EFFECT OF EICOSANOIDS ON CHOLESTEROL ACCUMULATION AND SUBENDOTHELIAL CELL PROLIFERATION IN THE HUMAN AORTA

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The obtaining of cultures of subendothelial cells of the human aorta and their use as a cell model to study in vitro the principal manifestations of atherosclerosis and the investigation of the antiatherosclerotic action of various compounds have enabled an effective search to be made for the factors which control atherogenesis and ways of its pharmacologic correction [3, 5, 8]. In this connection it is interesting to investigate the effect of eicosanoids on a culture of intimal cells [9]. In regulating atherogenesis the study of the role of prostacycline (PGI_2) and thromboxane A_2 (TXA_2), the ratio between whose concentrations is regarded as an important factor in the regulation of the circulation, deserves particular attention. An imbalance between these agents leads to the development of circulatory disturbances of a hemostatic and vasomotor kind. It is not yet clear whether they act on atherogenesis, an important factor in the pathogenesis of regional circulatory disturbances.

The aim of this investigation was to analyze the effect of PGI_2 and TXA_2 on a primary culture of intimal cells isolated from regions of the human aorta affected by atherosclerosis.

EXPERIMENTAL METHOD

Autopsy material was obtained 2-2.5 h after sudden death from unhospitalized men aged 40-60 years. Subendothelial intimal cells were isolated by dispersion with collagenase and cultured as described previously [3, 8]. The culture fluid contained medium 199, 2 mM glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin, 2.5 μg/ml of fungisone, and 10% delipidized embryonic calf serum (all reagents were from Gibco, England). Incorporation of ^{3}H-thymidine into DNA of the cultured cells and the cholesterol concentration in them were estimated as described previously [6, 8]. A 7-day culture of intimal cells was used; it was incubated with the test agents for 24 h. Since PGI_2 and TXA_2 are very unstable agents, their stable analogs – carbacycline and U46619 (Upjohn Co., USA) – were used. The numerical results were subjected to statistical analysis by the Student Fisher test and the nonparametric Wilcoxon–Mann–Whitney test.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the direction of action of carbacycline and U46619 on the principal characteristics of the functional state of the subendothelial intimal cells, namely the cholesterol concentration and incorporation of ^{3}H-thymidine into their DNA, was diametrically opposite. Whereas carbacycline in concentrations above 50 ng/ml caused a dose-dependent decrease in the cholesterol concentration and proliferative activity of the cells, thereby exhibiting antiatherosclerotic activity, U46619 provoked proliferation of the cells and accumulation of cholesterol in them very actively. Consequently, the shift of equilibrium between PGI_2 and TXA_2 toward the latter, which is considered to be one factor in the pathogenesis of disturbances of the coronary and cerebral circulation [4, 9], leads to the development of regional circulatory disturbances due not only to vasomotor and thromboembolic

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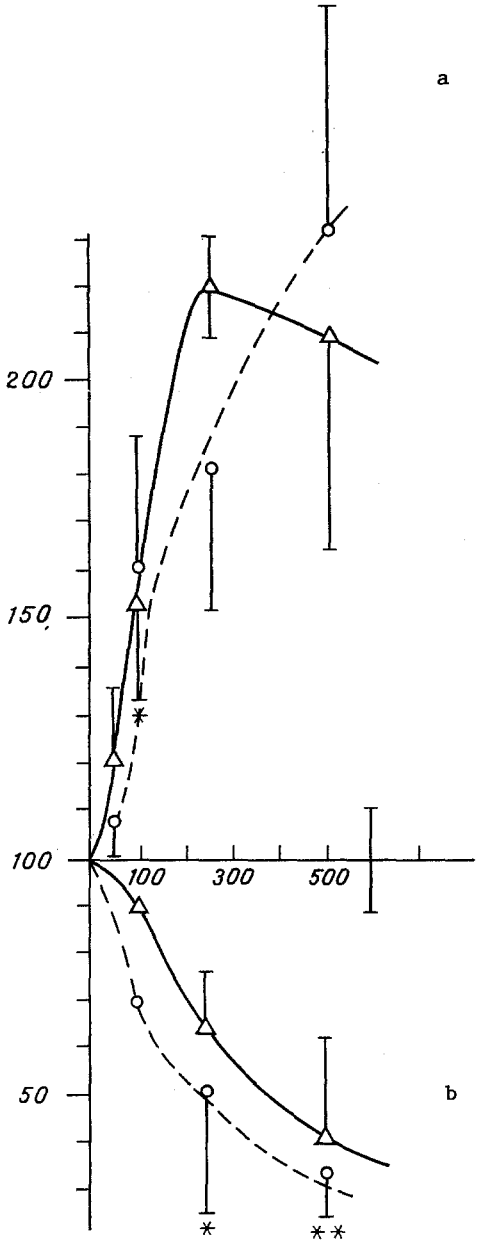

Fig. 1. Effect of U46619 (a) and carbacycline (b) on cholesterol concentration in intimal cells (continuous line) and ^{3}H-thymidine incorporation (broken line). Abscissa, concentration of agents (in mg/ml); ordinate, effect (in % of control).

disorders, but also to direct intensification of atherogenesis and associated changes in the morphologic and functional state of the circulatory bed. An imbalance between these agents is observed, as we know, in most patients with cardiovascular diseases [9]. In view of the data, it can be tentatively suggested that this kind of imbalance is one of the risk factors of atherosclerosis.

The well-defined direct action of eicosanoids on cells of the test culture provides good grounds for a search for approaches to its pharmacologic modulation. In this connection calcium antagonists, whose antiatherosclerotic effect has been described previously [6], were studied. The modulating action of verapamil, which blocks calcium transport through the plasma membrane, and of diltiazem, which acts mainly on calcium release from the intracellular depots, on the effects of PGI_2 and TXA_2 was studied. When these agents were used in relatively low concentrations, in which by themselves they had no significant antiatherosclerotic effect, their combinations in pairs (carbacycline—verapamil and carbacycline—diltiazem) sharply depressed both the cholesterol concentration in the cells and their pro-

TABLE 1. Interaction between Carbacycline and Calcium Antagonists on a Culture of Intimal Cells from the Human Aorta

Preparation	Cholesterol conc. µg/mg protein	^{3}H-thymidine incorp. counts/mg protein
control	61,2±9,3	255,4±39,0
Carbacycline (200 mg/ml)	54,0±6,2	212,8±33,6
Verapamil (10^{-5} M)	46,4±7,1	164,8±24,9*
Carbacycline +verapamil	22,2±2,1*	98,4±16,6*
Diltiazem (10^{-5} M)	57,0±10,3	192,9±47,6
Carbacycline +diltiazem	34,1±8,0*	76,8±21,1

Legend. Here and in Tables 2 and 3: *$p < 0.05$.

TABLE 2. Interaction between U46619 and Calcium Antagonists on a Culture of Intimal Cells of the Human Aorta

Preparation	Cholesterol conc. µg/mg protein	^{3}H-thymidine incorp. counts/mg protein
control	240,5±28,0	81,2±2,3
U46619 (250 ng/ml)	645,0±126,0*	125,8±7,8
Verapamil (10^{-5} M)	180,2±41,6	29,0±9,3*
U46619 + verapamil	292,2±56,4	31,2±3,3
Diltiazem (10^{-5} M)	220,0±47,2	37,9±10,7*
U46619 + diltiazem	340,1±82,9	50,6±10,6

TABLE 3. Effect of Lipo-Oxygenase Inhibitors on Cholesterol Accumulation and Proliferation of Intimal Cells of the Human Aorta

Preparation	Lowering of cholesterol conc. %	Lowering of ^{3}H-thymidine incorp. %
control	100	100
Compound 1 (10^{-4} M)	67,1±1,4*	45,7±0,7*
Compound 2 (10^{-4} M)	89,0±1,3*	55,7±0,3*

liferation (Table 1). Conversely, when combinations of these calcium antagonists with U46619 were investigated, both verpamil and diltiazem (the latter to a lesser degree) in low concentrations significantly limited the atherogenic effect of thromboxane (Table 2).

Thus calcium antagonists can be regarded as pharmacologic agents capable of modifying the effect of eicosanoids on atherogenesis, thereby abolishing unfavorable consequences of the development of an imbalance between PGI_2 and TXA_2 under pathological conditions. Besides the direct antiatherosclerotic action of calcium antagonists, the effect described above can be regarded as an important indication for their use in the treatment of atherosclerosis, even more since the modulating effect of calcium antagonists is exhibited in the presence of lower concentrations than their direct action on subendothelial cells.

Another possible approach to pharmacologic regulation of processes of atherogenesis is the effect of arachidonic acid on metabolic processes in the intimal cells. We accordingly studied the action of two compounds synthesized at the Institute of Fine Organic Chemistry, Academy of Sciences of the Armenian SSR, from this point of view: N-(4-hydroxy-3-methoxybenzylidine)phenethylamine (compound 1) and 4-hydroxy-3-methoxybenzaldehyde phenylhydrazone (compound 2). These agents are powerful inhibitors of lipo-oxygenase, blocking arachidonic acid metabolism by the lipo-oxygenase pathway [2]. As Table 3 shows, both compounds dose-dependently lowered the proliferative activity of the intimal cells and their concentrations of cholesterol esters. This effect may be linked with increased

intracellular synthesis of the main product of the cyclo-oxygenase pathway of arachidonic acid metabolism in the blood vessels, namely PGI_2 which, as was shown above, possesses an antiatherosclerotic action. This increase was caused by a decrease in consumption of arachidonic acid as a result of inhibition of lipo-oxygenase activity. On the other hand, we know that leukotrienes can be formed in the lipo-oxygenase pathway of arachidonic acid metabolism in blood vessels [7]. Since the latter, like TXA_2, when they act on the cell, cause the calcium level to rise, and their effect is blocked by calcium antagonists, it can be tentatively suggested that leukotrienes also possess more or less distinct "atherogenic" properties. Consequently, the effect of lipo-oxygenase inhibitors on intimal cells described above may be due also to inhibition of leukotriene formation in them.

The cascade of arachidonic acid metabolites is thus one element in the endogenous regulation of atherogenesis, and the direction of their influence is largely determined by equilibrium between functionally heterogeneous groups of eicosanoids. Their action is evidently based on an effect on calcium homeostasis of the cells. This applies not only to TXA_2 and leukotrienes, but also to PGI_2, which may lead to reduction of the cytoplasmic calcium concentration on account of elimination of calcium from the cells via the sodium-calcium antiport [1]. The positive interaction with calcium antagonists, preventing the entry of calcium into the cytoplasm, may also be connected with this component of this mechanism.

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EFFECT OF SUBCHRONIC ADMINISTRATION OF PHENAZEPAM AND SYNTHETIC ANTIOXIDANTS ON CEREBRAL CORTICAL SYNAPTIC MEMBRANE FUNCTION OF RATS EXPOSED TO LONG-TERM STRESS

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It is generally accepted that membrane lipids can actively influence the functional state of membrane-bound enzymes, such as Na, K-ATPase [10, 13], adenylate cyclase [9, 12], and monoamine oxidase (MAO) [14], which play an important role in synaptic transmission. Any modification to the lipid bilayer of biological membranes leads to a disturbance of one of the main functions of the lipids, which is to create a definite hydrophobic environment around the

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