Testing of serum atherogenicity in cell cultures: questionable data published

Bewertung der Serumatherogenität in den Zellkulturen: fragliche Daten veröffentlicht

Abstract

In a large series of studies was reported that culturing of smooth muscle cells with serum from atherosclerosis patients caused intracellular lipid accumulation, while serum from healthy controls had no such effect. Cultures were used for evaluation of antiatherogenic drugs. Numerous substances were reported to lower serum atherogenicity: statins, trapidil, calcium antagonists, garlic derivatives etc. On the contrary, beta-blockers, phenothiazines and oral hypoglycemics were reported to be pro-atherogenic. Known antiatherogenic agents can influence lipid metabolism and cholesterol synthesis, intestinal absorption or endothelium-related mechanisms. All these targets are absent in cell monocultures. Inflammatory factors, addressed by some antiatherogenic drugs, are also not reproduced. In vivo, relationship between cholesterol uptake by cells and atherogenesis must be inverse rather than direct: in familial hypercholesterolemia, inefficient clearance of LDL-cholesterol by cells predisposes to atherosclerosis. Accordingly, if a pharmacological agent reduces cholesterol uptake by cells in vitro, it should be expected to elevate cholesterol in vivo. Validity of clinical recommendations, based on serum atherogenicity testing in cell monocultures, is therefore questionable. These considerations pertain also to the drugs developed on the basis of the cell culture experiments.

Keywords: atherosclerosis, serum, cell culture, cholesterol

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Zusammenfassung

In einer großen Studienserie wurde berichtet, dass Kultivierung der Glattmuskelzellen mit dem Serum von Atherosklerosekranken eine intrazelluläre Lipidansammlung verursachte, während Serum von gesunden Kontrollsubjekten keine derartige Wirkung hatte. Die Zellkulturen wurden für die Bewertung anti- und pro-atherogener Wirkung von Arzneimitteln und anderer Substanzen verwendet. Mehrere Wirkstoffe verminderten angeblich die Serumatherogenität in den Zellkulturen: Statine, Trapidil, Calciumantagonisten, Knoblauchderivate und andere. Betablocker, Phenothiazine und orale Hypoglykämika wirkten hingegen pro-atherogen. Es ist jedoch bekannt, dass anti-atherosklerotische Arzneimittel auf folgende Punkte einwirken konnen: Lipidstoffwechsel und Cholesterinsynthese, intestinale Resorption von Lipiden und die endothothelassoziierten Mechanismen. Alle diese Angriffsziele sind in den Zellmonokulturen nicht vorhanden. Entzündungserscheinungen, die von einigen anti-atherosklerotischen Wirkstoffen moduliert werden können, werden auch nicht reproduziert. In vivo ist das Verhältnis zwischen der Cholesterinaufnahme von Zellen und der Atherogenese umgekehrt: z.B. veranlagt bei der familiären Hypercholesterinnämie eine vom LDL-Rezeptordefekt bedingte unzureichende Clearance von LDL-Cholesterin zur Atherosklerose. Wenn ein pharmakologischer Wirkstoff die Cholesterinaufnahme von Zellen senkt, sollte er in vivo den Cholesterinspiegel im Blut erheben. Die Zuverlässigkeit der aufgrund obenge-

nannter Zellkulturstudien formulierten klinischen Empfehlungen erscheint also fraglich. Das betrifft auch die Arzneimittel, die auf der Basis der Zellkulturexperimente entwickelt wurden.

Schlüsselwörter: Atherosklerose, Serum, Zellkultur, Cholesterin

Letter to the Editor

Strategies to treat atherosclerosis pharmacologically should take its multifactorial etiology into account [1]. Atherogenesis involves many cell types interacting with each other and with extracellular matrix [2]. Therefore, results obtained in studies on a single cell type should be considered with caution when extrapolated to the whole body. A large series of studies, having become internationally known in 1986 after the publication in The Lancet [3], has been continued until today [4]. Cultures of smooth muscle cells from human aorta and, in some studies, of peritoneal macrophages, were used as an in vitro model for testing of serum atherogenicity and anti- or pro-atherogenic action of various substances. The following, among other things, was reported: within 24 hours of cultivation with diluted (40%) sera of coronary atherosclerosis patients, the total intracellular cholesterol (Ch) increased twofold to fivefold [5]. Low density lipoproteins (LDL) from patients with coronary atherosclerosis caused a twofold to fourfold rise in cholesteryl esters in cultured human blood monocytes and intimal smooth muscle cells isolated from the normal aorta [6], [7]. Cultivation with the sera or LDL from healthy individuals failed to induce intracellular lipid accumulation [5], [6]. Furthermore, calcium antagonists (verapamil, nifedipine, darodipine, isradipine, diltiazem, etc.) reduced the Ch level in cultured cells and, at the same time, lowered the incorporation of ^{3}H-thymidine by the cells, which was interpreted as decreased cell proliferation [8]. It was concluded that calcium antagonists manifested a direct antiatherosclerotic effect in culture [8]. Furthermore, beta-blockers (propranolol, alprenolol, metoprolol, atenolol, pindolol, and timolol) caused a 1.5- to 2-fold rise in Ch level of cultured cells and stimulated their proliferation [8]. Apart from direct admixture of a tested substance to the culture medium, an "ex vivo" model was used: within 2–4 h after an oral administration of a beta-blocker (propranolol), plasma became atherogenic i.e. its addition to the cell culture medium induced intracellular Ch accumulation and stimulated proliferation of the cultured cells. At the same time, blood plasma of patients receiving calcium antagonists acquired antiatherosclerotic properties manifested by its ability to lower the intracellular Ch and to inhibit cells proliferation in a culture [8]. Another example: garlic extract added to the medium, where smooth muscle cells from atherosclerotic plaques had been cultured, significantly reduced the Ch level in the cells and inhibited their proliferation. Blood serum taken from patients 2 hours after an oral administration of 300 mg of garlic powder significantly lowered Ch accumulation in the cultured cells [9]. Remarkably, infiltration of cultured cells by lipids was reported to be associated with their increased proliferation: in the cultures of smooth muscle cells taken from zones of fatty infiltration and fatty streaks in the human aortic intima, the thymidine index exceeded the normal value 4.5- and 3-fold respectively [10]. Pharmacological agents, modifying intracellular lipid accumulation, influenced cell proliferation in the same direction [11]. In general pathology, however, fatty infiltration is seen as a manifestation of cell damage (degeneration) [12], which can hardly be expected to come along with enhanced proliferation.

Furthermore, recommendations for practice were formulated on the basis of cell culture experiments [13], including drug dosage: "To decrease atherogenic potential of serum and to maintain it at a low level, verapamil should be administered at a dose of 40 mg 5 times daily with a 4- to 5-hour interval between doses." [13] This recommendation should be seen within the scope of a broader question, whether data from cellular models may be used directly for clinical recommendations without clinical trials. Cellular systems in pharmacological research are essential for initial evaluation of drugs. However, their use to predict a response of the whole body is limited. A response of cellular systems to an agent influencing transport across the plasma membrane can have opposite effects in cells compared to the whole body [14]. As an explanation for atherogenicity of serum from patients with coronary atherosclerosis, a causative role of Ch-containing immune complexes was maintained [15]. However, among the mechanisms of atherogenesis induced by immune complexes, discussed in the literature, are inflammatory phenomena such as release of proinflammatory cytokines from macrophages, increase in adhesion molecules and dysfunction of endothelial cells [1], [16], [17], [18], which are not reproduced in the cell monocultures. Known action mechanisms of antiatherogenic or lipid-lowering drugs include regulation of Ch synthesis, lipid metabolism in the liver, intestinal absorption of lipids or endothelial functions [1], [19], [20]. All these targets are absent in the cell cultures. In addition, drugs like statins have numerous "pleiotrophic effects" that can be beneficial [1] but are not reproducible in the cultures. Inflammatory phenomena, addressed by some antiatherogenic agents [2], [21], are also not reproduced by this model. In vivo, the relationship between Ch uptake by cells and atherogenesis is inverse rather than direct. For example, in familial hypercholesterolemia, caused by abnormality of lipoprotein receptors, ineffective clearance of LDL-Ch from serum causes hypercholesterolemia and predisposes to atherosclerosis [22]. Moreover, up-regulation of LDL receptors (and, correspondingly, of the LDL-Ch uptake by cells) is one of the therapeutic strategies for atherosclerosis [23]. Accordingly, if a pharmacological agent reduces Ch uptake by cells in vitro, it should be

expected to cause serum Ch elevation in vivo. In other words, pharmacologic agents displaying an "antiatherogenic" effect in cell cultures should be expected to have a pro-atherogenic effect in vivo. Therefore, conclusions and recommendations, formulated on the basis of the cell culture studies discussed above, can be based on misunderstanding. Moreover, an anti-atherosclerotic drug Allicor, a derivative of garlic, was developed on the basis of the cell culture experiments. Anti-atherosclerotic efficacy of Allicor was confirmed by clinical trials [24], [25]. Discussing the mode of action of the Allicor, both articles refer to the cell culture study [8]. In fact, the results of the trials [24], [25] are at variance with those of the cell culture experiments: if garlic indeed lowers the intake of Ch by the cultured smooth muscle cells [9], it might cause elevation of serum Ch in vivo. It is written in [25] with reference to a review [26], obviously implying effectiveness of garlic: "Lipid-lowering properties of garlic-based drugs and preparations are studied rather well" [25]. However, it is stated in [26] that there is increasingly less evidence for lipid lowering properties of garlic preparations. A later review on this topic concluded that evidence, based on rigorous clinical trials of garlic, is not convincing [27]. For hypercholesterolemia, the reported effects of garlic are small and may be of no clinical relevance [27]. Previously we discussed other trials on hypercholesterolemia, results of which have not been convincingly confirmed by other researchers [28]. The matter could be clarified by means of a large-scale independent trial. Note that Allicor is produced by INAT Farma fused with the Institute of Aterosclerosis Research (http://inat.ru), where the above-mentioned cell culture experiments have been performed. With pharmaceutical costs increasing faster than most other health care expenditures, studies should meet the needs of evidence-based treatments and not just the needs of the manufacturers [1].

The material from this paper was presented at several conferences (e.g., [29]); and the author was asked, why it is focused on reports of mostly one research group and the topic is not discussed in a broader perspective. In fact, there have been no other studies, where serum atherogenicity and atherogenic/antiatherogenic potencies of drugs were evaluated directly on cell cultures and practical recommendations given on the basis of such evaluations. Earlier experiments with culturing of smooth muscle cells from atherosclerotic plaques [30], culturing or incubation of different cells in lipoprotein-containing media (e.g., [31], [32]) included neither measurement of serum atherogenicity nor drug evaluations. In conclusion, a reason for submitting this paper to an open access journal is to draw the attention of the scientific community to the fact that doubtful information was published in numerous pharmacological, clinical and other editions (e.g. [7], [10], [12], [33], [34], [35]) including a recently published Russian-language handbook, and is still being referred to in more recent studies (e.g. [36], [37], [38]). However, the study [38] disagreed with the results of some experiments by the research group under discussion in this paper; and an artifact in these experiments was proposed as an explanation for the disagreement.

Notes

Competing interests

The author declares that he has no competing interests.

References

1. Gebbers JO. Atherosclerosis, cholesterol, nutrition, and statins--a critical review. GMS Ger Med Sci. 2007 Aug 16;5:Doc04. Available from: http://www.egms.de/en/journals/gms/2007-5/000040.shtml
2. Mundo-Sagardía JA, Figueroa Y, Altieri PI, Banchs HL, Escobales N, Crespo MJ. The atherosclerotic plaque. P R Health Sci J. 2008 Sep;27(3):241-6.
3. Chazov EI, Tertov VV, Orekhov AN, Lyakishev AA, Perova NV, Kurdanov KA, Khashimov KA, Novikov ID, Smirnov VN. Atherogenicity of blood serum from patients with coronary heart disease. Lancet. 1986 Sep 13;2(8507):595-8. DOI: 10.1016/S0140-6736(86)92426-8
4. Jargin SV. Testing of anti-atherogenic drugs and food components on cell cultures: assessment of reliability. P R Health Sci J. 2010 Mar;29(1):86-7.
5. Orekhov AN, Tertov VV, Pokrovsky SN, Adamova IYu, Martsenyuk ON, Lyakishev AA, Smirnov VN. Blood serum atherogenicity associated with coronary atherosclerosis. Evidence for nonlipid factor providing atherogenicity of low-density lipoproteins and an approach to its elimination. Circ Res. 1988 Mar;62(3):421-9.
6. Tertov VV, Orekhov AN, Sobenin IA, Gabbasov ZA, Popov EG, Yaroslavov AA, Smirnov VN. Three types of naturally occurring modified lipoproteins induce intracellular lipid accumulation due to lipoprotein aggregation. Circ Res. 1992 Jul;71(1):218-28.
7. Tertov VV, Orekhov AN, Martsenyuk ON, Perova NV, Smirnov VN. Low-density lipoproteins isolated from the blood of patients with coronary heart disease induce the accumulation of lipids in human aortic cells. Exp Mol Pathol. 1989 Jun;50(3):337-47. DOI: 10.1016/0014-4800(89)90043-9
8. Orekhov AN, Baldenkov GN, Tertov VV, Ryong LH, Kozlov SG, Lyakishev AA, Tkachuk VA, Ruda MYa, Smirnov VN. Cardiovascular drugs and atherosclerosis: effects of calcium antagonists, beta-blockers, and nitrates on atherosclerotic characteristics of human aortic cells. J Cardiovasc Pharmacol. 1988;12 Suppl 6:S66-8. DOI: 10.1097/00005344-198812006-00017
9. Orekhov AN, Tertov VV, Sobenin IA, Pivovarova EM. Direct anti-atherosclerosis-related effects of garlic. Ann Med. 1995 Feb;27(1):63-5. DOI: 10.3109/07853899509031938
10. Orekhov AN, Kosykh VA, Repin VS, Smirnov VN. Cell proliferation in normal and atherosclerotic human aorta. II. Autoradiographic observation on deoxyribonucleic acid synthesis in primary cell culture. Lab Invest. 1983 Jun;48(6):749-54.
11. Orekhov AN, Tertov VV, Kudryashov SA, Khashimov KhA, Smirnov VN. Primary culture of human aortic intima cells as a model for testing antiatherosclerotic drugs. Effects of cyclic AMP, prostaglandins, calcium antagonists, antioxidants, and lipid-lowering agents. Atherosclerosis. 1986 May;60(2):101-10. DOI: 10.1016/0021-9150(86)90002-X

12. Riede UN. Störungen des Stoffwechsels. In: Riede UN, Wehner H, eds. Allgemeine und spezielle Pathologie. Stuttgart, New York: Thieme; 1986. p. 78-124.

13. Orekhov AN, Pivovarova EM, Sobenin IA, Yakushkin VV, Tertov VV. Use of cell culture for optimisation of direct antiatherogenic therapy with verapamil. Drugs. 1992;44 Suppl 1:105-110. DOI: 10.2165/00003495-199200441-00020

14. Escobales N. Testing of anti-atherogenic drugs and food components on cell cultures: assessment of reliability. Author's reply. P R Health Sci J. 2010;29(1):87.

15. Tertov VV, Orekhov AN, Sayadyan KS, Serebrennikov SG, Kacharava AG, Lyakishev AA, Smirnov VN. Correlation between cholesterol content in circulating immune complexes and atherogenic properties of CHD patients' serum manifested in cell culture. Atherosclerosis. 1990 Apr;81(3):183-9. DOI: 10.1016/0021-9150(90)90065-Q

16. Virella G, Lopes-Virella MF. Atherogenesis and the humoral immune response to modified lipoproteins. Atherosclerosis. 2008 Oct;200(2):239-46.

17. Narshi CB, Giles IP, Rahman A. The endothelium: an interface between autoimmunity and atherosclerosis in systemic lupus erythematosus? Lupus. 2011 Jan;20(1):5-13. DOI: 10.1177/0961203310382429

18. Gotto AM Jr. Jeremiah Metzger Lecture: cholesterol, inflammation and atherosclerotic cardiovascular disease: is it all LDL? Trans Am Clin Climatol Assoc. 2011;122:256-89.

19. Kraemer BF, Miller JW. Dyslipidemias. In: Carruthers SG, Hoffman BB, Melmon KL, Nierenberg DW, eds. Melmon and Morrelli's clinical pharmacology: basic principles in therapeutics. 4th ed. New York: McGraw-Hill; 2000. p. 552-80.

20. Tomasoni L, Sitia S, Borghi C, Cicero AF, Ceconi C, Cecaro F, Morganti A, De Gennaro Colonna V, Guazzi M, Morricone L, Malavazos AE, Marino P, Cavallino C, Shoenfeld Y, Turiel M. Effects of treatment strategy on endothelial function. Autoimmun Rev. 2010 Oct;9(12):840-4. DOI: 10.1016/j.autrev.2010.07.017

21. Biasucci LM, Biasillo G, Stefanelli A. Inflammatory markers, cholesterol and statins: pathophysiological role and clinical importance. Clin Chem Lab Med. 2010 Dec;48(12):1685-91. DOI: 10.1515/CCLM.2010.277

22. Marais AD. Familial hypercholesterolaemia. Clin Biochem Rev. 2004 Feb;25(1):49-68.

23. Scharnagl H, März W. New lipid-lowering agents acting on LDL receptors. Curr Top Med Chem. 2005;5(3):233-42. DOI: 10.2174/1568026053544524

24. Sobenin IA, Pryanishnikov VV, Kunnova LM, Rabinovich YA, Martirosyan DM, Orekhov AN. The effects of time-released garlic powder tablets on multifunctional cardiovascular risk in patients with coronary artery disease. Lipids Health Dis. 2010 Oct 19;9:119. DOI: 10.1186/1476-511X-9-119

25. Sobenin IA, Andrianova IV, Demidova ON, Gorchakova T, Orekhov AN. Lipid-lowering effects of time-released garlic powder tablets in double-blinded placebo-controlled randomized study. J Atheroscler Thromb. 2008 Dec;15(6):334-8. DOI: 10.5551/jat.E550

26. Berthold HK, Sudhop T. Garlic preparations for prevention of atherosclerosis. Curr Opin Lipidol. 1998 Dec;9(6):565-9. DOI: 10.1097/00041433-199812000-00008

27. Pittler MH, Ernst E. Clinical effectiveness of garlic (Allium sativum). Mol Nutr Food Res. 2007 Nov;51(11):1382-5. DOI: 10.1002/mnfr.200700073

28. Jargin SV. Discussion of Evaluation of cholesterol-lowering and antioxidant properties of sugar cane policosanols in hamsters and humans. Appl Physiol Nutr Metab. 2009 Feb;34(1):75; discussion 76-7. DOI: 10.1139/H08-141

29. Jargin SV. Cell culture as a testing system for lipid-lowering substances. Abstracts of the 3rd Intercontinental Congress of Pathology. May 17-22, 2008, Barcelona, Spain. Virchows Arch. 2008 May;452 Suppl 1:S34.

30. Eskin SG, Sybers HD, Lester JW, Navarro LT, Gotto AM Jr, DeBakey ME. Human smooth muscle cells cultured from atherosclerotic plaques and uninvolved vessel wall. In Vitro. 1981 Aug;17(8):713-8. DOI: 10.1007/BF02628408

31. Brown MS, Goldstein JL, Krieger M, Ho YK, Anderson RG. Reversible accumulation of cholesteryl esters in macrophages incubated with acetylated lipoproteins. J Cell Biol. 1979 Sep;82(3):597-613. DOI: 10.1083/jcb.82.3.597

32. Goldstein JL, Anderson RG, Buja LM, Basu SK, Brown MS. Overloading human aortic smooth muscle cells with low density lipoprotein-cholesteryl esters reproduces features of atherosclerosis in vitro. J Clin Invest. 1977 Jun;59(6):1196-202. DOI: 10.1172/JCI108744

33. Orekhov AN, Andrianova IV, Rekhter MD, Tertov VV, Andreeva ER, Ragimov SE, Mironov AA. Beta-blockers: propranolol, metoprolol, atenolol, pindolol, alprenolol and timolol, manifest atherogenicity on in vitro, ex vivo and in vivo models. Elimination of propranolol atherogenic effects by papaverine. Atherosclerosis. 1992 Jul;95(1):77-85. DOI: 10.1016/0021-9150(92)90178-J

34. Orekhov AN, Tertov VV, Pivovarova EM. The effects of antihypertensive agents on atherosclerosis-related parameters of human aorta intimal cells. Cardiology. 1998;89(2):111-8. DOI: 10.1159/000006765

35. Orekhov AN. In vitro models of anti-atherosclerotic effects of cardiovascular drugs. Am J Cardiol. 1990 Dec 18;66(21):23I-28I. DOI: 10.1016/0002-9149(90)91260-D

36. Orekhov AN, Andreeva ER, Andrianova IV, Bobryshev YV. Peculiarities of cell composition and cell proliferation in different type atherosclerotic lesions in carotid and coronary arteries. Atherosclerosis. 2010 Oct;212(2):436-43. DOI: 10.1016/j.atherosclerosis.2010.07.009

37. Sobenin IA, Suprun IV, Karagodin VP, Feoktistov AS, Melnichenko AA, Orekhov AN. The interaction of plasma sialylated and desialylated lipoproteins with collagen from the intima and media of uninvolved and atherosclerotic human aorta. J Lipids. 2011;2011:254267. DOI: 10.1155/2011/254267

38. Chappey B, Beyssen B, Foos E, Ledru F, Guermonprez JL, Gaux JC, Myara I. Sialic acid content of LDL in coronary artery disease: no evidence of desialylation in subjects with coronary stenosis and increased levels in subjects with extensive atherosclerosis and acute myocardial infarction: relation between desialylation and in vitro peroxidation. Arterioscler Thromb Vasc Biol. 1998;18(6):876-83.

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