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APOPTOSIS IN CARDIOVASCULAR DISEASES

Goran Davidović[1], Goran Brajušković[2]
[1]Department of Cardiology, Internal Clinic, Clinical Centre Kragujevac, Kragujevac, Serbia and Montenegro, [2]Institute for Pathology and Forensic Medicine, Military Medical Academy, Belgrade, Serbia and Montenegro

APOPTOZA U KARDIOVASKULARNIM BOLESTIMA

Goran Davidović[1], Goran Brajušković[2]
[1]Odeljenje kardiologije, Interna klinika, Klinički centar Kragujevac, Kragujevac, [2]Institut za patologiju i sudsku medicinu, Vojno medicinska akademija, Beograd

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SAŽETAK

Apoptoza je poseban tip ćelijske smrti koji se po svojoj prirodi i biološkom značaju suštinski razlikuje od nekroze. To je genski visoko regulisan tip ćelijskog umiranja koji u većini slučajeva ima homeostatsku ulogu. Apoptoza je opisana kao u normalnim fiziološkim uslovima (tokom procesa embriogeneze, diferencijacije i u procesima razvoja i regulacije imunskog sistema) tako i u nizu patoloških stanja organizma. Poremećaji programirane ćelijske smrti i narušavanje tkivne homeostaze predstavljaju molekularno biološku osnovu mnogih oboljenja. Poslednjih deset godina, posebna pažnja se posvećuje ulozi poremećaja (stimulacije) procesa apoptoze u patogenetskim osnovama razvitka brojnih kardiovaskularnih onboljenja. U ovom radu, pokušali smo da na sistematski način prikažemo osnovne morfološke i molekularne karakteristike procesa apoptoze kao i značaj apoptoze u razvitku i lečenja kardiovaskularnih bolesti.

Ključne reči: apoptoza, kardiovaskularne bolesti, srčani mišić

ABSTRACT

Apoptosis is a special type of cell death and by its nature and its biological significance it is basically different from necrosis. It is a genetically highly regulated type of cell death which in most cases has a homeostatic role. Apoptosis has been described in physiologic processes (during embryogenesis, differentiation and immune system development and regulation) as well as in pathologic states. Disturbances in programmed cell death and tissue homeostasis form the molecular and biologic basis for many diseases. In the last ten years special attention has been given to disturbances in apoptosis in the development of many cardiovascular diseases. In this study we have attempted to show in a systematic fashion the morphologic and molecular process in apoptosis and its significance in the treatment of cardiovascular disease.

Key words: apoptosis, cardiovascular disease, myocard

INTRODUCTION

Programmed cell death (apoptosis) together with growth and differentiation are the basic part of the cell's life cycle (1). Homeostatic control of cell numbers is a dynamic process of opposites-cell proliferation and apoptosis. The role of cell death in the normal development of an organism was first described in the 1950s by Glucksmann and Saunders. The first sign of two morphologically different types of cell death were described by Kerr (2) during histo-chemical analysis of hepatic lysosomes in hepatic ischemia. He named this newly discovered form of cell death, which was morphologically different from the necrosis (till then, the only other known type of cell death) apoptosis. The term apoptosis comes from the Greek and means falling (like leaves from a tree).Kerr basically wanted to state with this term that this was a physiologic or „natural" death.

Apoptosis has been described in normal physiologic conditions during the process of embryogenesis, differentiation and immune system development and regulation (8, 9). Disturbances in programmed cell death and tissue homeostasis form the molecular and biologic basis for many diseases. There are many diseases that are attributed to apoptosis inhibition (3–6). Malignant diseases are characterized by tissue homeostasis disturbances with uncontrolled proliferation of neoplastic cells. At the same time, there are many diseases that occur because of apoptosis stimulation. Basically, they are neurodegenerative diseases such as Parkinson's and Alzheimer's diseases and amyotrophic lateral sclerosis, autoimmune diseases and viral infections. Progressive death of the CD4 + T lymphocyte by the process of apoptosis has been detected in HIV infected patients. There is also a large volume of studies that show that organic erectile dysfunction is due to a dysfunction of apoptosis in the corpus cavernosum. Because of this, apoptosis has been the main theme in molecular-biologic studies that are involved the research of patho-physiologic development of many human diseases.

CARDIOVASCULAR DISEASES ASSOCIATED WITH APOPTOSIS

During the past 10 years, the results of many studies show the importance of disturbances in apoptosis, usually of the stimulative type, in the development of cardiovascular diseases (10).The development of molecular-biologic technology has been able to show us cells in the process of apoptosis (11). „Apoptotic cell death is observed in a variety of cardiovascular diseases, including myocardial infarction, ischemia-reperfusion injury, end-stage heart failure, arrhythmogenic right ventricular dysplasia and adriamycin-induced cardiomyopathy (12, 13). Immunohistochemical analysis of endomyocardial biopsies from patients with congestive heart failure, acute myocardial infarction, ischemic cardiomyopathies, and myocarditis, have led to the identification of apoptotic cardiomyocytes (14). Besides the simple immunohistochemical detection of apoptotic proteins, the most available methods are the TUNEL assay, Western blotting and flow citometry (7, 12). All these methods are based on the morphologic and molecular specifics of cell death due to apoptosis.

Apoptosis as a normal, physiologic type of cell death is encoded in the embryologic development of invertebrates and man. Research in the embryologic development of the heart has show that „during cardiac development, apoptosis was suggested to be of importance in the formation of septal, valvular and vascular structures". This

Correspondence:
Assist. Professor Goran Davidović, MD, PhD
Kliničkі Centar Kragujevac
Zmaj Jovina br. 1, 34000 Kragujevac, Serbia and Montenegro
tel. +381–34–370–060-lok. 424
e-mail: gorandr@verat.net

research has „implicated the potential importance of either excessive or inappropriate apoptosis in congenital heart disease". An elevated level of apopotosis in the cardiac conducting system is one of the possible mechanisms in the development of heart block and also in the develment of Wolff-Parkinson-White syndrome (15).

Programmed cell death is a critical regulator of homeostasis in many tissues, including the vasculature. Atherosclerosis is a systemic disease involving the intima of the large and medium arteries. Traditional hypothesis of atherogenesis have suggested that injury of vascular endothelial cells is critical for development of atherosclerosis. But, many studies have identified increased apoptosis of vascular cells in atherosclerotic plaques compared with normal vessels. After the publication of these results, the scientific thinking is that „apoptosis may prove to play an essential role in atherosclerotic alterations of the vessel wall (15–17). Studies have also shown that that apoptosis in „atherosclerotic lesions is triggered by inflammatory processes, both via cell-cell contact and by cytokines and oxidized lipids (17)". Apoptosis was not found in all analyzed samples of primary atherosclerotic lesions but its evidence has been „strongly correlated with the presence of intimal hyperplasia." Today it is obvious that the apoptosis of vascular smooth muscle cells induced by macrophages promotes plaque rupture. The clinical implication of this knowledge is that apoptosis in atherosclerotic lesions may lead to reduced plaque stability. „Although the significance of apoptosis in athrosclerosis remains unclear, it has been proposed that apoptotic cell death contributes to plaque instability, rupture and thrombus formation" (18–20).

With regard to oxidative stress, apoptosis could be biologic continuum in the CHF continuum. Oxidative stress is an important stimulus for apoptosis. Not surprisingly, apoptotic cell death has been identified in human atherosclerosis (21, 22). Large areas of apoptosis were localised at the vascular smooth muscle cells adjacent to the endothelium (63), suggesting a possible role in destabilising the plaque and promoting it's rupture. It has ben suggested that NO, which is a radical, promotes apoptosis in vascular smooth cells (23, 24). Interestingly, TNF-a alone, or in combination with gamma-interferon and interleukin I, also exerts a pro-apoptotic effect on cultured smooth muscle cells that may involve both NO-dependent and NO-indepent mechanisms (21, 23). These observations underlie the importance of inflammatory cytokines generated by infiltrating T lymphocytes and macrophages (25).

The common view on how cardiomyocytes die during and after myocardial infarction has altered in recent years. For many years it has been considered that necrosis was the only type of cardiomyoctic cell death after MI. Today it is known that cardiomyoctic „loss" after the acute stage of MI is not only due to necrosis but also due to apoptosis. The fact that apoptosis has a role in tissue damage after MI has both pathologic and therapeutic implications. „Studies on ventricular myocytes of the hearts of patients who died of acute MI" showed that that the cardiomyocytes died by the process of apoptosis (26). In myocardial infarction in humans, apoptosis has been observed in three different regions: 1) in the core of the ischemic myocardial area, 2) in the border zone of the infarction, and 3) in the viable myocarium, remote from the ischemic area (27). In the ischemic area it might be a determinant of the final size of the infarct and it seems to depend on the presence of post-ischemic re-perfusion (28). Additionally, it has been suggested that apoptosis may be responsible for a significant amount of cardiomyocyte death during the acute ischemic stage, as well as for a progressive loss of surviving cells during the subacute and chronic stages (29).

Apoptosis affects at least 12% of myocytes in the peri-infarct border zone and 1% of the distant myocardium (30). The role of apoptosis in re-perfusion injury has recently been addressed in rat and rabbit models, where reperfusion was shown to accelerate the apoptotic cell death in cardiomyocytes (31, 32). Oxygen free radicals are implicated in the genesis of reperfusion damage providing a possible mechanistic link between the two processes. Apoptotic cell death may have a role in the remodelling of non-infarcted myocardium, as evidenced in human myocardial specimens sampled 110 days after infarction showing that 0.7% of the cardiomyocytes were apopototic (30).

Experiments on animal models confirmed the belief that the intensity of apoptosis „is the major determinant of infarct size" (26).

Heart failure is a heterogenous syndrome that can result from primary cardiomyopathies or myocardial infarction, hypertension and valvular heart disease. Currently, the main research of the molecular basis of heart failure is programmed cell death due to apoptosis. Ther is multiple experimental evidence that cardiomyocyte apoptosis is a causal component of ischemia-reperfusion injury. This is confirmed by in vivo studies in animal and human models and in vitro in isolated animal hearts and human cardiomyocyte cultures. The most rigorous data demonstrate apoptosis rates of 0.8-0.25% in patients with end -stage dilated cardiomypathy compared with 0.001–0.002% in controls (26, 33).

With reference to CHF, apoptosis is detected in canine models of pacing-induced heart failure and in heart failure due to chronic ischemic injury (34, 35), as well as in several models of pressure overload hypertrophy (36, 37). In man apoptosis is found in myocardial specimens from patients undergoing cardiac transplantation, yielding an apoptotic index of 0.2–0.4% (32, 38, 39).

Potential mehanisms for the induction of apoptotic cell death may involve mechanical factors (i.e. stretching) or elevated levels of neurohumoral factors, such as increased levels of angiotensin II, TNF-a and atrial natriuretic peptide (40, 41).

Even today, after the results of modern studies, the cause of the beginning of heart failure is an enigma although heart failure as a syndrome was described over 366 years ago.

An elevated level of apoptotic cardimyocytes was detected in biopsies of „right ventricular myocardium in the patients with arrhythmogenic right ventricular dysplasia“ (42, 43). Buzas et al. followed the expression of pro-apoptotic proteins, showed progressive cardiomyocytic death in biopsies of patients with dilative cardiomyopathy (44). At the same time, Kavantzas et al. did a comparative analysis of bioptical material and showed that „the numbers of apoptotic cells in dilated cardiomyopathy specimens were significantly lower by comparison with both those of hypertrophic cardiomyopathy and those of arrhythmogenic right ventricular dysplasia specimens“ (45).

In the past few years special attention has been payed to the process of apoptosis in transplant surgery, especially in immunosuppressive strategies and heart graft rejection or post transplant graft coronary vasculopathy (46–48). Myocarditis, pre-excitation syndromes, chronic hibernating myocardium, myocardial degeneration in thrombotic thrombocytopenic purpura, arrhythmias associated with sudden death, inflammatory-fibroproliferative disorders of the vessel wall are just some of the researched pathologic states in cardiology that seem to have their pathogenesis in apoptosis (15, 48). Research has also shown that apoptotic cell in cardiomyocytes have significant role in the development of alcoholic heart disease (49).

The number of cardiovascular diseases that either have apoptotic stimulation or derangement as part of their cause is growing larger every day. That is why research of the molecular basis of the apoptotic process is important in the diagnosis and treatment of these diseases.

THE MOLECULAR BASIS OF THE APOPTOTIC PROCESS

Apoptosis is an asynchronous process which involves individual tissue cells. The first morphologic sign of apoptosis is chromatin condensation most frequently in the nuclear margin. At the same time that condensation changes are occuring in the chromatin there similar changings occuring in the cytoplasm of this early apoptotic phase. This leads to a decrease in cell volume, increased cytoplasmic density, compression of intact organelles cytoskeletal destruction. The later phase of apoptosis is marked nuclear segmentation and cytoplasmic fragmentation. Pseudopods start making an appearance on the cell membrane. The terminal phase of the apoptotic process is characterized by apoptotic cell fragments that are surrounded by plasma membrane, these are called apoptotic bodies. Apoptotic bodies undergo phagocytosis from macrophages, dendritic cell and other parenchymal cells. The process of phagocytosis of apoptotic bodies is characterized by the absence of an inflammatory reaction. This represents the final phase of apoptosis, the phase of „phagocytic and intracellular degradation“ (50, 51). A morphologically visible condensation of chromatin is followed by breakage in the DNA chain into approximately 180 basic pairs. Endonuclease which is responsible for the internucleosomal fragmentation of the DNA chain has not been purified and fully characterized. It is known that this enzyme is Ca and Mg dependent and is inhibited by zinc ions (52). The stereotype of morphologic and biochemical changes occuring in apoptosis, irrespective of cell population and the stimulus that induces it, suggest that apoptosis is an active and genetically strictly regulated process of cell autodestruction.

The „decision“ of a cell to go into the process of apoptosis could be due to binding of the extracellular signal to death receptors that belong to the TNFR receptor family (tumour necrosis factor receptor).The most common ligands that can induce the apototic process are TNF-alpha (the ligand for the TNFR 1 receptor), Fas ligand (for the Fas receptor) and TRAIL. The death receptors, like the Fas receptor, possess cytoplasmic domains of about 80 aminoacids and because of their significance are called“ death domains“. The activation of these receptors induces a homophilic interaction between death domain receptors and homologous death domain intracellular proteins which are called adapters. That is, the death domains of the Fas receptors interact with the homologous domain of the protein adapter which is called FADD (Fas-associated death domain).The protein adapters also contain DED (death effector domains).These domains interact with homologous death effector domains of the caspase 8 enzyme. Caspase 8 is the first enzyme that is activated in the transmission of the extracellular death signal and induces a cascade reaction activating other apoptotic enzymes-caspases. A complex of proteins (Fas, Fadd protein procaspase 8) that induce the process of apoptosis is called DISC complex (Death Inducing Signaling Complex) (53, 54).

Besides extracellularly induced death, the apoptotic process can also be induced via intracellular death signals. Intracellular death signals are usually activated as a response to some type of cellular stress. Medications and other toxins, heat stress, radiation, hypoxia and viral infections are the most frequent activators of the intracellular death signal. The mechanism by which different types of stresses lead to the biochemical activation of apoptosis is still not thouroughly clear. It is believed that the intensity and duration of the stress are key factors in the cell's „decision“ to activate the suicide process (55).

Recent reports suggests an important role of the mitochondria in the induction of apoptotic cell death. Three proteins seem to be necessary for caspase activation:1)the release of cytochrome c into the cytoplasm,2)the release of a second protein, called apoptotic protease-activating factor (APAF)-1 which is considered to be the mammalian homologue of the pro-apoptotic factor CED-4 of the C-elegants, the nematode in which apoptosis was first characterized (56), and 3)the release of a third protein called PAF-3 which is considered identical to caspase-9 (57).

No matter what kind of stimulus (extra- or intracellular) induces apoptosis, the key factor in this reaction is a cascade of protein activation of cystein proteases-caspases. This cascade reaction of caspase activation is the final phase of apopotosis (58). All caspases are synthetized

in a non-reactive pro-enzyme form, their enzyme activity is in the form of a heterodimer. Two sub-units, one smaller (p10) and one larger (p20) are combine to form a heterodimer (55). The „target" molecules that these heterodimers act on nuclear lamina proteins, poli (ADP-ribose) polimerase, the protein products of the Rb gene, histone H1, nuclear matrix proteins, protein kinase C, different components of the cytoskeleton (actin and actin binding proteins like gelsolin and fodrin), etc. Due to their enzymatic activity, caspases inactivate inhibitors of apoptosis and also lead to cytoskeletal structure destruction.

Chromain disintegration, cytoskeletal changes and budding on the plasma membrane are the morphologically visible changes of the biochemical action of caspases during the process of apopotosis (59, 60). Caspase activation is a sure sign that the cell is on the way to suicide and that nothing can stop it (58).

Multiple extracellular and intracellular signals (apoptosis inducers) can induce the process of apoptosis where the final phase is caspase activation (apoptosis effectors). The results of multiple studies have shown that apoptosis is regulated by a significant amount of intracelleular regulators (apoptosis modulators). Proteins from the Bcl 2 family, p53 protein, protein products of certain oncogens and of cell cycle regulator gene, also cytochrome C, different transcription factors, certain phosphatases and kinases, also represent some of the known apoptosis modulators (33).

Protein products of the Bcl 2 gene family represent key intracellular modulators (regulators) of the apoptosis process. The primarily discovered member of the Bcl gene family was anti-apoptotic proto-oncogen Bcl 2 (B-cell leukemia/lymphoma 2). After the Bcl 2 proteins, a whole family of homologous proteins were discovered some of have stimulative and others which have inhibitory effects on the apoptotic process. The first pro-apoptotic homologue to be discovered was Bax (Bcl2-associated X)protein, it is a immunoprecipitate of the Bcl2 protein. All the members Bcl2 protein family contain at least one of four highly conserved Bcl2 homologous (BH) domains (BH1-BH4). Bcl2 homologous domains are responsible for the process of homo- and hetero-dimerization of the Bcl2 protein family. Thanks to these BH domains, heterodimers are formed by proteins that have an opposite effect on the apoptotic process (61). The antiapoptotic effect of the Bcl2 protein is based on its ability to bind to Bax protein (in the form of heterodimers) and in this way lead to the formation of Bax/Bax homodimers (62).Because of its significance, the relationship between proapototic and anti-apototic homo- and hetero-dimers is considered a type of autonomous „cell rheostat" that decides what response a cell will have from apoptotic stimulation (61–63).

During the induction of the apoptotic process, the activated Bax proteins in the form of homodimers translocate onto the mitochondrial membrane and lead to the formation of membrane channels. Cytochrome C is released from the mitochondria into the cytosol (54). Because of its positive effect on caspase 3 activation cytochrome C is also called Apaf 2 (APAF-apoptotic protease activating factor). In the presence of adapter molecule protein Apaf 1, cytochrome C and adenosine triphosphate (ATP) proteolytic activation of pro-caspase 9 occurs (Apaf 3). The protein interaction between Apaf 1 and Apaf 3 molecules occur because of their homologous sequences called CARD (caspase recruitment domains).In the abscence of cytochrome C this interaction is blocked due to the structural conformation of Apaf 1. The binding of cytochrome C to Apaf 1 leads to its conformational change so that CARD domains are available to interact with the homologous domains of procaspase 9. The result of this interaction is the proteolytic activation of procaspase 9. In the apoptotic cascade, activated caspase 9 activates caspase 3 which irreversibly leads to the final phase of apoptosis. The controlled release of cytochrome C into the cytosol is necessary to start the final executor process of apoptosis. Because of this Koemer et al have called the mitochondria the regulator of life and death (55, 64).Results of experimental in vitro studies on cardiomyocites have shown that hypoxia and metabolic inhibition induce apoptosis throw the mitochondrial pathway (26).

The most well known modulator with a stimulatory effect on the process of apoptosis is the protein product of the p53 gene. The tumor supressor gene, p53 is located on 20kb sequence of chromosome 17 (17p13.1 locus). It's been shown that the p53 protein as a transcription factor has different effects: a) as a transcription supressor gene whose protein products are needed for the normal propagation of the cell throw the cell cycle; b) as a transcription activator gene whose protein products are not only involved in stopping the cell cycle in the G1 phase (eg.WAF1/CIP1) but also are involved in the apoptotic process (65). The capability of neonatal cardiomyocytes to be involved in the process of programmed cell death is furthered along by the expression of p53 protein (15).

APOPTOSIS AS A THERAPEUTIC TARGET IN THE TREATMENT OF CARDIOVASCULAR DISEASES

As a genetically highly regulated process, apoptosis represents a potential therapeutic target in the modern treatment of cardiovascular disease. Since apopotosis of cardiomyocytes has been detected in the majority of cardiovascular diseases, special attention has been given to discovering anti-apoptotic molecules that would have a cardioprotective effect (66, 67).The strategy in the past few years has developed in two directions, one in the direction of synthetising molecules that would effectively inhibit proapoptotic intracellular molecules and the other which would be capable of stimulating or de novo expression of antiapoptotic molecules in cardiomyocytes (48).

In the last few years multiple studies have shown that caspases as effector enzymes of death represent attractive targets in the modern treatment of diseases involving apoptosis including cardiovascular diseases. The injection of pan caspase inhibitor z-VAD. fmk in animal models has shown its self to be effective in reducing the level of

apopotosis in cardimyocytes in animals with heart failure after a myocardial infarction (68).

Caspase 3 inhibitors represent ideal synthetic molecules that could be used in the future in the treatment of acute myocardial infarction and other cardiac diseases. Certain pharmaceutical companies have already synthetised caspase inhibitors whose efficacy is still being tested in the pre-clinical phase. Caspase inhibitors IDN-1965 and IDN-6556 (Idun Pharmaceutical Inc.) have been capable of reducing the size of lesions in heart injury. Caspase inhibitor 3 MX 1013 which was made by Maxim Pharmaceuticals Inc. has been shown to be systemically efficacious in acute MI in animal models (68). The results of the newest studies by Balsam et al have shown that treatment with caspase-3 inhibitor improved survival and prevented ventricular dilation and dysfunction after permanent coronary artery occlusion (69).

Myocardial expression of Bcl2, an apopototic protein that inhibits cytochrome C release, reduces infarct size by 48–64% (33). On the basis of this information, stimulation of the anti-apoptotic Bcl2 molecule in cardiomyocytes could have a protective effect.

In studies of new therapeutic ways to treat ischemic heart damage, besides caspase inhibitors, there are other anti-apoptotic agents such as anti-oxidants, Ca-channel blockers, insulin-like growth factor 1 and the poly-adenosine diphosphate-ribosesynthetase inhibitors (70). Special attention is being given to the role of anti-oxidants since oxidative stress, via damage to mitochondrial electron transport and mitochondrial DNA, leads to cardiomyocyte hypertrophy and induces the process of apoptosis (71). Most of the therapeutic implications in reducing cardiomyocyte apoptosis are still in the experimental phase.

Certain therapeutic modalities which are being used in the treatment of cardiovascular disease have an indirect effect on cardiomycyte apoptosis. Studies on the effects anti-oxidant based therapies on arteriosclerosis have shown that vitamins C and E regulate the apoptotic process in cardiomyocytes (20). In chronic heart failure, activation of the immune system occurs, which results in the production and release of pro-inflammatory cytokines. These pro-inflammatory cytokines have a stimulative effect on the apoptotic process in cardiomyocytes. Several animal studies and also some clinical studies have suggested that down-regulation of inflammation may improve cardiac performance. Immunomodulatory agents with a primary effect on pro-inflammatory cytokines could potentially represent effective blockers of cardiomyocyte apoptosis.

A recent study (72) has shown that carvedilol, administered in vivo to rabbits subjected to ischemia and re-perfusion, induces a 77% reduction in the number apoptotic myocytes in the ischemic area. This effect of carvedilol seems to be specific and independent from its cardio-protective effect due to beta-blocade, in fact, propranolol at similar blocking dosage caused only a 39% reduction in the number of apoptotic myocytes. Interestingly, carvedilol and not propranolol, inhibited the activation of stress activated protein kinase (SAPK) and of the Fas protein. Fas is a „death receptor“ detectable in several organs, including the heart. It is structurally related to the cytokine receptor TNF-RI and it may be activated by the Fas ligand or by agonist-like antibodies. Interestingly, Fas-induced apoptosis can be blocked by anti-oxidants (72); moreover, the oxidative stress which occurs during ischemia and reperfusion has been demonstrated to activate SPAK whose signalling pathway is also implicated in apopotosis induction.

Therefore, it is conceivable that carvedilol's antiapototic effect can be, at least in part, due to its unique anti-oxidant activity.

In the future, novel drugs might also result from an understanding of apoptotic pathways in chronic disorders (73–75). Hepatocyte growth factor (HGF) is a cytokine whose multipotent actions are mediated by c-Met receptor. Experimental studies have shown that the administartion of HGF protein reduces infarct size and increases cardiac performance in a rat model of acute ischemia/re-perfusion. A positive effect has been shown on the chronic ischemic myocardium of dogs. Jin et al. believe that „clinical trials are need to investigate the therapeutic potential of HGF for post-infarction heart failure in humans (76)“.

In the last few years, special attention is being given to embryonic and adult stem cell research which could be used in the treatment of many cardiovascular diseases (77). Also, in the research of cardiomyocyte apoptosis and discovery of other therapeutic modalities in the treatment of cardiovascular disease, the most modern techniques of molecular biology are being utilised, such as knock-out and transgenic mice, high-throughput genomics and proteomics (78). The list of new techniques, molecules and agents is growing longer every day. Nitric acid synthetase inhibitors, ACE inhibitors, angiotensin II receptor antagonists, adrenergic receptor antagonists and small molecule inhibitors of p38 are just some on the list (79).

Instead of a conclusion, in the post-genome era, we are witnesses to the strong development of „gene therapy“ which will lead to epochal changes in the treatment of patients that currently have incurable diseases. In gene therapy, viral vectors have been the most effective as far as the heart, as a target organ, is concerned and the research is intense (80). The developments of apoptosis-based therapies are giving hope that we are finally on the edge of discovering more precise and efficacious modalities in the treatment of cardiovascular diseases (81).

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