Relationship between Oxidized Fibrinogen and Hemostasis Disturbances and Endothelial Dysfunction in Myocardial Infarction

Yu. I. Ragino, V. A. Baum, Ya. V. Polonskaya, E. V. Sadovski, S. R. Baum, and Yu. P. Nikitin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 4, pp. 389-391, April, 2008
Original article submitted April 3, 2007.

Oxidative modification of fibrinogen in acute myocardial infarction increased 1.3-fold compared to that in CHD and 1.5-fold surpassed that in the control without CHD. Elevated content of oxidized fibrinogen correlated with increased levels of LPO products, von Willebrand factor, and fibrin degradation products, with accelerated leukocyte and platelet aggregation, and reduced content of NO metabolites in the plasma. Independent associations of oxidized fibrinogen with myocardial infarction and typical thrombogenic and hypercoagulation hemostasis disorders and endothelial dysfunctions were revealed.

Key Words: *oxidized fibrinogen; myocardial infarction; oxidized lipids and proteins; hemostasis disturbances; endothelial dysfunction*

Oxidative stress, a pathogenetic component of atherosclerotic cardiovascular diseases including CHD and myocardial infarction (MI), leads to pathological oxidative modification of biomolecules [6,14]. Oxidation of proteins changes their physicochemical properties and conformation due to intermolecular crosslinking and partial defragmentation and aggregation, thus impairing physiological functions of protein molecules [13].

The data obtained in the studies of the role of fibrinogen in the development of inflammatory and antherothrombotic states, including increased adhesion and aggregation of platelets, blood viscosity, adhesion of leukocytes to endothelial cells via binding of intermolecular adhesion molecules [11,12] theoretically substantiated the urgency of evaluation of the role of oxidative modifications of fibrinogen (OF) in the pathogenesis of cardiovascular diseases. It was demonstrated that OF potentiated activation of blood clotting factors and platelet and erythrocyte aggregation and enhanced secretion of cytokines by macrophages, which led to pronounced thrombogenic disorders of hemostasis [1,8,9].

Here we studied the relationship between OF and hemostasis disturbances, endothelial dysfunction, and activity of LPO processes in CHD and acute MI.

MATERIALS AND METHODS

We analyzed blood plasma from 87 men (3 groups). Group 1 comprised 32 patients with CHD and acute MI, age 38-62 years (mean 53.2±2.0). MI was diagnosed on the basis of criteria approved by European Society of Cardiology and American College of Cardiology (2000) including typical pain attack, typical ECG signs in 2 and more subsequent leads, and dynamic changes in enzyme levels. Group 2 included 28 patients aging 38-65 years (mean 54.9±2.40) with CHD and a history of MI. Control group consisted of 27 individuals without CHD (age 35-60 years, mean 50.6±2.4). Patients with diabetes

Research Institute of Therapy, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** ragino@online.nsk.su. Yu. I. Ragino.

0007-4888/08/1454-0412

mellitus were not included in the study. The groups were equivalent by patients' age, smoking status, and hypertension.

The following parameters of hemostasis were analyzed: leukocyte-platelet aggregation [4], hemolysate-aggregation test, fibrinogen level, content of soluble fibrin-monomer complexes (SFMC), antithrombin III, activity of XIIa-dependent fibrinolysis, prothrombin index, and international standardized ratio as described elsewhere [2]. Tekhnologiya Standard kits were used.

The content of LPO products in the plasma was evaluated by the concentration of TBA-reactive products on a Hitachi F-300 spectrofluorometer. The degree of oxidative modification of plasma proteins was determined spectrophotometrically [5]. OF was assayed after reaction with 2,4-dinitrophenylhydrazine [7]. Endothelial function was evaluated by plasma content of NO metabolites (spectrophotometrically after plasma deproteinization and reduction of NO_3^- to NO_2^- with cadmium granules), von Willebrand factor, and endothelin-1 (ELISA with Acis and Biomedica Group kits).

The data were processed statistically using correlation and linear regression analyses, one-way ANOVA, and Dunnett test for multiple comparison.

RESULTS

The groups differed by the degree of oxidative modifications in lipids and proteins (Table 1). For instance, in patients with CHD and in patients with MI, the content of LPO products 1.1- and 1.3-fold surpassed the control level. The difference between the CHD and MI groups were also significant (by 1.2 times, $p<0.05$). The level of oxidized proteins did not differ between the CHD and MI groups, but in both groups this parameter 1.2-fold surpassed the control value ($p<0.05$). Plasma fibrinogen content was elevated in both CHD and MI patients (by 1.1 times, $p<0.05$) compared to the control. Since fibrinogen is very sensitive to oxidation [8], evaluation of its oxidative modification revealed differences between the three groups. The maximum level of OF was noted in acute MI (1.3- and 1.5-fold higher than in CHD patients and controls, respectively; $p<0.05$), which suggests that OF is a more valuable and specific parameter for MI than plasma fibrinogen concentration.

Evaluation of hemostasis parameters in CHD and MI showed that leukocyte-platelet aggregation was accelerated by 1.1 times, the content of SFMC was higher by 1.2 times (which attested to active thrombin formation process), and fibrinolytic activity was reduced 1.5-fold compared to the control. Vascular, platelet, and coagulation hemostasis and oxidation processes are closely related to vascular endothelium and its function [10,14]. For instance, the level of endothelin-1 was elevated by 1.5 times ($p<0.05$) in both MI and CHD. At the same time, the content of NO metabolites in MI was lower than in CHD and in the control (by 1.3 and 1.7 times, respectively) and the content of von Willebrand factor was higher (by 1.3 and 1.5 times, respectively; $p<0.05$).

Correlation analysis revealed direct relationships of OF content with CHD and MI, activity of LPO processes, content of von Willebrand factor and SFMC, and reverse relationship with the level of NO metabolites and the time of leukocyte-platelet aggregation (Table 2). No correlation was found between OF and the level of fibrinogen and oxidative modification of plasma proteins. Regression analysis showed independent association of OF

TABLE 1. Parameters of Oxidative Modifications, Hemostasis, and Endothelial Function in Blood Plasma from Examined Individuals ($M \pm m$)

Parameter	Control	CHD, history of MI	Acute MI
Plasma content of LPO products, nmol MDA/mg protein	2.6±0.1	2.9±0.1*	3.4±0.2*+
Oxidized plasma proteins, U/ml plasma	3.6±0.2	4.2±0.2*	4.4±0.3*
Plasma fibrinogen, g/liter	3.3±0.1	3.6±0.1*	3.6±0.1*
OF, U/mg fibrinogen/ml plasma	12.1±0.7	15.5±0.9*	17.8±1.0*+
Leukocyte-platelet aggregation, sec	8.7±0.2	7.8±0.2*	7.7±0.2*
SFMC, g/liter	0.073±0.003	0.087±0.004*	0.092±0.004*
Activity of XIIa-dependent fibrinolysis, min	22.5±1.7	34.8±3.1*	32.5±3.9*
Plasma content of endothelin-1, pmol/liter	311.0±60.0	482.0±83.0*	460.0±54.0*
Plasma content of von Willebrand factor, U/ml	1.39±0.09	1.61±0.10*	2.08±0.09*+
Plasma content of NO metabolites, μmol/liter	7.4±0.3	5.7±0.3*	4.3±0.3*+

Note. $p<0.05$ compared to: *control, +CHD.

TABLE 2. Correlation and Regression Analyses of the Relationship between OF and Studied Parameters

Studied parameters	Pearson coefficient	Kendall coefficient	Standardized coefficient Beta
CHD	0.245**	0.167**	—
MI	0.278**	0.189**	0.397**
LPO intensity	0.159*	0.113*	—
Oxidized proteins	—	—	—
Endothelin-1	—	—	—
von Willebrand factor	0.328**	0.240**	0.235**
NO metabolites	-0.185*	-0.192*	0.282*
Fibrinogen	—	—	—
SFMC	0.249*	0.231*	0.377*
Antithrombin III	—	—	—
Leukocyte-platelet aggregation	-0.179*	-0.162*	-0.289*
Hemolysate-aggregation test	—	—	—
Activity of XIIa-dependent fibrinolysis	—	—	-0.325*
Prothrombin index	—	—	—
International standardized ratio	—	—	—

Note. *p<0.05, **p<0.01; dash: data are insignificant.

with MI, rate of leukocyte-platelet aggregation, activity of XIIa-dependent fibrinolysis, and levels of SFMC, von Willebrand factor, and NO metabolites (Table 2), which attests to a relationship of OF with thrombogenic and hypercoagulation disturbances of hemostasis and with endothelial dysfunction, which are most pronounced in acute MI. The mechanisms of the potentiating effect of OF on endothelial dysfunction probably include both the direct cytotoxic effect on endotheliocytes (similarly to other oxidized molecules [14], and an indirect effect mediated via von Willebrand factor during their interaction on IIb/IIIa glycoprotein receptors of platelets [10].

The study was partially supported by President's grant No. MD-539.2007.7.

REFERENCES

1. A. V. Aseichev, O. A. Azizova, and B. A. Zhambalova, *Byull. Eksp. Biol. Med.*, **133**, No. 1, 51-54 (2002).
2. Z. S. Barkagan and A. P. Momot, *Diagnostics and Controlled Therapy of Hemostasis Disorder*s [in Russian], Moscow (2001).
3. P. P. Golikov and N. Yu. Nikolaeva, *Biomed. Khim.*, **50**, No. 1, 79-85 (2004).
4. A. A. Gromov, A. V. Rabko, M. V. Kruchinina, and V. A. Baum, *Vestn. Novosib. Gos. Univer.*, **4**, No. 2, 31-38 (2006).
5. E. E. Dubinina, S. O. Bupmistrov, D. A. Khodov, and G. E. Porotov, *Vopr. Med. Khimii*, **41**, No. 1, 24-26 (1995).
6. V. Z. Lankin, A. K. Tikhadze, and Yu. N. Belenkov, *Kardiologiya*, No. 7, 48-62 (2000).
7. *Yu. I. Ragino, V. A. Baum, Ya. V. Polonskaya, and A. A. Gromov*, *Klin. Lab. Diagn.*, No. 1, 13-16 (2007).
8. E. V. Roitman, O. A. Azizova, Yu. A. Morozov, and A. V. Aseichev, *Byull. Eksp. Biol. Med.*, **138**, No. 9, 277-279 (2004).
9. M. Belisario, D. Di, A. Pelagalli, *et al., Biochimie*, **79**, No. 7, 449-455 (1997).
10. G. R. De Meyer, M. Hoylaerts, M. M. Kockx, *et al., Arterioscler. Thromb. Vasc. Biol.*, **19**, No. 10, 2524-2534 (1999).
11. E. Ernst and K. L .Resch, *Ann. Intern. Med.*, **118**, No. 12, 956-963 (1993).
12. H. Koukkunen, K. Penttila, A. Kemppainen, *et al. Ann. Med.*, **33**, No. 1, 37-47 (2001).
13. E. R. Stadtman and R. L. Levine, *Ann. N. Y. Acad. Sci.*, **899**, 191-208 (2000).
14. R. Stocker and J.F.Keaney, Jr., *Physiol. Rev.*, **84**, No. 4, 1381-1478 (2004).