GENE CONTROL OF PROGRAMMED MYOCARDIAL CELL DEATH IN ACUTE MYOCARDIAL INFARCTION, ITS DYNAMICS IN TREATMENT AND PROSPECTS FOR THERAPY

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The aim of the investigation was to study gene regulation of apoptosis in acute myocardial infarction and during the treatment, and assess the effect of levocarnitine (Elcar) on gene expression of Fas-dependent apoptosis.

Materials and Methods. We examined 28 patients with Q-myocardial infarction included in the study within the first 24 h of the disease and followed up during the treatment course. The patients were divided into two groups: group 1 (n=10) consisted of patients with standard treatment (anti-aggregants, ACE inhibitors, and anti-anginal agents — if indicated); group 2 (n=18) — those patients, who received standard treatment with Elcar (levocarnitine) intravenously, in 5% glucose normal saline, at the dose of 3.0 g a day for three days. For the following 7 days the patients were given Elcar *per os*, 4.0 g a day. A control group (group 3) (n=18) consisted of healthy subjects.

All patients underwent general examination, ECG, echocardiography, genetic research: we determined mRNA gene expression of mFas and sFas using real-time polymerase chain reaction thrice in each patient — on admission, after 1 week treatment, and at the end of the second week of treatment.

Results. Apoptotic gene expression in patients with Q-infarction was found to be increased compared to the norm, the increase lasting for a longer period than acute myocardial infarction itself. This fact enables to explain the cause of prolonged apoptosis by the sustained activity of apoptosis-inducing genes. The use of levocarnitine promotes the normalization of an increased level of gene expression, and has a cardioprotective effect that is important in acute myocardial infarction treatment.

Key words: apoptosis in myocardial infarction; apoptosis genes; levocarnitine.

The discovery of cell apoptosis mechanisms and the study of the role of gene disorders in ontogenesis, pathogenesis of cardiovascular and other diseases are regarded to be the breakthrough in modern biology and medicine.

Programmed myocardial death in acute myocardial infarction has been found to accompany cell death of necrosis in the central infarction area and to occur much more (many times) frequently in peri-infarction area [1–3]. Apoptosis also persists in a post-infarction period for several weeks and even months causing the extension of myocardial infarction and ventricular remodeling, cardiac failure increasing the risk of arrhythmogenesis and post-infarction complications. The process of programmed cell death is controlled by genetic system — from the first, initial period (initiation) to the final stage (degradation). This process has been found to involve over 25 genes, which can have different effects on apoptosis. The ultimate result depends on the imbalance between pro- and anti-apoptotic effects of these genes. That is why it is necessary to know the gene activity state. Secondly, there are different pathogenic types of apoptosis, e.g. Fas-dependent type of onset and the course of apoptosis. Another type is related to the disorders of mitochondrial genetic system, there are also other types: cytokine, peroxidase types, etc.

Programmed cell and tissue death control is certainly to require different therapeutic interventions. It is impossible

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to solve this important task without cooperative studies of clinicians-cardiologists, geneticists and immunologists. It will enable to 1) search for possible ways of genetic control of induced apoptosis in patients with myocardial infarction; 2) assess the efficacy of partial and combined techniques to control cell genetic apparatus.

The aim of the investigation was to study gene regulation of apoptosis in acute myocardial infarction and during the treatment, and assess the effect of levocarnitine (Elcar) on gene expression of Fas-dependent apoptosis.

In the course of the study the following problems were to be solved: 1) quantitative characteristics of gene activity of the main (receptor) way of apoptosis in acute myocardial infarction (the so called Fas-dependent apoptotic pathway) at different stages of the disease development; 2) the study of dynamics in the treatment process of two types of Fas gene: membrane-bound mFas (CD95, FasR) and soluble sFas (FasL, CD178); 3) quantitative assessment of the effect of levocarnitine (Elcar) on Fas gene expression. According to some reports, levocarnitine is able to inhibit the apoptotic process. This agent has an antioxidant effect, increases myocardial tolerance to oxygen deficit (hypoxia), enhances cardiac contractile function [4–10].

Materials and Methods. We studied the activity of apoptotic genes by determining mRNA content of the genes under study in blood after lysis of cell and nuclear membranes, mRNA content depending on the total number of genes and their efficiency. mRNA level in blood was determined by its amplification (accumulation) in the course of real-time polymerase chain reaction using a special device DT Lite 4 (DNA-Technology, Russia) and calculating with due consideration of photofluorescent reaction of primers specific for mRNA under study. We took into account the number of cycles required to amplify a primer to the amount, which resulted in fluorescence. This period is called a crossing point (Cp), and enables to calculate the amplificate amount. For this purpose we used special formulas suggested by biotechnologists. The calculation method known as REST (relative expression softlove tool) — a computer program to calculate relative expression of nucleoproteins — devised by German and English geneticists has won the high rate of popularity [11, 12].

A group of experts who tested this gene expression calculation technique indicated the data accuracy, repeatability of the program and its feasibility to calculate intergroup and individual correlation [3].

All procedures related to the study of apoptosis gene expression were carried out in the laboratory of Scientific Research Institute of Molecular Immunology, Lobachevsky State University of Nizhni Novgorod.

When determining the gene expression, the mentioned program uses a relative value R derived from the following formula: $R=E^{(\Delta Cp\ target\ gene\ -\ Cp\ ref\ gene)}$.

In the formula *E* (efficiency) expresses the value, which mRNA concentration is increased by within a cycle; *Cp* is a number of cycles before a fluorescence curve; *ref gene* — reference (control) gene; Δ — the difference between mean Cp in a group of patients and the mean reference gene.

The determined R value shows how far a target gene is more active by expression compared to a resting gene. Numeric R value is non-dimensional. The determination of gene absolute value in blood is considered to have no advantages over the relative expression informativity. All data are given in comparison with the level of "housekeeping" gene (ubiquitin gene) regarded the most stable and taken as a reference point in the so called normalized results. Expression was analyzed by comparing averaged mRNA expression (mFas and sFas forms of Fas gene) in groups of patients and groups of healthy subjects. When studying gene dynamics we used the comparison of initial and obtained data. Statistical data were considered significant if $p<0.05$. The data obtained were "normalized", i.e. correlated with "housekeeping" gene level.

During the hospitalization period the patients were given the standard treatment — angiotensin inhibitors, anti-anginal and anti-platelet agents. The patients underwent ECG and echocardiography in dynamics and biochemical studies (C-reactive protein, creatine phosphokinase MB, troponin-I, blood lipids, etc.).

We examined 28 patients with myocardial infarction, among them there were 16 males and 12 females. Mean male age was 62 years, and mean female age — 68 years.

On admission the severity was assessed by TIMI value, its mean group values among male patients were equal to 4, among female patients — 4.2. Anterior myocardial infarction was found in 16 patients, inferior — in 9, circular — in 3.

We also examined 18 healthy subjects (donors and staff of medical treatment facilities) as a control group.

The study complies with the declaration of Helsinki (adopted in June, 1964 (Helsinki, Finland) and revised in October, 2000 (Edinburg, Scotland)) and approved by the Ethics Committee of Nizhny Novgorod State Medical Academy. Written informed consent was obtained from all patients.

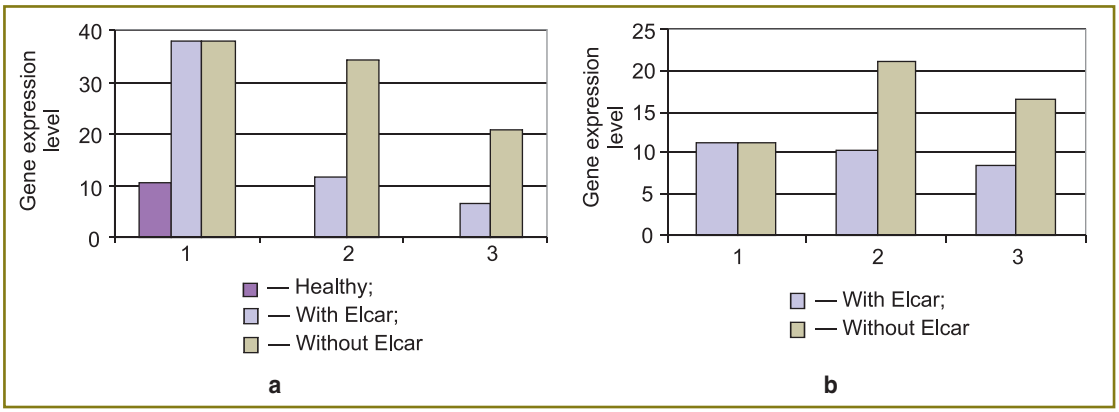
The dynamics of Fas gene expression was studied in 10 patients with myocardial infarction who had undergone a 10-day course of levocarnitine therapy (Elcar, Pik-Pharma, Russia). Elcar is supposed to inhibit apoptosis.

Total 156 test findings of patients and healthy subjects were studied.

Results and Discussion. The expression level of mRNA gene mFas of healthy subjects normalized by reference gene level is 4.2. The expression of this gene in a group of patients with large focal myocardial infarction on day 1 appeared to be 9.56, i.e. 2 times

Dynamics of apoptosis gene expression in myocardial infarction treatment (R value)

Groups under study	mFas			sFas		
	Before treatment	1 week later	2 week later	Before treatment	1 week later	2 week later
Treatment group — patients without Elcar therapy (n=18)	38	34.05	20.82	11.39	21.25	16.56
Elcar therapy patients (n=10)	38	10.33	8.46	11.39	10.33	8.46
Control group (n=18)		10.41			15.24	



The expression of mFas gene (*a*) and sFas gene (*b*) in healthy subjects and patients with acute myocardial infarction against Elcar administration and without Elcar: *1* — on admission; *2* — 1 week later; *3* — 2 weeks later

as high. In a group of healthy subjects mRNA sFas expression was 4.5, in a group of patients — 8.87. The findings of restudies of mRNA Fas expression carried out 1 and 2 weeks later demonstrate functional activity of Fas genes in patients in the course of treatment, and according to consensus instructions American Heart Association and American College of Cardiologists, European Society of Cardiology and Russian Society of Cardiology they consistent with the first — acute, and the second — subacute periods of myocardial infarction (See the Table and the Figure).

The Table shows R values — a relative value of gene expression (mRNA mFas and sFas genes) in patients with acute myocardial infarction during an acute and early post-infarction period, and in healthy subjects, whose findings were taken for norm. The Table and the Figure also show the monitoring results of gene expression under the influence of standard treatment of patients with acute and early subacute periods (weeks 1 and 2), and against the combination therapy with levocarnitine (Elcar) included.

The represented relationship between mFas и sFas apoptosis gene expression duration has been shown for the first time; it proves the necessity to inhibit apoptosis in order to protect myocardium using gene therapy in myocardial infarction.

Due to the “crisis” of genetic engineering techniques under clinical conditions [13–16], pharmacological control of gene activity (expression) can be an alternative to a genetic engineering method, and the use of levocarnitine inhibiting mitochondrial disorders — an essential addition to the known pharmacological agents to protect myocardium in infarction.

Conclusion. Activation (expression) of genes participating in the development and maintaining Fas-dependent apoptotic pathway in patients with large focal myocardial infarction is increased compared to the norm (expression level in healthy subjects). Apoptotic gene over-expression persists not only during peracute and acute (developing infarction) periods but also in an early post-infarction period that is certain to be the cause of a long-lasting apoptosis in these patients.

Levocarnitin (Elcar) taken in early myocardial infarction has an effect on apoptosis reducing the expression of Fas-pathway apoptotic genes and producing a cytoprotective effect.

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Conflict of Interests. The authors have no conflict of interest related to the study.

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