Immunohistochemical and Electron-Microscopic Characteristics of Secretory Cardiomyocytes in Experimental Myocardial Infarction

Deneysel Miyokard İnfarktüsünde Salgılayıcı Kardiyomiyositlerin İmmünhistokimyasal ve Elektron-Mikroskopik Özellikleri

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Abstract

Objective: To study immunohistochemical and electron-microscopic features of secretory cardiomyocytes in experimental myocardial infarction (MI).
Methods: Totally 15 hearts of dogs with experimental MI were studied. For electron-microscopic study materials were collected from left atrium, and its several parts (appendix, lateral and frontal walls). For immunohistochemical analysis of atrial natriuretic factor (ANF) incubation in monoclone immune serum was used (standard monoclonal serum and primary mice antibodies, Immunon). For statistic processing we used Chi-square test (criterion of Pearson agreement).
Results: Immunohistochemical and electron-microscopic investigation after 24 hours from experimental myocardial infarction indicated increase of specific activity of secretory cardiomyocytes and after 48 hours decrease of secretion of ANF while cardiomyopathy appears. After 72 hours, blockade of ANF secretion with decompensation of secretory cardiomyocytes occurred.
Conclusion: Imunohistochemical investigations and analysis of submicroscopic structures of secretory cardiomyocytes after experimental MI showed, that cells were functionally active 24 hours after myocardial infarction with further (48 hours and 72 hours of MI) decrease in amount and impairement of activity. *(Anadolu Kardiyol Derg 2003; 3: 299-302)*
Key Words: Experimental myocardial infarction, atrial natriuretic factor

Özet

Amaç: Bu çalışmada deneysel miyokard infarktüsünde (Mİ) salgılayıcı kardiyomiyositlerin immünhistokimyasal ve elektron-mikroskopik özelliklerini araştırmayı amaçlamıştık.
Yöntem: Deneysel Mİ'yı olan toplam 15 köpeğin kalpleri araştırılmıştır. Elektron-mikroskopik inceleme için materyaller sol atriyumdan ve onun farkli bölgelerden (apendiks, lateral ve frontal duvarları) toplandı. Atriyal natriüretik faktörün (ANF) immünhistokimyasal analizi için monoklonal immün serum kullanıldı (standard monoklonal serum ve primer fare antikorları, Immunon). İstatistiksel analizi için Ki-kare kriteri (Pearson kriteri) kullanıldı.
Bulgular: İmmünhistokimyasal ve elektron-mikroskopik inceleme deneysel Mi'dan 24 saat sonra salgılayıcı kardiyomiyositlerin spesifik aktivitelerinin arttığını, ancak 48 saat sonra kardiyomiyopatinin ortaya çıkması ile beraber ANF salgılamasının azaldığını gösterdi. Salgılayıcı kardiyomiyositlerinin dekompansasyonu ve ANF blokajı ise 72 saat sonra ortaya çıkmıştır.
Sonuç: İmmünhistokimyasal incelemeler ve salgılayıcı kardiyomiyositlerin submikroskopik yapılarının analizi miyokard infarktüsünün 24. saatinde hücrelerin fonksiyonel olarak aktif olduklarını, ancak daha sonra (Mİ'in 48.saati ve 72. saati) sayısal olarak azaldıklarını ve aktivitelerinde bozulma olduğunu gösterdiler. *(Anadolu Kardiyol Derg 2003; 3: 299-302)*
Anahtar kelimeler: Deneysel miyokard infarktüsü, atriyal natriüretik faktör

Introduction

In 1981 investigators from Canada discovered, that intravenous introduction of extract of atrium tissues causes abundant growth of sodium and urinary excretion (1, 2). Today, chemical structure of atrium natriuretic (ANF) and its synthetic analogs are established (3-5). This factor has a number of varied biological properties. Numerous facts testify that increase of pressure in atriums and stretching of atrium walls cause increase in sodium and urinary excretion, and change vessel tonus (6, 7). Electron-microscopic study discovered so-called "specific granules" close by morphological-functional properties to granules of endocrine cells, which produce peptide hormones (8-10).

Purpose of investigation – immunohistochemical and electron-microscopic study of ANF at experimental myocardial infarction.

Material and Methods

Totally 15 hearts of dogs with experimental myocardial infarction were studied. Thoracotomy through III-IV intracostal area was held on dogs after 24, 48 and

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72 hours from induction of experimental infarction by putting a ligature on intraventricular artery. For electron-microscopic study we used materials collected from left atrium, and its several parts (auriculum, lateral and frontal walls). Thin pieces of material were cut into 1mm width tissue bands and then were fixed in solution of a glutaralaldehyde and paraformaldehyde by Gluert (1975), dehydrated in acetone in increasing concentration and poured in EPON-812 solution by Iohonsen (1973). In every case half-thin cuts were exposed to the dyeing by toluene blue by method of Lunn (1965). Ultrathin cuts were cut on ultratom LKB-3 (Sweden) and studied on electron microscope JEM-100 S (JEOL, Japan).

For immunohistochemical analysis of ANF incubations in monoclonal immune serum, in diamino benzene and peroxide of horseradish were used. Standard monoclonal serum and primary mice antibodies (Immunon) were used. Secretory granules and ANF-positive cells were colored to brown or black color.

For statistical processing we used Chi-square test (criterion of Pearson agreement).

Results and Discussion

Immunohistochemical investigations of secretory cardiomyocytes after 24 hours from experimental myocardial infarction showed intensive increase of ANF secretion in all researched regions. This was peculiar for the auriculum of left atrium. Secretion of secretory granules were characterized by high indexes approximately twice above of intact values.

Electron-microscopic investigation revealed same facts. Karyolemma of secretory cardiomyocytes had typical structure and karyoplasm displayed condensed chromatin. Nuclei contained 1-2 nucleoli and sarcoplasm revealed all organelles. Basically laminar complex localized in paranuclear area and in basal parts of cytoplasm. Diameter of secretory granules (SG) varied from 150 to 350 nm. Light strips were defined around granules and matrix was represented with small-granular and small-blocked inclusions. Analysis of submicroscopic structures of secretory cardiomyocytes let us to suppose, that after 24 hours from experimental myocardial infarction cells were functionally active. In active functioned cardiomyocytes one part of electron-dense granules were in condition of desaggregation, but at the same time granules were enlarged, density of core reduced and rim under membrane disappeared (Fig.1).

The investigated material was characterized by increased amount and expansion of rough endoplasmic reticulum. Cellular contacts were represented with insertion discs, which were desmosome and fissural contacts.

After 48 hours from experimental myocardial infarction secretion of ANF impaired, all this happened while nonspecific alternative-dystrophic cardiomyopathic breachs appeared in all regions of atriums. This was different from previous period of investigation with fall of the amount of positive cardiomyocytes in subendocardial, subpericardial, and intramural muscular stratums. Atrial natriuretic factor-positive substratum in this cells appeared in form of coarse amorphous block structures. We did not observe signs of significant extrusion.

Electron-microscopically, after 48 hours from myocardial infarction nuclei of secretory cardiomyocytes were characterized by hyperchromatous nuclei material. Hypertrophy of laminar complex occurred. Secretory granules were polygon shaped, the most part of them were distributed in perinuclear parts of cytoplasm. Diameter of secretory granules yielded to the same parameters in cardiomyocytes of I group and varied in range of 200-250 nm. Light stripes were registered around granules. Small granular and block complexes of osmiofill substance concentrated in matrix of secretory granules (Fig.2).

Amount of granules in different cells fluctuate in a marked degree. Matrix in considerable part of granules stayed in degranulated condition. Increase of amount and enlargement of canals of rough endoplasmic reticulum, hypertrophy of cisterns and vesicles with osmiofill were also typical for this cardiomyocytes. Amount of mitochondria in this cells was reduced, but their size were enlarged, maintained lightened matrix and cristae were partially destroyed or smoothed.

Immunohistochemical parameters of ANF after 72 hours from experimental myocardial infarction were registered at the lowest level. Unlike intact indexes, amount of ANF-positive cells decreased approximately twice, small accumulations of these cells were seen only in subpericardial areas like small islands.

In cytoplasm of secretory cardiomyocytes amount of immunopositive secretory product was significantly reduced as compared with normal state or previous periods of investigation. Intensity of extrusion was minimal.

Analogous morphofunctional tendencies were typical for electron-microscopic picture of secretory cardiomyocytes. Nuclei of cells were enlarged, chromatin condensed, karyolemma formed numerous di-

verticulums and invaginations. Hypertrophy of Golgi complex, as in previous groups, occurred. Secretory granules were polygonal shaped, with diameter varied from 200 to 250 nm and significant variation in density. The most part of secretory cardiomyocytes were in the condition of degranulated matrix (Fig.3).

Immunohistochemical parameters of ANF-secretion of cardiomyocytes in different parts of atria are represented in details in Table 1.

Immunohistochemical picture of ANF during myocardial infarction is represented in Fig. 4.

So, immunohistochemical and electron-microscopic investigation after 24 hours from experimental myocardial infarction indicate increase of specific activity of secretory cardiomyocytes and decrease of secretion of ANF after 48 hours while cardiomyopathy appears. After 72 hours, blockade of ANF secretion with decompensation of secretory cardiomyocytes took place.

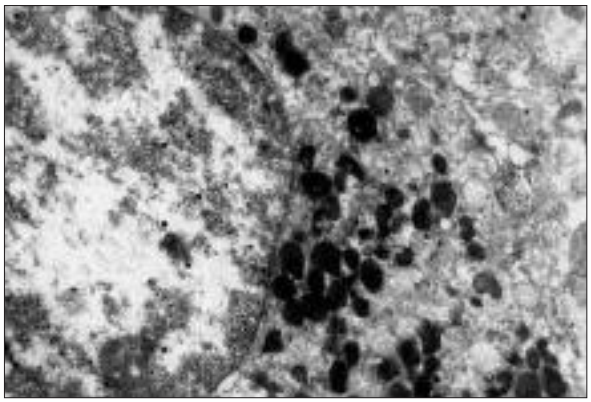

Figure 1: Ultrastructure of secretory granules of cardiomyocytes 24 hours after experimental myocardial infarction. Oval and polygonal secretory granules. (Maximized x 10000).

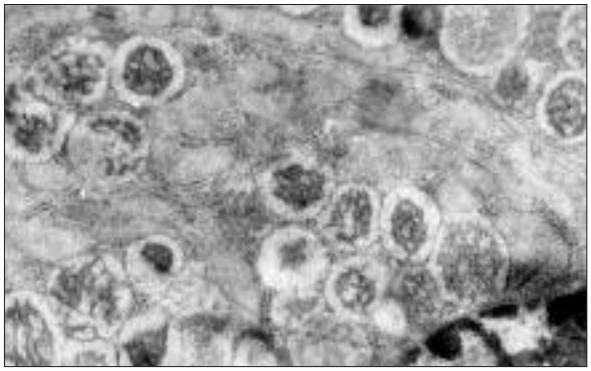

Figure 2: Process of extrusion in secretory granules 48 hours after myocardial infarction. (Max. x 15000).

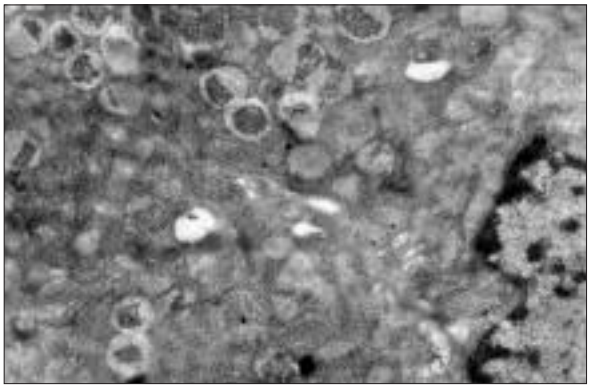

Figure 3: Episodic secretory granules 72 hours after experimental myocardial infarction. (Max. x 10000).

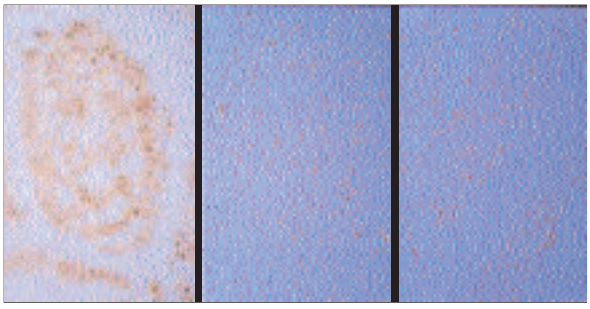

Figure 4: Immunohistochemical positive cardiomyocytes after 24, 48 and 72 hours from experimental myocardial infarction.

Table 1: Immunohistochemical indexes after experimental myocardial infarction

Index	Atrium	Standard	After 24 hours	After 48 hours	After 72 hours
Average amount of ANF "+" – cells in 1 mm^2	right left	196-200 130-138	240-250 160-170	200-210 120-125	101-106 103-109
Amount of substratum in ANF + cells	right left	2.3-2.4 1.8-2.0	3.3-3.6 3.2-3.3	1.8-2.0 1.9-2.0	1.0-1.1 1.1-1.2
Average amount of SG	right left	300-315 250-270	440-450 431-442	270-283 264-278	136-141 161-169
Intensivity of secretion, points	right left	2.0-2.1 1.5-1.7	3.7-3.8 3.5-3.6	1.7-1.8 1.8-1.9	0.08-0.09 0.09-0.01

ANF – atrial natriuretic factor, SG – secretory granules

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...Haşhaş pembesi ve mor damarlı bulutların Körfez'in batısında kat kat yığılışına dalmışım. Yukarlarda, şiddetli bir rüzgar, onları önüne katmış sürüyor. Şimdi İnciraltı dolaylarında barut siyahına dönüp kalınlaştırdıysa, birazdan Narlıdere tarafında tel tel çözerek, pirinç sarısına kaydırıyor. Deniz göğün tersine, iyice durgunlaşmış....

Atilla İlhan: Fena Halde Leman. Can Yayınları 1991