Development of Antiatherosclerotic Drugs on the basis of Cell Models: a Comment

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Abstract. The large research series has become internationally known in the 1980s and continues until today. Cultures of cultured or incubated smooth muscle cells or monocytes/macrophages were used for the measurement of capacities of substances to enhance or lower intracellular cholesterol deposition, which was interpreted as anti- or pro-atherogenic action. The LDL from patients with coronary atherosclerosis caused a 2-4 times elevation of cholesterol contents within the cultured cells. Incubation with sera or LDL from healthy subjects did not induce cholesterol accumulation. Various drugs and natural substances were found to possess pro- or anti-atherogenic potencies. Anti-atherogenic effect of different preparations of plant origin was reported: if an agent induced intracellular lipid accumulation it was regarded atherogenic and vice versa. However, if a pharmacological agent lowers the uptake of lipids by cells in a culture, it should be expected to increase the blood cholesterol level in vivo. It can be reasonably assumed that a drug, inhibiting cholesterol uptake by cultured cells, would elevate the blood cholesterol level in vivo. By analogy with familial hypercholesterolemia, it might contribute to atherosclerosis: lipids would be deposited into the intercellular/subendothelial space of the vascular wall, in sites with damaged endothelial barrier and vulnerable plaques. The agents supposedly having an anti-atherogenic potency in a cell culture might reduce cholesterol uptake diffusely by entire cell populations contributing thereby to hypercholesterolemia. On the contrary, atherosclerosis is a focal disease, affecting primarily damaged sites of the vascular lining, which would be favored by hypercholesterolemia.

1. INTRODUCTION

The large research series has become internationally known in the 1980s and continues until today [1-13]. In brief, cultures of smooth muscle or monocyte-derived cells have been used for evaluation of the ability of different substances to enhance or diminish cholesterol deposition in the cells, incubated with the serum from patients with atherosclerosis, which has been interpreted as anti- or pro-atherogenic action. Among other things, the following was reported: after 24 hours of incubation with diluted sera from patients with coronary atherosclerosis, the contents of total intracellular cholesterol in the cultured cells increased 2 to 5 times. The LDL from patients with coronary atherosclerosis caused a 2-4 times elevation of cholesterol contents within the cultured cells. Incubation with sera or LDL from healthy subjects did not induce cholesterol accumulation by the cultures [6,7]. According to a personal communication from Dr. Aksenov at the 77th Congress of the European Atherosclerosis Society in Istanbul (2008), the "cell cultures" did not grow. Therefore, it would be correct to define these cells, surviving for several days or weeks in serum-containing media [8], not as cell cultures but as incubated cells.

2. DISCUSSION

The cell model approach was used for evaluation of sex hormones: the estrogens (not otherwise specified) and testosterone were reported to reduce intracellular cholesterol accumulation by cultured normal and atherosclerotic "intimal mesenchymal cells" in parallel with the ³H-thymidine incorporation interpreted as a decrease in cell proliferation [9]. Note that considerable lipid infiltration of cells is regarded in general pathology as degeneration that can hardly be expected to be accompanied by an increase in cell proliferation. Surprisingly, dihydrotestosterone, the active

metabolite of testosterone, had an opposite effect: it increased intracellular cholesterol accumulation and DNA synthesis in cultured intimal mesenchymal cells. In monocytes/macrophages, the intracellular cholesterol accumulation was inhibited by estrogens. On the contrary, androgens increased cholesterol accumulation and ^3H -thymidine incorporation [9]. A later communication confirmed that both testosterone and dihydrotestosterone increased intracellular cholesterol content in cultured monocytes/macrophages [8]. However, in a recent article by the same research group, the addition of each of the three hormones (estradiol, testosterone, dihydrotestosterone) at a physiological concentration (1 nM) to the culture medium reduced the cholesterol accumulation in monocytes/macrophages [5].

Using the same cell model, various drugs and natural substances were found to possess pro- or anti-atherogenic potencies [3]. For example, an anti-atherogenic effect of different preparations of plant origin was reported on the basis of experiments with cell cultures [10-13]: if an agent induced lipid accumulation by the cultured cells it was regarded to be atherogenic and vice versa. However, as discussed previously [14-17], if a pharmacological agent lowers the uptake of lipids by cells in a culture, it should be expected to increase the blood cholesterol level in vivo. So, in familial hypercholesterolemia and some other conditions [15], an abnormality of lipoprotein receptors, accompanied by a decreased cholesterol uptake by cells from blood, results in accelerated atherosclerosis [18,19]. The blood level of atherogenic LDL largely depends on the functioning of LDL-receptors. In cultures, many cells rely on LDL receptors mechanism for cholesterol supply [20]. Lipoprotein receptors both of classical and of scavenger types have been found on macrophages and smooth muscle cells [21-24]. It appears to be probable that the role of scavenger receptors in the uptake from blood of modified (oxidized) LDL is analogous to that of classical receptors with regard to native LDL at least under certain conditions. So, the basal expression of the scavenger receptors LOX-1 is relatively low; but it can be induced by inflammatory cytokines and other pathological stimuli related to atherogenesis, e.g. higher concentrations of oxidized LDL [24,25]. It can be reasonably assumed that a drug, supposedly acting directly upon blood atherogenicity [3], or through receptor-mediated mechanisms, inhibiting cholesterol uptake by cultured cells, would elevate the blood cholesterol level in vivo. By analogy with familial hypercholesterolemia, it might contribute to atherosclerosis: lipids would be deposited into the intercellular/subendothelial space of the vascular wall, in vulnerable sites with the endothelial barrier damaged by hemodynamic forces or otherwise, within pre-existent vulnerable plaques – so as it happens in vivo in conditions of progressive atherosclerosis. This is the philosophical paradox apparently disregarded by the authors of [1-13]: the agents supposedly having an “anti-atherogenic” potency in a cell culture might reduce cholesterol uptake diffusely by entire cell populations contributing thereby to hypercholesterolemia. On the contrary, atherosclerosis is a focal disease, affecting primarily damaged sites of the vascular lining, which would be favored by hypercholesterolemia.

Another example: Allicor was reported to lower serum atherogenicity (the ability of serum to induce cholesterol accumulation in cultured cells) by 30% in 211 asymptomatic men aged 40-74 years [4]. Allicor is a garlic powder preparation registered in Russia as an anti-atherosclerotic dietary supplement. It is written in [26] with reference to the review [27], implying the anti-atherosclerosis efficiency of garlic: “Lipid-lowering properties of garlic-based drugs and preparations are studied rather well” [26]. However, it is stated in [27] that there is increasingly less evidence for lipid lowering properties of garlic preparations, whereas “other aspects of garlic drugs, such as direct effects on vessel walls (aortic elasticity, effects of antioxidant properties on early steps in atherosclerosis formation) or anti-platelet aggregation effects, are still awaiting further elucidation in clinical studies” [27]. A later review concluded that evidence, based on rigorous clinical trials of garlic, had not been convincing [28]. For hypercholesterolemia, the reported effects of garlic are small and may be of no clinical relevance [28]. However, the question is under discussion [29]; therefore, reliability of published reports is critical, the more so as the literature is abundant today. Some results obtained in vitro or ex vivo by the same researchers, such as anti-

atherogenic effects of mushroom extracts [12], canned fish [30], pine needles [31], “prolonged and pronounced antiatherosclerotic effect of wheat seedings”, “significant antiatherogenic effect” of green tea [4] and recommendations for practice [32], including drug dosages [1], based on the cell model experiments discussed above, appear doubtful. If even garlic is efficient, its supposed action mechanisms include plasma lipid lowering, anticoagulant properties and improvement of endothelial function [27,33]. These mechanisms are not reproduced in cell cultures. In particular, endothelium-related mechanisms are of importance for the regulation of lipid entrance into the vascular wall. There is no endothelial barrier in a cell culture. A well-known problem in laboratory investigations is how to put a biological barrier into a test tube [34]. This problem has not been solved in the experiments under discussion [1-13].

3. CONCLUSION

In conclusion, the use of cultures or incubated cells for prediction of body responses is limited [15-17]; and drug doses [1] should not be calculated solely on the basis of cell model experiments. However, the cell models have been used since the last 30 years [1-13] for testing of supposedly anti-atherogenic drugs and food supplements. Research quality and possible influence by the industry [16] should be taken into account defining inclusion criteria for studies into meta-analyses and reviews. Scientifically inadequate methods can be used for the purpose of official registration of drugs and dietary supplements. As a result, drugs with unproven effects can be offered to the elderly and other patients misinformed not only by advertising but also by some publications supposed to be scientific.

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