Studying the dynamics of the programmed cell death in the fetal heart-comparative study between the atria and ventricles

Received for publication, April 2, 2010
Accepted, May 21, 2010

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Abstract

Introduction: Cell death, with the growth and differentiation is part of the cell cycle. This study tries to evidentiate apoptotic cells at the level of the heart, and to appreciate the level of Apoptotic Index in different phases of cardiac development in order to compare the amplitude of the phenomenon in different cavities of the heart.

Materials and methods: We studied myocardial tissue samples collected from human aborted fetuses, with the age between 16 and 40 weeks, resulting from spontaneous abortions without cardiac malformations or injuries. The samples were stained conventionally with hematoxylin-eosin (H-E), Masson's tricrome and immunohistochemical method. We used a complete kit, ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit, based on terminal deoxynucleotidyl transferase (TdT).

Results and discussion: We made a comparison between the intensity of programmed cell death in atrial and ventricular myocardium and we found a higher density of apoptotic cells in the ventricular myocardium. This is visible by direct examination of histological images acquired by immunohistochemical staining Tunnel. Our finding was confirmed, once again, by statistical processing.

Conclusions: Apoptotic phenomenon increases with fetal age, both the ventricles and the atrial myocardium. Apoptotic index in the ventricular myocardium increased progressively from 0.223% at 16 weeks to 3.65% ± 0.08% to 40 fetal weeks. In the atrial myocardium apoptotic index increased progressively from 0.131% ± 0.07% to 1.131% ± 0.08% between weeks 16 and 40 of gestation. At the atrial level, the phenomenon is significantly increased between weeks 19-22 and after the 26th week (p (t) <0.05).

Keywords: heart development, apoptotic index

Introduction

Apoptosis-related concepts have become essential in understanding many aspects of structure and functioning of the heart.

Although cell death by apoptosis is often beneficial for the heart, when is exaggerated it can have abnormal consequences.

Cell death, with the growth and differentiation is part of the cell cycle (STEVEN A. & al.[1], 2000).

Homeostatic control of cell number results from a dynamic balance between proliferation and cell death.

There are two different mechanisms of cell death, necrosis and apoptosis. Apoptosis, or programmed cell death, is a normal cell response to different stimuli, so is the consequence of intervention of some mechanisms controlled by a genetic program, in the absence or presence of a pathologic agent (BAE S. & al. [2],2003; ŠOOCHAN & al. [3],2005).
The characteristics of apoptosis allow these phenomenon to be involved in tissue remodeling without destroying the integrity of the organ development (THOMAS [4], 1999).

A marker of apoptosis is activation of an endonuclease that break the nuclear deoxiribonucleic acid (DNA) in fragments having regular sizes.

Later, the apoptotic cell suffers extracellular degeneration and then the phagocytosis of apoptotic cells occurs and bodies with engulfment by neighboring macrophages.

In contrast to necrosis, apoptosis does not cause inflammation.

Absence of inflammatory process spares surrounding cells, which will not be harmed (JACOBSON MD. & al.[5], 1997; NAGATA S.[6], 1997).

The foetal heart contain different proportions of cells with the classic features of apoptosis, including cytoplasmic, nuclear and DNA fragmentation (HARDY K.[7], 1997).

This study tries to evidentiate apoptotic cells at the level of the heart, and to appreciate the level of Apoptotic index in different phases of cardiac development in order to compare the amplitude of the phenomenon in different cavities of the heart.

**Material and method**

We studied myocardial tissue samples collected from human aborted fetuses, with the age between 16 and 40 weeks, resulting from spontaneous abortions without cardiac malformations or injuries (Figure 1).

We collected myocardial tissue samples with a size of about 1 cm³ thickness, from the right and left ventricular wall and the atrial wall.

![Figure 1. Heart from aborted fetus at the age of 30 weeks. (Constanta Clinical University Hospital)](image)

Tissue samples were fixed with 10% buffered formaldehyde and embedded in paraffin. From each paraffin block were cut 4 to 6 sections of 5μm thickness and analyzed in order to identify apoptotic cells.

The histological samples were stained conventionally with Hematoxylin-eosin (H-E) (Figure 2) and Masson tricromic (Figure 3).
DNA fragmentation, the typical biochemical feature of apoptosis, was detected immunohistochemical with the use of “in situ” DNA end-labeling method (MC CARTH¥& al. [8], 1998).

We used a complete kit, ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit, based on terminal deoxynucleotidyl transferase (TdT), an enzyme more selective and specific than DNA polymerase, which allow us to make the distinction between the apoptosis and necrosis. Apoptotic cells were stained brown, dark brown, while the necrotic ones were stained more diffuse.

Sections were dewaxed, transferred to xylene and rehydrated with decreasing concentrations of ethanol (100%, 95%, 70%). After rehydration, slides were incubated with proteinase K 20μg per millimeter, in phosphate buffered saline (PBS).

The endogenous peroxidase was inactivated by hydrogen peroxide 3%.
Staining procedure followed the manufacturer's instructions. The method is based on a preferential covalent binding of nucleotides biotinilat 3'-OH end of DNA (GAVRIELI Y. & al. [9], 1992), reaction catalysed by deoxinucleotidil terminal transferase (TdT).

Staining purposes with ApopTag kit are the multiple 3'-OH ends of DNA, generated by DNA fragmentation, typically localized in apoptotic nuclei.

Figure 4. Apoptotic cells with loss of membrane integrity and nuclear fragmentation in atrial myocardium

The cells with apoptotic nuclei and apoptotic bodies were stained dark brown (Fig. 4). Normal nuclei or in some stage of proliferation, which have an insignificant number of 3'-OH ends of DNA, were not stained with this kit.

They can be viewed by counterstain with methyl green, which stains nuclei normal in green – blue.

Cells that undergoes necrosis, in some cases may contain DNA ends resulting in their colour, but because of ApopTag staining method used is specific for apoptotic cells, necrotic cells will stain more diffusely.

Four to six sections from each specimen were examined. Sections were examined under light microscopy at law magnification (x100) and then, 10 random fields per section, each 7 050μm² in size, from the regions with apoptotic cells were examined at a higher magnification.

The degree of nuclear staining by immunohistochemical method was expressed by the apoptotic index, calculated using the formula:

\[
\text{Apoptotic index} = \left( \frac{\text{apoptotic nuclei}}{\text{total no. of nuclei}} \right) \times 100
\]

For statistical analysis we used the gestational age and average apoptotic index.

We applied Student t test (\(P \leq 0.05\) significant finding), in Microsoft ® Excel ® 2000 operating system Microsoft ® Windows ® 2000.

Results and discusions

Atrial and ventricular compartments of the heart expand and grow during development, leading to a high rate of cell proliferation. This justifies the low levels of apoptosis found us in this study.
Conclusions of the previous studies show a great variability in the intensity of the apoptotic phenomenon, which is probably due to technical limitations of terminal staining of DNA.

Statistical analysis allowed us to suggest that the apoptotic phenomenon increases with fetal age.

Considering the functional adaptive changes that occur in the cardiovascular system even before birth and anticipated ones after birth, we believe that apoptosis occurs as a normal process during the heart development.

Apoptotic phenomenon is more intense in the hearts of fetuses aged between 34 and 40 weeks as can be seen in the graphic below.

In the next phase of study, we made a comparison between the intensity of programmed cell death in atrial (Fig. 4) and ventricular myocardium (Fig. 5) and we found a higher density of apoptotic cells in the ventricular myocardium.

This is visible by direct examination of histological images acquired by immunohistochemical staining Tunnel.

Our finding was confirmed, once again, by statistical processing.

Figure 4. Atrial myocardium (fetus of 22 weeks). Staining DNA fragments with terminal TdT.10X.

Figure 5. We can observe the high density of apoptotic nuclei in the ventricular myocardium compared with atrial myocardium (top image), the fetus of 22 weeks. Staining DNA fragments with terminal TdT.10X.
Calculating the growth rate of Apoptotic Index, we can see the differences between ventricular and atrial myocardium (Graphic no.1).

We can see that the apoptotic index at ventricular level has a growth rate in the fifth month of pregnancy of 0.116% per week, the sixth month of pregnancy 0.06% per week, then in the seventh month of 0.086% per week, the eighth month was 0.223% per week and last month of pregnancy 0.176%.

At the atrial level the growth rate evolves from 0.013% per week in the fifth month to 0.063% per week in the sixth month, 0.031% per week in the seventh month, 0.086% per week in the eighth month and 0.086 % per week in the ninth month.

Our results are quite similar to those obtained by Kajstura et al. (Kajstura J & al. [10], 1995; Abdelwahid E & al. [11], 1999).

Making studies in mouse hearts, Kajstura found a growing percentage of apoptotic cells, as heart development progresses from fetal stage to the adult stage.

He also found a higher incidence of apoptotic phenomenon at the level of the right ventricle than to the left.

This finding led the authors to postulate that apoptosis plays a role in pre-and postnatal remodelling of the ventricles, which results in the appearance of a thinner-walled right ventricle than the left one.

**Conclusions**

The phenomenon of apoptosis is involved in the remodelling of the heart during the fetal period as a natural process.
Low levels of apoptotic cells, during development of the cardiovascular system (apoptotic index at the age of 16 weeks is 0.223% ± 0.07%) are due to the high rate of cell proliferation, which is necessary for growth and expansion of heart compartments.

Apoptotic phenomenon increases with fetal age, both the ventricles and the atrial myocardium. Apoptotic index in the ventricular myocardium increased progressively from 0.223% at 16 weeks to 3.65% ± 0.08% to 40 fetal weeks.

In the atrial myocardium apoptotic index increased progressively from 0.131% ± 0.07% to 1.131% ± 0.08% between weeks 16 and 40 of gestation.

The phenomenon is accelerating in early fetal period and in the last eight weeks of pregnancy, at the ventricular level.

At the atrial level, the phenomenon is significantly increased between weeks 19-22 and after the 26th week (p (t) <0.05).

The apoptotic phenomena is more intensive in the older group of foetuses with the age between 34 and 40 weeks and in the right ventricle than in the left one, a factor that might contribute to the thinning of the RV after birth.

Apoptotic index growth rate is different at the ventricular and atrial significantly higher in the ventricular myocardium.

It is also different at different fetal ages: significantly higher in fifth and eighth months of pregnancy, in both the atrial and ventricular myocardium; significantly lower in the months sixth and ninth in the ventricles and only the seventh month in the atria.

Apoptotic cell density is higher in ventricular than in atrial myocardium in fetuses to the same age (p (t) <0.05).

References