

## • LETTER TO THE EDITOR •

### Testing of Anti-atherogenic Drugs and Food Components on Cell Cultures: Assessment of Reliability

**A**therogenesis involves multiple cell types interacting with each other and with extracellular matrix (1). Therefore, results obtained on a single cell type should be considered with caution when extrapolated to the whole organism. A large series of studies, having become internationally known in 1986 (2), has been continued until today by Orekhov and co-workers. Cultures of smooth muscle cells from human aorta were used as a testing model for evaluation of serum atherogenicity and effectiveness of anti-atherogenic drugs and food components. The following was reported: after 24 hours of cultivation with 40 % sera from patients with coronary heart disease (CHD), the intracellular cholesterol level in the cultured smooth muscle cells increased twofold to fivefold; low density lipoproteins (LDL) from patients with CHD or diabetes mellitus caused a twofold to fourfold intracellular cholesterol elevation. On the contrary, the serum and LDL from healthy persons failed to induce lipid accumulation (3-4). Furthermore, after 24 hours, calcium antagonists (verapamil, nifedipine, darodipine, isradipine, diltiazem, etc.) reduced cholesterol level in cultured cells, whereas beta-blockers (propranolol, alprenolol, metoprolol, atenolol, pindolol, and timolol) caused a 1.5- to twofold rise of cholesterol content in the cells, cultured from an atherosclerotic plaque (5). In a handbook of therapeutics (6) these data were summarized, and clinical recommendations given on their basis. Among substances, demonstrating anti-atherogenic effectiveness *in vitro*, the following were listed: statins, trapidil, prostaglandin E<sub>2</sub>, dibutyryl cyclic AMP, calcium channel blockers, lipoxygenase and acetylcholinesterase inhibitors, carbacyclin; and among pro-atherogenic substances - beta-adrenergic blockers, phenothiazines, oral hypoglycemics etc. The same data were published in peer-reviewed journals (7-11). For example, clinical recommendations were published in a pharmacological journal (12), including dosages of drugs, calculated on the basis of cell culture experiments: "To decrease atherogenic potential of serum and to maintain it at a low level, verapamil should be administered at a dose of 40mg 5 times daily with a 4- to 5-hour interval between doses" (12). However, known action mechanisms of anti-atherogenic or lipid-lowering agents include regulation of cholesterol synthesis, lipid and lipoprotein metabolism in the liver, intestinal absorption, and influence upon the endothelium-related factors (13-14). All these targets are absent in cell cultures. Inflammatory mechanisms, influenced by some

anti-atherogenic agents (1), also cannot be reproduced in a cell monoculture. *In vivo*, dependence between cellular cholesterol uptake and atherogenesis is inverse rather than direct. For example, in familial hypercholesterolemia caused by abnormality of LDL-receptors (which are present also on the smooth muscle cells), inefficient clearance of LDL from the serum results in hypercholesterolemia and predisposition to atherosclerosis (15-16). Accordingly, if a drug reduces cholesterol uptake by cells *in vitro*, it should be expected to cause blood cholesterol elevation *in vivo* (17). Therefore, the conclusions and recommendations regarding atherosclerosis treatment and prevention, formulated on the basis of cell culture experiments discussed above, can be disproven by *reductio ad absurdum*: pharmacologic agents with an "anti-atherogenic" effect in cell cultures should be expected to have a pro-atherogenic effect *in vivo*.

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## Reply

In this letter, Dr. Jargin raises the important issue on whether studies from simple cellular models can be used to model the response of organs, systems, or the body to anti-atherogenic treatments. The use of cellular systems in pharmacological studies is critical in the initial evaluation of drugs. However, their use to predict the response of the body to a given agent is limited as the author correctly indicates.

The determination of the concentration of a drug inhibiting a given physiological pathway by 50% ( $IC_{50}$ ) in a cellular system bears very little connection to the response of complex units (i.e. body) where besides the direct cellular response to the drug there is an interplay between tissues or organs affecting the agent. A critical component in this issue would be the excretion and/or metabolic routes of the drug that affects its pharmacokinetics *in vivo*. For this reason, caution must be exerted when determinations of  $IC_{50}$  obtained in cellular systems are used to predict the response of the body system to a given drug.

Also, the response of cellular systems to an agent affecting the transport across the plasma membrane of a given substance could have opposite effects in the cell and the organism. For example, low transport of cholesterol into cells would be expected to generate hypercholesterolemia and atherosclerosis (1-3). Similarly, treatments with steroids that reduce glucose uptake into cells are well known to be diabetogenic (4). For these reasons, the development of pharmacological treatments must be in the end, based on *in vivo* animal studies followed by carefully designed clinical trials.

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