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Pharmacological control of myocyte proliferation by different farnesyltransferase inhibitors

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The mevalonate (MVA) pathway plays an essential role in cell proliferation. In fact, from MVA derive not only cholesterol (important for membrane assembly) but also two terpenes (farnesol F-OH and geranylgeraniol GG-OH), utilized for post-translational modifications of cellular proteins, some of which are involved in cell duplication (ras, rho, rap). As we performed, before, several experiments in order to assess the antiproliferative properties of statins (competitive inhibitors of HMG-CoA reductase), we tested BZA-5B, a first generation benzodiazepine peptidomimetic (10-50 μM) farnesyltransferase inhibitor (FTI) and three second generation FTIs, (SCH 56582 (1-30 μM) is a nonpeptidic non-sulphydril tricyclic FTI, while PD 152440 and PD 169451(1-50 μM) are peptidomimetic) on rat smooth muscle cell proliferation, evaluated both by cell count and ^{3}H-thymidine incorporation into nuclear DNA. All of them are able, even if at different concentrations, to dose-dependently decrease this process, without affecting cholesterol biosynthesis. Moreover, the second generation molecules seem to act in the middle-G1 phase of the cell-cycle (time-course experiments). Their inhibitory effect is not completely prevented by the addition of MVA, F-OH and GG-OH, as in the presence of statins, but only in part (BZA-5B) or not at all (PD 152440, PD 169451 and SCH 56582), suggesting probably different mechanisms of action of these molecules. To assess if their inhibitory effect on cell growth was related to the ability in interfering with protein prenylation, we evaluated BZA-5B, SCH 56582 and PD 152440 on ^{3}H-F-OH and ^{3}H-GG-OH incorporation into specific cellular proteins (41-72 KDa and 21-28 KDa), known to be physiologically prenylated. The obtained results strongly suggest the relevance of the MVA pathway in the control of cell growth, and thanks to the property of these molecules to directly influence cell proliferation, probably through a modulation of protein prenylation, they might become of particular interest, at least *in vitro* in atherogenesis and tumours.

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A RABBIT MODEL OF ATHEROSCLEROSIS WHICH MIMICS HUMAN SOFT, LIPID-RICH, PLAQUES

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Many animal models have been generated over the years, to study the progression of neointimal proliferation and its pharmacological prevention. In these models, however, the intimal thickening is primarily consisting of smooth muscle cells and so resembling to what observed during restenosis rather than atherosclerosis. In our study carotid arteries of NZ white rabbits were injured by electric current, using a bipolar microcoagulator. After surgery, animals were fed a 1.5% cholesterol diet; at different time points ranging from 3 days to three months rabbits were sacrificed and hystological analysis of carotids were performed. Moreover, before sacrifice, at the latest time points (60 and 90 days) carotids were analyzed angiografically by intravascular ultrasound (IVUS) using a 3.5 F catheter. Electrical injury destroyed all medial smooth muscle cells and denuded the injured segment of intact endotelium. After 15 days repopulation of the media was observed mostly by lipid rich macrophages and neointimal formation started. At longer time points macrophages in the media were progressively replaced by smooth muscle cells and macrophages densely packed with lipid droplets predominated within the neointimal formation. Intravascular ultrasound performed at 60 and 90 days after injury detected poorly echo-reflective intimal thickening resembling to human atheromatous plaques characterized by a thin fibrous cap and heavily infiltrated foam cells, frequently unstable and prone to rupture. In conclusion, a rabbit model of atherosclerosis was generated in which the formation of a lipid rich plaque is observed, thus providing a useful animal model for pharmacological treatment aimed to stabilize or promote regression of vulnerable atherosclerotic plaques.

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A NOVEL PATHWAY FOR FIBROBLASTS ACTIVATION AND IL-6 RELEASE: POSSIBLE IMPLICATIONS IN ATHEROMA FORMATION

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The physiological role of of purinergic receptors (PR) of the P2Y and P2X subtypes, activated by ATP, is still unknown, although their involvement in many cellular functions (proliferation, cytokine secretion, cell death) has been proposed. Little information is available about their presence in human cells. Human fibroblasts (HF) share several features with smooth muscle cells and are directly involved in chronic degenerative diseases such as atherosclerosis; moreover, they are exposed to ATP released by endothelium after a shear stress and by platelets during thrombus formation. We have investigated responses to extracellular nucleotides in HF, evaluating: a) immunoreactivity by Western blot with a specific anti $P2X_7$ antiserum; b) stimulation of plasma membrane depolarization (PMDep) by benzoyl-benzoyl-ATP (BzATP); c) changes in plasma permeability by fluorescent tracers YO-PRO and lucifer yellow (Ly); d) activation of Ca^{2+} influx by intracellular trapping of Fura2-AM and e) IL-6 release by immunoassay. HF express an atypical $P2X_7$ receptor, since stimulation with ATP or BzATP caused a slow but relevant uptake of Ly and no significant cytotoxicity (LDH release <3%). BzATP also induced a striking increase in cytoplasmic microvesicle formation not due to increased pinocytosis and fully reversible upon nucleotide removal. We labelled Bz-ATP-stimulated HF with a monoclonal antibody directed against a specific Golgi marker, observing an increased staining of the Golgi apparatus. Two known blockers of P2X receptors, oxoATP and PPADS, fully inhibited the morphological changes and partially (by 50%) the ion fluxes. The residual rise in intracellular Ca^{2+} and membrane depolarization was dependent on activation of a P2Y-type receptor. The pharmacological sequence for Ca^{2+} release was: CTP ≥ATP >>BzATP =GTP =UTP =ATPγS (278 ≥240 >>160 =163 =170 =152 nM). The pharmacological sequence for PMDep was BzATP >CTP >>ATP =ATPγS =2MeATP (100 >52 >>11 =12 =12%). Moreover, ATP triggered a large release of the pro-inflammatory cytokine IL-6 in cells pre-treated with PMA. We suggest that HF express a P2Y receptor and an atypical $P2X_7$ ATP-gated channel, involved in release of IL-6. This pathway could be an additional target for pharmacological intervention in atherosclerotic disease.

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RELATIONSHIP BETWEEN, CELL GROWTH, CHOLESTEROL ESTERIFICATION AND MDR P-GLYCOPROTEIN INHIBITORS.

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Recently, multidrug resistance (MDR) P-glycoproteins have been involved in the cholesterol transport from plasma membrane to the ER for esterification. Previous studies from our laboratory have shown that the MDR inhibitors progesterone (PG) and dehydroepiandrosterone (DHEA), decrease in a dose-dependent manner both cholesterol esterification and cell growth in different tumor cell lines. We have suggested that MDR1 by modulating intracellular cholesterol ester levels may influence the rate of cellular growth. To further investigate the mechanisms by which such effect is produced, we have studied cholesterol metabolism, cell growth and P-glycoprotein activity in mitogen-stimulated lymphocytes and during the growth of CEM, MOLT4 and L1210 cell lines in presence of PG (40μM), (DHEA 60μM) and RU-486 (2mM). The results have shown that the inhibition of P-glycoprotein activity by PG and DHEA was accompanied by reduced intracellular cholesterol esterification and cell growth in all cell lines, either after a short (3 hrs) or a prolonged incubation (48 hrs) with the drugs. In mitogen-stimulated lymphocytes the two hormones were able to exert an inhibitory effect on cholesterol esterification and cell growth, but not on P-glycoprotein activity. RU-486 inhibited P-glycoprotein activity without affecting cholesterol esterification and cell growth in both, cell lines and mitogen-stimulated lymphocytes. However, RU-486, besides its antagonistic activity on PG receptors, failed to prevent the cellular effects of PG. These data add more evidence for the possible involvement of cholesterol esterification in the modulation of rate of cell growth. They, moreover, suggest that in the regulation of intracellular cholesterol metabolism different mechanisms other than MDR1 P-glycoprotein pumps may be involved.