Effect of Pravastatin on Phenotypical Transformation of Fibroblasts and Hypertrophy of Cardiomyocytes in Culture

O. M. Moiseeva, E. G. Semyonova*, E. V. Polevaya*, and G. P. Pinayev*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 1, pp. 60-63, January, 2007
Original article submitted July 28, 2006

We studied the effects of pravastatin on the functional state of cardiomyocytes and cardiac fibroblasts in culture. Pravastatin in doses of 0.1-10.0 μM suppressed proliferation of cardiac fibroblasts and their phenotypical transformation into myofibroblasts and prevented the development of cardiomyocyte hypertrophy. Pleiotropic effects of pravastatin can be explained by inhibition of production transforming growth factor β_1 production.

Key Words: *myofibroblasts; cardiomyocyte hypertrophy; statins*

The problems of cardiomyocyte (CMC) hypertrophy and apoptosis are closely related to the development of interstitial and perivascular fibrosis, and hence, the type of structural pathological changes in the heart is more accurately denoted by the term "myocardial remodeling". Some drugs can not only induce regression of myocardial hypertrophy, but also modify stromal elements of the heart as a component of remodeling. However, the cell aspects of these structural changes are not studied.

We studied the effect of pravastatin (PS; hydroxymethylglutaryl-coenzyme A-reductase) on the functional state of CMC and cardiac fibroblasts in culture.

MATERIALS AND METHODS

Cardiomyocytes were obtained from the hearts of 2-4-day-old rat pups; cardiac fibroblasts were isolated by differentiated adhesion. Fibroblasts of passages 3-6 were used in the study.

Control CMC were cultured in DMEM supplemented with 5% FCS; CMC hypertrophy was modeled by adding 10% FCS to the medium.

Cardiomyocytes were visualized by EA53 monoclonal antibodies to α-actinin and rhodamine falloidin (Molecular Probes Inc.). VA type myofibroblasts were detected by immunofluorescent analysis using monoclonal antibodies to vimentin (Sigma) and α-smooth-muscle actin (clone 1A4; DAKO). Proliferative activity of cardiac fibroblasts was evaluated by an indirect immunohistochemical method with monoclonal antibodies to proliferating cell nuclear antigen (PCNA) and visualized by the LSAB2 system (DAKO). Immunofluorescent analysis of CMC and cardiac fibroblasts for transforming growth factor-β_1 (TGF-β_1) was carried out using original antibodies obtained at Department of Cell Cultures, Institute of Cytology.

Apoptosis was detected by the method of neutral single-cell DNA-comet assay and staining of DNA fragments with DAPI fluorescent dye [2].

The degree of CMC hypertrophy *in vitro* was evaluated by cytomorphometrical parameters and

V. A. Almazov Institute of Cardiology, Russian Ministry of Health; *Institute of Cytology, Russian Academy of Sciences, St. Petersburg. ***Address for correspondence:*** omoiseeva@front.ru. O. M. Moiseyeva

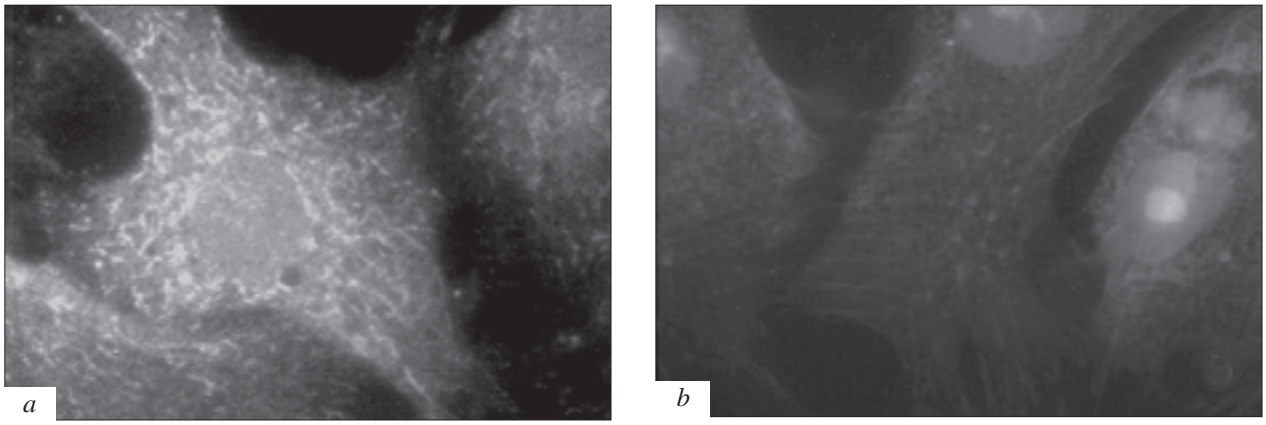


Fig. 1. Expression of TGF-β_1 in cultured rat cardiac fibroblast. *a*) control; *b*) PS, 10 μM. Here and in Figs. 2, 3: indirect immunohistochemical staining; phase contrast microscopy, ×1000.

by the intensity of protein synthesis evaluated by ^{3}H-leucine incorporation (autoradiography). The area of CMC was evaluated planimetricaly and expressed in arbitrary units. The intensity of protein synthesis in CMC was determined by ^{3}H-leucine incorporation by counting silver grains above the cell.

The effect of PS (lipostat; Bristol-Myers Squibb) on proliferative activity of fibroblasts was studied on a monolayer cell culture in a 96-well plate.

A total of 11 independent cultural experiments in 3 repetitions were carried out.

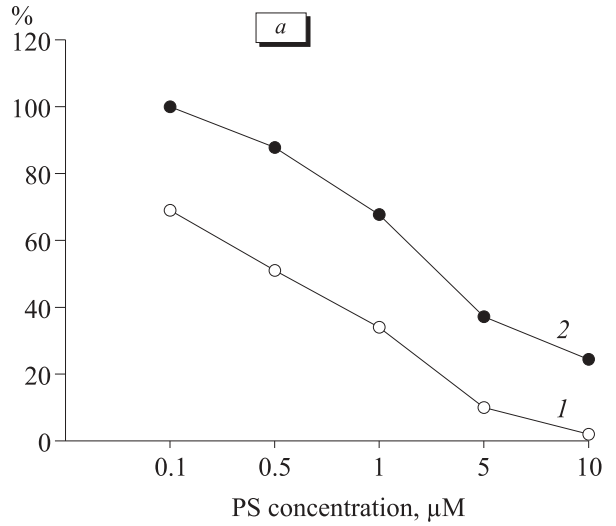
RESULTS

Coculturing of CMC and fibroblasts was associated with the development of CMC hypertrophy mainly due to chemical information exchange between the cells [1]. TGF-β_1 plays the major role among the auto/paracrine regulators of CMC hypertrophy; this factor is produced in both CMC and fibroblasts (Fig. 1). Phenotypical heterogeneity of fibroblasts appeared during coculturing of CMC and fibroblasts. A total of 42-69% fibroblasts (confirmed by positive staining for vimentin) actively synthesized smooth-muscle actin and were classified as myofibroblasts by the VA system [6]. Myofibroblasts are unique not only because they posses a potent receptor system making the cell sensitive to microenvironmental changes (mechanical stretching, auto/paracrine factors, and protein composition of the myocardial stroma). They can produce growth factors (TGF-β_1, fibroblast growth factor), proinflammatory cytokines and whereby not only regulate collagen synthesis, but also modify the hypertrophic growth of CMC. Myofibroblast release inflammation mediators (prostaglandin E_2, prostacyclin, platelet-activating factor, nitrogen oxide, and peroxides), cyto- and chemokines, which suggests their involvement in the regulation of local inflammatory reactions, while the presence of smooth-muscle actin suggests their participation in the regulation of myocardial contractile activity [5-7].

TABLE 1. Effect of PS on Cytomorphometric Parameters and Intensity of Protein Synthesis in Primary Culture of Rat CMC (*M*±*m*)

Variant of experiment	Parameter	Duration of experiment, days	
		2	9
Control	Cell area, arb. units	1071.3±168.5	2065.0±189.4
	^{3}H-leucine, number of silver grains	94.6±5.2	108.9±5.9
Experiment			
medium with 10% FCS	Cell area, arb. units	2659.0±376.2*	4567.1±421.8**
	^{3}H-leucine, number of silver grains	147.2±7.2*	250.9±9.6**
medium with 10% FCS+PS, 10 μM	Cell area, arb. units	1948.7±423.2	2557.1±225.0
	^{3}H-leucine, number of silver grains	97.5±4.8	117.6±8.8

Note. *p<0.01 compared to the control.



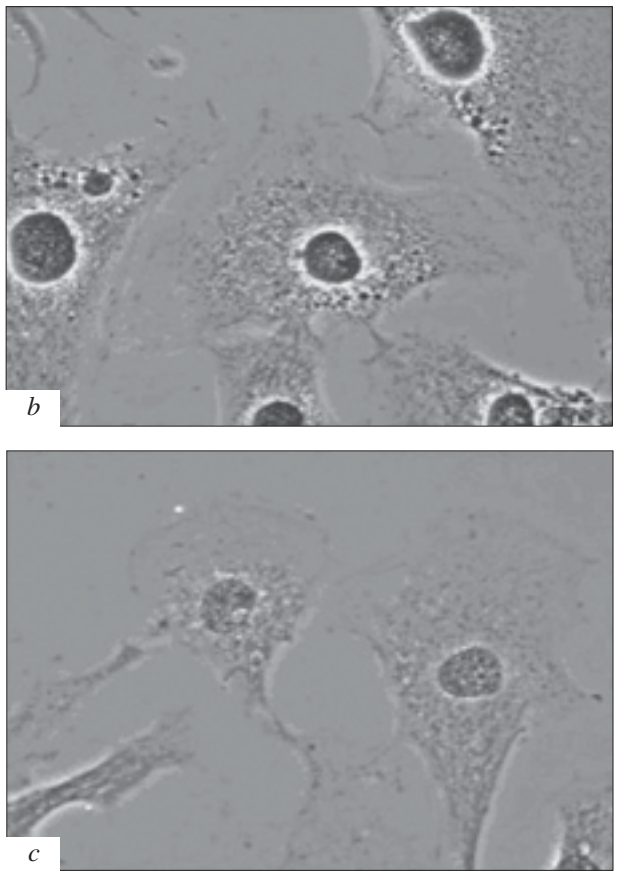
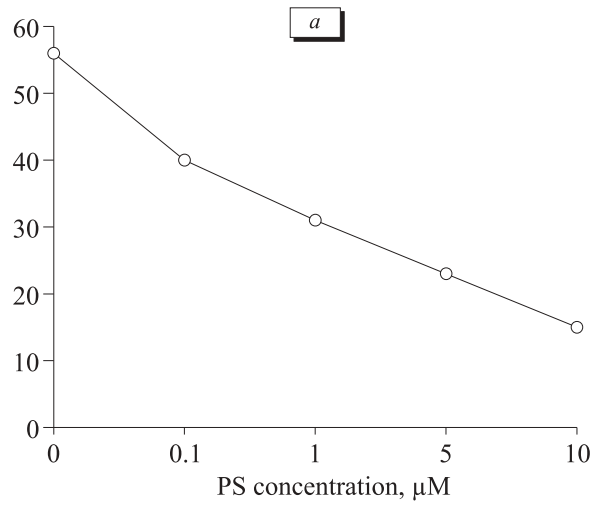


Fig. 2. Dose-dependent effect of PS on the number of $PCNA^+$ cardiac fibroblasts in culture. *a*: *1*) $PCNA^+$ cells; *2*) % of cells in culture; *b*: control; *c*: PS, 10 μM.



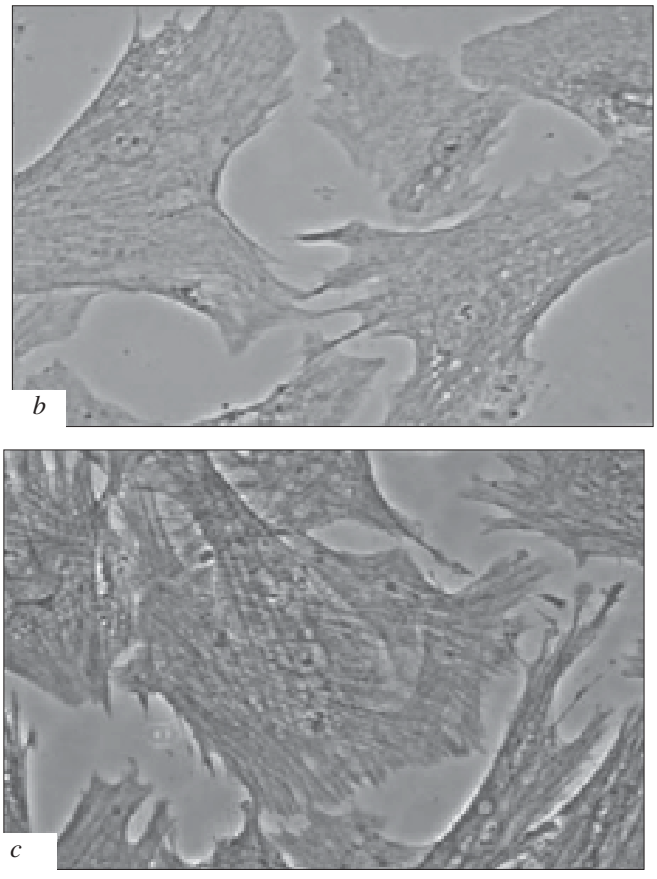


Fig. 3. Effect of PS on the expression of α-smooth-muscle actin in rat cardiac fibroblast culture. *a*) ordinate: percent of cells positively stained for α-smooth-muscle actin; *b*) control; *c*) PS, 10 μM.

Culturing of cardiac fibroblasts with PS was associated with a dose-dependent decrease in the number of cells in the culture and with a decrease in the number of cells in S phase of the cell cycle (PCNA-positive cells; Fig. 2). Moreover, addition of PS to culture medium led to a decrease in the number of cells expressing smooth-muscle actin (so-called myofibroblasts; Fig. 3).

Neutral DNA comet assay and staining with DAPI (fluorescent nuclear dye) confirmed the presence of DNA double-strand breaks and nuclear fragmentation after addition of 0.1 μM PS in 3% myo-

fibroblasts, and after addition of 1, 5, and 10 μM PS in 15, 36, and 40% cells, respectively. The decrease in the number of myofibroblasts in the culture can be explained by their apoptosis and the key role in the induction of this process is played by statins.

Intermediate metabolites of cholesterol biosynthesis are the critical components in the biochemical cascades regulating vital functions of the cells (proliferative activity and membrane integrity). In addition, they participate in the post-translation changes of minor Rho proteins, the main regulatory system responsible for the synthesis of cytoskeleton proteins. That is why CMC hypertrophy, migration and proliferation of vascular smooth-muscle cells, and increased permeability of endothelial cells in response to vasoactive agents are attributed to activation of Rho proteins [9].

By blocking Rho proteins modification, hydroxymethylglutaryl-coenzyme A-reductase inhibitors provide the "non-lipid" effects of statins [8].

Pravastatin did not change the viability of cultured CMC, but inhibited the development of their hypertrophy in culture (Table 1). This effect was presumably due inhibition of Rho proteins and superoxide anion generation [8]. It is also possible that the "antihypertrophic" effect of PS was due to a decrease in TGF-β_1 production (Fig. 1), which is in line with previous report [3].

The detected effect of fibroblasts on the development of CMC hypertrophy opens new potentialities for hypertrophic process control through modification of functional activity of stromal cells.

REFERENCES

1. O. M. Moiseyeva, E. G. Semyonova, E. V. Polevaya, *et al.*, *Tsitologiya*, **40**, No. 12, 1025-1030 (1998).
2. V. A. Tronov and I. I. Pelevina, *Ibid.*, **38**, Nos. 4-5, 427-439 (1996).
3. L. M. Blanco-Colio, A. Villa, M. Ortego, *et al.*, *Atherosclerosis*, **161**, No. 1, 17-26 (2002).
4. P. J. Lijnen, V. V. Petrov, and R. H. Fagard, *Am. J. Hypertens.*, **16**, No. 7, 604-611 (2003).
5. V. V. Petrov, R. H. Fagard, and P. J. Lijnen, *Hypertension*, **39**, No. 2, 258-263 (2002).
6. D. W. Powell, R. C. Mifflin, J. D. Valentich, *et al.*, *Am. J. Physiol.*, **277**, No. 1, Pt. 1, C1-C19 (1999).
7. G. Serini and G. Gabbiani, *Exp. Cell. Res.*, **250**, No. 2, 273-283 (1999).
8. M. Takemoto and J. K. Liao, *Arterioscler. Thromb. Vasc. Biol.*, **21**, No. 11, 1712-1719 (2001).
9. G. P. Van Nieuw Amerongen and V. W. van Hinsbergh, *Ibid.*, No. 3, 300-311.