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Cell Culture as a Testing System for Anti-Atherogenic Substances: A Brief Communication

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Abstract

In the series of publications started in 1986 and continued until today (several presentations at the EAS-2008 Congress in Istanbul) was reported that culturing of smooth muscle cells, macrophages or other cells with the serum from patients with coronary heart disease caused lipid infiltration *in vitro*. Cell culture was used for testing anti-atherogenic drugs. Several substances have been reported to be efficient against atherogenicity: statins, trapidil, prostaglandin E2, dibutyryl cyclic AMP, calcium antagonists, lipoxygenase inhibitors, carbacyclin etc. On the contrary, beta-blockers, phenothiazines, oral hypoglycemics etc. were shown to be atherogenic. Some natural products were demonstrated by the same method to possess anti-atherogenic efficiency: garlic, black elder berries, fragmented grape stems, hop cones and others. Relevance of these data for practice is discussed.

Key words: Atherosclerosis, lipid-lowering drugs, cell culture.

Cell culture as a testing system for anti-atherogenic substances

One of the first publications was in *The Lancet* in 1986 by E.I. Chazov et al. from Cardiology Research Center in Moscow (Chazov et al. 1986), according to which "sera from 62 of 68 patients with coronary heart disease (CHD) caused a two- to fivefold elevation in the cellular cholesterol in primary cultures of subendothelial cells taken at autopsy from the intima of human aorta. The sera from 33 of 42 healthy subjects did not show atherogenic properties". Results reported in later publications are even more convincing: "Within 24 hours of cultivation with 40 % sera of patients suffering from coronary atherosclerosis, the total intracellular cholesterol level increased twofold to fivefold. Cultivation with the sera of healthy subjects had no effect on the intracellular cholesterol level" (Orekhov et al. 1988), and more specific: "low density lipoprotein (LDL) from patients with coronary atherosclerosis caused a twofold to fourfold rise in cholesterol in cell culture. Native LDL from healthy subjects failed to induce intracellular lipid accumulation" (Tertov et al. 1992). The ability of the serum from patients with CHD to cause lipid infiltration of cells *in vitro* was named atherogenicity; and the method has been used by the same group of scientists for testing of anti-atherogenic drugs, food components and other substances. EAS Congresses. A brief summary follows.

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There followed an avalanche of publications and reports on meetings, including the 14th International Symposium on Atherosclerosis (Rome 2006), 76th (Helsinki 2007) and 77th (İstanbul 2008). Extract from garlic added to the "atherogenic" serum significantly reduced lipid infiltration and proliferation of cultured smooth muscle cells in vitro. Interestingly, the same effect was observed "*ex vivo*": blood serum taken two hours after oral administration of garlic "caused substantially less cholesterol accumulation in cultured cells" (Orekhov et al. 1995). Many substances have been reported to be efficient against serum atherogenicity: dibutyryl cyclic AMP, calcium antagonists and others, for example: "Within 2-4 h after a single dose of oral administration of beta-blocker (propranolol), patients' blood plasma turned atherogenic, i.e., its addition to the culture induced cholesterol accumulation and stimulated proliferation" (Kireev et al. 2006). The method was applied for evaluation of hormones: estrogens and testosterone reduced serum atherogenicity and suppressed proliferation of cultured cells (Kireev et al. 2006, Aksenov et al. 2008). Interestingly, dihydrotestosterone (DHT), an active intracellular form of testosterone, had an opposite effect (Kireev et al. 2006).

In the two-volume Handbook of Clinical Angiology, edited recently in Russia, these data are summarized. Among substances, demonstrating anti-atherogenic properties *in vitro,* the following are named: statins, trapidil, prostaglandin E2, dibutyryl cyclic AMP, calcium antagonists, lipoxygenase and acetylcholinesterase inhibitors, carbacyclin; among pro-atherogenic substances - beta-blockers, phenothiazines, oral hypoglycemics etc. Recommendations for practice are formulated (Pivovarova et al. 2004).

Apart from garlic, numerous natural substances have been shown by the same method to be efficient against serum atherogenicity: leech salivary gland secretion, components of tea, black elder berries, calendula and violet flowers (Gorchakova et al. 2006) as well as "grape seeds extract, fragmented grape stems, hop cones" (Korennaya et al. 2006) etc. Consume of squid liver fat and krill meat also produced "a marked reduction of blood serum atherogenicity" (Shagaeva et al. 2006). Extracts from 13 different mushrooms were shown to be efficient against serum atherogenicity: "Cultivation of atherosclerotic cells during 24 hr in the presence of serum from healthy subjects who had had mushroom meals resulted in a 21-30% decrease in the cellular cholesterol level. The atherogenic serum obtained from CHD patients after dietary mushroom consumption partly (30-41%) lost its ability to increase the cellular cholesterol content" (Ryong et al. 1989).

Physiological validity of serum atherogenicity was demonstrated: significant correlation was found between serum atherogenicity and "increase of intima-media thickness of common carotid arteries". "Spontaneous upraise of serum atherogenicity during follow-up was contingent with the progression of atherosclerosis (P=0,008)"; "The complete removal of serum atherogenic potential in treated patients was contingent with atherosclerosis regression (P=0,014)" (Orekhov et al. 2006)

Furthermore, the authors reported circulating LDL-containing immune complexes to be a pathogenetic factor of atherosclerosis (Orekhov et al. 1995). Cholesterol within such immune complexes was named "immune cholesterol". It was reported that the immune cholesterol level in the blood can be used as a predictor of coronary atherosclerosis (confirmed by angiography), diagnostic accuracy being 78 %. For extra-coronary, atherosclerosis accuracy was even higher. Besides, a correlation was found between concentration of immune cholesterol and anti-LDL antibodies, on one side, and serum atherogenicity *in vitro* - on the other side (Kacharava et al. 1993). Removal of immunoglobulins G and M from the serum

lowered its atherogenicity, and removal of circulating immune complexes – largely eliminated it. The authors concluded that circulating immune complexes are one of the main sources of lipids, infiltrating the vessel wall in atherosclerosis (Tertov et al. 1990). These data are not in agreement with publications on the autoimmune theory of atherosclerosis reporting a low concentration of circulating lipoprotein-antibody immune complexes (containing 0,1-0,2 % of apo-B-associated plasma lipoproteins at the most), whereas atherogenic potential of immune complexes was attributed to damaging of the endothelium (Klimov et al. 1990). The endothelium was absent in the cell culture.

The authors claim to have developed the "novel principle of direct anti-atherosclerotic therapy based on inhibition of cholesterol deposition in arterial wall" (Orekhov et al. 2006) without explaining mechanisms of its action directly on the serum. Known anti-atherogenic substances can act on cholesterol synthesis in tissues, on lipid and lipoprotein metabolism in the liver, on intestinal absorption of lipids or on the endothelium-related mechanisms (Marinetti, 1990). All these targets are absent in the cell cultures used by the authors. Furthermore, cultured cells are devoid of endothelial barrier, of endothelial receptor-mediated mechanisms, surface adhesion factors and athero-protective genes regulating lipid metabolism *in vivo*. Other structures and mechanisms participating in atherogenesis, such as coagulation and anticoagulation systems, hemodynamic factors (arterial hypertension, endothelial damage) are also not reproduced in the cell culture.

Moreover, in the absence of the endothelial barrier, dependence between cellular cholesterol uptake and atherogenesis should be inverse rather than direct. For example, atherosclerosis can be caused by an inherited abnormality of endothelial receptors, when hypercholesterolemia results from diminished cholesterol uptake by the cells (Marinetti, 1990) Accordingly, if a pharmacological substance reduces cholesterol uptake by the cells in vitro, this substance can be expected to cause cholesterol elevation in blood in vivo and therefore to possess a pro-atherogenic rather than anti-atherogenic effect. Finally, studying serum "atherogenicity", it is not to forget that dyslipidemia in many CHD patients can be found only after meals: so-called postprandial lipid abnormalities (Slyper 1992). Diurnal fluctuations of serum lipid levels were not taken into account in the experiments with the cell cultures. Therefore, it should be concluded that cell culture as a testing system for anti-atherogenic substances is not representative for the whole organisms and that results and recommendations for practice formulated on the basis of experiments with the cell cultures are not sufficiently substantiated.

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