BIOPHYSICS AND BIOCHEMISTRY

Fibrinogen and Its Oxidized Form Induce Interleukin-2 Production in Cultured Endothelial Cells of Human Vessels

O. A. Azizova, E. V. Maksyanina, Yu. A. Romanov, A. V. Aseichev, and O. N. Scheglovitova

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Oxidized fibrinogen was more potent than native fibrinogen in inducing interleukin-8 production in primary culture of human endothelial cells. The optimal concentration of oxidized fibrinogen was 3 mg/ml. The optimal time of UV irradiation was 17 min. Secretion of interleukin-8 was maximum during culturing of endothelial cells in a serum-free medium.

Key Words: *oxidized fibrinogen; interleukin-8; culture of vascular endothelium*

Recent studies showed that activation of free radical processes plays a role in the pathogenesis of cardiovascular diseases and their complications [3,13]. Oxidized low-density lipoproteins (LDL) are involved in the main stages of atherogenesis: impairment of endothelial permeability, transformation of macrophages and smooth muscle cells into foam cells, proliferation of smooth muscle cells, production of cytokines [3,5, 13], and dysfunction of blood cells [1,4,5]. Oxidized LDL appear in tissues and blood flow during oxidative stress [6,14]. It should be emphasized that other blood proteins also undergo oxidative modification. Fibrinogen (FG) is involved in blood coagulation. Increased FG content is an independent risk factor for atherosclerosis and its complications [9,10,15]. The pathogenetic mechanisms of these disturbances in patients with high FG content are poorly understood.

FG is very sensitive to free radicals in the blood [12]. We hypothesized that oxidized FG (OFG) can modulate functions of blood cells and vessels. Our previous studies showed that OFG induces platelet aggregation [2] and potentiates ADP-induced aggregation. We showed that OFG intensifies production of reactive oxygen species in zymosan-stimulated leukocytes. The effects of OFG on functional activity of endothelial cells (EC) remain unknown. Here we studied the ability of OFG to stimulate interleukin-8 (IL-8) production by cultured EC from human blood vessels. IL-8 acts as a chemokine recruiting neutrophils and platelets.

MATERIALS AND METHODS

EC were isolated from human umbilical vein [11]. The cells were grown in culture flasks with medium 199 containing 20 mM HEPES, 10% fetal bovine serum (FBS), 2 mM glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco), and 50 μg/ml endothelial growth factor of human nervous tissue. The medium was replaced every other day. The monolayer of the primarily culture was trypsinized (0.05% trypsin and 0.02% versen), passed in 24-well plates, and assayed on day 5.

Laboratory for Biophysical Bases of Pathology, Institute of Physicochemical Medicine, Russian Ministry of Health, Moscow. ***Address for correspondence:*** oazizova@mail.ru. Azizova O. A., aseychev@mail.ru. Aseichev A. V.

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OFG was obtained by UV-irradiation on a UV light-generating FEK-56PM device. FG (Sigma) was dissolved in phosphate buffer and irradiated in 24-well plates over various periods of time. The solution was mixed on a magnetic stirrer at a flow rate not causing foaming. The degree of oxidative modification of FG was estimated by the decrease in fluorescence of aromatic amino acids (Fig. 1) [2].

The initial and irradiated forms of FG were added to the culture medium containing or not containing FBS (dilution 1:2-1:3). The medium was sampled after 6 and 24 h, respectively.

The amount of IL-8 was measured by enzyme immunoassay using special test systems (Proteinovyi Kontur) [11].

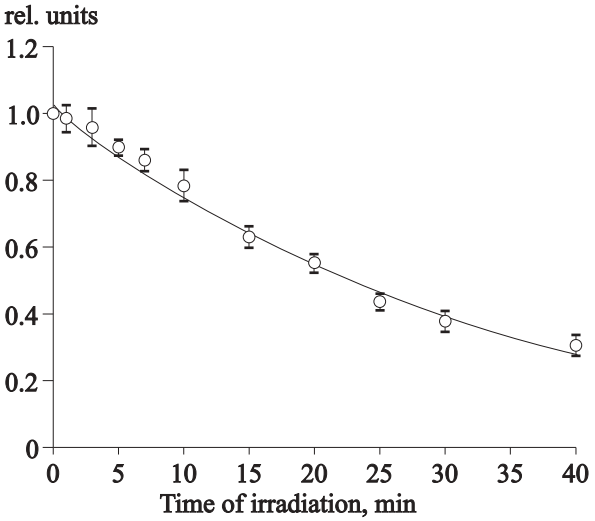

Fig. 1. Changes in fluorescence of aromatic amino acid residues depending on the time of UV irradiation.

RESULTS

The intensity of IL-8 production depended on the concentration of FG (Fig. 2). IL-8 secretion peaked after 6-h incubation with 1.4 mg/ml FG and 24-h incubation with 3 mg/ml FG.

We studied the ability of OFG to induce production of IL-8 in EC. Final concentrations of irradiated FG corresponded to doses inducing the maximum production of IL-8 (1.4 and 3 mg/ml). The intensity of IL-8 production in EC after treatment with OFG and oxidative modification of 10% aromatic amino acid residues (5-min irradiation) was 10-30% higher than that induced by FG (Fig. 3, *a*). IL-8 production was most intensive in the early period after induction (6 h).

Increasing the degree of FG oxidation (10, 22, and 40% amino acid residues after irradiation for 5, 10, and 17 min, respectively) led to intensification of IL-8 production (Fig. 3, *b*). The intensity of IL-8 secretion increased by 200% after irradiation with oxidative modification of 40% aromatic amino acid residues. IL-8 production was most intensive in the culture medium not containing FBS. Increasing the time of irradiation improved the ability of FG to induce IL-8. In these experiments the final concentration of FG was 3 mg/ml. Activation of IL-8 production was less pronounced under the influence of FG in lower concentrations.

Adhesion of monocytes to vascular EC leading to thickening of the intima is the initial pathogenetic stage of atherosclerosis [3,5,13]. Previous studies showed that adhesion of monocytes to the endothelium results from the interaction of complementary cell adhesion molecules ICAM-1 and MCP-1 and chemokine IL-8. It was also found that FG induces expression of ICAM-1 and MCP-1 in EC [7,8]. Therefore, FG-induced thickening of the vascular intima is related to expression of ICAM and MCP-1 interacting with mono-

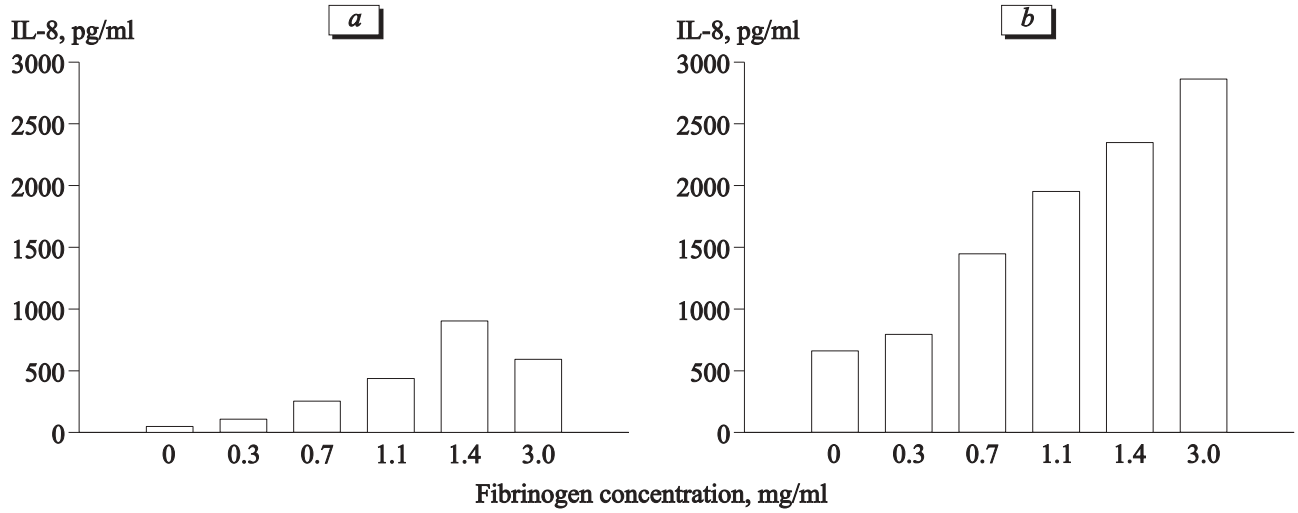

Fig. 2. Production of IL-8 in culture of human vascular endothelial cells at various concentrations of fibrinogen: 6 (*a*) and 24 h after induction of IL-8 (*b*).

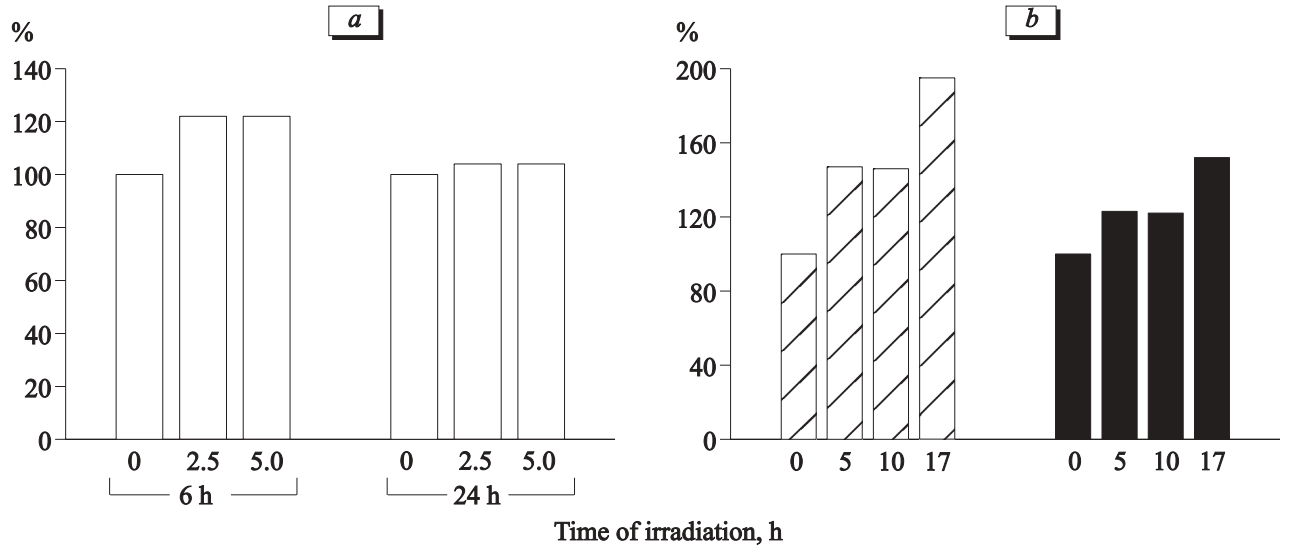

Fig. 3. Production of IL-8 in the culture of human vascular endothelial cells after induction with UV-irradiated fibrinogen: 6 and 24 h after induction (*a*); without (shaded bars) and with fetal bovine serum (dark bars, *b*). Samples were taken 6 h after induction.

cytes. Our experiments showed for the first time that FG initiates production of the chemoattractant IL-8 by EC, which also can recruit monocytes. We conclude that FG plays a role in the development of atherosclerosis by inducing IL-8 synthesis in EC. The intensity of IL-8 production increased by 1.5-2.0 times after treatment of EC with OFG. The data suggest that OFG plays an important role in the pathogenesis of cardiovascular diseases.

REFERENCES

1. O. A. Azizova and I. I. Vlasova, *Byull. Eksp. Biol. Med.*, **116**, No. 11, 485-487 (1993).
2. A. V. Aseichev, O. A. Azizova, and B. A. Zhambalova, *Ibid.*, **133**, No. 1, 51-54 (2002).
3. Yu. A. Vladimirov, O. A. Azizova, A. I. Deev, and A. V. Kozlov, *Itogi Nauki Tekhniki. Ser. Biofizika*, **29**, 100-132 (1991).
4. O. A. Azizova, O. N. Panasenko, and T. V. Vakhrusheva, *Free Radic. Biol. Med.*, **7**, No. 3, 251-257 (1989).
5. J. A. Berliner and J. W. Heinecke, *Ibid.*, **20**, 707-727 (1996).
6. H. Fickl, V. Vanantwerpen, G. A. Richard, and R. Vonderwalt, *Atherosclerosis*, **124**, 75-81 (1996).
7. S. L. Hartley and J. T. Powell, *Biochem. J.*, **341**, 739-744 (1999).
8. S. L. Hartley, J. Sturge, and J. T. Powell, *Vascul. Biol.*, **20**, 652-667 (2000).
9. M. Jink, C. H. Hennekens, and P. M. Ridker, *J. Am. Coll. Cardiol.*, **33**, 1347-1352 (1994).
10. W. B. Kannel, P. Wols, P. Castelli, and R. B. D'Agostino, *JAMA*, **258**, 1183-1186 (1993).
11. O. N. Scheglovitova, Yu. A. Romanov, E. V. Maksianina, *et al.*, *Rus. J. Immunol.*, **6**, 367-376 (2001).
12. E. Shaster, I. A. Willians, M. Linn, and R. L. Levine, *Free Radic. Biol. Med.*, **17**, 429-437 (1994).
13. D. Steinberg, S. Parthasarathy, T. E. Carew, *et al.*, *N. Engl. J. Med.*, **320**, 915-924 (1989).
14. L. P. L. Vandervijver, R. Steyger, G. Vanpoppe, and J. M. A. Boer, *Atherosclerosis*, **122**, 245-253 (1996).
15. J. M. B. Yarnell, I. A. Baker, P. M. Swetnam, and D. Baiton, *Circulation*, **83**, 836-844 (1991).