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Levocarnitine Normalizes Elevated Blood Level of Soluble Fas mRNA in Patients with Acute Myocardial Infarction

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Fas-induced apoptosis plays an important role in the mechanisms of tissue injury in myocardial infarction. The level of membrane Fas mRNA was elevated during the postinfarction period in the blood of patients and did not change in response to levocarnitine. The mRNA level of soluble Fas, inhibiting Fas-dependent apoptosis, remained normal during the first 7 days, but increased 14 days after myocardial infarction, which corresponded to previously detected increase of serum level of soluble Fas molecules. Addition of levocarnitine, inhibiting Fas-dependent apoptosis, to therapy caused no changes in the level of soluble Fas mRNA, presumably because of similar effects of soluble Fas and levocarnitine on the apoptotic processes in myocardial infarction.

Key Words: *apoptosis; myocardial infarction; soluble Fas mRNA*

Apoptosis plays an important role in tissue damage processes in myocardial infarction (MI) [11]. Several approaches to apoptosis initiation are known; one of them is Fas-dependent. Fas molecule (CD95) is represented by two forms, membrane and soluble. Membrane Fas (mFas) is present on the surface of many immune system cells and some others [6]; it was detected on cardiomyocytes and myocardial granular cells playing the main role in ventricular remodeling after MI [9]. mFas mediated transmission of apoptotic signal after binding to Fas ligand (FasL, CD178). Soluble Fas (sFas), a result of alternative splicing of pre-mRNA, is secreted into the pericellular space and acts as apoptosis inhibitor interfering mFas and FasL interactions [2,3]. Experiments on laboratory mice have demonstrated that the increase in sFas level during postinfarction period improves animal survival, suppresses apoptosis of actively proliferating granular cells in the myocardium, and promotes the formation of postinfarction cicatrix enriched with vessels and contractive cells [10].

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High levels of sFas are detected in patients during and after MI, the highest serum sFas levels recorded in patients with transmural infarction (*Q*-infarction) with extensive disorders in ECG and echocardiographic abnormalities [1]. The results attest to important role of high sFas concentrations in tissue remodeling after MI. However, the source of high sFas concentrations in the blood of MI patients remains unclear.

We analyzed blood levels of mFas and sFas mRNA in patients after acute MI receiving standard therapy and levocarnitine inhibiting the Fas-dependent apoptosis [13].

MATERIALS AND METHODS

Peripheral blood samples from 19 patients with acute MI treated at Municipal Clinical Hospital No. 5 were used. Group 1 patients (n=10) received standard therapy, group 2 patients (n=9) received standard therapy and 1.5 g levocarnitine (Elkar) twice a day (intravenous drip infusion on days 1-3 and *per os*). Control group consisted of 10 healthy volunteers. All patients and volunteers signed informed consent to participation in the study.

Peripheral blood (0.5 ml) was collected on admission to hospital and on days 7 and 14. Peripheral

blood was mixed with an equal volume of lyzing solution (4 M guanidine thiocyanate, 250 mM sodium acetate, 0.5% Triton X-100) and stored at -20°C until use. Nucleic acids were extracted from a 200-µl sample with phenol:chloroform mixture (1:1; 200 µl), precipitated by isopropranolol, and washed in 75% ethanol. The precipitate was dissolved in 20 µl nuclease-free water. The resultant preparation was used in inverse transcription reaction using MMLV revertase (AmpliSens) according to the instruction. Poly-T-primer was used in the reaction.

The relative content of mFas antigen mRNA (mFas mRNA) and sFas antigen mRNA (sFas mRNA) was evaluated by real-time PCR as described previously [4] with minor modifications. The PCR was carried out twice with each sample: threshold cycles (Ct) for mFas mRNA and UBC mRNA recorded in one reaction, sFas mRNA and UBC mRNA in the other. The reaction mixture contained PCR buffer, 5 U TaqF polymerase (InterLabService), 0.4 mM each dNTP, 10 pM each primer, 5 pM each probe specific to the studied mRNA. The primary structure of the primers and probes are presented in Table 1. PCR was carried out in DTlite nucleic acid analyzer (DNA-Technology) as follows: 15 min at 94°C and 50 cycle of the following protocol: 30 sec at 94°C, 40 sec at 55°C, and 45 sec at 72°C.

The resultant Ct values were used to calculate the levels of mRNA as described previously [8]. The levels of mRNA were compensated for with consideration for the reaction efficiency and normalized by UBC mRNA level. The data were compared by nonparametric methods for related (Wilcoxon's sign ranked test) and unrelated groups (Mann–Whitney *U* test) using Statistica 8.0 software. The results were presented as the median, 25Q and 75Q, with the minimum and maximum values.

TABLE 1. Primers and Probes Used in Real-Time PCR

Primer	Primary structure (5'→3')
Fas-F	ACCAAATGTGAACATGGAAT
Fas-R	TTCCTTTCTCTTCACCCAA
sFas-R	TTCCTTTCTCTTCACTTCC
Fas-Z	ROX-AGATCTAACTTGGGGTGGCTTTGTCTTCTT-BHQ2
sFas-Z	FAM-AGAGGAAGTGAAGAGAAAGGAAGTACAGA-BHQ1
UBC-F	CACAGCTAGTTCCGTCGCA
UBC-R	GAAGATCTGCATTGTCAAGT
UBC-Z	R6G-ATTTGGGTCGCAGTTCTTGTTTGTGGAT-BHQ1

RESULTS

Blood levels of mFas mRNA in MI patients on admission to hospital were significantly (p=0.026) higher than in controls. The level of mFas mRNA remained high in group 1 patients during the subsequent 2 weeks (Fig. 1). In group 2 patients, blood levels of mFas mRNA also surpassed the normal during the first 2 weeks of hospital treatment (Fig. 1).

The levels of sFas mRNA were virtually unchanged in MI patients on admission to the hospital. Two-week observation of group 1 patients showed that sFas mRNA level remained normal during week 1 and increased significantly after 14 days (p=0.036). In group 2, the level of sFas mRNA in the peripheral blood remained normal during the entire period of observation; that is, levocarnitine treatment modified the time course of sFas mRNA. Blood levels of

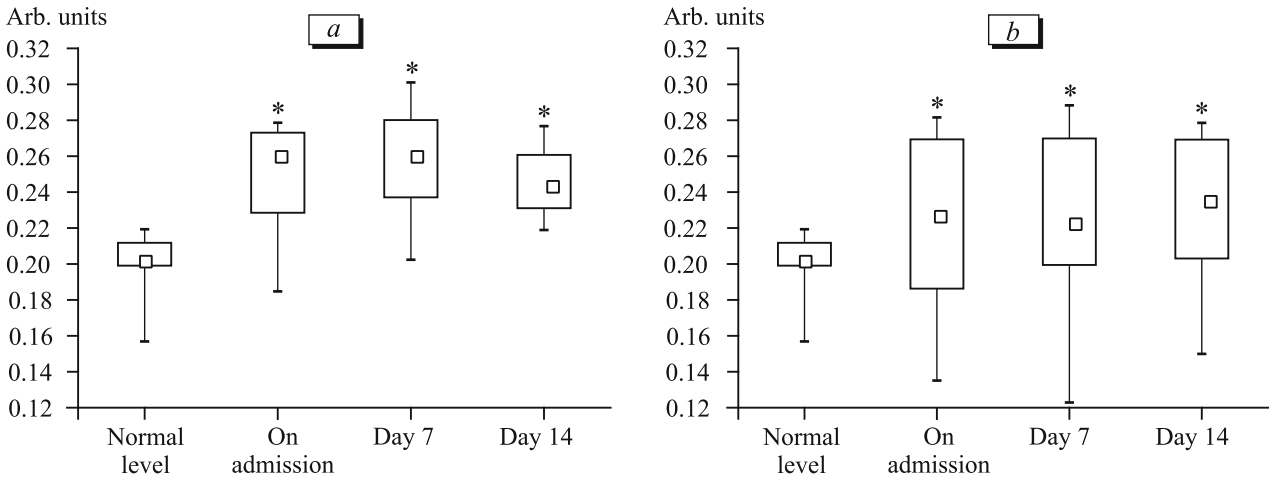

Fig. 1. Peripheral blood levels of mFas mRNA in MI patients, groups 1 (*a*) and 2 (*b*). *p<0.05 in comparison with the control.

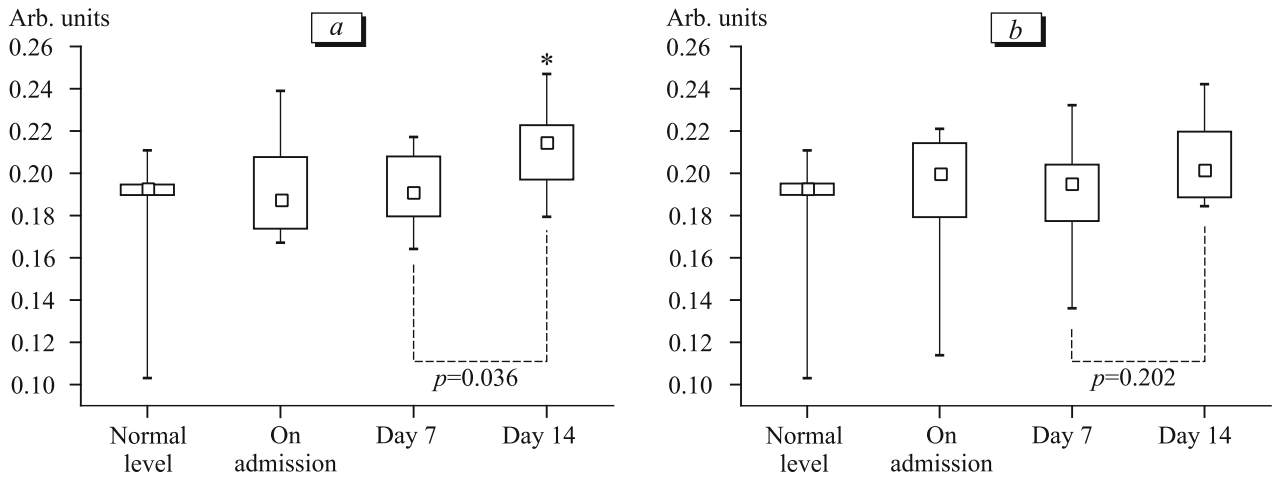

Fig. 2. Peripheral blood levels of sFas mRNA in MI patients, groups 1 (*a*) and 2 (*b*). *Between sFas mRNA levels on days 7 and 14 after infarction.

sFas mRNA did not increase during week 2 of the postinfarction period (Fig. 2).

MI is associated with an increase in the counts of Fas^+ lymphocytes and neutrophils [7]. High level of mFas mRNA in the peripheral blood of patients during 2 weeks of the postinfarction period was in good agreement with the data on high level of $CD95^+$ cells in the blood of MI patients. This increase was recorded in the patients receiving standard therapy and therapy supplemented with levocarnitine. The level of sFas mRNA increased only under conditions of standard therapy and only by day 14 of the postinfarction period, but not in patients treated with levocarnitine, in whom it remained stable during the entire period of observation.

In addition to high blood content of $CD95^+$ cells, MI patients had high serum levels of sFas [5,12]. In a previous study, we detected just a trend to an increase of serum sFas level during day 1 after MI, while the most marked increase in this parameter in patients receiving standard therapy developed on day 10 of the postinfarction period [1]. High level of serum sFas was presumably explained by high level of sFas mRNA in peripheral blood cells and hence, higher production and release of this soluble protein into the pericellular space and blood.

Antiapoptotic activity of sFas is realized via its competitive interaction with mFas on the cell surface. The increase in mFas expression on the membrane can be induced by stress exposure, *e.g.* hypoxia, a pathogenetic component of MI development [15]. The increase in sFas level under these conditions is a factor protecting the myocardial cells from Fas-dependent apoptosis. However, protection is realized only 10-15 days after MI and seems to be aimed at the maintenance of actively proliferating myocardial granular cells carrying surface mFas ready to trigger apoptosis [9]. This protection is important for ventricular remodeling after MI.

Levocarnitine, another inhibitor of Fas-induced apoptosis, stabilizes the structure of cardiomyocyte mitochondrial membranes, facilitates transport of fatty acids to the mitochondrial matrix, where they serve as a source of energy, and blocks the release of cytochrome C from mitochondria [13,14]. The inhibition is not direct, but mediated via the mitochondrial apoptotic pathway related to Fas-induced pathway [6]. The level of sFas mRNA remains normal in MI patients treated with levocarnitine; this fact indicates common mechanisms in the action of these two antiapoptotic factors. The normal level of sFas mRNA against the background of high level of mFas mRNA remains stable due to the stabilizing effect of levocarnitine on the structure and function of blood cell mitochondria. Levocarnitine produces simultaneous apoptosis-inhibiting effect on cardiomyocyte mitochondria and proliferating granular cells involved in myocardial repair.

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REFERENCES

1. S. S. Belousov, N. A. Sakharnov, N. B. Presnyakova, *et al.*, *Vestn. Nizhegorodsk. Univer. im. N. I. Lobachevskogo*, No. 4, 96-99 (2010).
2. V. V. Novikov, I. V. Evsegneeva, A. V. Karaulov, and A. Yu. Baryshnikov, *Ross. Bioter. Zh.*, **4**, No. 2, 100-105 (2005).
3. V. V. Novikov, A. V. Karaulov, A. Yu. Baryshnikov, *et al.*, *Mol. Med.*, No. 4, 27-33 (2009).
4. D. V. Novikov, M. Yu. Lebedev, N. A. Sakharnov, *et al.*, *Vestn. Nizhegorodsk. Univer. im. N. I. Lobachevskogo*, Nos. 2-3, 211-215 (2012).

5. N. N. Petrishchev, L. V. Vasina, and A. V. Lugovaya, *Vestn. Sankt-Peterburgsk. Univer.*, Ser. 11, No. 1, 14-24 (2008).
6. S. V. Ryzhov and V. V. Novikov, *Ross. Bioter. Zh.*, **1**, No. 3, 27-33 (2001).
7. S. N. Tereshchenko, G. V. Barmotin, A. A. Sokolovskaya, *et al.*, *Uspekhi Klin. Immunol. Allergol.* [in Russian], Ed. A. V. Karaulov, Vol. 1, Moscow (2001), pp. 179-190.
8. S. Cikos, A. Bukovska, and J. Koppel, *BMC Mol. Biol.*, **8**, 113 (2007).
9. Q. Z. Feng, Y. S. Zhao, and E. Abdelwahid, *Coron. Artery Dis.*, **19**, No. 7, 527-534 (2008).
10. Y. Li, G. Takemura, K. Kosai, *et al.*, *Circ. Res.*, **95**, No. 6, 627-636 (2004).
11. P. A. Krijnen, R. Nijmeijer,C. J. Meijer, *et al.*, *J. Clin. Pathol.*, **55**, No. 11, 801-811 (2002).
12. A. Mielczarek-Palacz, Z. Kondera-Anasz, J. Sikora, *et al.*, *Wiad. Lek.*, **62**, No. 4, 219-225 (2009).
13. M. C. Mutomba, H. Yuan, M. Konyavko, *et al.*, *FEBS Lett.*, **478**, No. 1-2, 19-25 (2000).
14. E. Oyanagi, H. Yano, M. Uchida, *et al.*, *Biochem. Biophys. Res. Commun.*, **412**, No. 1, 61-67 (2011).
15. M. Tanaka, H. Ito, S. Adachi, *et al.*, *Circ. Res.*, **75**, No. 3, 426-433 (1994).