Apoptosis and Human Ischemic Myocardial Damage, Including Conduction System

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Abstract

DNA fragmentation was observed by TUNEL method in damaged-cardiomyocytes (coagulation necrosis and contraction band necrosis) of human acute myocardial infarction. Both sinus and atrioventricular node-cells showed DNA fragmentation in 2 of 13 autopsy cases without clinical cardiac complications. Apoptosis plays an important role in ischemic myocardial cell damage and in cardiac conduction system.

Key words: TUNEL, acute myocardial infarction, apoptosis, conduction system, tissue transglutaminase.

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It has been well known that apoptosis is one major type of cell death and that it is associated with DNA fragmentation (internucleosomal cleavage of DNA) [1].

A few techniques, including the TUNEL method [2], DNA agarose gel electrophoresis [3] and immunohistology [4], have been used successfully to demonstrate apoptosis in histologic studies. In this study on ischemic myocardial damage by using these techniques, we tried to examine whether the typical necrosis is or is not completely independent from apoptosis. The TUNEL method can detect the early stage of myocardial cell death (cell-nuclei with fragmented DNA) which can not be demonstrated by the routine histologic method of H&E staining. In this study, we tried to examine the changes of sinus node cells and A-V node cells by the TUNEL method in order to examine whether DNA fragmentation occurs, or not during the agonal stage of a dying human with any disease.

Materials and Methods

Tissue samples

Nineteen autopsy cases of acute myocardial infarction (14 men and 5 women, ranging in age 58-93 years) were examined. Three cases with no particular myocardial changes were used as controls. Cardiac tissue blocks of the sinus node and A-V node taken out from 13 autopsy cases (6 men and 7 women, ranging in age 48-79 years) without cardiac complications were also examined. All cardiac tissues were fixed in 10% neutral buffered formalin, dehydrated in graded alcohols, cleared in xylene, and embedded in paraffin. Sequential sections were cut 6 μ thick for light microscopic examination and immunohistochemistry.

Techniques reported by James [5] and by James [6] were used for taking out tissue-blocks containing the sinus node and the A-V node.

TUNEL method

DNA nick end labelling was performed by the modified method (TUNEL method) reported previously by Gavrieli et al.[2] In brief; after being deparaffinized with xylene bath and washed in distilled water (DW), sections were incubated with 20 μg/ml proteinase K for 15 min at room temperature (RT), and then washed in DW four times. After the endogenous peroxidase was inactivated by covering sections with 2% H_2O_2 for 5 min at RT, the sections were rinsed with DW and immersed in TDT buffer (30 mM/L Tris-HCl buffer, pH 7.2, 140 mM/L sodium cacodylate, 1 mM/L cobalt chloride). Deoxynucleotidyl transferase (0.3 U/ml) and biotynilated dUTP in TDT buffer were then added to cover the sections and incubated in a humid atmosphere at 37°C for 60 min. The reaction was terminated by transferring the slides to the buffer containing 300 mM/L sodium chloride and 30 mM/L sodium citrate for 15 min at RT. The sections were then rinsed with DW, covered with 2% aqueous solution of bovine serum albumin for 10 min at RT, rinsed in DW, and immersed in phosphate buffered saline (PBS) for 5 min. The sections were covered with streptavidin-peroxidase complex, incubated for 10 min at 37°C, washed in DW, immersed in PBS

for 5 min in PBS, and stained with 3-3' diaminobenzidine tetrahydrochloride for 30 min at RT.

Immunohistology

Mouse monoclonal antibody for Transglutaminase II (mAbTG) (Clone CUB 7402) was purchased from Neo Markers (4880 Greekwood Drive, Fremont, CA 94555, USA). Immunohistologic staining was performed by the modified procedure reported previously [4].

After deparaffinization, the sections were irradiated by microwave for 5 min 5 times and treated with 2% H_2O_2 to inactivate endogenous peroxidase activity, washed in phosphate buffered saline (PBS) and then covered with 5% rabbit serum for 30 min at room temperature to block nonspecific binding of the antibody. The sections were then incubated with mouse anti-tTG antibody overnight at 4°C in 100% humidity, washed with PBS, and incubated with biotinylated rabbit anti-mouse IgG + IgA antibody (Nichirei Co, Ltd Tokyo, Japan) for 60 min at room temperature. After being washed with PBS, sections were incubated with preformed streptavidin-horseradish peroxidase complex for 10 min at room temperature and visualized with 3-3' diaminobenzidine tetrahydrochrolide.

Results

Acute myocardial infarction foci

The areas having typical morphological features of coagulation necrosis were selected in H&E-stained preparations, where the damaged myocardial cells preserved cell outline with or without nuclei and the cytoplasm appeared more eosinophilic, whereas cytoplasmic striations became less evident or disappeared (Fig. 1a). The myocardial cell-nuclei at the corresponding areas above stained dark brown by TUNEL staining. Even nuclear ghosts (unstained nuclei) of myocardial cells, which were not detected by H&E staining, but the location of which was estimated by lipofuscin granules in the myocardial cells, were stained strongly positive by the TUNEL method (Fig. 1b). The areas of the typical contraction band necrosis were also selected in H&E-stained preparations (Fig. 2a). The nuclei at the corresponding areas above stained strongly positive by the TUNEL staining method (Fig. 2b).

The characteristic morphological features in apoptosis, such as, shrinkage of cells, condensation of chromatin under the nuclear membrane, nuclear fragments, apoptotic bodies and phagocytosis by neighbors, were never observed. Neutrophils infiltrating the infarcted foci were not stained positive by the TUNEL method.

Sinus node and A-V node cells

The nuclei of nodal cells were not remarkable in all cases in H&E stained-preparations, whereas the nuclei of both the sinus node cells (Fig. 3a) and A-V node cells (Fig. 3b) were positive with TUNEL staining in 2 of 13 autopsy cases and nuclei of either type of node cells were positive with the TUNEL method in 4 of 13 cases.

Immunohistologic examination

In cases of acute myocardial infarction, the surviving myocardial cells expressed strong activity of tTG. In a few layers of surviving myocardial cells around a small artery, in those cells just beneath the endocardium and in islands of surviving cells in infarcted myocardial cell-foci, a positive reaction was observed diffusely in their cytoplasm (Fig. 4). Capillary endothelial cells were positive mainly in the areas of surviving myocardial cells. Smooth muscle cells of large vessels were positive in places, with anti-tTG weakly positive in other cases.

In sinus node cells and A-V node cells, the expression of tTG was observed in nodal cell-cytoplasm diffusely, stronger than normal nyocardial cells. Endothelial cells in nodal cell areas were also stained with anti-tTG.

Discussion

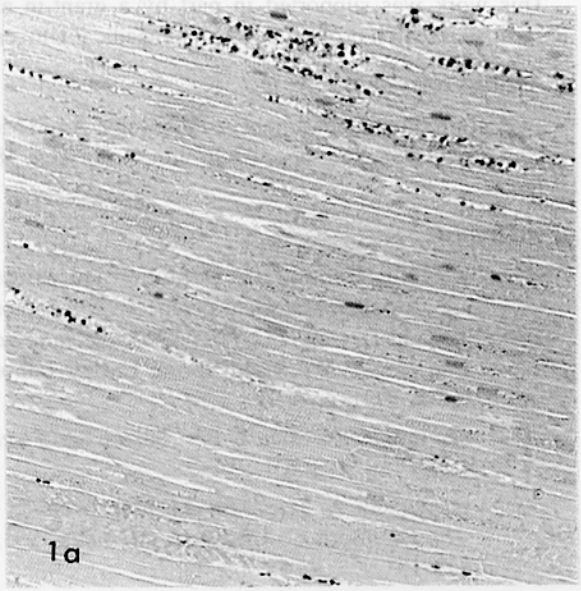
Contraction band necrosis has been well known to be caused by a combination of ischemia and reperfusion [7]. Gottlieb et al. [8] have reported that ischemia/reperfusion produced apoptosis in rabbit cardiomyocytes. In this study, the nuclei of the myocardial cells with contraction band necrosis stained strongly with the TUNEL method. This finding, therefore, is not incompatible with the previous

Figure 1. A 72 year-old-female patient with acute myocardial infarction. a) Myocardial cells with typical coagulation necrosis are present. Their nuclei are weakly stained with hematoxylin. (H&E stain. X 150) b) The myocardial cells at the corresponding area of Fig. 1a). Positive reaction with TUNEL method was observed in their nuclei (TUNEL stain. X 150).

Figure 2. A 72-year-old-male patient with acute myocardial infarction. a) A surviving myocardial cells are adjacent to the necrotic cells having a morphological feature of contraction band necrosis (lower hal portion). (H&E stain. X 150) b) Nuclei of surviving myocardial cells (upper half portion) at the corresponding area of Fig. 2a) are negative with TUNEL method, whereas nuclei of necrotic cells showing contraction band necrosis is strongly positive with TUNEL stain (TUNEL stain. X 150).

Figure 3. A 52 year-old-female patient with pancreas necrosis. a) Sinus node cells were positive with TUNEL stain. (TUNEL stain. X 150) b) A-V node cells were positive with TUNEL stain (TUNEL stain. X 150).

Figure 4. Same patient in Fig. 1). Surviving myocardial cells just beneath the endocardium express strong tTG activity in their cytoplasm. Endothelial cells of capillary are also positive with anti-tTG (anti-tTG stain. X 150).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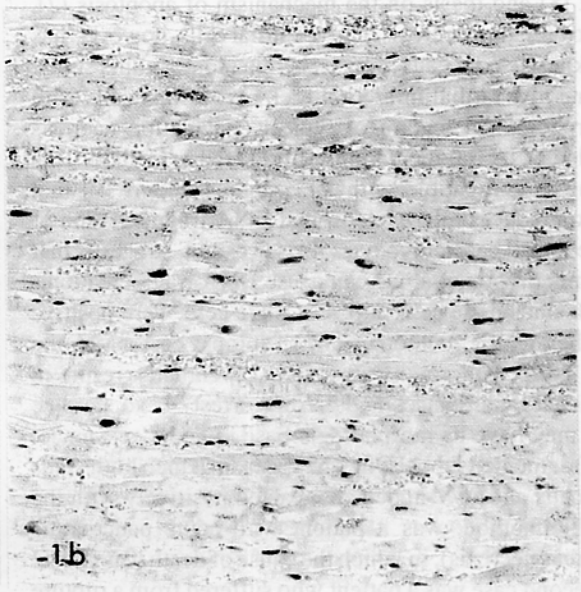
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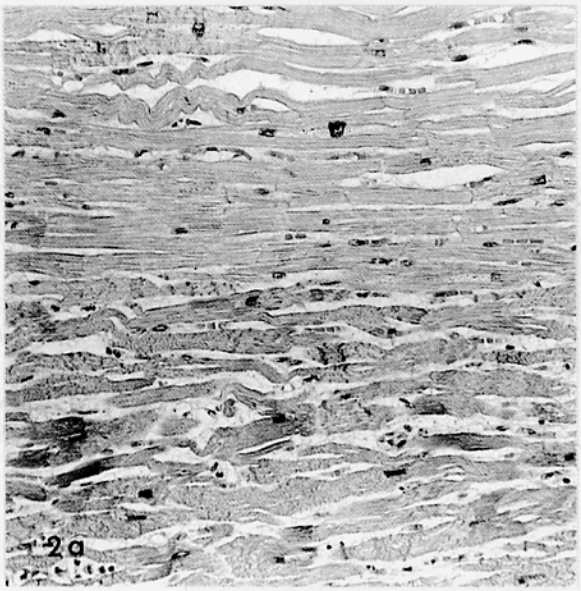
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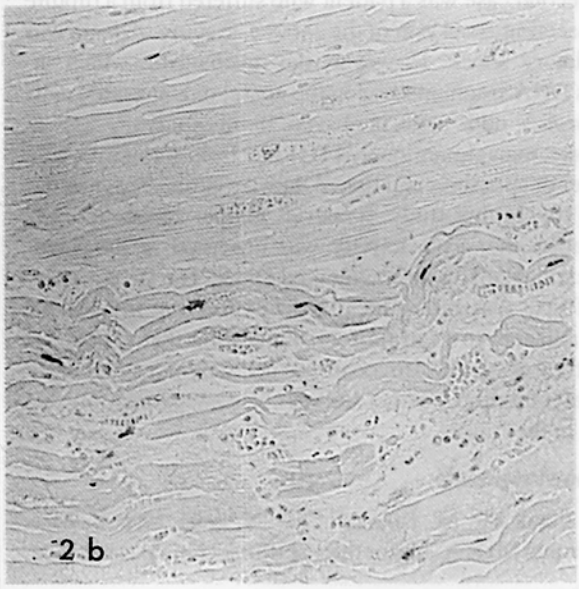
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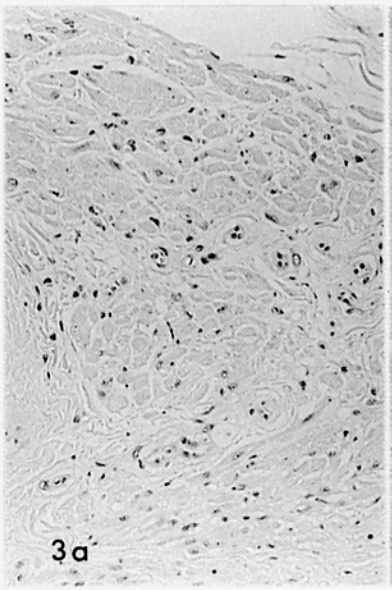
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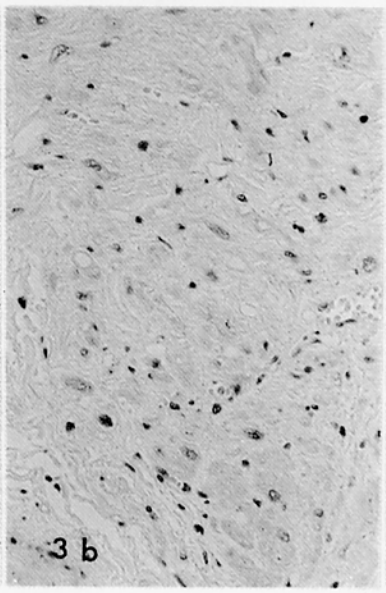
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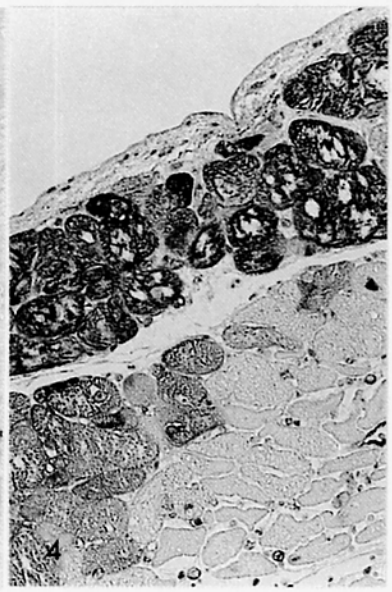
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report [8] and thesis on the mechanism of apoptosis [7]. The coagulation necrosis of myocardial cells, however, has been known to be caused by ischemia alone. In this study, in acute myocardial infarction foci, myocardial cells with a morphological feature of typical coagulation necrosis revealed DNA fragmentation by the TUNEL method. This finding is compatible with the previous report [9] that hypoxia induces internucleosomal DNA fragmentation of rat neonatal cardiomyocytes in vitro and suggests that ischemia (hypoxia) alone can cause DNA fragmentation.

Both sinus node cells and A-V node cells were positive with the TUNEL method in 2 of 13 autopsy cases. May the nodal cells with fragmented DNA eventually result in functional disturbances and cardiac arrest? If so, cardiac death may have its morphologic base in nodal cell-nuclei with fragmented DNA. One of 2 cases, in which both sinus node cells and A-V node cells reacted positively with the TUNEL method, was a patient with acute pancreatitis (pancreas necrosis), in which the cause of death was shock. The second case was a patient who suffered from a rupture of dissecting aneurysm of the aorta. The cause of death was circulatory shock. However, the mechanism, which induces DNA fragmentation in the nuclei of sinus node cells and/or A-V node cells, is obscure in this study.

Tissue transglutaminase (tTG) has been known to be a marker of apoptosis and linked with apoptotic body formation [10]. In this study, however, neither apoptotic body formation, nor tTG activity was observed in damaged-myocardial cells with fragmented DNA. The strong activity of tTG was rather localized in surviving myocardial cells adjacent to necrotic foci, suggesting that tTG is not directly linked with (myocardial) cell death in acute myocardial infarction. Further studies are needed regarding this point in the future.

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