling in the necrotic myocardium. The remodelling of the surviving left ventricular myocardium is differently expressed in the regions adjacent to and remote from the infarcted tissue. Wall thinning with side-to-side slippage of myocytes and cellular hypertrophy are greater in the border zone [22]. These changes have been attributed to the greater stress on this area with respect to the remaining myocardium [1]. The distribution of apoptosis in the infarcted ventricle found here appears to reflect the variations in loading associated with the loss of a large portion of functioning tissue. In this regard, a small amount of apoptosis was found in papillary muscles stretched beyond their maximal length [5]. In this load dependent model apoptotic myocyte cell death was coupled with depressed mechanical performance and side-to-side slippage of cells within the tissue [5].

In conclusion, the results of this investigation demonstrated that there are different mechanisms of myocyte cell death in the infarcted human myocardium. Some myocytes are affected by apoptosis and necrosis and some others by apoptosis alone. Although this difference may be purely coincidental, the possibility can be advanced that the apoptotic component of myocyte cell death in the infarcted myocardium can be decreased or prevented by therapeutic intervention.

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